DOI: 10.1002/anie.201302365

Modern Agriculture

4-Hydroxyphenylpyruvate Dioxygenase Inhibitors in Combination with Safeners: Solutions for Modern and Sustainable Agriculture

*Hartmut Ahrens, Gudrun Lange, Thomas Müller, Chris Rosinger, Lothar Willms, and Andreas van Almsick**

crop protection agents · drug discovery · herbicides · HPPD inhibitors · safeners

Dedicated to the Bayer company on the occasion of its 150th anniversary

Inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) prevent plant carotenoid pigment formation, which in turn leads to chlorophyll degradation. This "bleaching" herbicide mode of action provides weed-control products for various crops, such as rice, corn, and cereals. Combinations with suitable safeners allow the full exploitation of the potential of this compound class to selectively control major weed problems, including rapidly increasing cases of resistance against other important herbicide classes.

1. Introduction

World food security is threatened by crop losses because of weeds, pathogens, and animal pests. It is estimated that, if these were left unchecked, weeds would have the highest impact on global crop yields (34% reduction).[1] Control of weeds is therefore of utmost importance to secure food supply for the increasing world population.

Since around the middle of the last century, synthetic herbicides have gained increasing importance in weed control, and replaced or augmented mechanical control measures. This is also true for weed control in noncrop areas (e.g. railways), where weeds also interfere with human interests. Herbicides that control weeds without injury to the crop are termed "selective", whereas others are termed "nonselective". However, the introduction of crop varieties that are genetically modified (GM) to tolerate nonselective herbicides (especially glyphosate[2]) has blurred this distinction.

In certain cropping systems, farmers have not only reduced mechanical weed control, but also reduced crop rotation and the number of herbicides used. All these factors can cause selection pressure on the populations of some weed species. Over several years, this has led to fields containing weeds that are resistant to previously effective herbicides.

Significant examples of resistance toward important modes of action are known, such as acetyl coenzyme A carboxylase (ACCase) and acetolactate synthase (ALS) and more recently the most important herbicide glyphosate.[3] Therefore, there is a rising demand for new solutions to control a broad spectrum of weeds, including resistant biotypes, combined with excellent selectivity in the crops. The 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor bleaching herbicide mode of action offers such solutions.

Safeners, first discovered in the mid-20th century, are chemicals that induce crop plants (but not weeds) to more rapidly degrade herbicides. In doing so, they ensure that the herbicides maintain weed control but with little or no injury to the crop. Thus, these provide an alternative selectivity technology to the more recently developed GM approach. Approximately 15 safeners have been commercialized, although several of these have already been superseded.

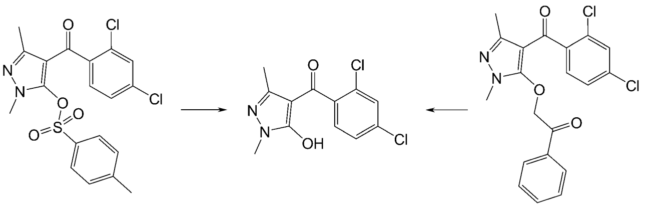
This Minireview outlines the history of the discovery of HPPD inhibitors and describes how Bayer CropScience AG (BCS) discovered its own HPPD-inhibiting compounds. Furthermore, the Minireview gives an overview of safeners that were developed by BCS and combined with HPPD herbicides to achieve the necessary crop selectivity. These examples show the high value and opportunities of the combination of HPPD inhibitors with safeners as outstanding solutions for modern and sustainable agriculture.

2. Discovery of HPPD Inhibitors

2.1. Chemistry

In the early 1970s, Sankyo developed pyrazolynate (**1**, Scheme 1), which they launched in 1980. This was followed by

[*] Dr. H. Ahrens, Dr. G. Lange, Dr. T. Müller, Dr. C. Rosinger, Dr. L. Willms, Dr. A. van Almsick
Small Molecules Research, Bayer CropScience AG
Industriepark Höchst, 65926 Frankfurt a.M. (Germany)
E-mail: andreas.almsick@bayer.com



1 pyrazolynate

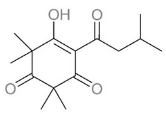
3 active compound (drug)

2 pyrazoxyfen

Scheme 1. Rice HPPD herbicides pyrazolynate (**1**) and pyrazoxyfen (**2**) and their metabolism to active compound **3**.

the development of pyrazoxyfen (**2**), which Ishihara launched in 1985. Both were developed for weed control in rice. The two compounds are pro-drugs and release the free hydroxypyrazole **3**, which is herbicidal and binds to the HPPD enzyme. These were the first compounds known to inhibit HPPD and were able to control annual as well as perennial weeds in paddy fields at application rates of 3–4 kg a.i./ha[*].[4–6]

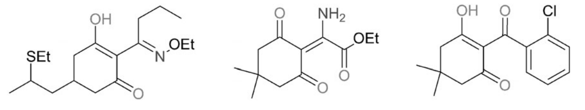
In 1982, Stauffer (a legacy company of ICI and now Syngenta) discovered triketone-type HPPD inhibitors, which are related to the natural product leptospermone (**4**, Figure 1), which has weak herbicidal activity toward HPPD.[7]



4

Figure 1. Structure of leptospermone (**4**).

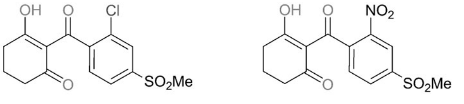
However, Stauffer aimed to prepare other functional mimics of the oximino moiety of sethoxydim (**5**, Figure 2), an ACCase herbicide. After first observations of encouraging herbicidal activity of compound **6**, further structural variations afforded the 2-chloro benzoyl derivative **7** with even better herbicidal activity and surprising bleaching effects.[8]



5 **6** **7**

Figure 2. Structures of sethoxydim (**5**), lead structure **6**, and the first triketone HPPD inhibitor **7**.

Intensified research on this subject led to the development and launch of the corn HPPD herbicides sulcotrione (**8**; post-emergence herbicide; Figure 3) and later mesotrione (**9**; pre- and post-emergence herbicide). Both mainly control broadleaf weeds and, unlike pyrazolynate (**1**), they are the actual HPPD inhibitors (not pro-drugs).[4]



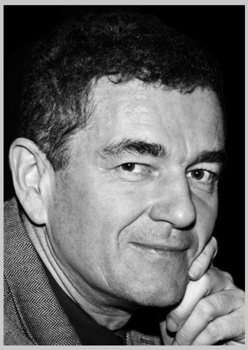
8 **9**

Figure 3. Structures of sulcotrione (**8**) and mesotrione (**9**).

In the late 1980s, Rhone-Poulenc (a legacy company of BCS) identified an interesting new lead structure that, through optimization, led to isoxaflutole (**10**; first patented in 1991;[9] Scheme 2). This development established a new class of HPPD inhibitors, this time with an isoxazole heterocycle. Isoxaflutole was the first HPPD-inhibiting herbicide for cross-spectrum control (broadleaf and grass weeds) and also

---

[*] a.i. = active ingredients; means always the compound regardless of whether or not it is a pro-drug



Andreas van Almsick gained his doctorate from the University of Wuppertal, Germany, in 1990 for studying enzymes in organic synthesis. He has worked as a chemist in Research for over 20 years, first with Schering in Berlin (from 1991), and later with AgrEvo, Aventis, and Bayer CropScience in Frankfurt (since 1996). He specialized in research on HPPD herbicides and identified tembotrione and tefuryl-trione.

