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Reactivity studies of 3-alkoxy-7-amino-4-chloroisocoumarins (β-amyloid peptide inhibitors) *versus* different classes of amines

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3-Alkoxy-7-amino-4-chloroisocoumarins have been shown to lower the β-amyloid secretion (a major component of the senile plaques involved in Alzheimer's disease). This paper reports the characterization of new adducts resulting from the reaction between the isocoumarin synthon and different classes of amines. This study allows on the one hand, a better understanding of the biological molecular processes by which such isocoumarin derivatives may interact with enzyme nucleophiles and, on the other hand, brings out the chemical potential of these synthons to generate new polycyclic derivatives.

Introduction

We have recently reported the remarkable properties of the 3-alkoxy-7-amino-4-chloroisocoumarin scaffold **I** (Fig. 1) to inhibit the secretion of the β-amyloid peptide, a major component of the senile plaques involved in Alzheimer's disease.[1,2] These new isocoumarin analogues appear to be very promising compounds, or at least useful tools, in order to help the understanding of this pandemic neurodegenerative disease. These isocoumarin derivatives are clearly distinct from the β-amyloid peptide secretion inhibitors described so far,[3] mainly because they are not peptides or pseudopeptides, and also because they are the first inhibitors to lower the β-amyloid secretion without affecting the Notch signaling pathway.[1,2] This biological property is of interest for Alzheimer's disease therapy since the Notch pathway is notably involved in embryogenesis.

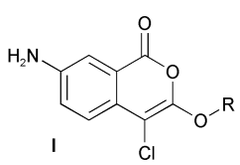
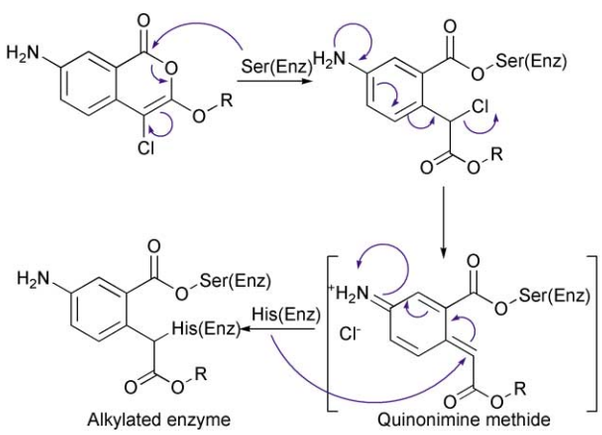


Fig. 1

The current hypothesis that supports the observed biological activity of these isocoumarin derivatives is based on their chemical reactivity towards various nucleophiles. These derivatives have mainly been shown to act as protease suicide-inhibitors according to a mechanism underlined in Scheme 1, in which the quinonimine methide intermediate is believed to play a major role in enzyme inactivation.[4] Consequently, one of the major obstacles for the use of such isocoumarin derivatives as possible drug candidates for Alzheimer's disease, could be their high reactivity *versus* numerous proteins, implying a lack of biological specificity.

In order to obtain a better understanding of why and how such isocoumarin derivatives react with biological nucleophiles, we studied the chemical reactivity of these compounds towards three classes of amines which mimic the nucleophile functions included in enzymes or other biological residues. Isolation and identification of the obtained adducts brought out the high degree of versatility of such 3-alkoxy-7-amino-4-chloro-isocoumarin analogues. Since the synthesis of 5-amino-4-chloro-3-substituted-isocoumarin has been already reported,[5]



Scheme 1

we describe in this paper the reactivity of 3-alkoxy-7-amino-4-chloroisocoumarins towards a primary (benzylamine), a secondary (pyrrolidin-3-ol) and a heteroaromatic (pyridine) amine.

