Tetrahedron 74 (2018) 3238–3245

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Cascade reactions leading to the mechanism of action of vinaxanthone and xanthofulvin, natural products that drive nerve repair

Anders M. Eliasen [a], Matthew R. Chin [b], Abram J. Axelrod [c], Ruben Abagyan [d], Dionicio Siegel [d, *]

[a] California Institute for Biomedical Research, La Jolla, CA 92037, USA
[b] Gilead Sciences, Inc., Foster City, CA 94404, USA
[c] Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Center Research, New York, NY 10021, USA
[d] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA

ARTICLE INFO

Article history:
Received 5 January 2018
Received in revised form
23 February 2018
Accepted 27 February 2018
Available online 26 March 2018

Keywords:
Methodology
Total synthesis
Biosynthesis
Natural product
Regeneration
SUCNR1
Succinate receptor
Allosteric
GPCR

ABSTRACT

The natural products vinaxanthone and xanthofulvin promote regeneration in animal models of spinal cord injury and corneal transplant. However, inhibition of the initially described biological target of these compounds, semaphorin 3A, does not fully account for the recovery demonstrated *in vivo* following administration of the natural products. Through chemical synthesis substantial quantities of both natural products have been accessed with early reaction development paving the way for synthesizing both compounds. The success of a model system, first disclosed herein, translated to the syntheses of both natural products. Following from this we also report for the first time the discovery of a new target of the natural products, the succinate receptor 1 (SUCNR1). Both natural products function as positive allosteric modulators of SUCNR1. As the first known allosteric modulators of SUCNR1, the compounds represent powerful new tools to understand the pharmacology of SUCNR1 and its control of growth and cellular defense.

1. Introduction

It can be difficult to predict *in vitro in vivo* correlation (IVIVC) phenomena, as the transition from *in vitro* assays into preclinical testing frequently yields unforeseen effects. These effects can be increased toxicity, loss of activity, or only partial recapitulation of the effects based on the *in vitro* hypotheses. An additional, and highly desirable, phenomenon is enhancement of the expected phenotypic response through unforeseen synergistic off-target effects. However, deconvolution of these multiple effects is required to capitalize on these rare, serendipitous discoveries. We have found this is the case for the natural products vinaxanthone and xanthofulvin (Fig. 1), which induce a strong regenerative response in rodent models of spinal cord injury and corneal transplantation. Access to the compounds to permit this was achieved through reaction development that led to the creation of useful laboratory preparations, total synthesis.

Both natural products were initially identified as semaphorin 3A (Sema3a) inhibitors, capable of inhibiting the protein-protein interaction of Sema3a with its receptor, the plexin-neuropilin-1 complex.[1–3] Semaphorins have membrane-bound and soluble forms, are upregulated following injury to neurons in the central nervous system (CNS), and are found in high concentrations within fibroblasts at the site of injury.[4–6] Similar to other guidance cues, Sema3a plays an inhibitory role in axonal guidance, angiogenesis, and cellular movement. Towards validating the hypothesis that Sema3a inhibition provides a long sought target for CNS injury full spinal cord transection model in rats were examined with both natural products.[7,8] From these studies it was found that xanthofulvin and vinaxanthone enhance axonal regeneration, promote remyelination, increase angiogenesis, and attenuate lesion scarring. The combination of these effects leads to an improved functional recovery. Relatedly, administration of vinaxanthone following corneal transplant improved innervation into the transplanted

* Corresponding author.
E-mail address: drsiegel@ucsd.edu (D. Siegel).

https://doi.org/10.1016/j.tet.2018.02.068
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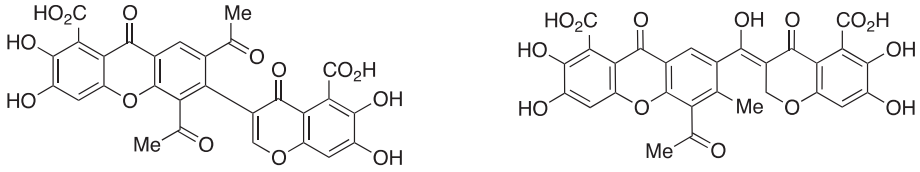


Fig. 1. Structures of vinaxanthone (**1**) and xanthofulvin (**2**).

