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Simultaneously electrogenerated diene and dienophile : a unique
access to novel polyfunctionalized 1,4-benzoxazine derivatives as
neuroprotective agents

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Abstract : New insights into the scope of a multistep one-pot electrochemical synthesis of polyfunctionalized 2-alkylamino-1,4-benzoxazine derivatives are delineated. This cascade sequence, wherein both cycloaddition partners are generated *in situ*, at room temperature, under metal-free conditions, should be useful to generate libraries of heterocycles which constitute the molecular framework of medicinally relevant compounds. In this respect, 2-alkylamino-1,4-benzoxazine derivatives proved to be potent neuroprotective agents *in vitro*, on immortalized HT22 hippocampal cell cultures, and *in vivo*, in an animal model mimicking the cerebral palsy in human newborns.

Keywords : anodic oxydation, enamine, azaquinone, cascade reactions, neuroprotective agents

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1. Introduction

The electrochemical methodology becomes more and more attractive compared to conventional chemical procedures, due to increasing environmental problems. It is especially suited for preventive environmental protection because the practically mass-free electrons are used as reagents. It allows indeed the formation of organic compounds without the production of ecologically critical waste which has been to be disposed [1]. The reaction conditions are usually mild using ambient temperature, normal pressure and often alcoholic solvents.

In particular, the electrochemical methods can be used to generate highly reactive species which can be engaged in synthetically useful follow-up reactions [2-8]. Among them, electrochemically induced cycloadditions have proved to be a promising tool for organic chemistry [9-15]. Various unstable electrogenerated dienes [16-19] or dienophiles [20] have been successfully used in Diels-Alder reactions leading to the desired cycloadduct in high yield, with a good stereocontrol.

Recently, we reported the high catalytic performance of the redox mediator 3,4-azaquinone $\mathbf{1}_{ox}$ in the chemoselective indirect electrochemical oxidation of primary aliphatic amines, under metal-free conditions [21]. The catalytic cycle produced the reduced catalyst $\mathbf{1}_{red}$ and an alkylimine as the product of amine oxidation (Scheme 1). However, in the case of $(R^1)_2CHCH_2NH_2$ amines, the catalytic process ceased after a few turnovers, as the catalyst was trapped through [4+2] cycloaddition reaction with the simultaneously electrogenerated tautomeric enamine form of the alkylimine extruded during the catalytic process. This unexpected reaction allowed the rapid construction of polyfunctionalized 1,4-benzoxazine derivatives [22]. This cascade sequence, wherein both cycloaddition partners are generated *in situ*, at room temperature, under metal free conditions, allowed the inverse-electron-demand Diels-Alder (IEDDA) reaction of an *o*-quinone imine diene with a secondary alkylamine dienophile, two chemically nonaccessible unstable entities. Because the reactivity of *o*-

quinone imine as an azadiene for IEDDA reactions represented uncharted terrain, though similar reactions with *o*-quinone monoimides [23-28] and *o*-quinone monooximes [29] are known, we then decided to explore further its potential for [4 +2] cycloaddition reactions with variously substituted enamines. Especially, to increase the molecular diversity, we attempted to generate enamines in which the substituents on the amino group were different from those linked to the double bond. For this purpose, the amine $(R^1)_2CHCH_2NH_2$ was catalytically oxidized by 3,4-azaquinone **1_{ox}** in the presence of a second primary aliphatic amine R^2NH_2 (Scheme 2). Then, we showed that the **1_{ox}**-mediated cascade reactions led to the construction of complex 1,4-benzoxazine derivatives **2**, in a regiospecific manner, and allowing diastereospecific heterocyclic annellation [30].

In this paper, we pursue our investigations concerning the extension of the multistep one-pot electrochemical synthesis of 2-alkylamino-1,4-benzoxazine derivatives, because this cascade reaction should be useful to generate libraries of heterocycles which constitute the molecular framework of medicinally relevant compounds. Furthermore, as a result of their structural similarity with a series of topologically different 8-alkylamino-1,4-benzoxazine neuroprotective agents published earlier [31, 32], we report briefly, for comparison, the results of *in vitro* and *in vivo* assays of some substituted 2-alkylamino-1,4-benzoxazine derivatives.

2. Experimental

Chemicals were commercial products of the highest available purity and were used as supplied. Reduced catalysts **1_{red}** and **3_{red}** were synthesized as earlier reported [33]. Compounds **2b-f** were prepared according to our published procedures [30]. All apparatus, cells and electrodes were identical with those described previously [34]. The experimental

conditions for *in vitro* and *in vivo* biological assays are extensively detailed in references 31 (immortalized HT22 hippocampal cell cultures) and 32 (neonatal mice).

[(R,S)-3,3-dimethyl-5-hydroxy-2-isobutylamino-3,4-dihydro-2H-1,4-benzoxazin-6-yl](phenyl)methanone 2a. 3,4-alkylaminophenol **3a_{red}** (142.5 mg, 0.5 mmol) and isobutylamine (1.0 mL, 10 mmol) were added to a 0.02 M solution of tetraethylammonium perchlorate (TEAP) (1.15 g, 5 mmol) as the supporting electrolyte in MeOH. The resulting solution was then oxidized under nitrogen, at room temperature, at a mercury pool whose potential was fixed at 0.0 mV vs SCE (initial current 50 mA). After exhaustive electrolysis (8h, n=16), that is, when a negligible current was recorded (1 mA), the solution was neutralized with dry ice and the solvent was removed under reduced pressure. The brown oil residue was then poured into diethyl ether (20 mL). Insoluble TEAP was filtered off and the filtrate was evaporated under reduced pressure, at 30°C. Flash chromatography of the residue on silica gel with toluene as the eluent afforded 2-alkylamino-1,4-benzoxazine **2a** in 62% yield (110 mg, 0.31 mmol), together with 2-hydroxy-1,4-benzoxazine **4** in 7% yield (10.5 mg, 0.03 mmol), as yellow solids. Spectroscopic data for products **2a** and **4** have been reported earlier [22, 30].