Results

Reactivity of 7-amino-4-chloro-3-alkoxyisocoumarins towards benzylamine

We first considered the reactivity of benzylamine with 7-amino-4-chloroisocoumarin (**I**). When isocoumarin **1** was treated with 3 equivalents of benzylamine in acetonitrile at room temperature for a few hours, a new major product **2** was detected by TLC and isolated and purified in 64% yield by flash chromatography on silica gel. Structural identification of compound **2** was at first surprising, since its structure included three carbonyl functions. To explain the formation of **2**, we proposed the following mechanism outlined in Scheme 2. The isocoumarin lactone ring was first opened by the benzylamine primary amine resulting in the formation of an unstable α-chloro ester. This compound underwent a second nucleophilic attack by the same benzylamine amino group, which reacted with the ester function leading transitorily to the unstable 4-chloroisoquinoline-1,3-dione derivative. Because of the presence of the amine group at the 7-position, the 7-amino-4-chloroisoquinoline-1,3-dione rearranged to form an unstable

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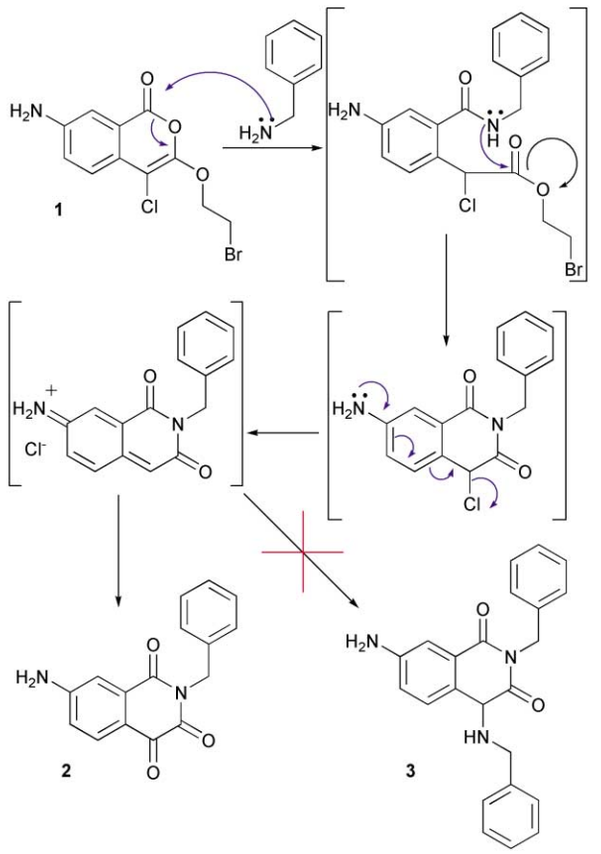


Scheme 2

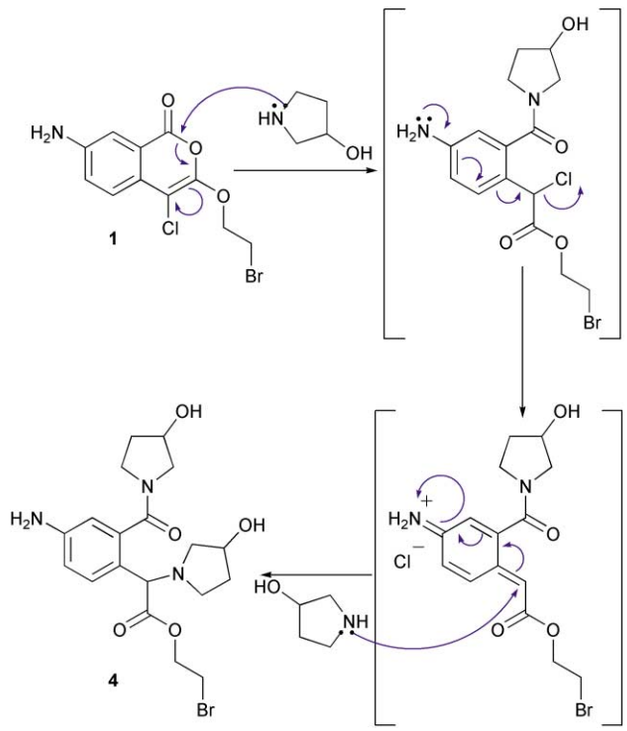
reactive quinonimine intermediate. Surprisingly, the benzylamine nucleophilic attack on the electrophilic carbon at the 4-position did not occur to give **3**, instead under these experimental conditions, the quinonimine intermediate was oxidized, leading to the isoquinoline-1,3,4-trione compound **2** isolated in 64% yield.