Hartmut Ahrens studied chemistry in Kiel and joined the group of Prof. Hoppe in Münster for his PhD in stereoselective synthesis. He then worked at Central Research of Akzo Nobel on projects involving functional chemicals and surfactants. Since 1999, he has worked in herbicide research in Frankfurt, first with AgrEvo and Aventis and since 2002 with Bayer CropScience.

Chris Rosinger gained his doctorate from the University of North Wales, Bangor, in 1982 for studying cold-stress plant physiology, and then worked on a similarly themed post-doctorate at the University of Manitoba, Canada. He has worked as a biologist in herbicide research for 28 years, first with Schering in the UK, and later with AgrEvo, Aventis, and Bayer CropScience in Frankfurt, Germany (since 1994). Currently, he specializes in safener and rice-herbicide research.

Thomas Müller graduated from the J.-W. Goethe University, Frankfurt, in 1996. In 1999, he received his PhD on natural product synthesis from the Max–Planck Institut (MPI) für Kohlenforschung, Mülheim a. d. Ruhr, and then did post-doctorate research at the Ohio State University, Columbus Ohio, US. He has worked as a chemist in process and herbicide research for 12 years, first with Aventis and then with Bayer CropScience. Currently, he specializes in safener and phytotonics research.

the first herbicide that was used for pre-emergence application to corn. A possible synthetic access to isoxaflutole (Scheme 2) starts with 2-chloro-4-trifluoromethyl benzoic acid (**11**). The aromatic nucleophilic substitution with sodium thiomethylate and the following oxidation of the thioether to the sulfone furnishes the substitution pattern of the benzoyl moiety in the isoxaflutole molecule. The acid chloride of benzoic acid **13** acylates the magnesium enolate of *tert*-butyl 3-cyclopropyl-3-oxopropionate (**15**), the product of which is decarboxylated in the next step. After a condensation reaction with triethyl orthoformiate, a final ring closure with hydroxylamine yields the target product isoxaflutole.[4]

Isoxazole-type HPPD inhibitors are a new type of prodrug that are rapidly metabolized in soil and in plants to the corresponding diketonitriles, which are the entities with the herbicidal activity (Scheme 3).[10]

In 2006, BASF commercialized the new hydroxypyrazole topramezone (**20**, Figure 4). In post-emergent application, topramezone controls a wide spectrum of annual grass and broadleaf weeds. The excellent tolerance in corn is caused by a more rapid metabolism as well as by a lower sensitivity to the HPPD target enzyme in the crop.[11]

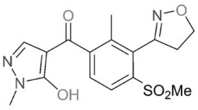
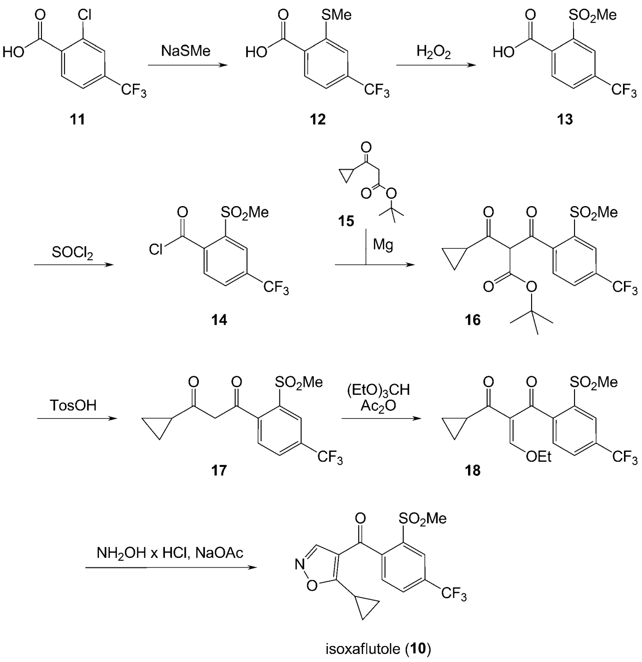

20

Figure 4. Structure of topramezone (**20**).

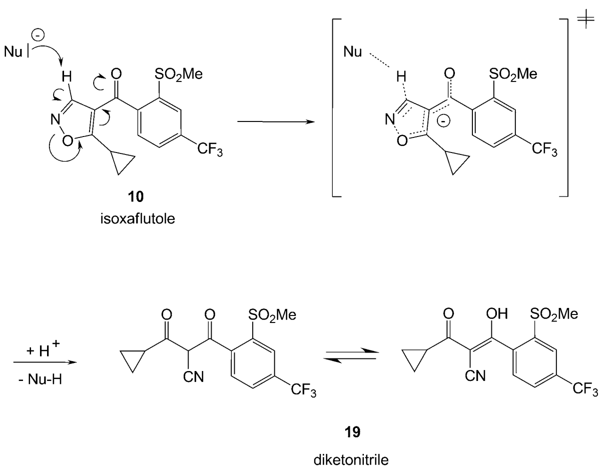
Since 2008, a further hydroxypyrazole, pyrasulfotole (**21**) from BCS, is available for post-emergent control of a wide range of broadleaf weeds. This herbicide enabled for the first time HPPD weed control in cereals (wheat, barley, and triticale). Pyrasulfotole was found in 1999 by combination of the 2-methanesulfonyl-4-trifluoromethyl benzoyl moiety of isoxaflutole with the 5-hydroxy-1,3-dimethylpyrazole heterocycle **22** of pyrazolinate (Scheme 4).[6]

Lothar Willms studied chemistry at the University of Bonn and received his PhD on phosphorus organic chemistry in 1979. He worked as a chemist in research and in different managerial positions in research with Hoechst, Roussel-Uclaf, AgrEvo, Aventis, and now with Bayer CropScience. He is specialized in herbicide and safener SAR.

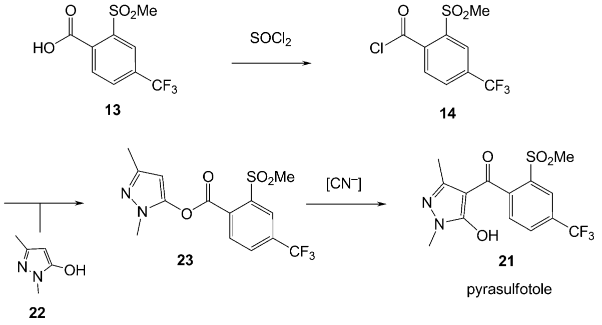
Gudrun Lange studied chemistry at the University of Freiburg. She analyzed the self-association of a protein combining X-ray scattering and kinetic modeling for her PhD (Max-Planck Groups, Hamburg). Since 1990, she worked as a protein crystallographer at the EMBL, the University of York, and at Hoechst Pharma, elucidating structures of proteins with fragments and ligands. In 2000, she moved to Aventis (now Bayer CropScience), focusing on structure-based design and the relationships within protein families.