[1-hydroxy-5a-morpholino-5a,6,7,8,9,9a-hexahydro-5aH-phenoxazin-2-yl](phenyl)methanone 5. 3,4-Aminophenol **1_{red}** (114.5 mg, 0.5 mmol) and 1-morpholino-1-cyclohexene (0.43 mL, 2.5 mmol) were added to a 0.02 M solution of TEAP (1.15 g, 5 mmol) as the supporting electrolyte, in MeOH. The resulting solution was then oxidized under nitrogen, at room temperature, at a mercury pool whose potential was fixed at + 50 mV vs SCE (initial current 10 mA). After exhaustive electrolysis (6h, n=2), that is, when a negligible current was recorded (0.5 mA), the solution was treated according to the above mentioned procedure. Flash chromatography of the residue on silica gel with toluene/acetone 98/2 v/v as the eluent

afforded the expected phenoxazine derivative **5** in 50% yield (98.5 mg, 0.25 mmol) as a yellow solid (mixture of two unassigned diastereoisomers A and B in a 2:1 ratio).

Diastereoisomer A: ^1H NMR (300 MHz, CDCl_3) δ : 1.40-2.10 (m, 8H), 2.75-2.85 (m, 4H), 3.50-3.60 (m, 1H), 3.65-3.75 (m, 4H), 3.97 (s, 1H), 6.35 (d, $J = 9\text{Hz}$, 1H), 6.95 (d, $J = 9\text{Hz}$, 1H), 7.40-7.60 (m, 3H), 7.70 (d, $J = 8\text{Hz}$, 2H), 12.7 (s, 1H).

Diastereoisomer B: ^1H NMR (300 MHz, CDCl_3) δ : 1.40-2.10 (m, 8H), 2.75-2.85 (m, 4H), 3.50-3.60 (m, 1H), 3.65-3.75 (m, 4H), 3.92 (s, 1H), 6.45 (d, $J = 9\text{Hz}$, 1H), 7.05 (d, $J = 9\text{Hz}$, 1H), 7.40-7.60 (m, 3H), 7.70 (d, $J = 8\text{Hz}$, 2H), 12.78 (s, 1H).

^{13}C NMR (mixture of A and B) (75 MHz, CDCl_3) δ : 20.6, 22.3, 22.5, 23.7, 27.7, 28.7, 29.3, 33.8, 44.6, 48.2, 55.1, 67.5, 91.7, 96.5, 108.2, 108.8, 112.5, 121.1, 121.5, 123.6, 125.3, 128.1, 128.2, 128.9, 131.2, 131.4, 138.1, 138.6, 148.0, 148.9, 151.9, 152.1, 200.5, 200.6.

MS (Desorption Chemical Ionization) m/z 395 (MH^+).

[1,5a-dihydroxy-5a,6,7,8,9,9a-hexahydro-5aH-phenoxazin-2-yl](phenyl) methanone 6. 3,4-Aminophenol **1**_{red} (114.5 mg, 0.5 mmol) and 1-pyrrolidino-1-cyclohexene (0.41 mL, 2.5 mmol) were added to a 0.02 M solution of TEAP (1.15 g, 5 mmol) as the supporting electrolyte in MeOH. The resulting solution was then oxidized under nitrogen, at room temperature, at a mercury pool whose potential was fixed at 0.0 mV vs SCE (initial current 37 mA). After exhaustive electrolysis (3h, $n=2.2$), that is, when a negligible current was recorded (1 mA), the solution was treated according to the above mentioned procedure. Flash chromatography of the residue on silica gel with toluene/acetone 98/2 v/v as the eluent afforded the phenoxazine derivative **6** in 55% yield (89.4 mg, 0.275 mmol) as a yellow solid (mixture of two unassigned diastereoisomers A and B in a 2:1 ratio).

Diastereoisomer A: ^1H NMR (300 MHz, CDCl_3) δ : 1.40-2.05 (m, 7H), 2.25-2.35 (m, 1H), 3.00 (dd, $J = 4\text{ Hz}$, $J = 11\text{ Hz}$, 1H), 4.00 (s, 1H), 4.35 (s, 1H), 6.45 (d, $J = 9\text{Hz}$, 1H), 7.08 (d, $J = 9\text{Hz}$, 1H), 7.40-7.60 (m, 3H), 7.70 (d, $J = 8\text{Hz}$, 2H), 12.78 (s, 1H).

Diastereoisomer B: ^1H NMR (300 MHz, CDCl_3) δ : 1.40-2.05 (m, 7H), 2.25-2.35 (m, 1H), 3.30 (dd, $J = 4$ Hz, $J = 11$ Hz, 1H), 4.10 (s, 1H), 4.35 (s, 1H), 6.45 (d, $J = 9$ Hz, 1H), 7.02 (d, $J = 9$ Hz, 1H), 7.40-7.60 (m, 3H), 7.70 (d, $J = 8$ Hz, 2H), 12.72 (s, 1H).

^{13}C NMR (mixture of A and B) (75 MHz, CDCl_3) δ : 22.3, 22.4, 23.6, 23.9, 28.7, 29.3, 33.7, 35.7, 54.1, 55.1, 95.9, 96.5, 108.6, 108.8, 112.8, 113.0, 119.5, 121.5, 124.5, 125.4, 128.2, 128.9, 131.4, 131.5, 138.2, 138.3, 146.8, 148.1, 152.1, 152.4, 200.5.

MS (Desorption Chemical Ionization) m/z 326 (MH^+).

3. Results and discussion

3.1. Chemical Investigations

3.1.1. Attempted Electrosynthesis of *N*-substituted-2-alkylamino-1,4-benzoxazine Derivatives

To extend the scope of the reaction, we attempted to synthesize 2-alkylamino-1,4-benzoxazine derivatives bearing a substituent on the nitrogen atom of the oxazine ring. For this purpose, we envisioned a modification of our earlier reported procedure [22], in which the 3,4-aminophenol $\mathbf{1}_{\text{red}}$ was replaced by a 3,4-alkylaminophenol $\mathbf{3}_{\text{red}}$ ($\text{R} = \text{Bu}^i$, Pe^i or 2-hydroxyethyl, Scheme 3). Under the optimum conditions previously reported [30], the cyclic voltammogram of compound $\mathbf{3a}_{\text{red}}$ (2 mM, $\text{R} = \text{Bu}^i$), in deaerated MeOH containing tetraethylammonium perchlorate as the supporting electrolyte (20 mM) and an excess of isobutylamine (40 mM), showed, at a dropping mercury electrode, an oxidation peak Pa due to a diffusion-controlled two-electron process at -80 mV vs SCE, the sweep rate being 0.5 Vs^{-1} . As can be seen in Figure 1, a cathodic peak Pc appeared on the reverse sweep at -180 mV vs SCE, illustrating the partial reversibility of the two-electron transfer that could be assigned to the 3,4-alkylaminophenol $\mathbf{3a}_{\text{red}}$ /3,4-alkyliminoquinone $\mathbf{3a}_{\text{ox}}$ redox couple. However, the redox potential E'° could not be accurately evaluated under our experimental conditions, as the system (Pa, Pc) did not fulfill all the diagnostic criteria required for a

reversible process, at least when v was $\leq 500 \text{ V s}^{-1}$: the ratio of the height of Pa over that of Pc never reached unity ($i_{\text{Pc}}/i_{\text{Pa}} \sim 0.7$) and the value of $E_{\text{Pa}} - E_{\text{Pc}}$ (E being the peak potential) was found to be higher than 30 mV. Note that, in the reverse sweep, a second reduction peak Pc' was recorded at a more negative potential (- 1750 mV vs SCE), due to the irreversible two-electron reduction of the carbonyl group of the benzophenone skeleton [30].

