Bioorganic & Medicinal Chemistry 24 (2016) 435–443

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Tubulin modulating antifungal and antiproliferative pyrazinone derivatives

Andrew E. Taggi [a,*], Thomas M. Stevenson [a], James F. Bereznak [a], Paula L. Sharpe [a], Steven Gutteridge [b], Robert Forman [c], John J. Bisaha [a], Daniel Cordova [b], Martina Crompton [c], Lora Geist [a], Patrick Kovacs [a], Eric Marshall [a], Ritesh Sheth [a], Courtney Stavis [a], Chi-Ping Tseng [a]

[a] DuPont Crop Protection, Discovery Chemistry, 1090 Elkton Road, Newark, DE 19714, USA
[b] DuPont Crop Protection, Chemical Genomics, 1090 Elkton Road, Newark, DE 19714, USA
[c] DuPont Crop Protection, Discovery Biology, 1090 Elkton Road, Newark, DE 19714, USA

ARTICLE INFO

Article history:
Received 23 June 2015
Revised 25 August 2015
Accepted 26 August 2015
Available online 28 August 2015

Keywords:
Fungicide
Tubulin
Pathogen
Synthesis
Cell line
Agriculture

ABSTRACT

A novel class of synthetic tubulin polymerization disruptors, based on a substituted pyrazin-2-one core, has been discovered. These molecules have proven to be potent broad spectrum fungicides, with activity on agriculturally important ascomycete and basidiomycete pathogens. They have also been found to be particularly potent against human rhabdomyosarcoma cells. Using an efficient synthetic route, the agricultural and medicinal activity was explored.

1. Introduction

It is projected that the world will need to increase food production by 60% in order to feed a global population expected to be in excess of 9 billion people by 2050. At the same time, it is estimated that every year global crop yields are reduced by 20–40% by agricultural pests.[1] Because of the ability of plant pathogens to rapidly develop resistance, it is imperative that new classes of broad spectrum disease control agents, with novel modes of action, be discovered in order to prevent catastrophic yield losses, which can approach 80% in some circumstances.[2] In order to discover a fungicide with activity on a broad range of taxonomic classes of fungi, one must target an essential pathway that is fairly well conserved across the pathogens of interest. Historically, some of the most successful fungicides have inhibited critical processes such as mitochondrial electron transport,[3] sterol biosynthesis,[4] and tubulin polymerization.

Tubulin is a member of a superfamily of globular proteins which exist in six subtypes, including the most common α- and β-tubulin. α- and β-tubulin polymerize into heterodimers and then ultimately into larger structures which govern critical cellular processes, such as mitosis. Interference with the dynamic equilibrium between polymerization and depolymerization of tubulin has historically been an effective strategy for treating diseases characterized by rapid cellular proliferation, such as cancer.[5] Conceptually, there is much similarity between the proliferation of cancer cells and the rapid cellular propagation of a fungal infection. It is therefore not surprising that tubulin polymerization disruptors have been successfully used in agricultural settings since the 1960s, starting with methyl benzimidazole carbamates (MBCs)[6], and more recently with zoxamide[7] and the quinolinyloxyacetamides[8] (which have yet to be exploited commercially).

With tubulin polymerization modulators playing such an important role in agriculture and medicine, it is imperative that novel chemotypes of known classes of molecules, as well as molecules that bind to completely different binding sites, be discovered and studied. In this paper, we will discuss the synthesis and biological evaluation of a new class of tubulin modulating molecules, based on the pyrazin-2-one core, for both medicinal and agricultural use.

* Corresponding author.
E-mail address: andrew.e.taggi@dupont.com (A.E. Taggi).

http://dx.doi.org/10.1016/j.bmc.2015.08.038
0968-0896/

2. Results and discussion

2.1. Chemistry

When we started our work in the area of tubulin polymerization modulators, we were aware of the triazolopyrimidines (**1**, Fig. 1) from American Cyanamid and eventually BASF[9] as well as subsequent research programs at Bayer[10] and Syngenta.[11] In particular, we were drawn to the 2-pyrazolyl-pyrimidines (**2**),[12] as they had opened a new vein of opportunity by moving away from the bicyclic core, into a linear arrangement. Additionally, the internal imine in the pyrido[2,3-*b*]pyrazines (**3**) suggested the possibility of a carbonyl to replace one of the pyrimidine nitrogens. To satisfy the need for another sp^2 center in the core, which would be the attachment point for the alkyl side chain, the pyrimidine nitrogen was conceptually moved over by one position, resulting in the pyrazin-2-one core (**4**).[13]

