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Total Synthesis and Neuritotrophic Activity of Farinosone C and Derivatives

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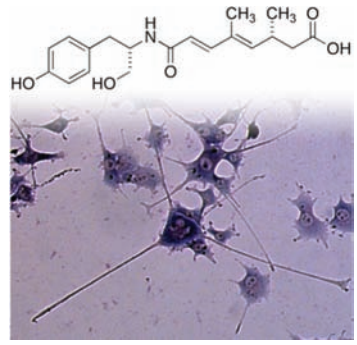
ABSTRACT



An efficient synthesis of all four possible stereoisomers of the neurotrophic amide farinosone C was developed. The absolute and relative configuration was assigned by comparison of synthetic material with a derivatized authentic sample of the natural product. Several derivatives allowed for the generation of preliminary structure activity relationships concerning neurite outgrowth in PC-12 cells, unravelling L-tyrosinol-amide as the pharmacophore.

Neurodegenerative diseases such as Alzheimer's or Parkinson's are becoming increasingly prevalent in the aging societies of industrialized countries.[1] The financial and social burden placed on patients and their families is considered severe. Current pharmacotherapy is symptom oriented and slows down the loss of cognitive function, but causal treatments remained unknown up to now.[1]

Progression of Alzheimer's disease is paralleled by neuritic atrophy and loss of synaptic function. Therefore, some emerging therapeutic approaches attempt to maintain neuronal function with the aid of neurotrophic factors such as nerve growth factor (NGF). However, clinical trials with these proteins were disappointing as a major problem was the delivery across the blood brain barrier.[1] One possible strategy of circumventing this problem would be the use of small organic molecules capable of mimicking or enhancing NGF action in the brain.[2]

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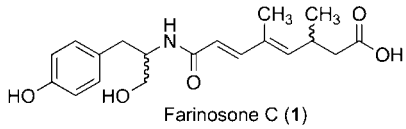
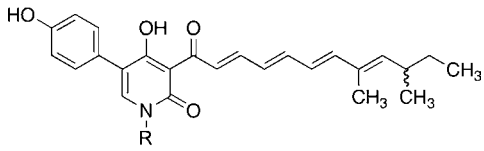
Farinosones A−C (**1**−**3**) were isolated from the entomologic fungus *Paecylomyces farinosus* and displayed neuritogenic properties in the PC-12 cellular assay.[3] Farinosone C (**1**) appears to be a dead-end byproduct in the pyridone alkaloid biosynthesis of this organism.[4] However, the absolute and relative configuration of **1**−**3** is unknown, and no total synthesis has been published. In this communication, we report the total synthesis of all four possible stereoisomers of farinosone C (**1**), the assignment of the relative and absolute configuration of the natural product, as well as preliminary structure activity relationship (SAR) data for this compound class concerning neuritogenic activity in the PC-12 assay.



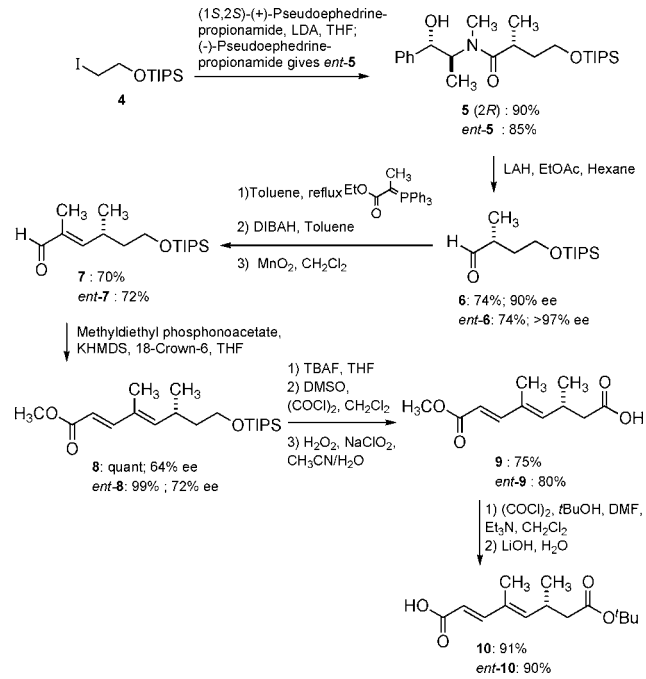
Farinosone C (**1**)



R = H: Farinosone A (**2**)
R = OH: Farinosone B (**3**)

The total synthesis of farinosone C (**1**) started with the polyketide side chain (Scheme 1): We used a stereoselective enolate alkylation reaction with commercially available pseudoephedrine propionamide and readily accessible TIPS protected 2-iodo ethanol **4** as reported by Myers and coworkers.[5] Both enantiomers **5** and *ent*-**5** were obtained and converted into the corresponding aldehyde **6** by direct reductive cleavage[6] resulting in excellent yields and enantiomeric excess (up to 97% ee).[6] We then proceeded with a Wittig reaction to introduce the (*E*) configured double bond, followed by reduction of the ester with DIBAH and subsequent oxidation to aldehyde **7** using activated manganese dioxide. The next double bond was introduced via a Horner−Wadsworth−Emmons (HWE) reaction giving the (*E*,*E*) configured esters of both enantiomers **8** and *ent*-**8** in