Scheme 2. Synthesis of isoxaflutole (**10**).

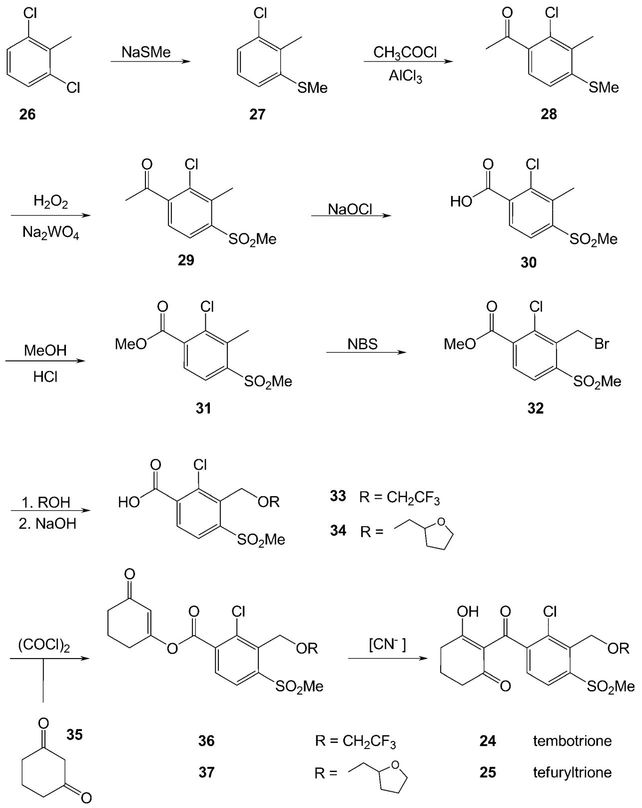


Scheme 3. Metabolism and prodrug concept of isoxaflutole (**10**).



Scheme 4. Synthesis of pyrasulfotole (**21**).

Intensive variation of the benzoyl moiety at AgrEvo (a legacy company of BCS) led to special side chains in the 3-position and the new active ingredients tembotrione (**24**) and tefuryltrione (**25**). Since 2007, tembotrione is commercialized for the control of broadleaf and grass weeds in corn production, whereas three years later tefuryltrione was launched for weed control in rice.[4,12] Chemical synthesis of these compounds begins with an aromatic nucleophilic substitution in 2,6-dichlorotoluene (**26**) with a methylthio group. Friedel–Crafts acylation followed by thioether oxidation and hypochlorite oxidation of the acetyl group by haloform reaction yields the benzoic acid **30**. This compound can be brominated at the methyl group (after esterification) under conditions favoring radical substitution to the key intermediate **32**. This species can be substituted in the benzylic position with a broad range of nucleophiles, particularly alcohols such as trifluoroethanol and (tetrahydrofuran-2-yl)methanol, leading to the new benzoyl moieties **33** and **34** through saponification. Final steps in the synthesis of tembotrione and tefuryltrione involve esterification of the acid chloride with 1,3-cyclohexanedione (**35**) and rearrangement of the enolester (Scheme 5).[13]



Scheme 5. Synthesis of tembotrione (**24**) and tefuryltrione (**25**).

It should be mentioned that all HPPD inhibitors exist in tautomeric forms as shown for diketonitrile **19** in Scheme 3. An overview of HPPD inhibitors including the timeline of their commercialization is shown in Figure 5.

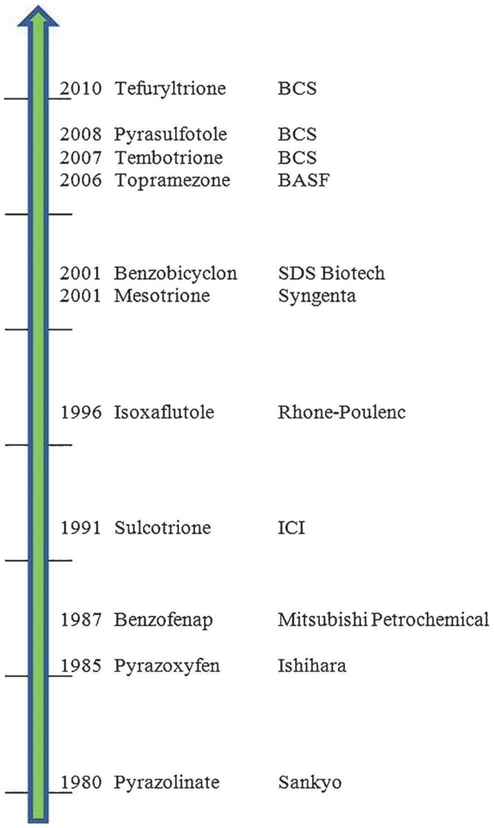
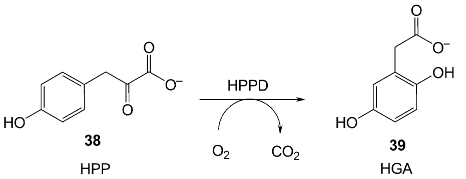


Figure 5. Timeline of first marketing of HPPD inhibitors.

2.2. Mode of Action

The enzyme HPPD, which is targeted by the herbicide, catalyzes the oxidative decarboxylation and the rearrangement of *p*-hydroxyphenylpyruvate (HPP, **38**) to homogentisate (HGA, **39**; Scheme 6).[14]



Scheme 6. HPPD-catalyzed conversion of HPP (**38**) to HGA (**39**).

This conversion is an early step in the tyrosine degradation pathway. More importantly, HGA is the key precursor for the biosynthesis of tocopherols and plastoquinone. Tocopherols are antioxidants required for plant growth and stress tolerance. Plastoquinone is a crucial co-factor in photosynthesis and in the biosynthesis of carotenoids. These pigments protect plants from detrimental effects of singlet oxygen, which is produced under excessive sunlight. The inhibition of carotenoid biosynthesis by HPPD inhibitors

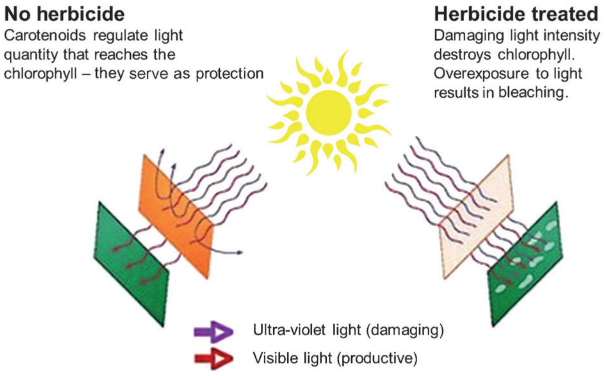


Figure 6. HPPD inhibitors destroy the UV shield of plants.

results in the bleaching and subsequent death of treated plants (Figure 6).[15]