When the controlled potential of the mercury pool was fixed at 0.0 mV vs SCE, that is at a potential for which $\mathbf{3a}_{\text{red}}$ could be oxidized to the alkyliminoquinone form $\mathbf{3a}_{\text{ox}}$ (Figure 1), the anodic current remained unchanged for a certain time, consistent with steady state catalytic behavior. Accordingly, a value of 16 was found for the total number of electrons (n) transferred per molecule of $\mathbf{3a}_{\text{red}}$ in the catalytic process (8 turnovers). Further, the catalytic process ceased and substituted 2-alkylamino-1,4-benzoxazine $\mathbf{2a}$ could be isolated from the exhaustively oxidized solution in 62% yield, together with 7% of the accompanying 2-hydroxy by-product $\mathbf{4}$ resulting from the conversion of $\mathbf{2a}$ on silica gel [30]. Whatever the nature of the 3-alkylamino chain of the starting material $\mathbf{3}_{\text{red}}$, our attempts to prepare the desired N-substituted-2-alkylamino-1,4-benzoxazine derivatives were disappointing, leading to the unwanted benzoxazine $\mathbf{2a}$. These results confirmed that the catalytic oxidation of isobutylamine proceeded through the ionic transamination process earlier reported for the autorecycling oxidation of activated benzylamine [35]. This mechanism is also the accepted process for catalytic deamination by amine oxidases cofactors [36-39]. The occurrence of this mechanism was substantiated when the controlled potential electrolysis was spontaneously stopped after the transfer of two electrons per molecule of $\mathbf{3a}_{\text{red}}$. Then, 3,4-aminophenol $\mathbf{1}_{\text{red}}$ was isolated in 70% yield, along with 8% of the recovered starting material $\mathbf{3a}_{\text{red}}$, a result which implied $\mathbf{1}_{\text{red}}$ as the efficient catalyst for isobutylamine oxidation. Then, the IEDDA reaction occurred between the electron-poor 3,4-azaquinone heterodiene $\mathbf{1}_{\text{red}}$ and the electron-rich enamine dienophile, the more electron-rich carbon atom of the enamine adding to the

nitrogen atom of **1_{ox}**, giving the 2-alkylamino-1,4-benzoxazine derivative **2a** (Scheme 3). These results highlighted a limitation of our electrochemical procedure that uses both *in situ* generated diene and dienophile, which failed to produce N-substituted-2-alkylamino-1,4-benzoxazine derivatives.

3.1.2. Electrosynthesis of 9a-Nonsubstituted-phenoxazine Derivatives **5** and **6**

Another limitation of our electrochemical procedure was evidenced when we used a cyclic alkylamine generated from the **1_{ox}**-mediated catalytic oxidation of cyclohexylamine derivatives. Using 2-methylcyclohexylamine as the amine substrate, we found that the cascade reaction led to heterocyclic annellation in a diastereospecific manner, while the same experiment performed with cyclohexylamine did not afford the corresponding phenoxazine derivative [30]. This result proved to be general with amines which did not possess a β -tertiary carbon atom. This prerequisite prohibited the efficient use of such amines in the cascade reaction leading to the 1,4-benzoxazine derivatives. This observation was rather amazing as related phenoxazine derivatives were previously successfully isolated from the IEDDA reactions between stable *o*-quinone monoimides and 1-morpholino-1-cyclohexene [23]. At this point, we suspected that, with cyclohexylamine, our cascade sequence also provided the desired phenoxazine. However, this subsequently decomposed due to the basicity of the medium consecutive to the excess of amine present in the electrolysis solution. So, to get a better understanding of our results, we focused on the [4+2] cycloaddition of commercially available 1-morpholino-1-cyclohexene and 1-pyrrolidino-1-cyclohexene with the electrogenerated 3,4-azaquinone **1_{ox}** (Scheme 4).

The cyclic voltammogram of compound **1_{red}** (2 mM), in deaerated MeOH containing tetraethylammonium perchlorate as the supporting electrolyte (20 mM), and an excess of 1-morpholino-1-cyclohexene (10 mM), at a dropping mercury electrode, showed an oxidation

peak Pa at -20 mV vs SCE, the sweep rate being 0.5 Vs^{-1} , in agreement with the two electron oxidation of $\mathbf{1}_{\text{red}}$ to $\mathbf{1}_{\text{ox}}$. As can be seen in Figure 2, the partial reversibility of the two electron process was illustrated by the appearance of a cathodic peak Pc on the reverse sweep at -120 mV vs SCE. In the reverse sweep, a second reduction peak Pc' was recorded at a more negative potential (-1600 mV vs SCE) due to the irreversible two-electron reduction of the carbonyl group of the benzophenone skeleton. Note that the height of Pa was lower than that expected for a two-electron process because the basicity of the alkylenamine was not sufficient to quantitatively produce the monoanionic species of $\mathbf{1}_{\text{red}}$, which is the sole form that can be oxidized to 3,4-azaquinone $\mathbf{1}_{\text{ox}}$. Nevertheless, the reaction could go to completion by equilibrium displacement.

When E was fixed at $+50$ mV vs SCE, that is at a potential for which $\mathbf{1}_{\text{red}}$ could be oxidized to the 3,4-iminoquinone form $\mathbf{1}_{\text{ox}}$, a coulometric value of 2.0 ± 0.1 was found for n, consistent with a two-electron transfer. Close inspection of the exhaustively oxidized solution revealed that electrogenerated 3,4-azaquinone $\mathbf{1}_{\text{ox}}$ was trapped with 1-morpholino-1-cyclohexene to give the substituted phenoxazine **5** in 50% yield (Scheme 4), as a mixture of two unassigned diastereoisomers (ca 2:1 ratio). The progress of the electrolysis was followed by monitoring the UV-vis absorption spectrum (Figure 3). Before electrolysis, $\mathbf{1}_{\text{red}}$ was present as the major neutral form ($\lambda_{\text{nm}} = 310$), together with the minor monoanionic form ($\lambda_{\text{nm}} = 350$, shoulder in Figure 3). After applying the potential, a decrease in the UV-vis absorption band shown by the monoanionic form of $\mathbf{1}_{\text{red}}$ at 350 nm was observed, while the band at 310 nm slightly increased and a new band at 260 nm developed. The new bands at 310 and 260 nm could be assigned to the phenoxazine derivative **5**, as corroborated after recording the UV-vis absorption spectrum of the isolated product.

