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Flavaglines: Discovery From Plants Used in Traditional Chinese Medicine, Synthesis and Drug Development against Cancer and Immune Disorders

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Abstract: Flavaglines, a family of compounds coming from plants used in Traditional Chinese Medicine, exhibit a broad range of biological effects including anticancer, antiviral, cardioprotectant and anti-inflammatory activities. They exert their action by targeting the scaffold proteins called prohibitins-1 and-2, and the mRNA helicases eIF4A and DDX3. Flavaglines are densely functionalized cyclopenta[b]benzofurans that have attracted the attention of some of the most eminent organic chemists. This review provides an overview of the biosynthesis, total synthesis and pharmacological activities of flavaglines, which recently culminated with the entrance of a synthetic derivative, Zotatifin, into clinical trials against advanced solid tumors refractory or intolerant to standard treatments.

Keywords: Natural products; Traditional Chinese Medicine; eIF4A; prohibitins; cancer; cardioprotection; total synthesis; medicinal chemistry.

1. INTRODUCTION

The plant genus Aglaia found in the tropical and subtropical rainforest of southern China, the Indo-Malayan region, some Pacific Islands and Northern Australia is characterized by benzo[b]oxepines, cyclopenta[b]benzofurans (also called flavaglines) and cyclopenta[bc]benzopyrans [1]. These trees have a long history in traditional medicine. In China, the branches and leaves of Aglaia odorata are often decocted or made into externally applied ointment to treat pain in rheumatic joints, injuries from falls, superficial infections and toxic swellings. Its flowers are recommended against distress of chest and diaphragm, common cold and cough [2]. The bark decoction of Aglaia elliptica, is used to treat tumors, whereas its flowers are applied to wounds [3].

In India, Aglaia roxburghiana is traditionally used in Ayurvedic medicine against inflammation, leprosy, throat infections, bilious and febrile complaint, and also to treat snake and scorpion bites [4, 5]. Aglaia elaeagnoidea and Aglaia Lawii are used to treat diarrhea, skin diseases, bacterial infection, headaches, liver diseases and tumors [6].

In Thailand, Aglaia odorata is used as a tonic, febrifuge and a remedy for menorrhagia during the menopause [7].

Rocaglamide (1, Figures 1 and 2) was isolated in 1982 from a plant used in Traditional Chinese Medicine (TCM), Aglaia elliptifolia by Taiwanese scientists, King and collaborators [8]. These authors identified the structure of rocaglamide and demonstrated its anticancer activity in a murine model of leukemia. Since then, more than 100 flavaglines have been isolated from Aglaia species, and recently from trees of the gender Dysoxylum [1, 9, 10-12].

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In the 1980’s, this compound and some of its natural analogs were found to delay tumor growth in mice at the National Cancer Institute. But, as they did not eradicate these tumors, this investigation was interrupted.

In 2004, Marian and collaborators dubbed “flavaglines” this family of flavonol–cinnamate-derived cyclopenta[b]benzofurans as they stem from the metabolism of flavonoids in plants of the genus Aglaia. Importantly, this team discovered that flavaglines induce the death of cancer cells without affecting normal cells [13].

Thereafter, flavaglines were found to display anti-inflammatory, neuro- and cardioprotectant activities, suggesting that they not only display anticancer properties but also protect the organism against the adverse effect of chemotherapies.

The discovery in 2008 and 2012 that these drugs target the initiation factor of protein synthesis eIF4A [14-17] and prohibitins (PHBs) [18] greatly stimulated this field of research, leading to the advancement in 2019 of a synthetic flavagline, zotatifin (also called eFT226) into of phase 1/2 clinical trial against advanced solid tumors refractory or intolerant to standard treatments [19]. Next year flavaglines were found to also target the mRNA helicase DDX3 [20].

2. BIOSYNTHESIS

The coexistence of cinnamic amides 12, aglaforbesins 15, aglains 16 and flavaglines in the plants of the genus Aglaia, led Proksch and coll. to propose the biosynthetic pathway presented in Scheme 1 [21]. A [3 + 2] condensation between a hydroxyflavone 11 and a cinnamic amide 12 generates benzo[b]oxepines 13, which can be reduced to diols 16 or undergo acyloin rearrangement to afford flavaglines 17. The presence of flavaglines in these plants probably stems from their potent insecticide activity [21-24].