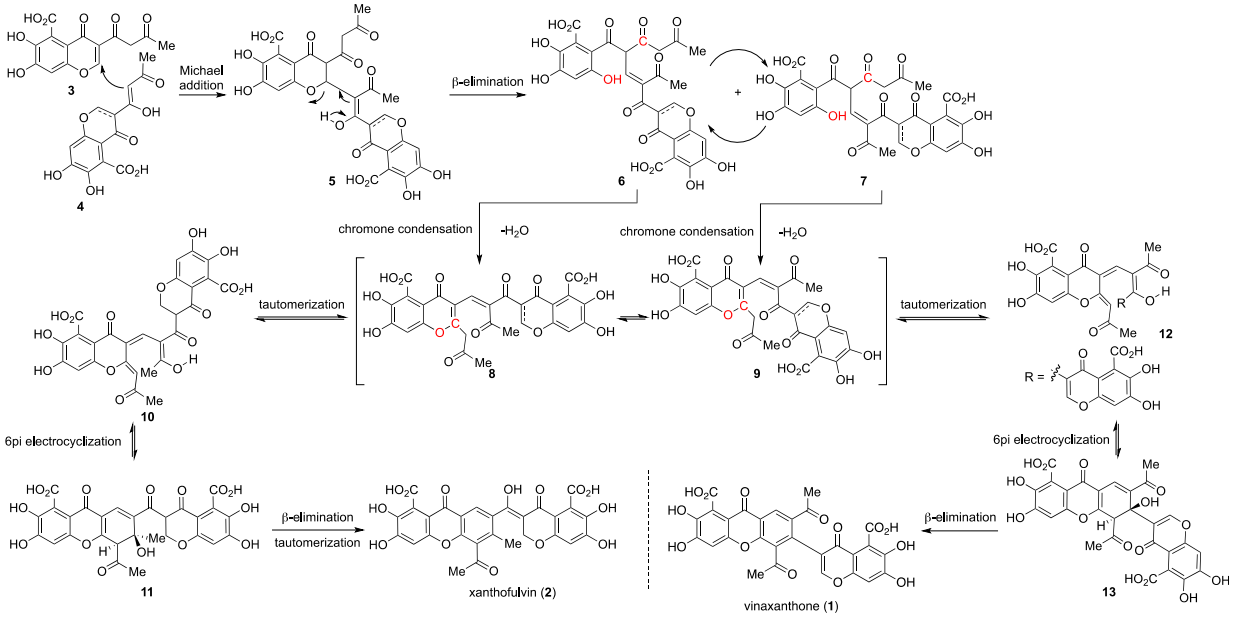
tissue.[9] However, other Sema3a inhibitors do not elicit this type of response, suggesting unknown, positive off-target interactions. Additionally, advanced investigation into the effects of Sema3a on regeneration determined that decreased or absent Sema3a activity alone was not responsible for the diverse beneficial effects the compounds induced following spinal cord injury.[10] These findings taken together suggest other biological target(s) exist for vinaxanthone and xanthofulvin and that these are important components to the complete mechanism of action of these natural products.

2. Results and discussion

Initially, access to both vinaxanthone and xanthofulvin was limited to fermentation using *Penicillium* sp. SPF-3059, a fungus utilized by Sumitomo Dainippon Pharma. A laboratory preparation of vinaxanthone was reported in 2007, however, a late stage Diels-Alder reaction with a concomitant oxidation/aromatization sequence in the synthesis proved variable and incompatible with analog synthesis and could not be utilized to access xanthofulvin.[11] To provide improved access our laboratory has developed a fully synthetic approach to both vinaxanthone and xanthofulvin.[12] Additionally, our synthetic platform can furnish analogues to interrogate the role of each carboxylic acid and phenol substituents in CNS tissue regeneration. This matrix of analogues has elucidated a greater understanding of structure features enabling damage repair.[13] Early studies surrounding the syntheses of the natural products were based on hypothetical dimerization-like reactions that were proposed to occur spontaneously with a non-enzymatic union of two simpler compounds to directly prepare the larger, targeted natural products proceeding through the proposed cascade sequence shown in Scheme 1.

Reaction development was inspired by the proposed cascade sequence and focused on the synthesis of triketone (**14**) as a simplified surrogate for dehydropolivione (**3**) (Fig. 2). This was required for optimization as using the highly polar compound would provide a poor readout of reaction efficiency due to isolation/purification challenges. The synthesis of triketone **14** starting from vinylogous amide **15** required the introduction of an acetoacetate group, which was sought in a single operation (Scheme 2). The vinylogous amide (**15**) has two reactivity attributes as it serves as the framework to stitch the chromone ring together through direct acetoacetylation. The reaction was investigated using several acetoacylating reagents, acyl-ketene equivalents. Acyl transfer reaction onto enaminone **15** would first result in phenolic esterification, ester **17**, followed by spontaneous *O* → *C* transfer via the vinylogous amide acting as a nucleophile to furnish **20**, then after loss of dimethylamine the desired compound **14**.

This transformation was first attempted with diketene and only generated the expected product in 2% yield and proved unable to be improved (Entry 1, Table 1). However, triketone **14** was able to be characterized by single crystal x-ray diffraction and under these conditions it was shown to be in the enol form (Fig. 3). Given the low yield different acetoacetylation reagents were examined to improve the conversion of enaminone **15** to triketone **14**. Thermolysis of furan-dione (Entry 2) in toluene at reflux furnished



Scheme 1. Proposed biosynthesis of vinaxanthone (**1**) and xanthofulvin (**2**) through nonenzymatic, cascade reactions.