A convenient extension of the Wessely–Moser rearrangement for the synthesis of substituted alkylaminoflavones as neuroprotective agents in vitro

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A CONVENIENT EXTENSION OF THE WESSELY-MOSER REARRANGEMENT FOR THE SYNTHESIS OF SUBSTITUTED ALKYLAMINOFLAVONES AS NEUROPROTECTIVE AGENTS *IN VITRO.*

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Abstract : A series of 8-alkylamino-5,7-dihydroxyflavones was prepared from chrysine *via* a seven step sequence. The synthesis of their 6-alkylamino isomers could be subsequently accomplished through a convenient extension of the Wessely-Moser rearrangement. These compounds were found to be efficient neuroprotective agents *in vitro*.

Keywords : Antioxidants, Neuroprotective agents, Radical scavengers, Substituent effects.

There is now substantial evidence accumulating to suggest that oxidative stress may play a role in the progression of many neurodegenerative pathologies of the central nervous system, including Parkinson's disease and Alzheimer's disease.[1] Consequently, supplementation with exogenous antioxidants can represent an important therapeutic potential to minimize central nervous system damage. So, there is considerable interest in the discovery and development of efficient antioxidants.

In the course of our search for new neuroprotective agents,[2,3] we recently reported the synthesis of novel 8-alkylamino-3-substituted-1,4-benzoxazine derivatives **I** as well as 3-alkylamino-2,4-dihydroxy-benzophenones **II** (Figure).[4-6]
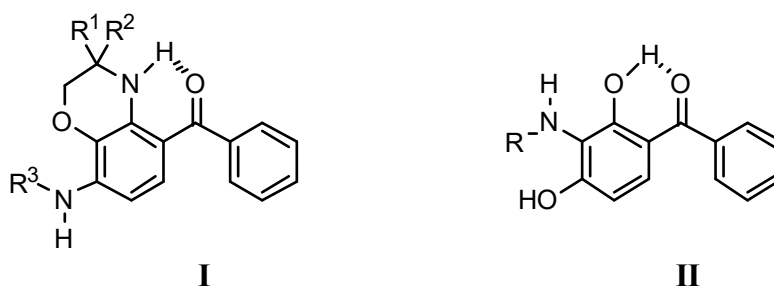
Figure

From their capacity to inhibit oxidative stress-mediated neuronal degeneration *in vitro*, these compounds were found to be potent neuroprotective agents with activity close to that of standard α-tocopherol.[7,8] From these studies, substituted-1,4-benzoxazines **I** were identified as the most promising compounds for therapeutic potential owing to their very low intrinsic cytotoxicity.[8,9] Although 3-alkylaminophenols **II** were found to be at least ten fold more toxic than the series of 1,4-benzoxazines, they

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remained potent neuroprotective agents.[7] So, we envisioned potentially useful structural modifications in order to enhance antioxidant activity. We thought that it would be then possible to determine a concentration range for which antioxidant effects could be achieved without the manifestation of intrinsic toxicity. Toward this goal, we planned to synthesize 6-alkylamino-5,7-dihydroxyflavones **10a-e** (Scheme 1). Flavonoids, in particular polyhydroxyflavones such as quercetin, morin, myricetin and kempferol, are indeed well known to exhibit antioxidant activity.[10-12] This raised the question of whether or not 6-alkylaminoflavones **10a-e** in general would be more promising derivatives than their benzophenone analogues **II**.

In this paper, we wish to report the synthesis of 6-alkylamino-5,7-dihydroxyflavones **10a-e** that has been successfully accomplished from their 8-alkylamino isomers through a convenient extension of the Wessely-Moser rearrangement. We also disclose the neuroprotective properties of these compounds as part of our continuing research efforts to find safe and effective neuroprotective agents.

