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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.

There is now substantial evidence accumulating to suggest that oxidative stress may play a pivotal 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 synthetic 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-dihydroxybenzophenones (**II**) (Scheme 1).[4,5] 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.[6,7] From these studies, substituted-1,4-benzoxazines (**I**) were identified as the most promising compounds for therapeutic potential as a result of their low intrinsic cytotoxicity in vitro.[7,8] Although 3-alkylaminophenols (**II**) were found to be at least 10-fold more toxic than the series of 1,4-benzoxazines, they remained potent neuroprotective agents.[6] On this basis, we envisaged potential useful structural modifications in order to enhance antioxidant activity and in parallel to reduce the manifestation of intrinsic toxicity. In this aim, the synthesis of 6-alkylamino-5,7-dihydroxyflavones (**10a–e**) was undertaken (Scheme 1). Flavonoids, in particular polyhydroxyflavones, are well known to exhibit antioxidant activity.[9–11] 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, the synthesis of 6-alkylamino-5,7-dihydroxyflavones **10a–e** is reported from their original 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. Indeed, currently available methods to prepare 5,7-dihydroxy-6-nitroflavone from 2,4,6-trihydroxy-3-nitroacetophenone including Baker–Venkataraman rearrangement, synthesis from chalcones or via an intramolecular Wittig strategy, as well as newer methods using organolithium reagents or phase-transfer

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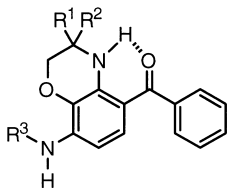
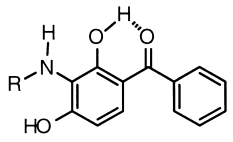
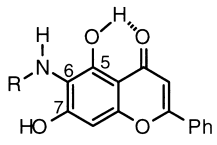


I



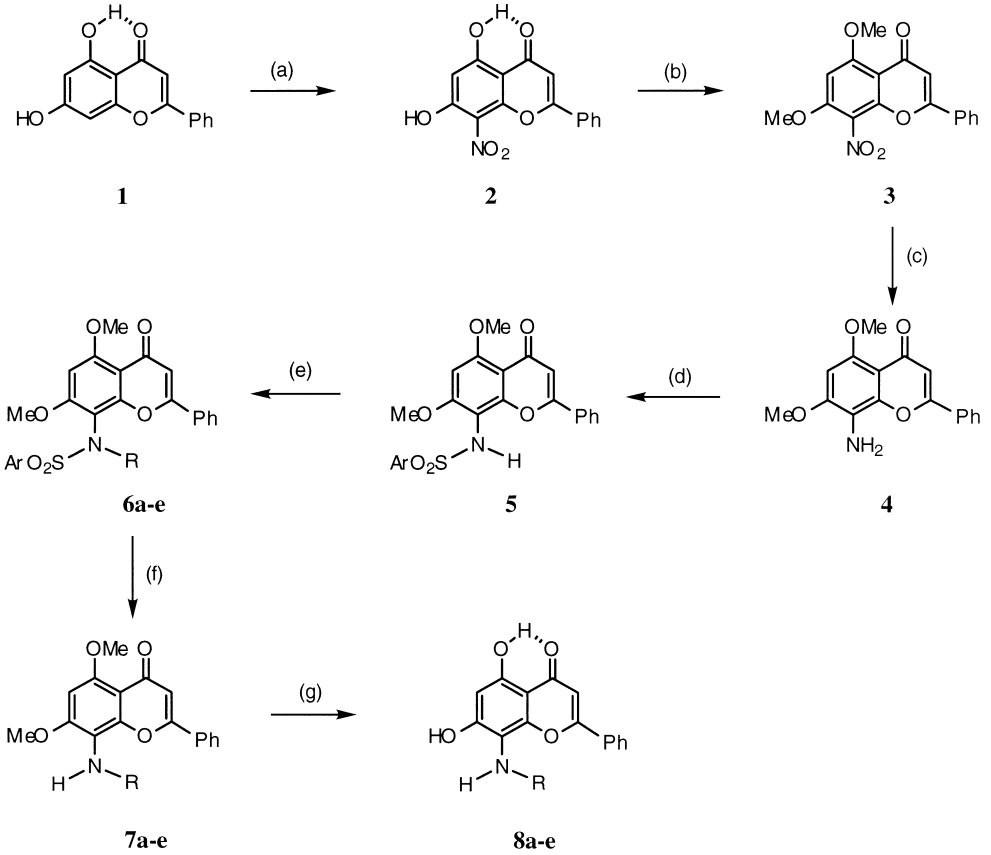
II



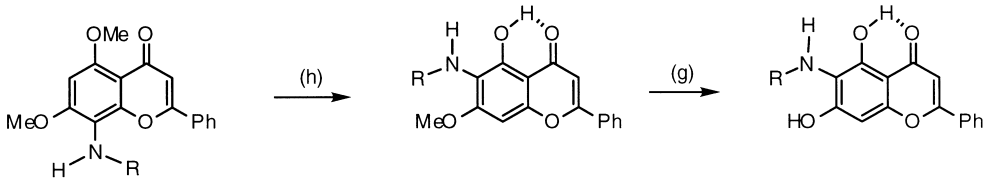
10a-e

Scheme 1. R=Pr^{i} (a); nPr (b); Bu^{i} (c); Pe^{i} (d); CH_2=$CHCH_2$ (e).