2.3. Crystal Structure and Structure–Activity Relationships

HPPDs are non-heme Fe^{II}-containing dioxygenases. The crystal structures of HPPDs show that they are homodimers.[16,17] Each monomer is formed by two domains that have similar topology. The domains consist of barrel-shaped β-sheets surrounded by α-helices. The active site is located within an open twisted β-sheet that is made of seven β-strands, and is formed by two strictly conserved histidines and a glutamate, which hold the catalytic iron. This octahedrally coordinated iron also binds the chelating moieties of HPPD inhibitors and presumably the oxygen atoms of the substrate in the transition state (Figure 7). The main driving forces for the binding of the inhibitor are the interaction of the chelating oxygen atoms with the cationic iron and the burial of hydrophobic inhibitors atoms in hydrophobic protein pockets. These two key interactions are exemplified well by the interactions of tembotrione (Figure 8), in which the aromatic ring is sandwiched between the side chains of two phenylalanine moieties. The substituent in the 3-position of the aromatic ring points into a large open pocket, which explains the high variability of substituents tolerated at this position.

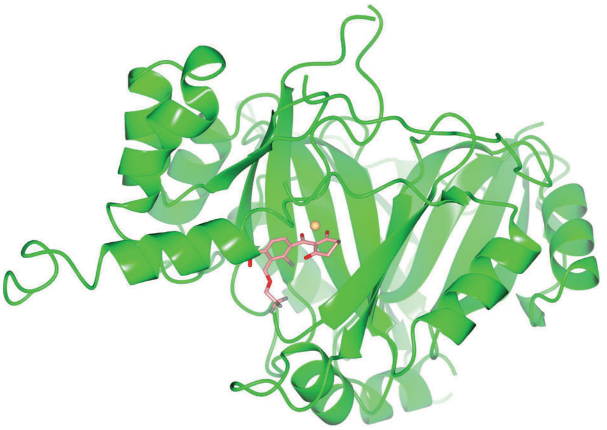


Figure 7. Schematic representation of the overall folding of the HPPD enzyme. The position of the iron atom is indicated by an orange sphere.

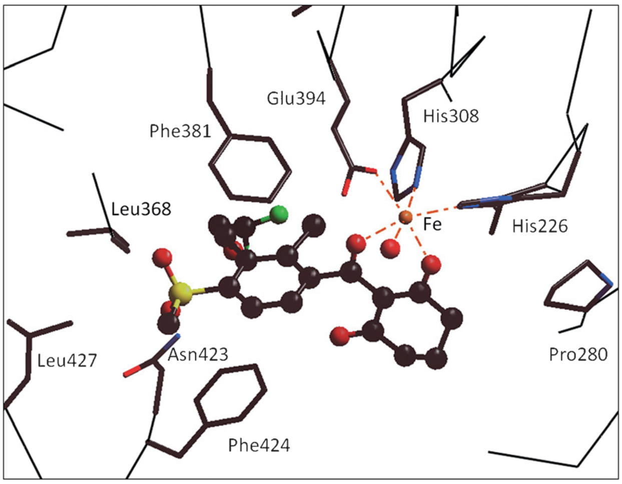


Figure 8. Crystal structure of tembotrione with HPPD from *Arabidopsis thaliana*. The active site is formed by two strictly conserved histidines (His226 and His308) and a glutamate (Glu394), which hold the catalytic iron ion. The aromatic ring of tembotrione, which contributes significantly to the hydrophobic effect, is sandwiched between the side chains of Phe381, Leu368, Phe424, and Leu427.

HPPD can assume two different conformations ("open" and "closed") and tembotrione binds to the open form. Many different HPPD inhibitors that have a cyclohexanedione, a hydroxypyrazole, or a diketonitril group as a chelating moiety also bind to the open form, which explains the similar structure–activity relationship (SARs) of these compound classes.

Generally, the analysis of structure–activity relationships in HPPD chemistry can be divided into two parts: the analysis of the aryl group and that of the chelating moiety. The chelating moiety is responsible for binding to the active site of the HPPD enzyme. For inhibition, different substitution patterns are tolerated at the aryl, but they help to adjust the physico-chemical behavior, such as the acidity and the polarity in order to allow good uptake and transport to the target site. Particularly good biological efficacy was found with 2,4- and 2,3,4-substitution patterns at the benzoyl moiety. At the 2-position, Cl, NO_2, SO_2Me, CF_3, and Me substituents give the best results. At the 4-position, substituents such as halogen atoms, and haloalkyl and alkylsulfonyl groups are favored. And at the 3-position, a multitude of substituents, including heterocycles and alkoxy, alkylsulfonyl, or haloalkoxyalkyl groups, lead to strong herbicides. Specific metabolism or the lack of metabolism also modifies the herbicidal potential of an inhibitor. For example, plant metabolism of the cyclohexanedione moiety leads to detoxification of the herbicide. Substitution at this moiety can block metabolism and could therefore increase herbicidal activity.[4]

3. Discovery of Safeners

3.1. Brief History of Safener Discovery

Safener research started after a fortuitous observation by Otto Hoffmann in 1947. As a herbicide researcher in the Gulf Oil company, he saw that tomato plants had suffered injury that he presumed to have been caused by the vapor drift of the herbicide 2,4-D in a warm glasshouse. However, plants previously treated with 2,4,6-trichlorophenoxyacetic acid appeared to be protected from the injury.[18] The research program into herbicide safeners that was triggered by this observation eventually led to the discovery of 1,8-naphthalic anhydride, a safener for the treatment of corn seeds. In the years following this discovery, several companies started research programs that led to commercial safeners (Figure 9).

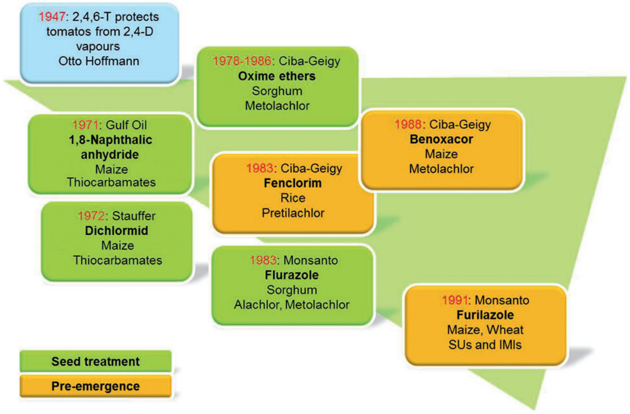


Figure 9. Early commercialized seed treatment and pre-emergence safeners.

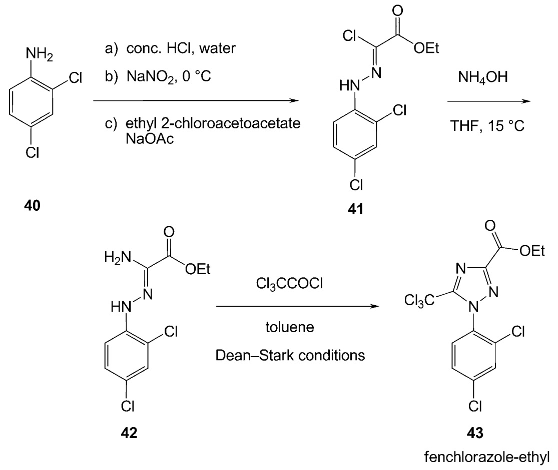
During this early research, further seed-treatment compounds were found and safeners were identified that could be used soil applied with pre-emergence herbicides. All these safeners were intended for use in monocot crops.

