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THE EFFECT OF PYRROLO[3,4-c]CARBAZOLE DERIVATIVES ON SPINAL CORD ChAT ACTIVITY

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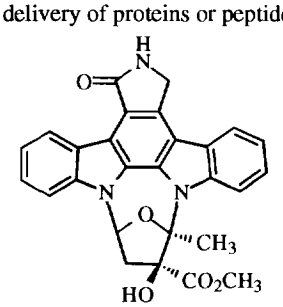
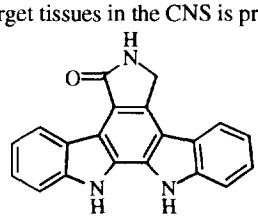
Abstract: Pyrrolo[3,4-c]carbazole derivatives were prepared as potential neurotrophic agents. The compounds were assayed for their ability to stimulate choline acetyltransferase (ChAT) activity in embryonic rat spinal cord cultures. These simplified K252a derivatives, although less potent and efficacious, have led to the identification of minimal structural requirements for K252a neurotrophic activity.

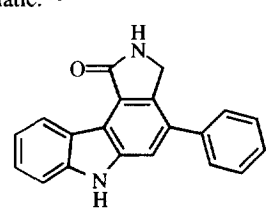
K252a, **1**, is an alkaloid produced by *Nocardiopsis* sp. K252[1] that was originally identified as a protein kinase C inhibitor. Subsequently, K252a was found to promote survival and/or differentiation in peripheral and central neuronal cultures.[2b,c] It was also reported to possess neurotrophic activity as measured by enhancement of choline acetyltransferase (ChAT) activity in embryonic rat spinal cord cultures.[2a] The increase in ChAT activity was similar to that observed with protein growth factors such as CNTF and IGF-I. An analog of **1** has been reported to be more potent and efficacious in enhancing ChAT activity in spinal cord culture and has also been shown to prevent developmentally programmed motoneuron death and the loss of ChAT activity in adult motoneurons *in vivo*.[3] The discovery and development of small organic molecules with neutrophic properties is an important and currently unmet therapeutic need.[4,5] Such molecules may be particularly useful in diseases where delivery of proteins or peptides to target tissues in the CNS is problematic.[4c]



1 (K252a)



2 (K252c)



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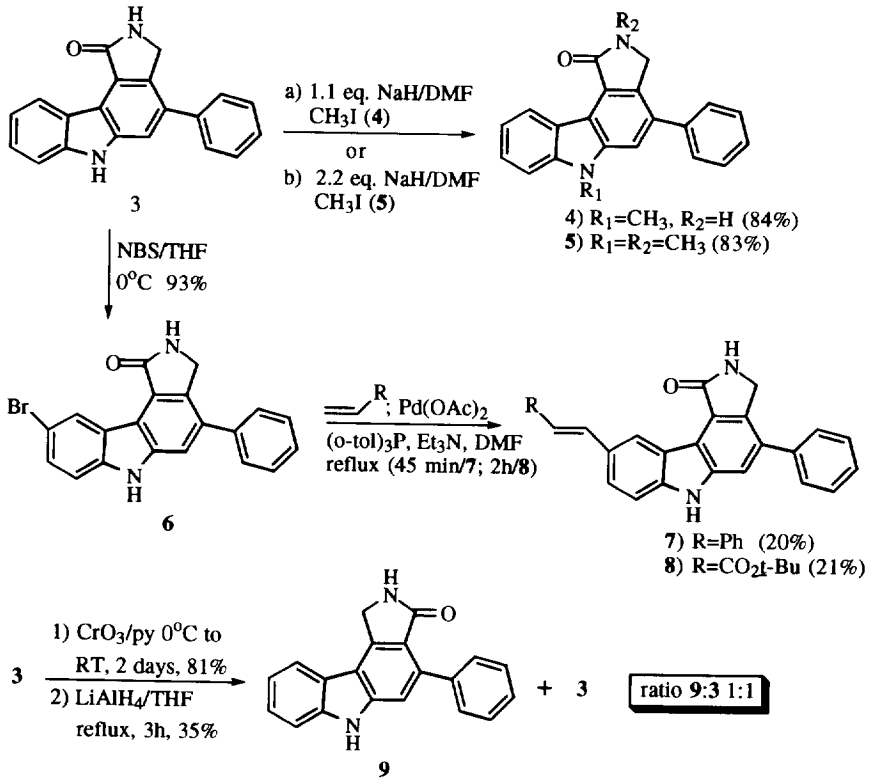
Currently, the only source of K252a is microbial fermentation.[1] Therefore it was an important objective to identify simplified derivatives of **1** that retain neurotrophic activity, and determine structural features important for ChAT enhancement. One further objective was to conduct an initial SAR survey to discover how modifications to this molecule influence *in vitro* activity. The natural product K252c[1], **2**[6c], and the known pyrrolocarbazole **3**[6a], synthetically more accessible analogs of **1**, were evaluated for efficacy *in vitro* using the rat spinal cord ChAT assay (Table 1). Both compounds demonstrated activity and **3** was selected for synthetic studies because it constituted a simpler active structure.