3. TOTAL SYNTHESES

The densely functionalized tricyclic core of flavaglines represents a considerable challenge that was first taken up by Barry Trost [25]. Since then, other approaches have been described and recently reviewed [26]. However, only two strategies have been extensively used in medicinal chemistry...
programs. The first of these particularly practical approaches was originally developed by Richard Taylor and improved by Markus Dobler (Scheme 2) [27-29]. It is based on a Michael reaction between a benzofuranone 18 and cinnamaldehyde, followed by the formation of a cyanohydrin 21 that can be deprotonated to react with the ketone and provide the flavagline backbone.

The second approach, inspired by the biosynthesis of flavaglines in plants, was developed by John Porco (Scheme 3) [30-33]. UV irradiation of a 3-hydroxyflavone 11 in the presence of a cinnamic ester or amide generates a photoexcited triplet biradical 24 capable of performing a [3 + 2] cycloaddition, to afford the aglain backbone (25). In the presence of a base, this adduct undergoes an α-acyloin rearrangement to generate the flavagline 26.

As part of our medicinal program to develop flavaglines with improved pharmacological properties, we considered using this strategy to prepare new derivatives. For this purpose, we developed two efficient syntheses of the cyclopentanone 37 substituted by the different aryl moieties necessary for the pharmacological activity (Scheme 5). The first approach relied on a gold(I)-catalyzed cyclopentanone formation that was originally described with tertiary silyl ethers [35]. This synthesis began with a Perkin condensation of acid 32 and benzaldehyde followed by the conversion to an acyl chloride and a Sonogashira coupling that afforded ketone 33 as a sole E isomer [36]. Condensation with lithiated trimethoxybenzene, molybdenum (VI)-catalyzed transposition and etherification of allylic alcohol 34 conveniently furnished the allylic ether 35. Gratifyingly, gold(I)-catalyzed annulation of alkyne 36 afforded the cyclopenteno 37 in 63% yield.

During the development of this synthesis, we serendipitously found that ReO₄ efficiently catalyzes the formation of cyclopentanone 37 directly from styryl-propargyl alcohol 34 [36]. This type of reaction has not been described before. It represents an interesting alternative to the Rautenstrauch rearrangement. Selective monodemethylation using BBr₃ efficiently afforded phenol 38 in a 75% yield (Scheme 5). Unfortunately, all our attempts to construct the flavagline scaffold from this compound, including variation around the strategy developed by Ragot and coll. were unsuccessful. This explains also why these authors didn’t use this method to synthesize compounds substituted with the methoxys groups necessary for a pharmacological activity.
These two methoxys were also found to modulate the chemical reactivity of flavaglines. Thus, we found that acylation of amine 39 with dimethylcarbamoyl chloride at reflux in dichloromethane afforded a mixture of carbamates 45 and 46 in a 83/17 ratio (Scheme 6) [37]. This unusual inversion of configuration of an aminoalcohol was not observed with flavagline 47. The mechanism of this reaction probably involves the benzylic carbocation 40, which may either afford the styrylurea 43 or undergo a ring closure to form the oxazolidinone dimethyliminium salt 41. The latter may also undergo a rearrangement into 43. Upon protonation and hydration 43 may generate the hydroxyureas 45 and 46 via the carbocations 42 and 44, with a strong preference for the thermodynamically more stable isomer 45.

This possibility to generate a stabilized carbocation was exploited to introduce an azide in position 1a from the cyclic sulfite 50 (Scheme 7) [38]. Reduction and acylation thereof provided the formamide 52 and the sulfonamide 53, which, unfortunately, were found to be pharmacologically inactive.