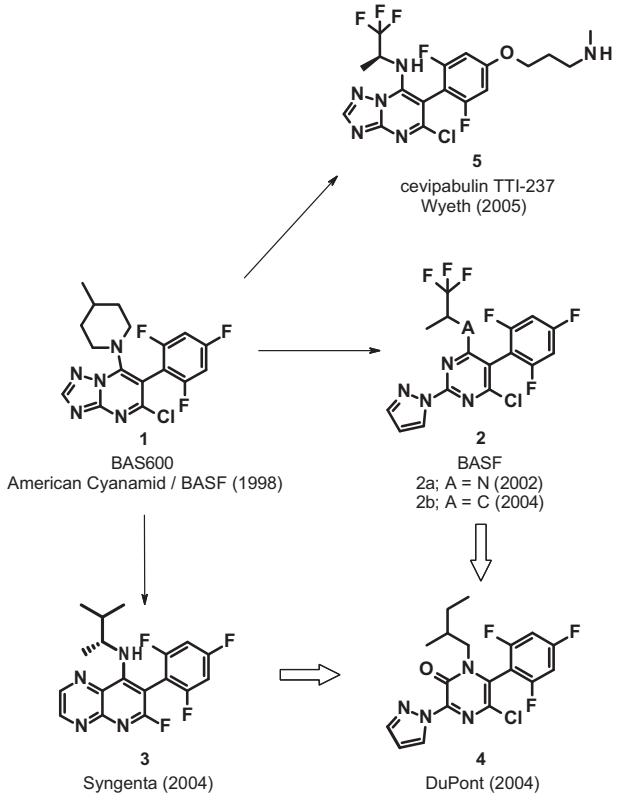
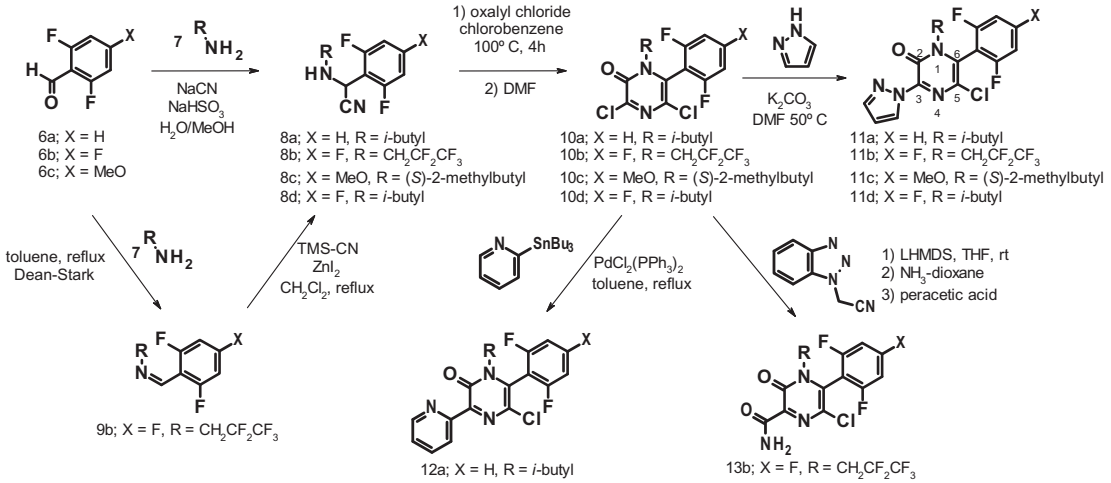


Figure 1. Different mono- and bicyclic tubulin modulating fungicides.

The optimization and evaluation of the pyrazin-2-ones was greatly simplified by the early adoption of a modular synthesis, which allowed us to specifically modify the four pendant functionalities in order to explore their biological relevance (Scheme 1). The synthesis usually began with a Strecker reaction between an aldehyde, an amine, and cyanide to establish the functionalities that would ultimately be in the 1 and 6 positions of the pyrazinone core. If necessary, the Strecker reactions could be catalyzed with sodium bisulfite or a Lewis acid such as indium(III) chloride.[14] In cases where the amine had poor nucleophilicity, such as anilines or fluorinated alkyl groups, a two-step procedure was employed where the imine was prepared under dehydrating conditions, and then subsequently allowed to react with a cyanide source to generate the α-aminonitrile.

The pyrazinone core was then formed by treating the α-aminonitrile (**8**) with oxalyl chloride at elevated temperatures. This reaction often suffered from yields in the range of 40–60%, with the remaining mass balance being composed of mono-chlorinated pyrazinone. It was subsequently found that a more efficient conversion to the dichloro pyrazinone could be achieved by the addition of a small amount of DMF to the reaction mixture *after* the α-aminonitrile had reacted with the oxalyl chloride. This procedure resulted in increased product yields, approaching 90% in some cases. Under the same conditions, use of oxalyl bromide resulted in the 3,5-dibromo-pyrazin-2-one, which could be used to explore alternate substitutions at the 5 position. Finally, the 3-chloro group could be displaced with a variety of nucleophiles, including NH containing heterocycles, such as pyrazole to form the final targets. The same chlorine could also be subjected to transition metal catalyzed cross-coupling reactions to yield targets with C-linked heterocycles. Amides could also be prepared from the same synthon by reaction with *N*-cyanomethyl-benzotriazoles and base followed by oxidation with peracetic acid.[15]

While in related art, the alkylaminoalkoxy side chain of cevipabulin (**5**) could be added through the nucleophilic displacement of the 4-fluoro on a 2,4,6-trifluorophenyl,[16] this was not possible in the pyrazinone system due to the concomitant displacement of the N-linked pyrazole. To overcome this difficulty,



Scheme 1. The general synthesis of pyrazin-2-one fungicides.