Reactivity of 7-amino-4-chloro-3-alkoxyisocoumarins towards pyrrolidin-3-ol

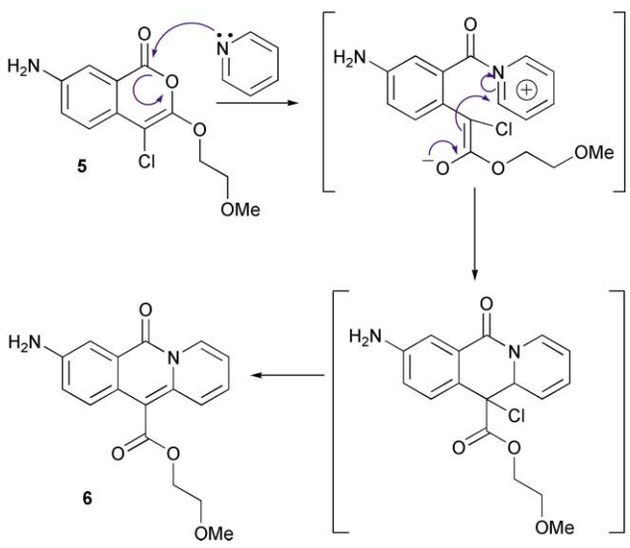
When isocoumarin **1** was treated with pyrrolidin-3-ol under the same experimental conditions, a new major product **4**, detected by TLC, was isolated in 43% yield after purification by flash chromatography. The suggested mechanism leading to this adduct is outlined in Scheme 3. At first, opening of the isocoumarin lactone ring was achieved by the secondary amine of the pyrrolidine, leading to the unstable α-chloro ester which rearranged into an unstable and very reactive quinonimine methide intermediate. Then the highly electrophilic carbon in the α-position of the ester group underwent a second attack by pyrrolidin-3-ol leading to diadduct product **4**.

Reactivity of 7-amino-4-chloro-3-alkoxyisocoumarins towards pyridine

When isocoumarin **5** was heated in pyridine at 90 °C for a few hours, a new major product **6** was detected by TLC, isolated and purified in 42% yield by flash chromatography. Compound **6** was fully characterized by LCMS, ^{1}H and ^{13}C NMR and elemental analysis. The proposed mechanism leading to this tricyclic adduct formation is described in Scheme 4. The suggested mechanism involved the nucleophilic attack of the isocoumarin lactone function by the heteroaromatic amine of the pyridine moiety,[6] leading to an unstable intermediate which immediately rearranged into the tricyclic derivative dihydropyrido[1,2-*b*]isoquinolin-6-one. Subjected to the experimental conditions (90 °C), this latter tricyclic compound



Scheme 3



Scheme 4

underwent a dehydrohalogenation reaction leading to the stable and fully characterized conjugated product **6**.

Discussion

The obtained results can be discussed at the chemical or biological level. Firstly, at the chemical level, the above study shows that 3-alkoxy-7-amino-4-chloroisocoumarin derivatives represent attractive and versatile synthetic building-blocks since they allow access, through original mechanisms, to a diversity of products, mainly polyheterocycles. The first step common to all the amines studied involves opening of the lactone ring by the nucleophile. Then, depending on the nature of the amine, the structures of the isolated products and their formation mechanisms differ. In the case of pyrrolidin-3-ol, lactone ring opening results in the formation of an α-chloro ester adduct which rearranges to an unstable quinonimine methide which presents a very electrophilic center at the α-position of the ester function. This electrophilic center represents a new target for