### 4. MECHANISM OF ACTION AND PHARMACOLOGICAL ACTIVITIES

Flavaglines mediate their biological activities by inhibiting the mRNA helicases eIF4A and DDX3 and also by modulating the activity of the scaffold proteins prohibitins-1 and -2 (PHB1/2) (Figure 3). eIF4A is a helicase that unwinds strongly structured mRNA. Only a small number of mRNAs, which mainly code for proteins involved in cancer, need this helicase for their translation. Flavaglines stabilize eIF4A complexed to mRNAs, which prevents the liberation and recycling of eIF4A, leading to an inhibition of its activity [39]. Importantly, this inhibition seems to affect every cancer cell type, including persistor cells that usually tolerate anticancer drugs and are at the origin of acquired resistance.
and cancer relapse. The inhibition of eIF4A also relieves the resistance to BRAF and MEK inhibitors in a murine model of metastatic melanoma, prevents the immune escape of tumor cells by blocking inhibitory checkpoints, abrogate taxane resistance in prostate cancer and synergizes with MEK inhibitors to kill NRAS-mutant cancer cell lines.

Recently, using the O-nitrobenzoxadiazole-conjugated derivative of rocalaglumide 54, Iwasaki and coll. found that flavaglines also target the helicase DDX3 [20]. Indeed this probe was found to perform a proximity-specific fluorescence labeling of both eIF4A and DDX3 (Figure 4).

In addition to these helicases, flavaglines also target prohibitins-1 and -2 (PHB1/2). These scaffold proteins exists as two isoforms in the animal kingdom where they regulate the activity of a myriad of signaling proteins [40-43].

PHBs are characterized by a stomatin/prohibitin/flotillin/HK/HIC (SPFH) domain that is present in an evolutionarily conserved family of proteins in archaea, bacteria and eukaryotes [44]. Thus, through their actions on PHBs, flavaglines inhibit the activation of KRAS and C-RAF, to block the C-RAF/MEK/ERK pathway critical to the survival of many cancer cell types [45, 46]. In bladder cancer, flavaglines inhibit PHB1 phosphorylation by Akt, leading to removal of PHB1 in mitochondria and also upregulate GADD45α to arrest cell cycle progression at the G2/M phase [47]. They also promote the expression of Axin 1 to inhibit Wnt/β-catenin signaling and combat intestinal tumorigenesis [48]. Moreover, flavaglines block mitophagy and energy productions by inhibiting the effects of PHB2 on the mitochondrial inner protease PARL and LC3-II and by disrupting the complex PHB/SH2D4/STAT3 in mitochondria [49]. Indeed, they prevent the interleukine-6-induced phosphorylation of STAT3 leading to a reduction STAT3 transcriptional activity and HIF1α stabilization in hepatocellular carcinoma.

Flavaglines can also induce apoptosis of cancer cells by activating AIF, and caspase-8 through yet uncharacterized mechanisms. In addition to their antitumor effects, flavaglines protect the heart of mice against the adverse effects of doxorubicin, a widely used anticancer medicine that induces a severe cardiotoxicity in patients. This cardioprotective effect is also mediated by prohibitins: flavaglines induce a cardioprotection by inducing a translocation of PHB1 and STAT3 to mitochondria, a phosphorylation of HSP27 and an overexpression of PHB1 in cardiomyocytes [50, 51]. Altogether, these data suggest that flavaglines could improve the efficacy of chemotherapies and alleviate in the same time their adverse effects.

In line with the use of Aglaia extracts in traditional Chinese medicine to treat inflammatory diseases, flavaglines protect mice in a model of Crohn’s disease in which inflammation is driven by epithelial ulceration of the colon [52]. Indeed, FL3 (6) decreased the inflammation of the colon and in the same time promoted the survival of the intestinal epithelial cells against the inflammatory stress.

Through their effects on eIF4A and/or PHBs, flavaglines have been shown to display potent antiviral activities against several types of viruses, including Chikungunya virus and coronaviruses [53-58].