3.2. Discovery of Safeners at the Bayer Company

3.2.1. Fenchlorazole-ethyl and Mefenpyr-diethyl

In order to achieve full crop selectivity of some of the highly effective HPPD herbicides, it was necessary to use products containing safeners. Of the BCS legacy companies, only the agriculture division at the Hoechst AG (subsequently AgrEvo and then Aventis CropScience) undertook the systematic synthesis and screening of safeners. This project started at the end of the 1970s. While the then existing safeners were applied to seeds and soil, the concept of safeners with foliar activity (leaf-active) was a highly attractive target. The objective was the protection of cereals against post-emergence ACCase-inhibiting herbicides (especially fenoxaprop-ethyl). The physico-chemical properties of leaf-active safeners would have to be different from soil-active compounds and would need systemicity features aligned with those of the corresponding foliar-acting herbicides.

Compounds to be tested for safening activity were sprayed after emergence on wheat plants, which were subsequently treated with fenoxaprop-ethyl. Compounds that reduced injury were subjected to secondary and tertiary tests with additional dose rates of safener and herbicide, and including grass weeds to ensure that herbicidal activity was maintained. This approach identified various compound classes with good lead potential, justifying chemical exploration and optimization and leading to the discovery of fenchlorazole-ethyl (**43**).[19] A synthesis route to this first foliar safener is shown in Scheme 7. The chlorohydrazone **41** can be prepared by Japp–Klingmann reaction from aniline **40** and ethyl 2-chloroacetoacetate.[20] Treatment of **41** with aqueous ammonia furnishes aminohydrazone **42**, which is subsequently converted to fenchlorazole-ethyl (**43**) with trichloroacetyl chloride under Dean–Stark conditions.



Scheme 7. Synthesis of fenchlorazol-ethyl (**43**).

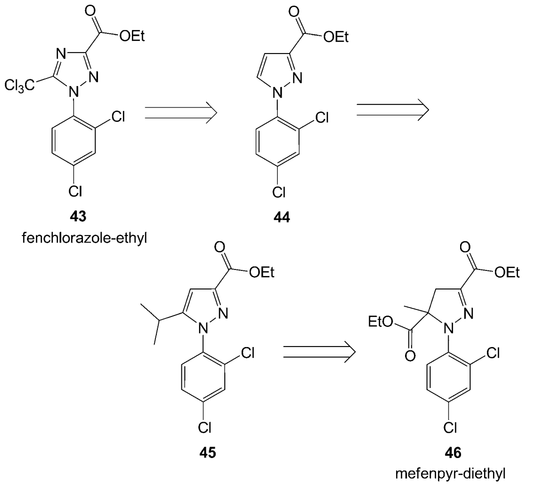
Fenchlorazole-ethyl[21] was launched with fenoxaprop-ethyl in the product Puma in 1989. Both these esters have good foliar-uptake characteristics but are rapidly de-esterified in the plant to phloem-mobile acids. The safening properties allowed selective use in wheat and rye. However, for safening of fenoxaprop in barley, a better safener was required.

Systematic variation of the central, heterocyclic core (including its substituents) of fenchlorazole-ethyl provided firstly the experimental pyrazole safeners **44**[22] and **45**[23] and finally the commercial safener mefenpyr-diethyl (**46**;[24] Scheme 8).

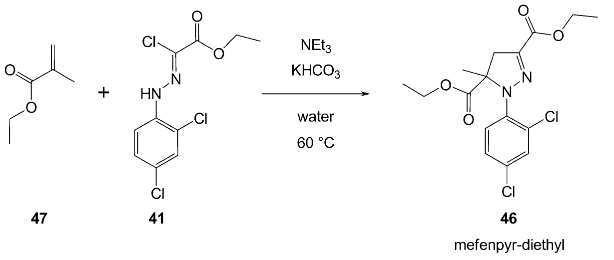
A possible synthesis route of the pyrazoline derivate mefenpyr-diethyl is based on the [3+2] dipolar cycloaddition of chlorohydrazone **41** and ethyl methacrylate (**47**) in the presence of triethyamine and $KHCO_3$ in water (Scheme 9).[25] This superior safener replaced the combination of fenchlorazole-ethyl and fenoxaprop-ethyl, but the product name Puma was retained.

3.2.2. Isoxadifen-ethyl

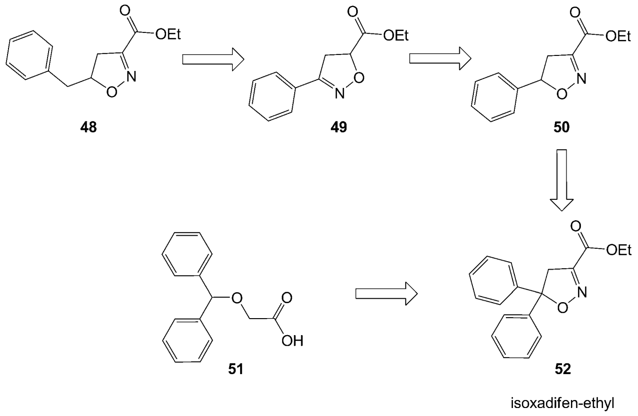
Fenoxaprop-ethyl also had good post-emergence efficacy against common grass weeds in rice. However, this crop was



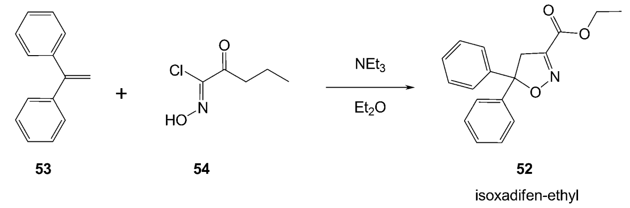
Scheme 8. Structure optimization from fenchlorazol-ethyl (**43**) to mefenpyr-diethyl (**46**).



Scheme 9. Synthesis of mefenpyr-diethyl (**46**).



Scheme 10. Structure optimization from lead structure **48** to isoxadifen-ethyl (**52**).



Scheme 11. Synthesis of isoxadifen-ethyl (**52**).

also injured by the herbicide. Unfortunately, while highly effective in wheat and barley, mefenpyr-diethyl could not safen rice against fenoxaprop-ethyl or other available herbicides. Such crop specificity is common for safeners,[26] so in the 1990s, researchers at the Hoechst company turned their attention to identifying a foliar safener to protect rice. Once again, fenoxaprop-ethyl was used as the target herbicide. Replacing the pyrazoline core from mefenpyr-diethyl by a similar substituted isoxazoline resulted in the safeners **48**,[27] **49**,[28] and **50**,[29] which were not sufficiently active for commercialization. However, combining the structural features of **50** and the experimental Monsanto safener **51**[30] led to the strong rice safener isoxadifen-ethyl (**52**;[31] Scheme 10).