Using 1-pyrrolidino-1-cyclohexene, $\mathbf{1}_{\text{red}}$ was exclusively present as its monoanionic form, due to the stronger basicity of the enamine compared to that of 1-morpholino-1-

cyclohexene. Accordingly, the height of Pa ($E_{pa} = -80$ mV vs SCE) was as expected for a two-electron process (Figure 2) and the UV-vis absorption spectrum recorded before electrolysis only showed the absorption band characteristic of the monoanionic form of **1_{red}** at 350 nm (Figure 4). Under the experimental conditions above mentioned, when E was fixed at 0.0 mV vs SCE, a coulometric value of 2.2 ± 0.1 was found for n, consistent with a two-electron transfer. After applying the potential, a decrease in the UV-vis absorption band shown by the monoanionic form of **1_{red}** at 350 nm was observed, while two new bands at 310 and 260 nm developed. Treatment of the exhaustively electrolyzed solution afforded the phenoxazine derivative **6** in 55% yield (Scheme 4), as a mixture of two unassigned diastereoisomers (ca 2:1 ratio). This resulted from hydrolysis of the labile pyrrolidino chain on silica gel [30,40]. The presumption that excess of amine should decompose the phenoxazine derivative was corroborated by exposure of the electrolysis solution, which contained phenoxazine **5**, to an excess of cyclohexylamine (20 mM). Then, a decrease in the UV-vis absorption bands at 310 and 260 nm was observed. Accordingly, the electrolysis solution became dark and no product could be isolated. A pathway leading to the presumably decomposition of the phenoxazine derivative **5** under basic conditions could be proposed, in which the opening of the oxazine ring would generate an oxidizable alkylaminophenolate producing an alkyliminoquinone which subsequently would polymerize (Scheme 5).

Finally, although our cascade sequence, that uses both electrogenerated 3,4-azaquinone **1_{ox}** and enamine, prohibited the efficient use of amines with β -secondary carbon atom, a variant in which the enamine is commercially available, or separately prepared, might complete the aforementioned procedure and avoid this limitation.

3.2 Biological Investigations

Various 1,4-benzoxazine derivatives have shown to display some interesting pharmacological properties including antibacterial, antiemetic, antirheumatic and antitumor activities. A few years ago, we have reported the neuroprotective activity of novel 8-alkylamino-substituted-1,4-benzoxazine derivatives. *In vitro*, these compounds were found to be very potent on immortalized HT-22 hippocampal cell cultures, with activity close to that of standard α -tocopherol [31]. From the combined results of both intrinsic cytotoxicity and neuroprotection, two compounds, S-24718 and S-24429, have been then selected for further *in vivo* biological evaluation (Table). Both compounds were found to be effective in an animal model of brain damage mimicking the lesions underlying cerebral palsy [32]. Since the 2-alkylamino-1,4-benzoxazine derivatives **2** were topologically different from S-24718 and S-24429, we thought that it would be interesting to know if these new benzoxazine derivatives also displayed neuroprotective activity.

The neuroprotective activity of 2-alkylamino-1,4-benzoxazine **2** was assessed *in vitro* on immortalized HT-22 hippocampal cell cultures, in a model of cell death by oxidative stress. At this point, it should be noted that oxygen, though essential for aerobic metabolism, can be converted to toxic metabolites such as superoxide, hydrogen peroxide, and hydroxyl radicals, collectively known as reactive oxygen species (ROS). When ROS generation exceeds the capacity of endogenous enzymatic and nonenzymatic antioxidant defense systems, tissues become vulnerable to damage, as the result of the widely accepted phenomenon called oxidative stress [41]. Compared to kidney or liver, the brain contains only low to moderate activities of endogenous enzymes (superoxide dismutase, catalase and glutathione peroxidase). In contrast, among the nonenzymatic endogenous defense systems, evidence is growing that glutathione is essential for the cellular detoxification of ROS in brain cells, and that alterations in brain glutathione metabolism might contribute to the occurrence

of oxidative stress [42,43]. Neuroprotective properties of 1,4-benzoxazine derivatives were estimated through their protective effects against L-homocysteic acid (L-HCA) cytotoxicity by evaluating the concentration producing 50% protection (PC_{50}). Exposure of HT-22 hippocampal cell lines to L-HCA induces a depletion in intraneuronal glutathione, which leads to the accumulation of ROS and, finally, to the programmed cell death by oxidative stress, with features of necrosis and apoptosis [44,45]. Previous cell culture toxicity studies have demonstrated that these toxic effects could be attenuated with antioxidants. The new series of 2-alkylamino-1,4-benzoxazine derivatives **2** showed significant *in vitro* neuroprotective activity with PC_{50} values between 0.41 and 10.0 μ M (Table). Interestingly, most of these compounds were found to be more active than S-24718 (PC_{50} = 15.8 μ M) and S-24429 (PC_{50} = 2.5 μ M), considered as the most attractive derivatives of the previously synthesized 8-alkylamino-1,4-benzoxazine series.

Our initial objective was to design targets that effectively protected neuronal cell lines *in vitro* from the toxicity induced by L-HCA, without causing any intrinsic toxic effects to the neurons. At this point, it should be underlined that all of the different classes of neuroprotective compounds tested to date are intrinsically cytotoxic. However, at more or less lower concentration, a neuroprotective effect can predominate. The intrinsic neurotoxic effects of each compound was evaluated by monitoring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reduction assay), which allows an evaluation of the “redox state” of the cells and emphasizes oxidative stress [46]. The maximum tolerated concentration tested lacking toxicity (MTC) was estimated for each tested compound. A safety index was then estimated as the MTC/PC_{50} ratio, in order to select candidate with the widest safety index *in vitro*. With regard to this parameter, all targeted 3,3-alkyl-substituted-1,4-benzoxazine derivatives **2a-c** and **4** were found to be at least 25-fold more toxic than benzoxazines S-24718 and S-24429. So, although exhibiting potent

neuroprotective activity, these derivatives could not be considered as promising agents for therapeutic potential, since the safety index values were lower than expected. Interestingly, introduction of phenyl groups at the 3-position of the 1,4-benzoxazine ring resulted in a large decrease in *in vitro* cytotoxicity (at least 10 times). The most striking example was given by the comparison of compounds **2c** (MTC = 5 μ M) and **2e** (MTC = 100 μ M), both bearing a 2-(N-methyldioxolan)methylamino substituent. Consequently, 2-alkylamino-1,4-benzoxazine **2e** (Table), with the highest safety index (200), was selected for further *in vivo* biological evaluation.