To evaluate the role of the two NH groups, selective methylation of the indole nitrogen of **3** was carried out with 1.1 equivalents of NaH and methyl iodide in DMF (Scheme 1) to give **4** (mp 230-232°C)[7] in 84% yield.

The position of this methyl group was conclusively established by NMR spectral comparison with the starting material (indole NH at 11.6 ppm, lactam NH at 8.7 ppm) and the dimethyl product **5** (mp 180-181°C), which was also obtained in good yield (83%) from **3** using a larger excess of base and alkylating agent.

SCHEME 1:



Regiospecific bromination of **3**, using 1.05 equivalents of NBS in THF at 0°C furnished **6** (mp 302-304°C) in 93% yield (Scheme 1). This allowed us to utilize Pd-mediated addition/elimination reactions to investigate the effect of other substituents at this position on the indole nucleus. Bromo derivative **6** underwent $Pd(OAc)_2$-mediated Heck reaction[8] with styrene and t-butyl acrylate to give the corresponding products **7** (mp >350 °C) and **8** (mp >350 °C), respectively, in 20% and 21% yield. Regioisomeric lactam **9** was prepared in two steps from **3**: i) CrO_3 oxidation (81%) to the corresponding imide (mp 265-266 °C, lit.[6b] 262-263°C) and ii) $LiAlH_4$ reduction in refluxing THF gave a 1:1 mixture of **9** (mp 269-270 °C, lit.[6b] 250°C decomp.) and **3** in 35% yield which was separated by flash chromatography.

Choline acetyltransferase (ChAT) activity measured in neuronal cultures has been used as an initial assessment of motoneuron survival, differentiation and/or enzyme regulation.[2a,b,5d] Spinal cords were dissected and dissociated from rat fetuses of embryonic age E14.5-15. The cells were then cultured and assayed for ChAT activity as previously described.[2a] The data in Table 1 represent the mean of at least two experiments (n=4 for

each determination). K252a was used as an internal control in each experiment. K252c proved to be less potent and efficacious compared to K252a. In addition to **2** and **3**, pyrrolocarbazoles **4** and **6** demonstrated activity in this screen; analogs **5**, **7**, **8** and **9** were inactive when tested up to 10 μM. Among the active pyrrolocarbazoles, the parent **3** and N-methyl analog **4** had efficacy similar to K252c, albeit at a 10 to 30-fold higher concentration. Compound **6** was noteworthy in that it stimulated ChAT activity at lower concentrations than the other pyrrolocarbazoles.

Table 1: Effect of Indolo- and Pyrrolocarbazoles on ChAT Activity in Spinal Cord Cultures

Compd	Maximal ChAT stimulation (concentration) % of Control[a]	Compd	Maximal ChAT stimulation (concentration) % of Control[a]
1	186±.03 (0.300 μM)	**6**	146±1.7 (1μM)
2	162±8 (1 μM)	**7**	inactive (10 μM)
3	167±9 (10 μM)	**8**	inactive (10 μM)
4	159±0.6 (10 μM)	**9**	inactive (10 μM)
5	inactive (10 μM)		

a) Maximum efficacy for enhancement of ChAT activity versus untreated control cultures, including the concentration of maximum measured effect. Values are mean ± SEM for at least 2 independent experiments

As a result of this work, the two most important objectives were achieved: a) identification of two simplified and more readily synthesized[6] structures (**2** and **3**) that retain neurotrophic activity *in vitro*; b) the demonstration that the lactam ring is a key region of the molecule for neurotrophic activity since N-methylation (**5**) or conversion to a regioisomer (**9**) eliminated enhanced ChAT activity. Bromination of **3** to give **6** improves the potency of the pyrrolocarbazole nucleus, but the vinyl substituents examined at this position (**7** and **8**) led to inactive analogs. The results obtained in this study as well as our earlier reports,[2a,b,3] provide evidence for the potential of organic molecules to act as neurotrophic agents. The mechanism(s) by which this group of molecules enhance ChAT activity is under active investigation[9], and it is known that this property is not related to inhibition of protein kinase C.[2a,9] Potential applications for small molecules with this activity include serious disorders for which there are currently no effective treatments, such as amyotrophic lateral sclerosis and Alzheimer's disease, as well as head and spinal cord trauma.[4]

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