The ethyl ester of isoxadifen was identified as the strongest candidate, combining good foliar uptake with rapid plant metabolism to the systemic isoxadifen acid.[33] Products containing isoxadifen-ethyl and fenoxaprop-P-ethyl (the active isomer) for application to rice include Ricestar, which was launched in 2002. Scheme 11 shows a simple one step synthesis of isoxadifen-ethyl through [3+2] bipolar cycloaddition of chloro-oxime **54**[32] and 1,1-diphenylethene (**53**) in the presence of triethylamine in diethylether.

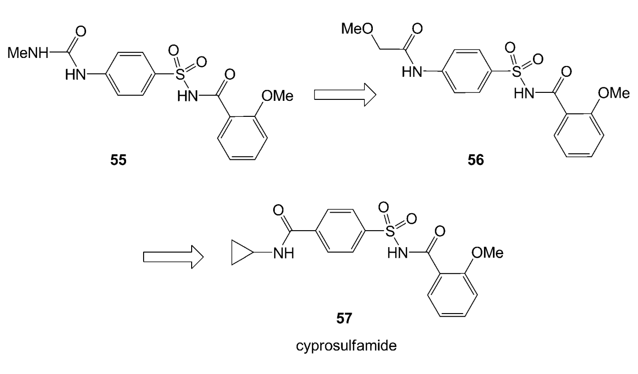
3.2.3. Cyprosulfamide

A Ciba–Geigy patent application,[34] published in April 1990, claimed a novel class of acyl-sulphonamide safeners with strong post-emergence efficacy in corn. AgrEvo prepared and tested some compounds described in this patent, such as the urea–sulfonamide safener **55**,[34] and confirmed its safening activity. Further exploration and optimization provided the experimental carboxamide safener **56**[35] and later the inverse acyl–sulfonamide cyprosulfamide (**57**; Scheme 12).[36]

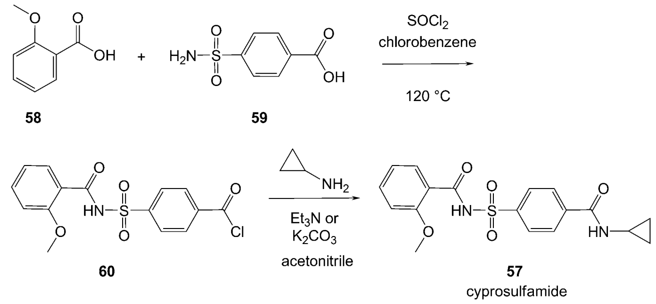
Treatment of **58** and **59** with thionylchloride in chlorobenzene at 120 °C produces the acid chloride **60**. Amide coupling with cyclopropylamine under standard conditions gives cyprosulfamide (Scheme 13).[37]

3.2.4. Safeners for Various Crops and Modes of Action

Mefenpyr-diethyl, isoxadifen-ethyl, and cyprosulfamide were first discovered using test models with one crop and one



Scheme 12. Structure optimization from lead structure **55** to cyprosulfamide (**57**).



Scheme 13. Synthesis of cyprosulfamide (**57**).

herbicide. However, in all three cases, it was found sooner or later that they could safen at least one other crop and/or inhibit many other herbicides with various modes of action. This was a ground-breaking discovery: it meant that with just a few safeners, portfolios of valuable herbicide products could be developed for cereals, corn, and rice.

Safeners work by increasing the speed at which the crop degrades (detoxifies) the herbicide.[26,38] This effect relies on the increased expression of the genes that code for the enzymes responsible for degradation (e.g. P450, GSTs, and ABC transporters). The increased expression of these genes can be investigated by measuring the amount of mRNA. Such studies have shown that increased gene expression is only seen in crops that respond to particular safeners. However, it is difficult to explain why some crops respond to safeners and others (and weeds) do not, because little is known about the receptors of safeners. On the other hand, because herbicides with different modes of action may share the same plant metabolism processes, the gene expression patterns caused by various safeners explain why multiple herbicides can be safened.

4. Products Combining HPPD Inhibitors and Safeners

Even though safeners were not originally developed to safen HPPD inhibitors, the modern BCS safeners with their ability to safen multiple modes of herbicide action are very useful to also safen this class of herbicides. However, it is too simple to believe that every HPPD inhibitor could be safened to 0% phytotoxicity. Certain HPPD-inhibiting compounds could not be safened at all, others only partially, and some fully. Because the level of herbicidal activity can vary from glasshouse to field (depending on for example light and soil), safening levels can also vary. Additionally, the different crop varieties may not respond to the herbicide and/or safener in the same way. This all means a lot of testing in the glasshouse and field to identify the best compound for development. However, in the best cases, even HPPD inhibitors with strong phytotoxicity could be safened fully and consistently (see Figure 10).

There are solutions for pre- and post-emergence treatments. Farmers tend to have good knowledge of the weed-control issues in the different crops in their various fields. They select herbicides for application either before (pre) or after (post) emergence of the crop from the soil, based on a range of agronomic factors. For example, are herbicides actually available for the pre- or post-emergence control of the weeds in that field? Does the farmer have time between sowing and crop emergence to spray all the fields before emergence? Also, do the pre-emergence treatments provide control for the full season or are post-emergence follow-up treatments needed? The farmer must decide which program provides the most cost-effective weed control for his particular situation, and this can change as agricultural research delivers new options.

Figure 10. Field testing of HPPD inhibitors without and with safener.

4.1. Isoxaflutole and Cyprosulfamide in Corn

In the late 1990s, Rhone–Poulenc Agriculture had identified a problem regarding crop selectivity with its pre-emergence corn herbicide isoxaflutole. Early recommended rates of approximately 100 g a.i./ha of this herbicide (as Merlin or Balance) provided excellent control of broadleaf and grass weeds, but crop injury was observed under certain conditions. A change to lower rates (ca. 75 g a.i/ha) overcame the crop injury problems, but weed control was not always sufficient. When AgrEvo and Rhone–Poulenc Agriculture merged to form Aventis CropScience in 1999, this problem was addressed by testing the "back catalogue" of AgrEvo safeners with isoxaflutole on corn before emergence. These tests led to the result that neither mefenpyr-diethyl nor isoxadifen-ethyl (or acid) were effective, but that the acyl–sulfonamides (discussed in Section 3.3) were highly interesting candidates. These tests in glasshouses and fields identified cyprosulfamide as the most effective compound. The combination of isoxaflutole and cyprosulfamide was developed by Aventis CropScience and Bayer CropScience, and was launched in 2009. Merlin and Balance were replaced by Merlin Flexx and Balance Flexx to signify the added dose flexibility provided by cyprosulfamide. An example of this effect is shown in Figure 11, where all corn varieties suffering unacceptably injury (>10%) by pre-emergence application of isoxaflutole alone are safened to acceptable levels by adding cyprosulfamide. As well as this pre-emergence effect,

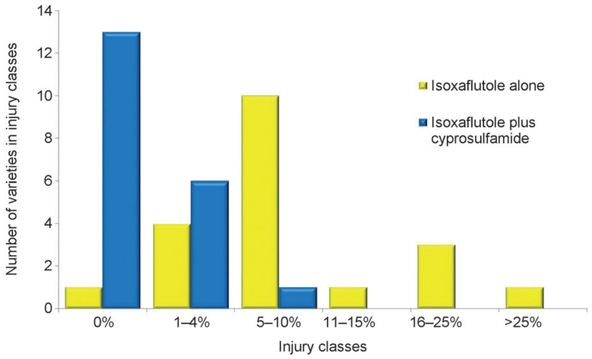
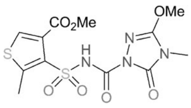


Figure 11. Pre-emergence corn injury resulting from treatment with 100 g a.i./ha isoxaflutole without and with 50 g a.i./ha of cyprosulfamide.