The neuroprotective activity of the benzoxazine derivative **2e** was then evaluated *in vivo*, in a well-characterized animal model of brain damage mimicking the lesions underlying cerebral palsy [47,48]. In a newborn mouse model, S-bromo-willardiine injection induces marked toxic effects in both cortex and white matter, two brain lesions found in patients with cerebral palsy. In this model, cotreatment with 2-alkylamino-1,4-benzoxazine **2e** protected in a dose dependent manner both the white matter and the cortical plate against the insult. Both doses (1 and 10 mg/kg) of **2e** or S-24718 were neuroprotective, while only the highest dose (10 mg/kg) of S-24429 yielded a significant protection in this model. Finally, 2-alkylamino-1,4-benzoxazine **2e** may be considered as a potential candidate for the treatment and prevention of cerebral palsy, and might be active in other neurodegenerative diseases.

Conclusion

In this paper, we have provided new insights into the mechanism and scope of the multi-step one-pot electrochemical synthesis of complex 2-alkylamino-1,4-benzoxazine derivatives. This cascade sequence, wherein both cycloaddition partners are generated *in situ*, at room temperature, under metal-free conditions, allows the IEDDA reactions of a 3,4-

azaquinone diene and an alkylamine dienophile, two chemically unstable entities. However, two limitations of our electrochemical procedure have been delineated. First, it failed to produce N-substituted-2-alkylamino-1,4-benzoxazine derivatives. Second, it prohibited the use of amines with β -secondary carbon atom. Nevertheless, in this case, a variant in which the enamine is commercially available or separately prepared might constitute an alternative procedure.

Despite of these two limitations, this cascade reaction remained attractive as it allowed the rapid construction of highly functionalized benzoxazine derivatives, under mild conditions. Furthermore, the electrochemical methodology we describe appears to be amenable to the design of a wide range of novel and biologically relevant heterocycles for biological screening purposes. In this respect, our method allowed the rapid *in vitro* screening of sixty 2-alkylamino-1,4-benzoxazine derivatives as neuroprotective agents, especially for the treatment and prevention of cerebral palsy.

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References

1. E. Steckhan, T. Arns, W.R. Heineman, G. Hilt, D. Hoormann, J. Jörisen, L. Kröner, B. Lewall, H. Pütter, *Chemosphere* 43 (2001) 63 and references therein.
2. K. D. Moeller, *Tetrahedron* 56 (2000) 9527 and references therein.
3. M.E. Niyazymbetov, D.H. Evans, *Tetrahedron* 49 (1993) 9627.
4. G. Hilt, *Angew. Chem. Int. Ed.* 41 (2002) 3586.
5. G. Hilt, *Angew. Chem. Int. Ed.* 42 (2003) 1720.
6. M. Durandetti, J.-Y. Nedelec, J. Perichon, *Org. Lett.* 3 (2001) 2073.
7. M.R. Shaaban, T. Fuchigami, *Tetrahedron Lett.* 43 (2002) 273.
8. A.P. Esteves, D.M. Goken, L.J. Klein, M.A. Lemos, M.J. Medeiros, D.G. Peters, *J. Org. Chem.* 68 (2003) 1024.
9. J. Mlcoch, E. Steckhan, *Tetrahedron Lett.* 28 (1987) 1081.
10. C. F. Gürther, S. Blechert, E. Steckhan, *Angew. Chem. Int. Ed. Engl.* 34 (1995) 1900.
11. C. F. Gürther, S. Blechert, E. Steckhan, *Chem. Eur. J.* 3 (1997) 447.
12. T. Peglow, S. Blechert, E. Steckhan, *Chem. Eur. J.* 4 (1998) 107.
13. U. Haberl, E. Steckhan, S. Blechert, O. Wiest, *Chem. Eur. J.* 5 (1999) 2859.
14. J.-I. Yoshida, K. Sakaguchi, S. Isoe *J. Org. Chem.* 53 (1988) 2525.
15. S. Yamamura, S. Nishiyama, *Synlett* 4 (2002) 533 and references therein.
16. T. Inokuchi, S.-I. Tanigawa, S. Torii, *J. Org. Chem.* 55 (1990) 3958.
17. M. Jinno, Y. Kitano, M. Tada, K. Chiba, *Org. Lett.* 1 (1999) 435.
18. J.H.P. Utley, S. Ramesh, X. Salvatella, S. Szunerits, M. Motevalli, M.F. Nielsen, *J. Chem. Soc. Perkin Trans. 2* (2001) 153.
19. N. Kise, R. Miura, N. Ueda, *Bull. Chem. Soc. Jpn.* 75 (2002) 2693.
20. J. Lorans, J.-P. Hurvois, C. Moinet, *Acta Chem. Scand.* 53 (1999) 807.
21. M. LARGERON, A. Neudörffer, M.-B. Fleury, *Angew. Chem. Int. Ed.* 42 (2003) 1026.