catalysis, met with total failure.[12–16] At this juncture, an alternative route which involved the functionalization of chrysine **1** was attempted 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). 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. Firstly, encouraged by the results of Wessely and Moser previously demonstrating the conversion of 5,7,8-trimethoxyflavone into the 5,6,7-trihydroxy compound on demethylation,[17] we thought that 8-alkylaminoflavones (**8a–e**) possibly could serve as the starting materials for the preparation of their 6-isomers **10a–e**. Secondly, compounds **8a–e** could prove interesting in exploring 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. Methylation of 8-nitrochrysine (**2**) with Me_2SO_4 and K_2CO_3, followed by reduction of the nitro group with stannous chloride in ethanol,[18] afforded **4** in roughly 60% overall yield. The latter was alkylated after protection with the 2,4-dinitrobenzenesulfonyl group.[19] *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



Scheme 2. (a) HNO_3 70%, AcOH, 65 °C, 1 h, 70%; (b) Me_2SO_4, K_2CO_3, DMF, room temperature, 3 h, 81%; (c) $SnCl_2.2H_2O$, EtOH, reflux, 5 h, 71%; (d) 2,4-$(NO_2)_2PhSO_2Cl$, pyridine, CH_2Cl_2, room temperature, 8 h, 88%; (e) RI, K_2CO_3, DMF, room temperature, 1–16 h, 83–87% or Pr^{i}OH, DEAD, PPh_3, THF, reflux, 3 h, 77%; (f) $HSCH_2COOH$, Et_3N, CH_2Cl_2, room temperature, 5–16 h, 71–94%; (g) $AlCl_3$, toluene, reflux, 68–92% or BBr_3, CH_2Cl_2, room temperature, 4–5 h, 50–70%.



7a-e **9a-e** **10a-e**

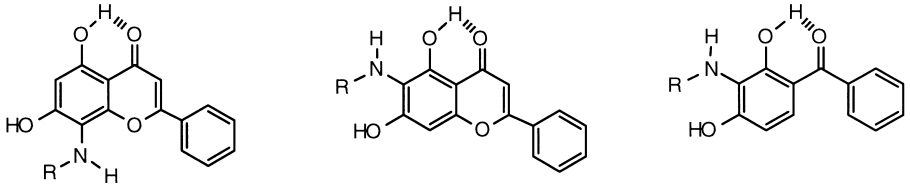
Scheme 3. (h) conc. HCl, reflux, 30–64 h, 60–74%.

alkylated under the Mitsunobu conditions,[20] 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).[19] 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.

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 established for some 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,[17] was essentially used for the elucidation of structures of flavonoids and was further extended to chromones, xanthones and derivatives.[21,22] Surprisingly, little attention has been devoted to this rearrangement as a surrogate of direct synthesis, especially when the latter was cumbersome and inefficient.[22,23] 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 all cases, we were unable to detect a trace of the expected 6-nitro isomer. Consequently, the conversion of 8-alkylamino derivatives **7a–e** by the action of boiling hydrochloric

Table 1. In vitro neuroprotective activity of compounds **8a–e** and **10a–e**



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

Compound	R[c]	Toxicity[a]				Protection versus 2 mM *L*-HCA[b]	
		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

[a]In vitro neurotoxicity monitored either by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reduction assay) or by quantification of cellular lysis after measurement of the lactate dehydrogenase (LDH) activity released from damaged cells into the culture supernatant.

[b]In vitro neuroprotective activity estimated through their protective effects against *L*-homocysteic acid (*L*-HCA) cytotoxicity.[25]

[c]Abbreviations: MTC, maximum tolerated concentration; TC_{50}, concentration producing 50% toxicity; PC_{50}, concentration producing 50% protection; Bu^i, isobutyl; Pe^i, isopentyl; *n*Pr, *n*-propyl; Pr^i, isopropyl.

acid was investigated. Then, 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 (Scheme 3).[24]

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 and compared with those of benzophenone analogues **II**.[25] The results are presented in Table 1.

Taking into account that optimum activity was displayed by alkyl substituents in the benzophenone series **II**,[6] only substituted alkylaminoflavones bearing isopropyl, *n*-propyl, isobutyl, isopentyl and allyl chains were evaluated. As reported in Table 1, 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. Based on the PC_{50} values, it appeared that 6-alkylaminoflavones (**10a–d**), as well as their benzophenone analogues **IIa–d**, demonstrated equivalent neuroprotective activities (for instance, compare **10a** and **IIa**). Furthermore, as the 6-alkylaminoflavones did not exhibit a reduced toxicity compared to their benzophenone analogues **IIa–d**, it could be concluded that alkylaminoflavone derivatives could not be considered as promising compounds for therapeutic potential.

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 was successfully accomplished through a convenient extension of the Wessely–Moser rearrangement. To the best of our knowledge, such a rearrangement has as yet not been reported for 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.

Acknowledgements

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24. **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, $J=6$ Hz), 1.50 (sextet, 2H, CH_2, $J=6$ Hz), 3.15 (t, 2H, N-CH_2, $J=6$ Hz), 6.35 (s, 1H, 6-H), 6.95 (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 0.85 (t, 3H, Me, $J=6$ Hz), 1.45 (sextet, 2H, CH_2, $J=6$ Hz), 3.20 (t, 2H, N-CH_2, $J=6$ Hz), 6.60 (s, 1H, 8-H), 6.95 (s, 1H, 3-H), 7.55 (m, 3H, H *meta* and H *para*, Ph), 8.05 (m, 2H, H *ortho*, Ph), 12.95 (s, 1H, 5-OH); MS (DCI): $m/z=312$ (MH^+).
25. In vitro biological assays are extensively detailed in refs 6 and 7.