61

Figure 12. Structure of thiencarbazone-methyl (**61**).

cyprosulfamide also allows isoxaflutole to be applied safely to corn early after emergence.

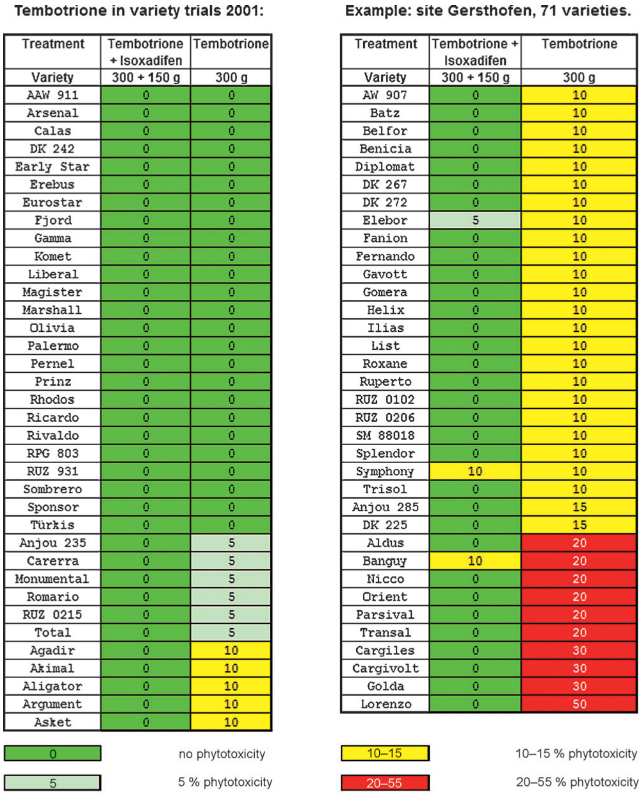
After the takeover of Aventis CropScience by the Bayer company in 2002, the combination of the Bayer ALS inhibitor thiencarbazone-methyl (**61**; Figure 12) with isoxaflutole and the safener cyprosulfamide was also developed for application in corn. This “three-way innovation” is now marketed as Adengo and Corvus.

4.2. Tembotrione and Isoxadifen-ethyl in Corn

The control of a wide spectrum of broadleaf and grass weeds combined with an outstanding selectivity in combination with the safener isoxadifen-ethyl in corn makes tembotrione special. At recommended rates of 75 to 100 g a.i./ha, tembotrione can result in strong control of common grass weeds, such as foxtails and woolly cupgrass, but also of the toughest broadleaved species, including glyphosate-, ALS-, or dicamba-resistant weeds. Even though tembotrione is mainly active after emergence, the compound stays quite active in the soil throughout the growing season. This activity provides some residual control of weeds until the corn canopy closes, but does not limit crop-rotation opportunities in soybeans or other crops in the following season.[11]

With regard to corn selectivity, Figure 13 shows how application of tembotrione alone can cause injury to some sensitive varieties at high rates. However, the addition of isoxadifen-ethyl at a 2:1 ratio reduces this crop damage to an acceptable level of less than 10 % in all varieties. For this reason, tembotrione products (Laudis and Soberan) include the safener isoxadifen-ethyl in a ratio of 2:1. As a result, the products provide a high degree of flexibility for the corn farmer, who does not need to be concerned about which variety he uses. As in the combination of isoxaflutole with cyprosulfamide, the safened tembotrione can also be used at the rates that are needed to control a broader spectrum of weeds, especially grasses.

Scheme 14 shows the metabolic pathway of tembotrione in plants. For corn, this degradation is much faster in the presence of isoxadifen-ethyl.[39] Also, the rate of metabolism was not effected in tembotrione-susceptible species of broadleaf and grass weeds.



Tembotrione in variety trials 2001:

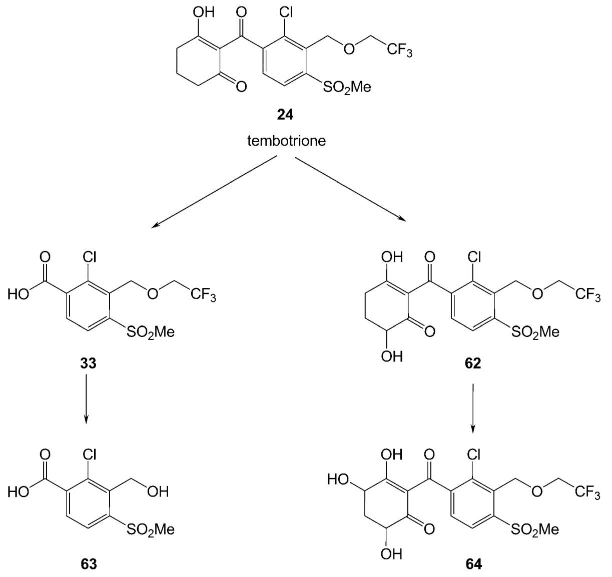
Treatment	Tembotrione + Isoxadifen	Tembotrione
Variety	300 + 150 g	300 g
AAW 911	0	0
Arsenal	0	0
Calas	0	0
DK 242	0	0
Early Star	0	0
Erebus	0	0
Eurostar	0	0
Fjord	0	0
Gamma	0	0
Komet	0	0
Liberal	0	0
Magister	0	0
Marshall	0	0
Olivia	0	0
Palermo	0	0
Pernel	0	0
Prinz	0	0
Rhodos	0	0
Ricardo	0	0
Rivaldo	0	0
RPG 803	0	0
RUZ 931	0	0
Sombrero	0	0
Sponsor	0	0
Türkis	0	0
Anjou 235	0	5
Carerra	0	5
Monumental	0	5
Romario	0	5
RUZ 0215	0	5
Total	0	5
Agadir	0	10
Akimal	0	10
Aligator	0	10
Argument	0	10
Asket	0	10

Example: site Gersthofen, 71 varieties.