a second nucleophilic attack by another pyrrolidine moiety, leading to product **4**. In contrast, in the case of benzylamine, the α-chloro ester intermediate preferentially undergoes nucleophilic attack at the carbonyl bond, giving rise to an α-chloroisoquinoline intermediate which rearranges into the quinonimine moiety. In this case, the carbon atom in the 4-position appears to be a weaker electrophilic center, which disfavours nucleophilic attack by a second benzylamine moiety. However, the unstable quinonimine moiety, highly sensitive to oxidation, was oxidized into the isoquinoline-1,3,4-trione **2**. Similar oxidations have been reported in the literature,[7] indeed the oxidation process seems to occur during the purification step on silica gel column chromatography which is carried out in the presence of air. In the case of pyridine, the α-chloro ester intermediate doesn't present an electrophilic center at the α-position of the ester group, but a nucleophilic center which reacts with the carbon atom in the 2′-position of the pyridinium moiety, leading to the tricyclic analogue **6**. It can be underlined that besides the isolated compounds, some decomposition of the starting material is also observed, which explains the relatively modest yield of the reaction.

In conclusion, 3-alkoxy-7-amino-4-chloroisocoumarin represents a new synthon which upon reaction with amines allows the generation of new substituted heterocycles such as 6-oxo-6*H*-pyrido[1,2-*b*]isoquinoline or isoquinoline-1,3,4-trione. The structural diversity of the adducts obtained seems to be under the control of the different electrophilicity or nucleophilicity states of the carbon at position 4 of the isocoumarin nucleus, induced by the various nucleophilic amine moieties.

Biologically speaking, the results obtained seem to confirm the high reactivity of 3-alkoxy-7-amino-4-chloroisocoumarin *versus* nucleophiles. Such high and unspecific reactivity with various biological nucleophiles, constitutive of enzymes or receptors, does not appear consistent with the possible use of such isocoumarin derivatives as therapeutic drugs.

Experimental section

All common chemicals and solvents were reagent grade or better. The purity of each compound was checked by ^{1}H and ^{13}C NMR spectroscopy, mass spectroscopy, thin-layer chromatography, melting point, and elemental analysis and results were consistent with the proposed structures. The ^{1}H NMR were recorded on either a Bruker AC-300 MHz or a Bruker AMX-400 instrument, and ^{13}C NMR on a Bruker AMX-72 MHz. *J* values are given in Hz. Elemental analyses were performed by Service Central d'Analyses du CNRS (Venaison, France) and were within ± 0.3% of the theoretical values. Thin-layer chromatographic analyses (TLC) were conducted on silica gel plates 0.2 mm thick ($60F_{254}$ Merck) with various mixtures as eluent. Preparative flash column chromatographies were carried out on silica gel (230–240 mesh, G60 Merck). Analytical purity was assessed by LCMS using a Waters 2790 system. Low resolution mass spectra were recorded on a Micromass ZMD mass spectrometer. Mass spectra were acquired under electrospray ionisation (ESI).

7-Amino-2-benzylisoquinoline-1,3,4-trione (2). To a solution of **1** (285 mg, 0.9 mmol) in MeCN (10 ml) was added benzylamine (2.8 mmol) and the reaction mixture was stirred at room temperature for 9 hours, and concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was washed with 5% $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and the solvent was removed under vacuum to give a residue which was purified by flash chromatography on silica gel to give **2** (161 mg, 64%). R_f (EtOAc–hexane [1 : 1]) 0.36; elemental anal. found: 68.59; H, 4.31; N, 9.98%. Calc. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99%; δ_H (400 MHz; $CDCl_3$) 7.82 (d, 1H, *J* 8.6, 8-H), 7.34 (d, 1H, *J* 2.4, 5-H), 7.33–7.24 (m, 3H, Ph), 6.94 (dd, 1H, *J* 2.4 and 8.6, 6-H), 6.93 (m, 2H, Ph), 5.06 (s, 2H, C*H*$_2$–Ph); δ_C (72 MHz; $CDCl_3$) 170.98, 162.72, 158.29, 155.84, 136.62, 131.72, 130.02, 128.11, 127.37, 126.88, 118.86, 117.79, 112.09, 43.29; *m*/*z* (ESI) 281.