Chemistry

At the outset, our synthetic strategy for the preparation of compounds **10a-e** involved the intermediary synthesis of 5,7-dihydroxy-6-nitroflavone, which appeared deceptively simple. This should be further reduced to 6-amino-5,7-dihydroxyflavone, and then alkylated by the appropriate methods leading to the expected compounds **10a-e** (Scheme 1).
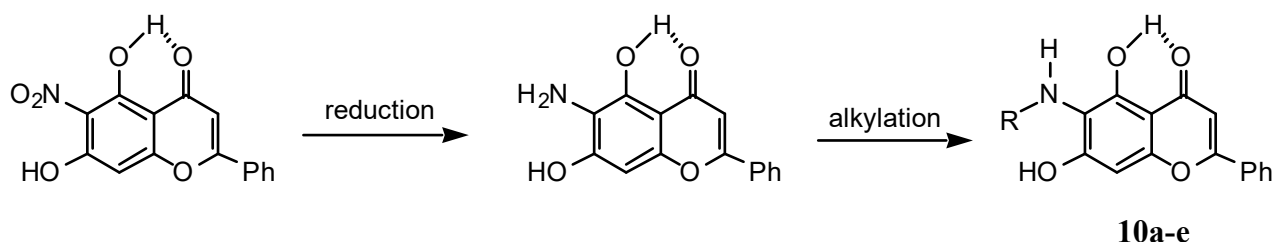
Scheme 1

Currently available methods to prepare 5,7-dihydroxy-6-nitroflavone from 2,4,6-trihydroxy-3-nitroacetophenone including Baker-Venkataraman rearrangement,[13] synthesis from chalcones[14] and synthesis *via* an intramolecular Wittig strategy[15] met with total failure. Furthermore, newer methods for directly converting 2,4,6-trihydroxy-3-nitroacetophenone into the required 1,3-diketone intermediate, such as organolithium reagents[16], or phase-transfer catalysis[17], proved to be ineffective. At this juncture, an alternative route which involved the functionalization of chrysine **1** was followed for the preparation of 5,7-dihydroxy-6-nitroflavone (scheme 2). Unfortunately, the nitration of chrysine **1** with fuming HNO_3 in acetic acid gave the desired 5,7-dihydroxy-6-nitroflavone as a minor product (2% yield), while the unwanted 8-nitro isomer **2** was isolated as the main product (70% yield).[18] Nevertheless, in view of the relative ease of preparation of 8-alkylamino-5,7-dihydroxyflavones from 8-nitrochrysine **2**, the synthesis of isomers **8a-e** was pursued for two reasons. First, encouraged by the results of Wessely and Moser which previously observed the conversion of 5,7,8-trimethoxyflavone into the 5,6,7-trihydroxy compound on demethylation,[19] we thought that 8-alkylaminoflavones **8a-e** possibly could serve as starting materials for the preparation of their 6-isomers **10a-e**. Second, compounds **8a-e** could be interesting to explore the relationship between the position of the alkylamino chain and the neuroprotective activity. Accordingly, 8-alkylaminoflavones **8a-e** were synthesized from 8-nitrochrysine **2** *via* a six step sequence, which involved