Scheme 1. Synthesis of Fragments **10** and *ent*-**10**



quantitative yield. After removal of the TIPS protecting group, partial racemization of the compounds was observed by HPLC analysis. Examination of the different intermediates and derivatives suggested the Wittig and HWE reactions as the responsible steps. As a separation of the isomers became feasible at a later stage of the synthesis, we did not change the protocols to avoid this partial racemization. A two-step oxidation[7] to the corresponding carboxylic acids **9** and *ent*-**9** followed by *tert*-butylation and selective saponification of the methyl ester afforded acids **10** and *ent*-**10** in excellent yields.

The carboxylic acids **10** and *ent*-**10** were directly used in the peptide coupling with D- or L-tyrosinol to give the amides **11** (Scheme 2). At this stage, the L-tyrosinol derived esters **11a** and **11c** were enriched in their diastereomeric purity up to a ratio of 40:1 by simple column chromatography. Cleavage of the *tert*-butyl esters with TFA yielded all four possible stereoisomers of farinosone C (**12a**−**d**), which were therefore prepared in overall yields up to 20% (ca. 90% average yield for every single step). The NMR spectra of compounds **12a**−**d** were indistinguishable, and no resolution of the stereoisomers of farinosone C could be obtained by analytical HPLC using various chiral stationary phases.

Therefore, we chose to use the methyl esters **13a**−**d**, which were prepared by derivatization of both synthetic samples **12a**−**d** and a sample of the natural product **1**. The methyl farinosones **13** could be separated by chiral HPLC, and in combination with the optical rotation, the absolute configuration of the natural product farinosone C (**1**) was therefore assigned as (1′*S*,3*R*) (**12a** = **1**).

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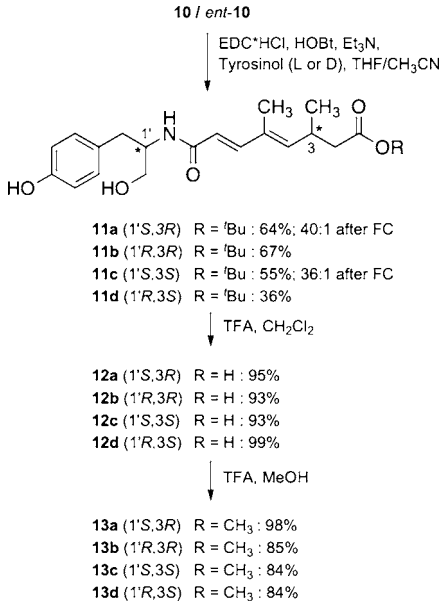
Scheme 2. Synthesis of Farinosones C and Derivatives



The establishment of structure activity relationships (SAR) of the compounds with regard to the induction and enhancement of neurite outgrowth was carried out using a standardized PC-12 cellular assay.[8,2a] PC-12 cells (DSMZ, Germany) were grown in DMEM[9] in culture flasks. Cell aggregates were separated by passage through a 21-gauge needle. The assays were conducted in collagene coated 24-well plates. Cell suspensions (10^5 cells/mL) were preincubated for 12 h and then incubated with the substances for two days with a final concentration of 50 μM (final amount of DMSO: 0.05%) with or without 10 ng/mL of NGF. All assays were performed in triplicate. After the incubation, cells were fixed with 1% glutaraldehyde in PBS and stained with Giemsa to visualize cell bodies and neurites or directly examined by phase contrast microscopy without staining. Cells from one to two areas of the single experiments were analyzed under a microscope, and from the data of the three experiments the mean score was obtained (>500 cells were examined for every single compound). The percentage of cells bearing neurites (more than one cell diameter in length) compared to total cells was calculated, and the significance of the data was determined by ANOVA with Dunnett's posttest. Samples with DMSO (0.05%) and NGF (10 ng/mL) were carried out on each plate as positive and negative controls. The results of these experiments are shown in Figure 1.

Clearly, farinosone C (**12a** = **1**) in its natural configuration both induced neurite outgrowth and enhanced the effect of NGF (Figure 1A). The carboxylic acid moiety had no influence on these effects, as both the methyl ester **13a** and