[4-Amino-2-(3-hydroxypyrrolidine-1-carbonyl)phenyl]-(3-hydroxypyrrolidin-1-yl)acetic acid 2-bromoethyl ester (4). To a solution of **1** (285 mg, 0.9 mmol) in MeCN (5 ml) was added pyrrolidin-3-ol (2.8 mmol) and K_2CO_3 (3.8 mmol). The reaction mixture was stirred at room temperature, and concentrated under reduced pressure. The residue was solubilized in EtOAc and the organic layer was washed with 5% $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and the solvent was removed under vacuum to give a residue which was purified by flash chromatography on silica gel to give **4** (172 mg, 43%). R_f (MeCN–MeOH [1 : 4]) 0.41; elemental anal. found: 49.89; H, 5.79; N, 9.30%. Calc. for $C_{19}H_{26}BrN_3O_5$: C, 50.01; H, 5.74; N, 9.21%; δ_H (300 MHz; MeOD) 7.55 (m, 1H, *J* 8.5, NH_2–C=C*H*–C–CO–N<), 6.89 (dd, 1H, *J* 2.4 and 8.5, NH_2–C–C*H*=CH), 6.73 (d, 1H, *J* 2.4, NH_2–C–CH=C*H*), 4.64–4.45 (m, 5H, 2 × C*H*–OH, O–C*H*$_2$–CH$_2$–Br, >N–C*H*–CO–O–), 3.89–3.61 (m, 4H, O–CH$_2$–C*H*$_2$–Br, CO–N–C*H*$_2$–CH$_2$–CH–OH), 3.45–3.35 (m, 4H, CO–N–C*H*$_2$–CH–OH, Ph–CH–N–C*H*$_2$–CH$_2$–CH–OH), 3.03–2.82 (m, 2H, Ph–CH–N–C*H*$_2$–CH–OH), 2.31–2.09 (m, 4H, 2 × CH$_2$–C*H*$_2$–CH–OH); δ_C (72 MHz; $CDCl_3$) 173.41, 167.23, 145.52, 136.86, 130.40, 123.24, 116.09, 114.62, 70.14, 68.65, 68.13, 59.87, 56.12, 53.57, 42.65, 39.88, 32.41, 31.12, 30.69; *m*/*z* (ESI) 456 (458).

8-Amino-6-oxo-6*H*-pyrido[1,2-*b*]isoquinoline-11-carboxylic acid 2-methoxyethyl ester (6). Compound **5** (1 g, 3.7 mmol) was solubilized in pyridine (11 ml), the reaction mixture was stirred at 90 °C for 7 hours, and concentrated under reduced pressure. The residue was solubilized in DCM and the organic layer was washed with 5% $NaHCO_3$ aqueous solution, dried over $MgSO_4$, and the solvent was removed under vacuum to give a residue which was purified by flash chromatography on silica gel to give **6** (480 mg, 42%). R_f (EtOAc–hexane [2 : 1]) 0.47; mp 110 °C; elemental anal. found: 65.39; H, 5.13; N, 8.98%. Calc. for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97%; δ_H (300 MHz; $CDCl_3$) 8.88 (d, 1H, *J* 7.7, 1-H), 8.00 (d, 1H, *J* 9.1, 10-H), 7.92 (d, 1H, *J* 9.5, 4-H), 7.81 (d, 1H, *J* 2.6, 7-H), 7.20 (dd, 1H, *J* 2.6 and 9.1, 9-H), 7.04 (qd, 1H, *J* 1.1 and 9.5, 2-H), 6.67 (td, 1H, *J* 1.1 and 7.7, 3-H), 4.62 (t, 2H, *J* 4.5, O–C*H*$_2$–CH$_2$–OMe), 4.07 (br s, 2H, N*H*$_2$), 3.79 (t, 2H, *J* 4.5, O–CH$_2$–C*H*$_2$–OMe), 3.47 (s, 3H, Me); δ_C (72 MHz; $CDCl_3$) 168.26, 159.39, 145.43, 136.51, 127.84, 127.50, 127.14, 126.82, 124.37, 124.32, 121.80, 113.15, 110.42, 104.65, 71.11, 64.92, 59.73; *m*/*z* (ESI) 313.

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