protection and deprotection steps as reported in scheme 2. Alkylation of 8-nitrochrysine **2** with Me_2SO_4 and K_2CO_3, followed by reduction of the nitro group with stannous choride in ethanol,[20] afforded **4** in roughly 60% overall yield. The latter was alkylated, after protection with the 2,4-dinitrobenzenesulfonyl group that could be further cleaved under mild conditions.[21] *N*-monosubstituted 2,4-dinitrobenzenesulfonamide **5**, readily prepared in 88% yield from 8-amino-5,7-dimethoxyflavone **4** and 2,4-dinitrobenzenesulfonyl chloride, could be efficiently *N*-alkylated with appropriate primary alkyl halides by the conventional methods, in roughly 85% yield. For the introduction of the isopropyl chain, compound **5** was alkylated under the Mitsunobu conditions,[22] leading to compound **6a** in 77% yield. Facile deprotection of *N,N*-disubstituted-2,4-dinitrobenzenesulfonamides **6a-e** was achieved by treatment with mercaptoacetic acid and triethylamine in dichloromethane, giving the required compounds **7a-e** (71-94% yields).[21] Finally, complete demethylation of compounds **7a-d** was successfully performed using 5 equiv. of $AlCl_3$, in toluene heated at reflux, to afford compounds **8a-d**, in yields ranging from 68 to 92%. In the specific case of compound **7e**, bearing an allyl chain, demethylation with 5 equiv. of BBr_3 in dichloromethane at room temperature led to compound **8e** in 70% yield.
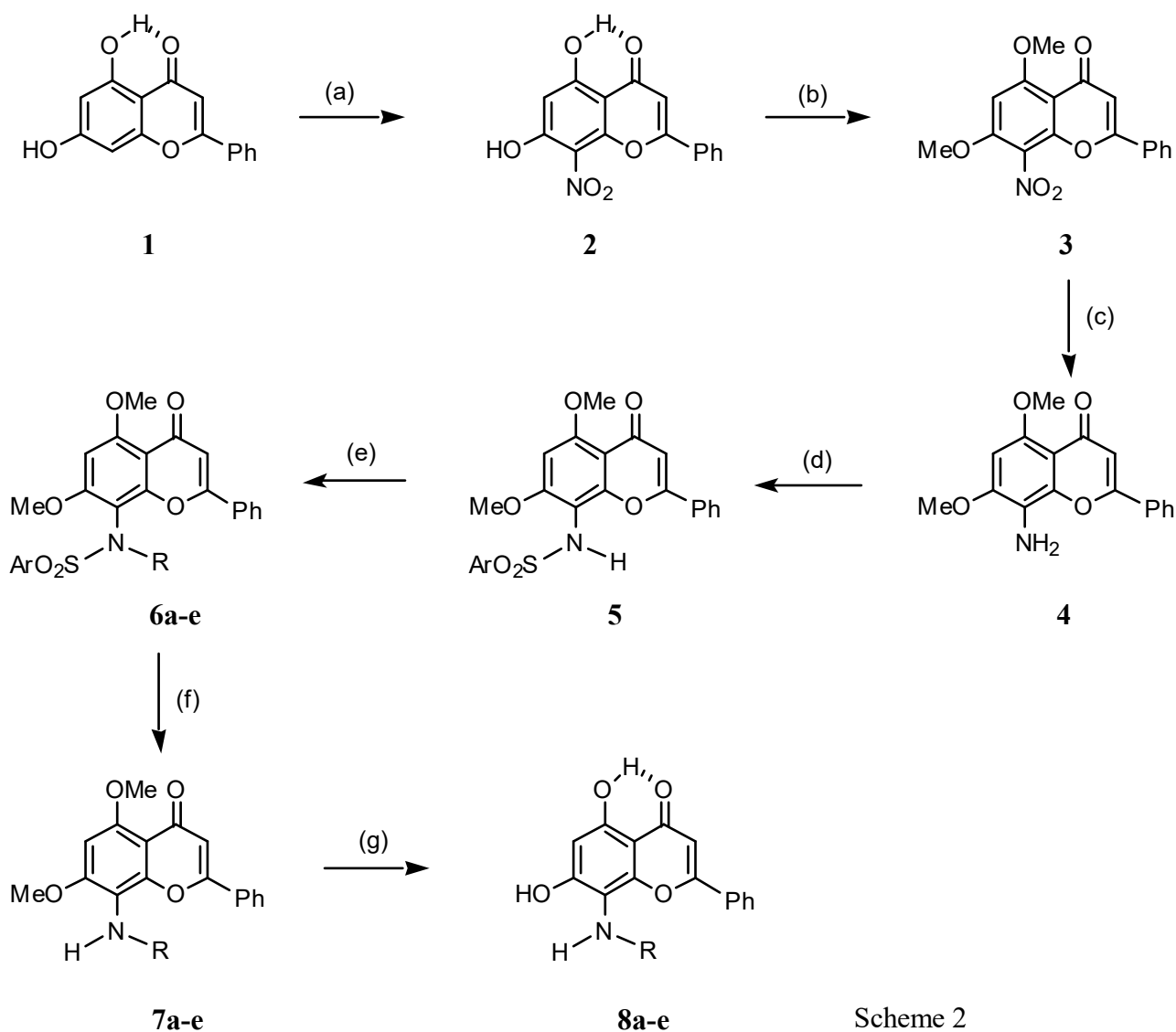
Scheme 2