**5. DEVELOPMENT OF ZOTATIFIN (EFT226)**

Founded in 2012 in California, eFFECTOR Therapeutics is a pharmaceutical company that developed the first flavagline to enter clinical trials [19]. When this project started, many patents had been filed by academic laboratories and several pharmaceutical companies (Bayer and its spin-off, IMD Natural Solutions (Germany), Infinity Pharmaceuticals (USA) and Pierre Fabre (France)) [59-62]. To generate some intellectual property, the chemists at eFFECTOR came up with the idea of replacing one of the carbons in the flavagline backbone with a nitrogen. In the course of their investigations, they noticed that compounds with a 40-degree aryl-aryl torsion as a preferred low-energy conformation tend to display higher potency (Figure 5).

Through their effects on eIF4A and/or PHBs, flavaglines have been shown to display potent antiviral activities against several types of viruses, including Chikungunya virus and coronaviruses [53-58].

**Figure 5.** Conformational analysis. At the lowest energy conformation, θ = 40° is optimal for the inhibition of eIF4A [19].

This observation has been used prospectively to select the compounds to synthesize. The isostere 56 with a nitrogen in position 8 was found to be 25-fold less cytotoxic than rocalaglumide in MDA-MB-231 breast cancer cells (Figure 6). In accordance with a previous report by another biotech company, Infinity Pharmaceuticals [61], the replacement of the methoxy in position 4’ by a nitrile (57) enhanced the activity. Shifting the nitrogen and the methoxy (57→58) improved further the cytotoxicity, but at the expense of Caco permeability and solubility. These two problems could be overcome by reducing the tertiary amide to a dimethylamine 59. By shifting the nitrogen to position 7 and reinstalling the methoxy that had been removed, a compound
as cytotoxic as rocaglamide with improved solubility and permeability was identified. This compound, eFT226 (10), has successfully passed US regulatory preclinical requirements and entered phase 1/2 clinical trial against advanced solid tumors refractory or intolerant to standard treatments, under the name zotatifin [19]. To develop this clinical candidate, more than 20,000 flavagline derivatives were synthesized.

Thanks to the basic dimethylamino side chain, eFT226 is much more soluble than rocaglamide (>20 mg/mL), which greatly facilitates intravenous administration. eFT226 (1 mg/kg, intravenous administration) inhibited tumor growth in a MDA-MB-231 triple negative breast cancer orthotopic xenograft model treated once weekly for 2 weeks.

Biophysical studies demonstrated that eFT226 enhances the binding of polypurine RNA motifs to eIF4A independently of ATP similarly to eIF4A1. The mutation of phenylalanine 163 to leucine, which inhibits the binding of eIF4A to flavaglines, reduced the antiproliferative effects of eFT226 by a factor of 60, demonstrating that eIF4A is the main target to mediate its anticancer properties. Up to now, the effect of this compound on PHB signaling has not been reported. Zotatifin is not expected to be efficient against every type of tumors. eFFECTOR’s scientist found that this drug is particularly effective in cancers where the kinase mTOR is driving mRNA translation, such as diffuse large B-cell lymphoma (DLBCL), and Burkitt lymphoma. In mouse models of these two types of lymphoma, eFT226 displayed impressive anticancer activities at low doses (1 mg/kg/week i.v.) [63].

The synthesis of zotatifin was achieved using Porco’s biomimetic synthesis to construct the flavagline scaffold prepared by lithiation of pyridine derivative 61 using classical reactions.

6. Conclusion and perspective

Since their discovery in 1982; flavaglines have attracted the attention of many organic chemists due to the challenge posed by the complexity of their structure. So far only two methods are used in medicinal chemistry: that of Taylor and Dobbler, and that of Porco.

Regarding the pharmacological studies of these compounds, these are dominated by their anticancer effects, with as a culmination the entry of Zotatifin in clinical trials against advanced solid tumors refractory or intolerant to standard treatments. In this area, the major obstacle is to identify how to combine flavaglines with other drugs to optimize their anticancer effects in patients. Although in vivo cytoprotective, antiviral, anti-inflammatory and immunosuppressive effects have been demonstrated, the exploration of these effects should increase in the coming years, even if it remains marginal for the moment compared to studies in oncology.

In addition to their potential, flavaglines have been shown to be useful as chemical tools to explore the function of prohibitins and eIF4A. To our knowledge, the involvement of these targets in the mechanism of action of traditional medicine preparations incorporating Aglaia extract has not yet been reported.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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