22. M. Langeron, A. Neudörffer, M. Vuilhorgne, E. Blattes, M.-B. Fleury, *Angew. Chem. Int. Ed.* 41 (2002) 824.
23. H.W. Heine, B.J. Barchiesi, E.A. Williams, *J. Org. Chem.* 49 (1984) 2560.
24. H.W. Heine, M.G. La Porte, R.H. Overbaugh, E.A. Williams, *Heterocycles* 40 (1995) 743.
25. K.C. Nicolaou, Y.L. Zhong, P.S. Baran, *Angew. Chem. Int. Ed.* 39 (2000) 622.
26. K.C. Nicolaou, K. Sugita, P.S. Baran, Y.L. Zhong, *Angew. Chem. Int. Ed.* 40 (2001) 207.
27. K.C. Nicolaou, P.S. Baran, Y.L. Zhong, K. Sugita *J. Am. Chem. Soc.* 124 (2002) 2212.
28. K.C. Nicolaou, K. Sugita, P.S. Baran, Y.L. Zhong, *J. Am. Chem. Soc.* 124 (2002) 2221.
29. D.N. Nicolaides, C. Bezergiannidou-Balouctsi, R.W. Awad, K.E. Litinas, E. Malanidou-Xenikaki, A. Terzis, C.P. Raptopoulou, *J. Org. Chem.* 62 (1997) 499.
30. E. Blattes, M.-B. Fleury, M. Langeron, *J. Org. Chem.* 69 (2004) 882.
31. M. Langeron, B. Lockhart, B. Pfeiffer, M.-B. Fleury, *J. Med. Chem.* 42 (1999) 5043.
32. M. Langeron, B. Mesples, P. Gressens, R. Cechelli, M. Spedding, A. Le Ridant, M.-B. Fleury, *Eur. J. Pharmacol.* 424 (2001) 189.
33. R. Larget, B. Lockhart, B. Pfeiffer, A. Neudorffer, M.-B. Fleury, M. Langeron, *Bioorg. Med. Chem. Lett.* 9, (1999), 2929.
34. M. Langeron, A. Neudorffer, M.-B. Fleury, *J. Chem. Soc. Perkin Trans 2* (1998), 2721.
35. M. Langeron, M.-B. Fleury, *J. Org. Chem.* 65 (2000) 8874.
36. M. Mure, J.P. Klinman, *J. Am. Chem. Soc.* 117 (1995) 8707.
37. Y. Lee, L.M. Sayre, *J. Am. Chem. Soc.* 117 (1995) 11823.
38. S. Itoh, N. Takada, S. Haranou, T. Ando, M. Komatsu, Y. Ohshiro, S. Fukuzumi, *J. Org. Chem.* 61 (1996) 8967.
39. V.L. Davidson, *Arch. Biochem. Biophys.* 428 (2004) 32.
40. K. Juhl, K.A. Jorgensen, *Angew. Chem. Int. Ed.* 42 (2003) 1498.
41. H. Sies, *Angew. Chem.* 98 (1986) 1061.

42. R. Dringen, *Prog. Neurobiol.* 62 (2000) 649.
43. R. Dringen, J. Hirrlinger, *Biol. Chem.* 384 (2003) 505.
44. T.H. Murphy, M. Miyamoto, A. Sastre, R.L. Schnaar, J.T. Coyle, *Neuron* 2 (1989) 1547.
45. R.R. Ratan, T.H. Murphy, J.M. Baraban, *J. Neurochem.* 62 (1994) 376.
46. T. Mosmann, *Immunol. Methods* 65 (1983) 55.
47. S. Marret, R. Mukendi, J.F. Gadisseux, P. Gressens, P. Evrard, *J. Neuropathol. Exp. Neurol.* 54 (1995) 358.
48. S. L. Tahraoui, S. Marret, C. Bodénant, P. Leroux, M.-A. Dommergues, P. Evrard, P. Gressens, *Brain Pathol.* 11 (2001), 56

Figure Legends

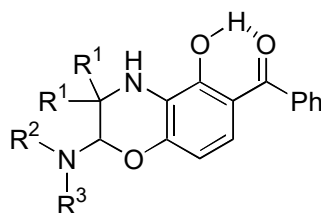
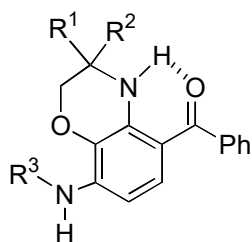
Figure 1. Cyclic voltammogram of **3a_{red}** (2mM) at a dropping mercury electrode, in deaerated MeOH containing tetraethylammonium perchlorate (20 mM) and isobutylamine (40 mM). Arrowheads indicate the direction of the potential sweep; $v = 0.5 \text{ Vs}^{-1}$. The vertical arrow indicates the initial potential point.

Figure 2. Cyclic voltammogram of **1_{red}** (2mM) at a dropping mercury electrode, in deaerated MeOH containing tetraethylammonium perchlorate (20 mM) and 10 mM of 1-morpholino-1-cyclohexene (dashed line) or 1-pyrrolidino-1-cyclohexene (continuous line). Arrowheads indicate the direction of the potential sweep; $v = 0.5 \text{ Vs}^{-1}$. The vertical arrow indicates the initial potential point.

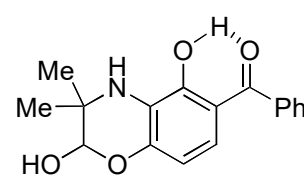
Figure 3. Spectrophotometric changes accompanying the electrochemical oxidation of **1_{red}** (2mM) at a mercury pool ($E = + 50 \text{ mV vs SCE}$), in deaerated MeOH containing tetraethylammonium perchlorate (20 mM) and 1-morpholino-1-cyclohexene (10 mM): (a) 0 (before electrolysis); (b) 1; (c) 2 mol of electrons. Cell thickness, 0.1 cm.

Figure 4. Spectrophotometric changes accompanying the electrochemical oxidation of **1_{red}** (2mM) at a mercury pool ($E = 0.0 \text{ mV vs SCE}$), in deaerated MeOH containing tetraethylammonium perchlorate (20 mM) and 1-pyrrolidino-1-cyclohexene (10 mM): (a) 0 (before electrolysis); (b) 1; (c) 2; (d) 2.2 mol of electrons. Cell thickness, 0.1 cm.

Table. *In Vitro* Neuroprotective Activity of 2*H*-1,4-Benzoxazin-6-yl-(phenyl)-methanones **2a-f** and **4**



2a-f



4

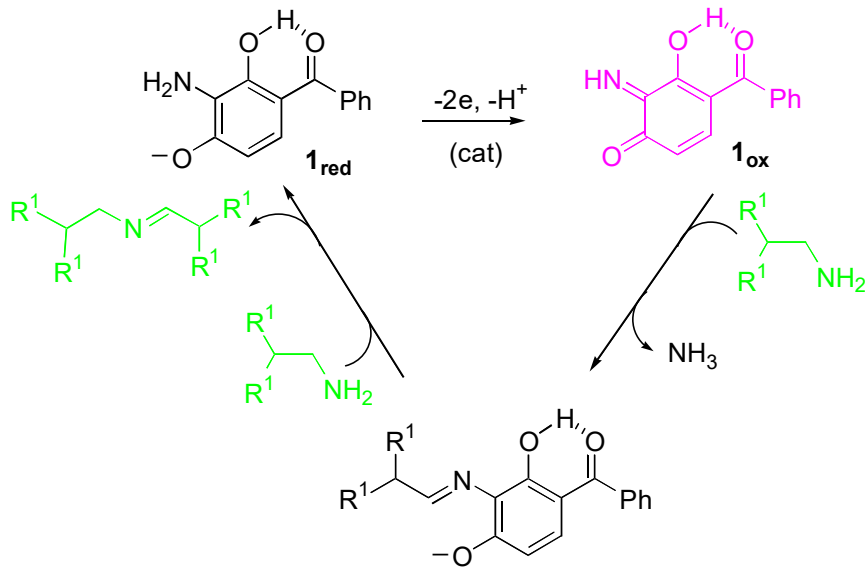
S-24718: R¹, R² = cyclopentyl; R³ = benzyl