(a) HNO_3 70%, AcOH, 65°C, 1h, 70%; (b) Me_2SO_4, K_2CO_3, DMF, room temperature, 3h, 81%; (c) $SnCl_2.2H_2O$, EtOH, reflux, 5h, 71%; (d) 2,4-$(NO_2)_2PhSO_2Cl$, pyridine, CH_2Cl_2, room temperature, 8h, 88%; (e) RI, K_2CO_3, DMF, room temperature, 1-

16h, 83-87% or Pr^iOH, DEAD, PPh_3, THF, reflux, 3h, 77%; (f) $HSCH_2COOH$, Et_3N, CH_2Cl_2, room temperature, 5-16h, 71-94%; (g) $AlCl_3$, toluene, reflux, 68-92% or BBr_3, CH_2Cl_2, room temperature, 4-5h, 50-70%.

Having established the seven step sequence for the synthesis of 8-alkylamino-5,7-dihydroxyflavones **8a-e**, we next turned our attention to the hypothetical conversion of these compounds to their 6-alkylamino isomers **10a-e**. It has been found for a long time that flavonoids having the 5,7,8-arrangement of methoxy groups suffered isomeric change, on demethylation, yielding a product with the 5,6,7-arrangement. This conversion, known as the Wessely-Moser rearrangement,[19] was essentially used for the elucidation of structures of flavonoids and was further extended to chromones, xanthones and derivatives.[23-26] Surprisingly, little attention has been devoted to this rearrangement, as a surrogate of direct synthesis, when the latter was cumbersome and inefficient.[26, 27] Furthermore, use of the Wessely-Moser rearrangement has been restricted in general to compounds bearing hydroxy or methoxy groups.

Initial attempts to perform the Wessely-Moser rearrangement on 8-nitroflavone **3** were to no avail: in no cases, were we able to detect a trace of the expected 6-nitro isomer. So, we decided to investigate the conversion of 8-alkylamino derivatives **7a-e**, by the action of boiling hydrochloric acid. In all cases, the isomeric change took place leading to 6-alkylaminoflavones **9a-e**, in good yields ranging from 60 to 74%. Finally, synthesis of 6-alkylamino isomers **10a-e** could be achieved through demethylation at the 7-position, under the conditions reported above[28] (scheme 3).

7a-e   (h)   **9a-e**   (g)   **10a-e**
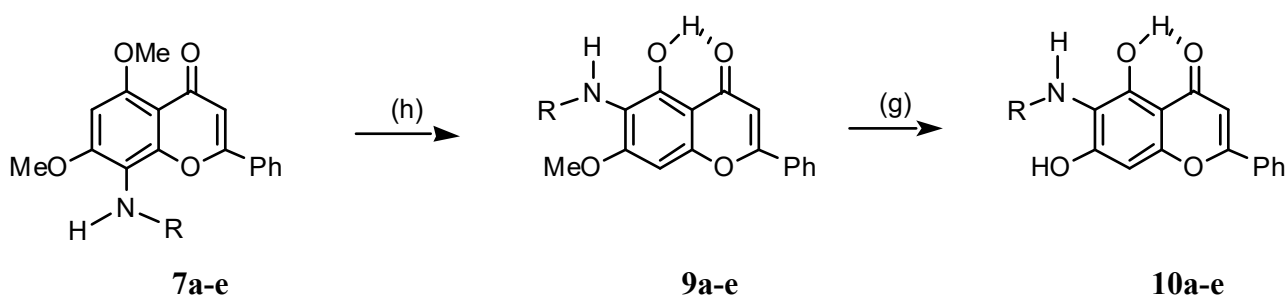
Scheme 3

