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# Biological evaluation of pyridone alkaloids on the endocannabinoid system

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ABSTRACT

Naturally occurring pyridone alkaloids as well as synthetic derivatives were previously shown to induce neurite outgrowth. However, the molecular basis for this biological effect remains poorly understood. In this work, we have prepared new pyridones, and tested the effect of thirteen 4-hydroxy-2-pyridone derivatives on the components of the endocannabinoid system. Investigation of binding affinities towards $CB_1$ and $CB_2$ receptors led to the identification of a compound binding selectively to $CB_1$ (**12**). Compound **12** and a closely related derivative (**11**) also inhibited anandamide (AEA) hydrolysis by fatty acid amide hydrolase. Interestingly, none of the compounds tested showed any effect on 2-AG hydrolysis by monoacylglycerol lipase at 10 μM. Assessment of AEA uptake did, however, lead to the identification of four inhibitors with $IC_{50}$ values in the submicromolar range and high selectivity over the other components of the endocannabinoid system.



## 1. Introduction

Hydroxy-2-pyridones constitute a compelling class of secondary metabolites,[1] which have been suggested to act as chemical mediators in interactions between entomopathogenic fungi and their insect hosts. Over the past years, a number of biological activities have been reported for these compounds, mostly focusing on their *in vitro* properties, such as cytotoxicity.[2] For example, ricinine, a 2-pyridone alkaloid first isolated from castor oil plants in the 19th century,[3] has long been known as an insecticide[4] and lately been reported to have stimulatory effects on the central nervous system.[5] Pronounced antifungal activity has been observed with the structurally more complex compounds ilicicolin H,[2d,6] apiosporamide,[7] and YM-215343.[2e] However, the molecular targets of 4-hydroxy-2-pyridones have generally remained elusive.[8] A recent study by the group of Waldmann, however, demonstrated an inhibitory effect on kinase activity, and a crystal structure of 3-acyl-4-hydroxy-2-pyridone complexed with wild-type mitogen-activated protein kinase 4 (MAP4K4) with the inhibitor residing in the ATP binding site supports this hypothesis.[9]

There have been numerous efforts directed towards the total synthesis of 4-hydroxy-2-pyridones, as it is difficult to obtain these alkaloids in pure form from natural sources.[10] The work of one of our groups has focused on a family of 3-acyl-4-hydroxy-2-pyridone alkaloids isolated from entomopathogenic fungi (Table 1).[2b,11] Chemical synthesis yielded the congeners pretenellin B, prebassianin B, farinosone A, militarinone D and another homologue of the militarinone family (HJJ-510). All of these substances show neuritogenic activity in the PC12 cell assay.[12] Based on these phenotypic observations, we became interested in the potential mechanism of action of these compounds.

The structurally related compound farinosone C triggers neurite outgrowth, and we have shown that structurally simplified analogs retain this activity.[13] In order to investigate the underlying biochemical pathways responsible for neurite outgrowth, these substances were tested for their ability to bind to cannabinoid receptors ($CB_1$ and $CB_2$). It had been previously reported that $CB_1$ receptor activation promotes neuritogenesis in Neuro-2A cells via a complex signalling pathway[14] and to restore neuritogenic activity in hyperglycemic PC12 cells.[15] One of the farinosone C analogs (BSL-34; Scheme 1) very effectively inhibits anandamide (AEA) uptake – most likely by interacting with the putative endocannabinoid membrane transporter, which is supposed to regulate the bidirectional shuttle of endocannabinoids across the plasma membrane or via interaction with cytoplasmic shuttle proteins.[16]

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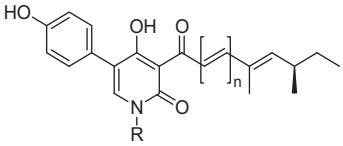
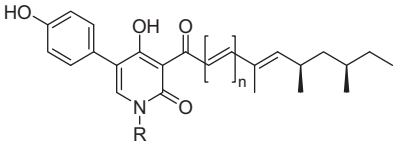
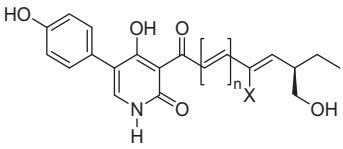
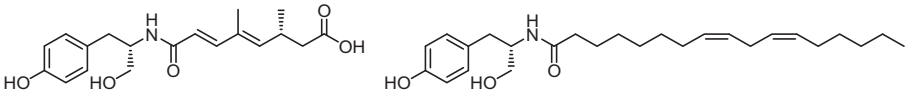
0968-0896/

**Table 1**
Family of pyridone alkaloids from entomopathogenic fungi.

| | | |
|---|---|---|
|  | R = H, n = 1 | **Pretenellin B** |
| | R = OH, n = 1 | Tenellin |
| | **R = H, n = 2** | **Prebassianin B** |
| | R = OH, n = 2 | Bassianin |
| | **R = H, n = 3** | **Farinosone A** |
| | R = OH, n = 3 | Farinosone B |
|  | **R = H, n = 1** | **Unknown (HJJ-510)** |
| | R = OH, n = 1 | Unknown |
| | **R = H, n = 2** | **Militarinone D** |
| | R = OH, n = 2 | Unknown |
|  | **X = $CH_3$, n = 1** | **Pyridovericin** |
| | **X = H, n = 2** | **Torrubiellone C** |



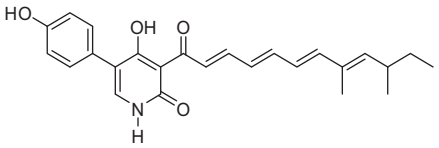
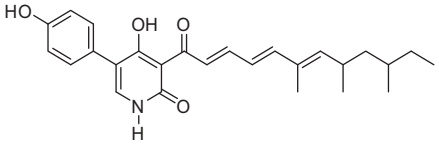
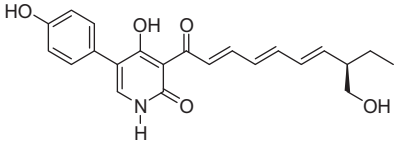
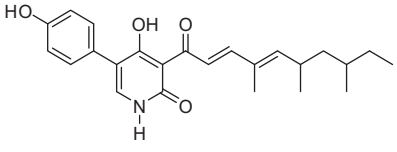

**Scheme 1.** Neuritogenic compounds farinosone C and BSL-34.

Based on these observations, we reasoned that AEA uptake inhibition might be the cause for the neuritogenic effects exhibited by BSL-34 because such inhibition would lead to an increase in the extracellular concentrations of AEA, which can in turn activate cannabinoid receptors. In addition, increasing levels of AEA might also affect TRPV1 activity, which plays a role in neuronal differentiation of SH-SY5Y human neuroblastoma cells.[17] Therefore, we investigated whether the neuritogenic effects of the pyridone alkaloids are related to activation of $CB_1$ and $CB_2$ and/or indirect modulation of AEA levels by inhibition of the degrading enzymes and/or specifically AEA uptake. All compounds tested – eight natural products, two truncated ester derivatives and three derivatives with ketone side chains of various length – are shown in Table 2.

## 2. Chemistry

The natural products tested in this study for their activity towards components of the endocannabinoid system were

**Table 2**
Chemical structure of the pyridone alkaloids tested on the different components of the ECS and known inhibitors for positive controls.

| Entry | Name | Structure |
|---|---|---|
| **1** | Farinosone A |  |
| **2** | Militarinone D |  |
| **3** | Torrubiellone C |  |
| **4** | HJJ-510 |  |

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