S-24429: R¹ = H; R² = Bu^t; R³ = phenylethyl

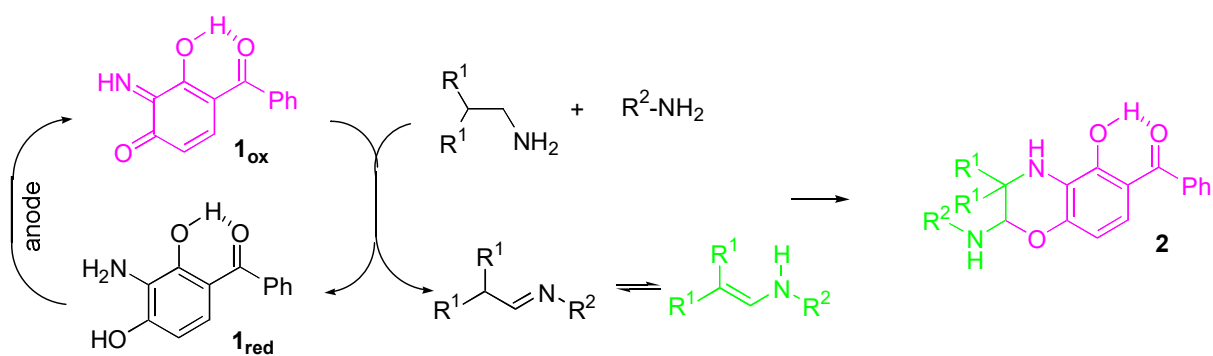
Compd	R ¹	R ²	R ³	Toxicity ^a	Protection vs 2mM L-HCA ^b	Safety index
				MTC(μM)	PC ₅₀ (μM)	MTC/PC ₅₀
S-24718				>250	15.8	>15.8
S-24429				>250	2.5	>100
2a	Me	H	Bu ⁱ	10	0.41	24.4
2b	Me	H	(CH ₂) ₂ -Ph	10	2.0	5.0
2c	-(CH ₂) ₅ -	Me		5	2.4	2.0
2d	Ph	H	CH ₂ -CH(Ph) ₂	>250	10.0	>25
2e	Ph	Me		100	0.5	200
2f	Ph	H	CH ₂ -CH(OMe) ₂	100	5.0	20
4	Me	-	-	10	0.97	10.3

^a *In vitro* neurotoxicity monitored by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT assay); ^b *In vitro* neuroprotective activity estimated through their protective effects against L-homocysteic acid (L-HCA) cytotoxicity; MTC, maximum tolerated concentration; PC₅₀, concentration producing 50% protection