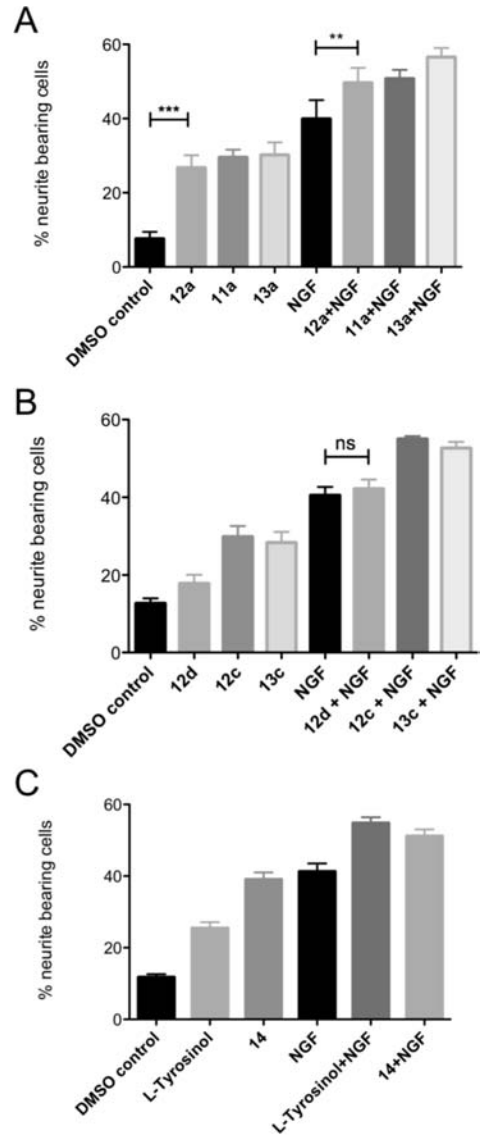


Figure 1. Induction and NGF-enhancement in the PC-12 assay, 12 h preincubation, followed by 2 days incubation with the compounds (conc. = 50 μM; NGF: 10 ng/mL). Values are reported as means ± SD of triplicate experiments: ***P < 0.001, **P < 0.01, ns = not significant, DMSO control: negative DMSO control.

the *tert*-butyl ester **11a** exerted similar activity. Therefore, this functional group is not part of the pharmacophore and could be used for further functionalizations. We then studied the influence of the configuration at C(3) in the side chain on activity (Figure 1B). Again, there was no influence of this position on the activity observed, as also compound **12c** (1′*S*,3*S*) and its ester **13c** showed an activity profile comparable to, e.g., **13a**. Strikingly, the activity of the compounds was drastically reduced when L-tyrosinol was substituted by the D-enantiomer, as (1′*R*)-**12d** was found to be less active. This effect is also evident when **12d** was assayed for NGF enhancement, where no increase of activity was found.

On the basis of these experiments, the pharmacophore could be delineated: the natural (1′*S*)-L-configuration of the tyrosinol fragment can be considered essential, whereas the

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(9) Dulbecco's modified eagles medium: 10% horse serum, 5% fetal calf serum, heat inactivated, 200 U/mL of penicillin, 200 μg/mL of streptomycin.

side chain only contributed little to the activity of the compounds. To substantiate this hypothesis, L-tyrosinol as well as the known[10] L-tyrosinol-propionamide **14** (Figure 2)

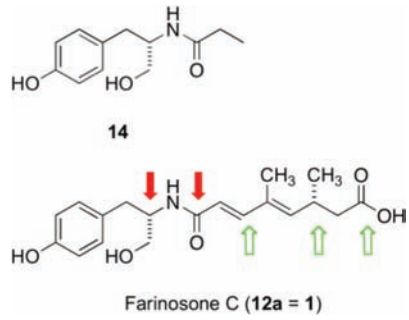

Figure 2. L-Tyrosinol-propionamide **14** and definition of the pharmacophore of farinosone C (filled: important positions; empty: less important positions).

were assayed as simplified analogues of farinosone C (Figure 1C). L-Tyrosinol itself induced neurite outgrowth and enhanced NGF action. This neuritotrophic behavior was found to be of similar potency as in the case of L-tyrosinol-propionamide **14** and other comparable compounds such as farinosone C (**12a**) and the esters **11a** and **13a**. In the absence of NGF, the induction of neurite outgrowth was found to be significant in the case of L-tyrosinol and considerably stronger for propionamide **14**. Therefore, the amide bond appears to be an integral part of the pharmacophore. Interestingly, these tyrosinol derivatives induced neurite outgrowth in the presence of a 15-fold excess of L-tyrosine which is present in DMEM. Farinosone C (**1** = **12a**) was not found to be toxic to PC-12 cells in the MTT assay in concentrations up to 50 μM.[3]

In this study, we reported the first total synthesis of all possible stereoisomers of farinosone C (**12a** = **1**). This led to the assignment of the absolute configuration of the natural product as shown for **12a**. By establishing preliminary structure activity relationships (SAR), the pharmacophore of these compounds was investigated, and it was demonstrated that the induction of neurite outgrowth and enhancement of NGF originates from the structural pattern of L-tyrosinol-amides. Whether the neurotrophic action of farinosone C in the PC-12 model is based on a persistent activation of PI3-K/PKB and MEK/ERK signaling pathways as observed for militarinone A[11] is unclear at present. As the carboxylic acid is available for further functionalization, it might be possible to identify the molecular target of these compounds by pull-down assays. These experiments are currently being carried out in our laboratories.

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Supporting Information Available: Experimental procedures, full spectroscopic data for all new compounds, and biological experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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