Evolution of artificial metalloenzymes for the Chemo - photo - enzymatic asymmetric synthesis of Indolone-based drugs

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Specific problem being addressed: We have recently used artificial metalloenzyme catalysis, or biocatalysis, to synthesize bioactive small compounds asymmetrically. In this case, our goal is to increase the production of both commercial and bioactive medicinal compounds using our previously established processes. We will use the large-scale protein expression and whole-cell reaction conditions that have already been attained for the reliable production of small drug molecules. The attainment of high turnover numbers (TON) is the primary focus of the study. Additionally, there will be a large reduction in the consumption of organic solvents.

Project Summary: Indolones are renowned for having a broad spectrum of biological activities. For synthetic chemists, chiral indolones in particular are crucial target molecules due to their uses in pharmacy and other domains. As a result, many groups have expressed interest in its synthesis, and numerous procedures for doing so may be found in the literature. (Picture 1). One of the earliest antifibrotic medications, nitedanib (Ofev®), was authorized for the treatment of idiopathic pulmonary fibrosis. It is currently approved for the treatment of systemic sclerosis-associated ILD (SSc-ILD) as well as other chronic fibrosing ILDs with a progressive pattern.

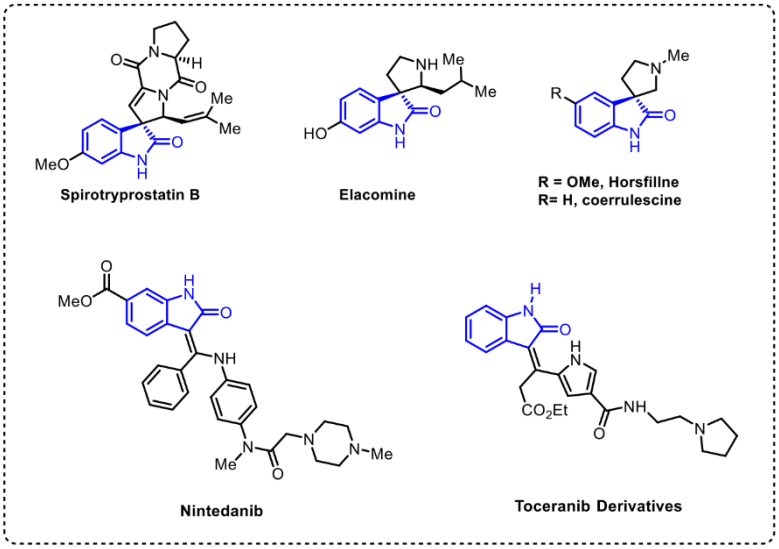

Fig 1. Indolone based drug target

An RTK inhibitor called toceranib (Palladia) destroys tumor cells and reduces the blood flow to the tumor. Toceranib inhibits the growth of tumors by generating antiangiogenic and antiproliferative effects. In dogs with cancer, toceranib also lowers regulatory T-cells (Treg), which may improve immune surveillance. Our goal is to use artificial metalloenzyme-catalyzed photo-enzymatic synthesis to carry out the large-scale biocatalytic synthesis of derivatives of nitenedanib and tecetane.

Table 1. Importance of indolones target drugs

Target molecules	Uses
Spirotryprostatin B	It is an indolic alkaloid found in the Aspergillus fumigatus fungus that belongs to a class of naturally occurring 2,5-diketopiperazines. It have been found to have anti-mitotic properties, and as such they have become of great interest as anti-cancer drugs
Elacomine	Elacomine are naturally occurring hemiterpene spirooxindole alkaloids isolated from the rootsof the shrub *Elaeagnus commutate.*
Nintedanib	Nintedanib is used for the treatment of idiopathic pulmonary fibrosis. Nintedanib competitively inhibits both nonreceptor tyrosine kinases (nRTKs) and receptor tyrosine kinases (RTKs).
Toceranib	A Toceranib is a receptor tyrosine kinase inhibitor and is used in the treatment of canine mast cell tumor also called mastocytoma. Toceranib is likely to act mostly through inhibition of the kit tyrosine kinase, though it may also have an anti-angiogenic effect.

We have designed and synthesized an Iridium based cofactor 1 (Ir-gen-1) which is photoactive and it is found to be highly efficient in catalyzing the formation of indolone derivatives from ethyl diazoacetate and acrylamide derivatives, when it is anchored within Sav variants. After getting the cofactor the artificial metalloenzyme was constructed in-situ and the desired asymmetric synthesis of indolones was achieved, the anchoring of the cofactor was confirmed via fluorescent activity study inside gel doc. Quantitative conversion of the starting material was observed in both cases when hosts were t-Sav S112A and K121A. As many of the drug molecules and natural products that contain indolone scaffold have an aromatic substituent, we propose that our methodology would be a good fit for the synthesis of such molecules and provide value to industries that perform their large-scale synthesis (Figure 1).

Table 2 : commercial value of some of the target molecules

Compound	Annual Sales	Reaction steps	Notes
Nintedanib	$ 2.743 billion	More than 6	Eco-friendly and large scale industrial synthesis can be achieved by biocatalysis
Toceranib	$ 30 million	More than 6	Eco-friendly and large scale industrial synthesis can be achieved by biocatalysis

A brief description regarding the importance of selected indolones based drugs is highlighted in table 1& 2. The required diazo derivatives can be easily obtained and easily used as the carbene precursor for the synthesis of the corresponding indolones, containing drug scaffolds.

Impact of this innovation: While asymmetric catalysis has long been a challenge for chemists, nature has used a number of enzymes to pretty robustly carry out such reactions on extremely complicated molecules. These enzymes do, however, have restrictions on the kinds of reactions they can carry out, their degree of flexibility, and the substrate specificity. The designed protein that catalyzes the process is what makes our method novel. This required a large amount of work to screen for different mutations in the active sites that could alter the geometry surrounding the metal core and contribute to asymmetric catalysis. Many pharmaceutical companies can use this technology to create medication compounds based on indolone.