Scheme 1



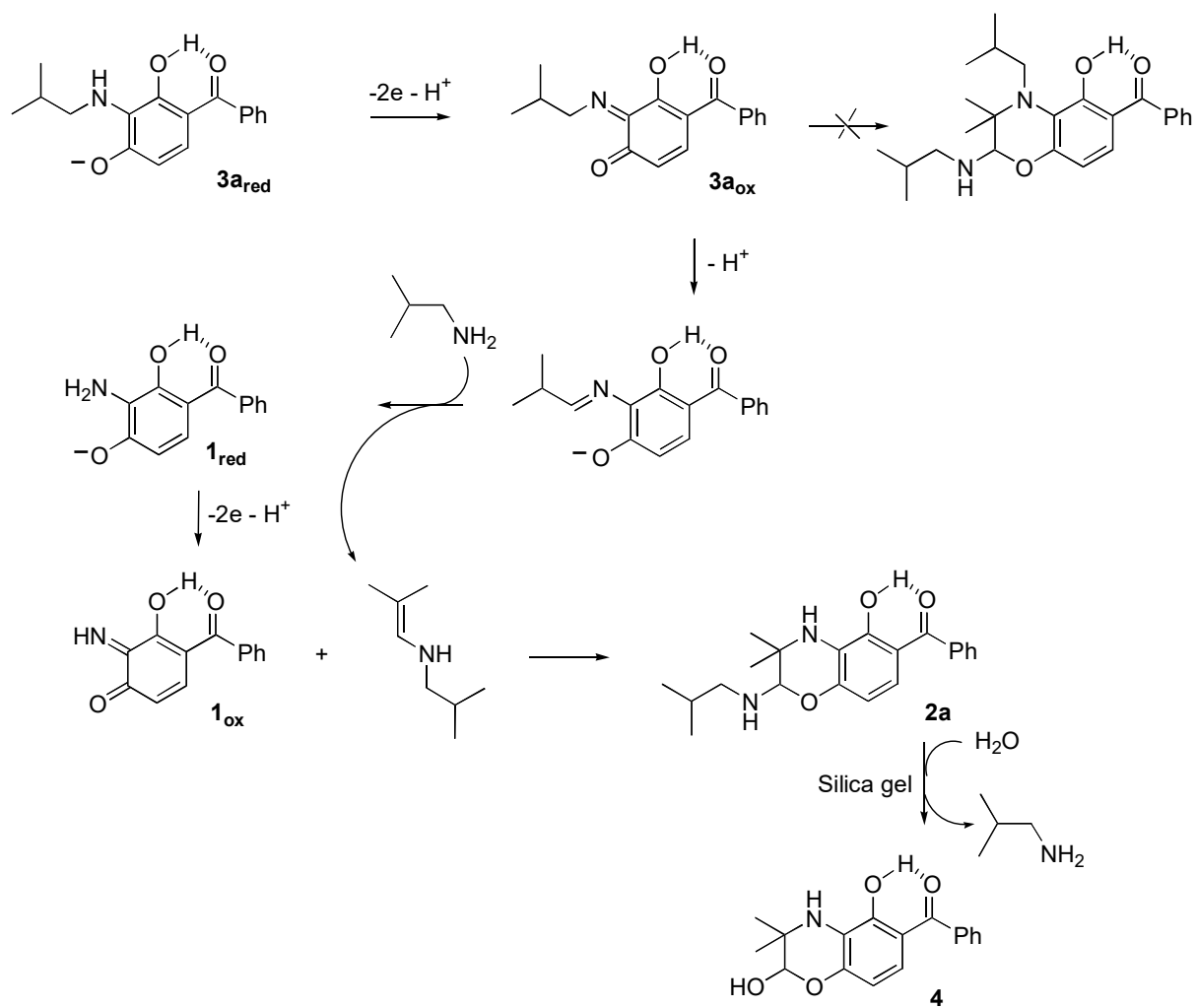
Scheme 2



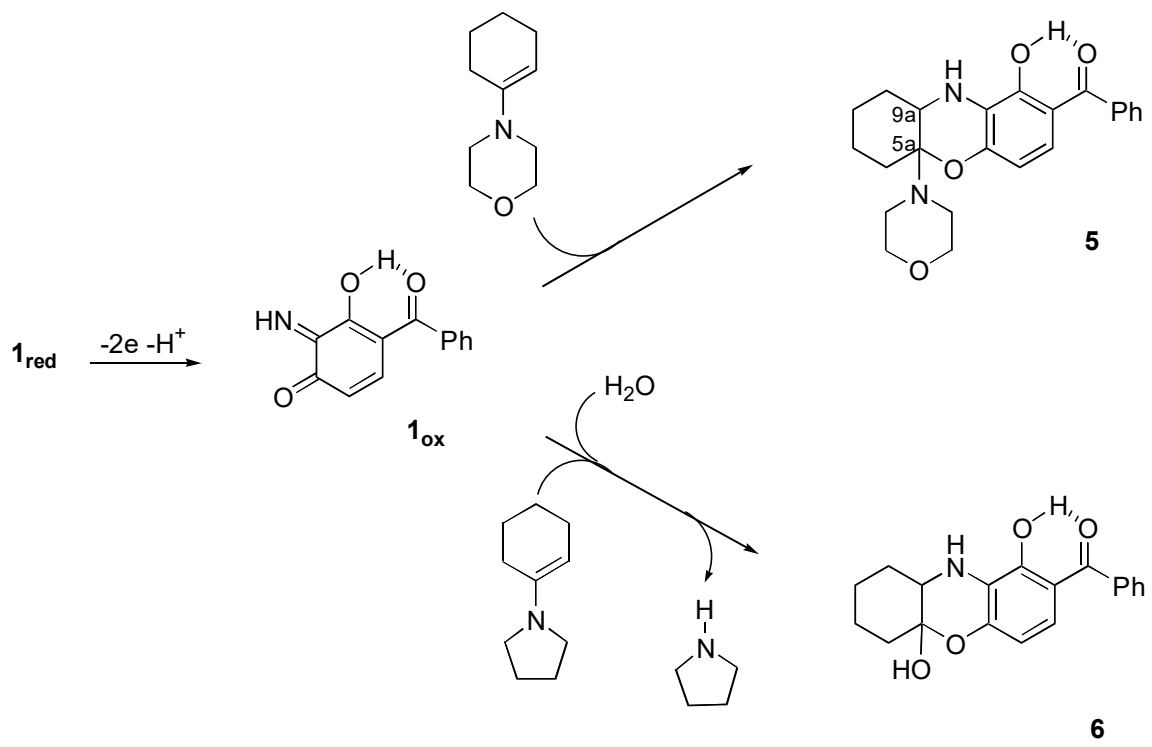
$R^1, R^2 = Me, Me; (CH_2)_5; Ph, Ph...$

$R^2 = Bu^i; (CH_2)_5; (CH_2)_2-Ph; (CH_2)_2-OMe...$

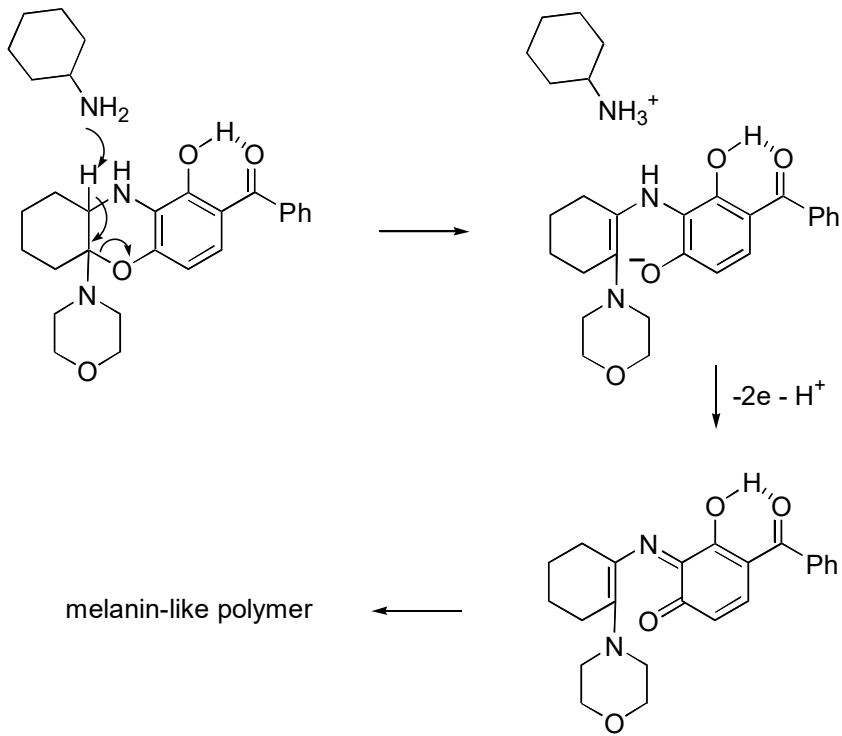
Scheme 3



Scheme 4



Scheme 5



QuickTime™ et un décompresseur
Photo - JPEG sont requis pour visualiser
cette image.

Figure 1

E. Blattes, M.-B. Fleury, M. LARGERON

QuickTime™ et un décompresseur
Photo - JPEG sont requis pour visualiser
cette image.

Figure 2

E. Blattes, M.-B. Fleury, M. Largeron

QuickTime™ et un décompresseur
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cette image.

Figure 3

E. Blattes, M.-B. Fleury, M. Largeron

QuickTime™ et un décompresseur
Photo - JPEG sont requis pour visualiser
cette image.

Figure 4

E. Blattes, M.-B. Fleury, M. Largeron