(h) conc. HCl, reflux, 30-64h, 60-74%.
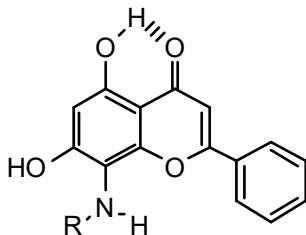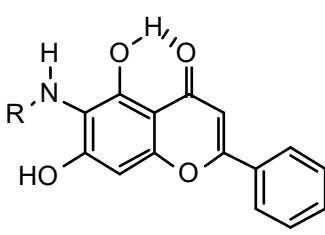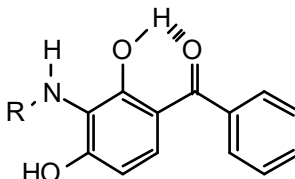
In vitro biological results

The intrinsic neurotoxicity as well as the neuroprotective activity of 8-alkylaminoflavones **8a-e** and 6-alkylaminoflavones **10a-e** were assessed *in vitro* on murine HT-22 hippocampal cell cultures[29] and compared with those of benzophenone analogues **II**. The results are presented in Table.

In order to determine potential pro-oxidant effects and to select a concentration range lacking intrinsic toxicity suitable for studying the neuroprotective activity of the compounds, the intrinsic neurotoxic effects of each compound was evaluated following two different methods. Neurotoxicity was monitored either by quantification of cellular lysis (death), after measurement of the lactate dehydrogenase (LDH) activity released from damaged cells into the culture supernatant, or by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reduction assay), that allows an evaluation of the "redox state" of the cells and emphazises oxidative stress. The maximum tolerated concentration (MTC), and the concentration producing 50% toxicity (TC_{50}), were estimated for each tested compounds using both MTT and LDH determination.

Neuroprotective properties of alkylaminoflavone derivatives were estimated through their protective effects against *L*-homocysteic acid (*L*-HCA) cytotoxicity. The latter has been reported to provoke, on immature primary neurons or on certain neuronal cell lines, oxidative-stress-mediated neuronal degeneration that could be attenuated with antioxidants. The concentration leading to 50% protection (PC_{50}) was estimated for both MTT and LDH determinations.

Taking into account that optimum activity was displayed by alkyl substituents in the benzophenone series **II**,[8] only were evaluated substituted alkylaminoflavones bearing isopropyl, n-propyl, isobutyl, isopentyl and allyl chains. As reported in Table, all tested flavonoids showed significant *in vitro* neuroprotective activity, with PC_{50} (MTT) values between 4.6 and 7.0 µM and PC_{50} (LDH) values between 4.2 and 10.0 µM. It could be noted that 6-alkylaminoflavones in general seemed to be slightly more active than their 8-isomers, (for example, compare **10b** and **8b)**. Nevertheless, in view of the PC_{50} values, it appeared that 6-alkylaminoflavones **10a-d**, as well as their benzophenone analogues **IIa-d**, gave similar results, (for instance, compare **10a** and **IIa)**. Furthermore, as they did not exhibit a lower toxicity than that showed by their benzophenone analogues **IIa-d,** it could be concluded that alkylaminoflavone derivatives could not be considered as more promising compounds for therapeutic potential.

Table. *In vitro* neuroprotective activity of compounds **8a-e** and **10a-e**.

8a-e   **10a-e**   **II**

| Compd | R | Toxicity | | | | Protection *vs* 2mM *L*-HCA | |
| | | MTC (µM) | | TC_{50} (µM) | | PC_{50} (µM) | |
		MTT	LDH	MTT	LDH	MTT	LDH
IIa	Pr^i	25	100	118	>250	5.5	5.9
IIb	nPr	25	100	78	>250	5.1	5.1
IId	Pe^i	10	50	20	82	4.0	5.5
8b	nPr	5	25	14	28	7.0	8.0
8c	Bu^i	10	10	12	12	4.6	4.2
8d	Pe^i	10	10	12	16	5.3	5.5
8e	CH$_2$=CHCH$_2$	5	10	18	11	>5	>10
10a	Pr^i	10	100	18	152	5.1	6.6
10b	nPr	5	100	10	176	5.2	5.5
10d	Pe^i	5	100	16	116	5.2	5.2

Abbreviations : *L*-HCA, *L*-homocysteic acid; MTC, maximum tolerated concentration; TC_{50}, concentration producing 50% toxicity; PC_{50}, concentration producing 50% protection; Bu^i, isobutyl; Pe^i, isopentyl; nPr, n-propyl; Pr^i, isopropyl.

In summary, we have synthesized a series of novel 8-alkylaminoflavone derivatives from chrysine *via* a seven step sequence. The synthesis of their 6-alkylamino isomers could be then successfully accomplished through a convenient extension of the Wessely-Moser rearrangement. To the best of our knowledge, such a rearrangement had not been as yet reported on alkylaminoflavones. Finally, the results of our biological evaluation revealed that replacement of the benzophenone skeleton by the flavone ring did not markedly affect the neuroprotective activity *in vitro*.

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28. **5,7-dihydroxy-2-phenyl-8-propylamino-4H-1-benzopyran-4-one *8b***: ^{1}H NMR (300 MHz, DMSO D_6): δ 0.90 (t, 3H, Me, nPr, J = 6 Hz), 1.45 (sextet, 2H, CH_2, nPr, J = 6 Hz), 3.14 (t, 2H,

N-CH$_2$, nPr, J = 6 Hz), 6.33 (s, 1H, 6-H), 6.96 (s, 1H, 3-H), 7.60 (m, 3H, H *meta* and H *para*, Ph), 8.10 (m, 2H, H *ortho*, Ph), 12.50 (s , 1H, 5-OH); MS (DCI) : m/z = 312 (MH$^+$).

5,7-dihydroxy-2-phenyl-6-propylamino-4H-1-benzopyran-4-one 10b: ^{1}H NMR (300 MHz, DMSO D$_6$): δ 0.87 (t, 3H, Me, J = 7 Hz), 1.43 (sextet, 2H, CH$_2$, J = 7 Hz), 3.20 (t, 2H, N-CH$_2$, J = 7 Hz), 6.62 (s, 1H, 8-H), 6.97 (s, 1H, 3-H), 7.56 (m, 3H, H *meta* and H *para*, Ph), 8.03 (m, 2H, H *ortho*, Ph), 12.50 (s , 1H, 5-OH); MS (DCI) : m/z = 312 (MH$^+$).

29. *In vitro* biological assays are extensively detailed in references 7 and 8.

A Convenient Extension of the Wessely-Moser Rearrangement for the Synthesis of Substituted Alkylaminoflavones as Neuroprotective Agents *in Vitro*

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10a-e

R = Pr[i], nPr, Bu[i], Pe[i], CH$_2$=CHCH$_2$
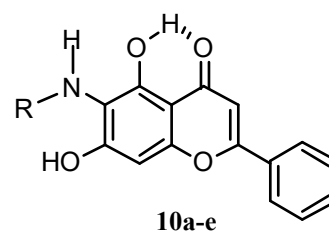
The synthesis of the title compounds, as neuroprotective agents, has been successfully accomplished *via* the Wessely-Moser rearrangement of their 8-isome