Treatment	Tembotrione + Isoxadifen	Tembotrione
Variety	300 + 150 g	300 g
AW 907	0	10
Batz	0	10
Belfor	0	10
Benicia	0	10
Diplomat	0	10
DK 267	0	10
DK 272	0	10
Elebor	5	10
Fanion	0	10
Fernando	0	10
Gavott	0	10
Gomera	0	10
Helix	0	10
Ilias	0	10
List	0	10
Roxane	0	10
Ruperto	0	10
RUZ 0102	0	10
RUZ 0206	0	10
SM 88018	0	10
Splendor	0	10
Symphony	10	10
Trisol	0	10
Anjou 285	0	15
DK 225	0	15
Aldus	0	20
Banguy	10	20
Nicco	0	20
Orient	0	20
Parsival	0	20
Transal	0	20
Cargiles	0	30
Cargivolt	0	30
Golda	0	30
Lorenzo	0	50

🟩 0	no phytotoxicity	🟨 10–15	10–15 % phytotoxicity
▨ 5	5 % phytotoxicity	🟥 20–55	20–55 % phytotoxicity

Figure 13. Tembotrione in different corn varieties with and without isoxadifen-ethyl.



Scheme 14. Metabolism of tembotrione in plants.

4.3. Pyrasulfotole and Mefenpyr-diethyl in Cereals

For more than 20 years, no new mode of action was developed for the control of broadleaf weeds in cereals. This changed in 2008 with the introduction of pyrasulfotole. This HPPD inhibitor is used as an innovative tool for resistance management and allows excellent post-emergence use on all types of wheat, barley, and triticale. Application rates of 25–50 g a.i./ha provide reliable weed control, especially in mixtures with photosystem II inhibitors. For example, the combination of pyrasulfotole and bromoxynil shows truly synergistic activity, which allows the control of even the toughest broadleaf weeds, including kochia, Russian thistle, China lettuce, and wild buckwheat.[6]

Even though pyrasulfotole is a typical herbicide for broadleaf weeds and has very limited herbicidal activity toward grass, the compound alone is unfortunately not safe enough for cereals crops. However, the tolerance of wheat to pyrasulfotole is much higher than that of for example weed such as chickweed. This difference is based on a much faster metabolic degradation in wheat. The main metabolite in plants is the nonherbicidal benzoic acid **13**. The somewhat slow rate of metabolic degradation in the crop can be increased by combination with the right safener, in this case mefenpyr-diethyl.[40] Crop tolerance is then guaranteed in all varieties and at full rates. As for tembotrione, there are no crop-rotation restrictions, and also very importantly for the farmer, pyrasulfotole can be tank-mixed with a variety of grass-control products for cereals, providing excellent compatibility and flexibility. In addition, BCS has developed products that contain a grass-control component such as fenoxaprop-P-ethyl as coformulation. Interestingly, in combination with Puma (combination of fenoxaprop-P-ethyl with mefenpyr-diethyl), the safener works for both herbicides.

Pyrasulfotole was introduced for the post-emergence application to cereals crops in 2008 under the trade names Huskie in the USA, Infinity in Canada, and Velocity in Australia (all are mixtures of pyrasulfotole, bromoxynil, and mefenpyr-diethyl). In South Africa, the same product was launched in 2011 as Resolve. Further products with pyrasulfotole are Precept (pyrasulfotole, MCPA, and mefenpyr-diethyl), Wolverine and Tundra (fenoxaprop-P-ethyl, pyrasulfotole, bromoxynil, and mefenpyr-diethyl) and Velocity M3 (thiencarbazone, pyrasulfotole, bromoxynil, and mefenpyr-diethyl).

5. Outlook

As already mentioned, many key herbicide classes face significant problems of resistant weed species, which is one of the main driving forces in current herbicide research. To overcome resistance, either new herbicides with new modes of action or new compound classes of existing modes of action are needed. Several approaches have been used to identify new compound classes as HPPD inhibitors. Experience has shown that high-throughput screening using biochemical assays quite often results in weakly binding, hydrophobic hits with a substantial molecular weight. An alternative approach to new structural classes leading to more polar compounds is to start with fragments and add lipophilic extensions. Early results of "SAR by crystallography" showed the advantage of polar fragments as starting points and the importance of a complete network of H-bonds.[41] Thus, one strategy at BCS was to first identify the polar fragments and to then add hydrophobic substituents in a second step in order to increase the affinity. As a consequence, the BCS fragment library contained mainly highly water-soluble, polar compounds, thus allowing screening with established biochemical assays at higher concentration in order to detect fragment binding.

The HPPD binding pocket (see Section 2.3), which includes binding sites for specific polar groups and for apolar substituents, is ideal for this fragment approach. A previously established enzyme-binding assay was used for the identification of the best fragments.[42] A total of 82 fragments with a molecular weight of less than 200 kDs were found to inhibit HPPD significantly. Figure 14 shows a selection of A) fragments binding to HPPD, and B) chelating fragments that do not inhibit HPPD. Interestingly, all binding fragments are potential chelators and include the fragments of well-known inhibitor classes, such the 1,3 cyclohexandione. Subsequent extensions of various fragments based on the crystal structures of HPPD led to a number of new inhibitor classes. Figure 15 shows the crystal structure of HPPD from *Arabidopsis thaliana* in complex with a new HPPD inhibitor that combines a heterocyclic fragment with the benzoyl moiety from isoxaflutole. Further exploration is currently ongoing.

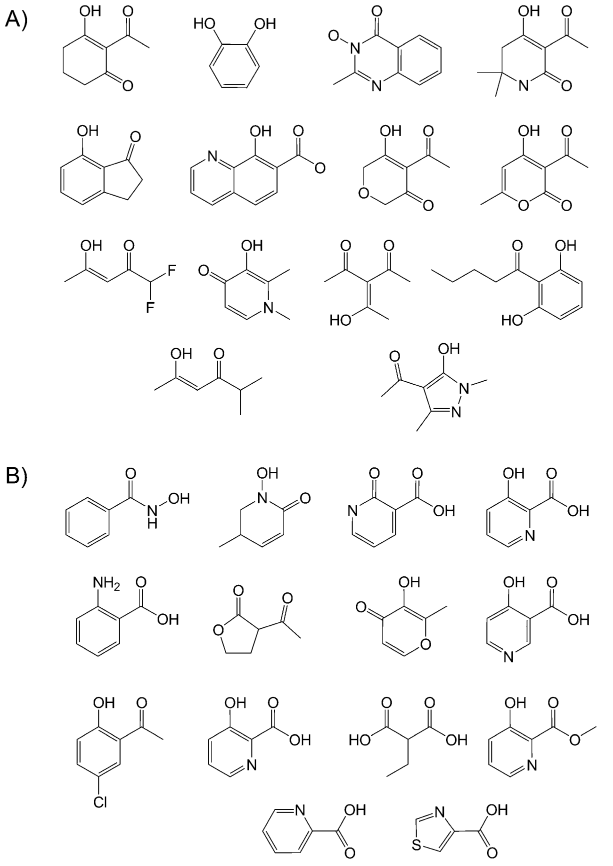


Figure 14. High-concentration screening allows the differentiation between chelating fragments that bind to HPPD (A) and those that do not bind to HPPD (B).

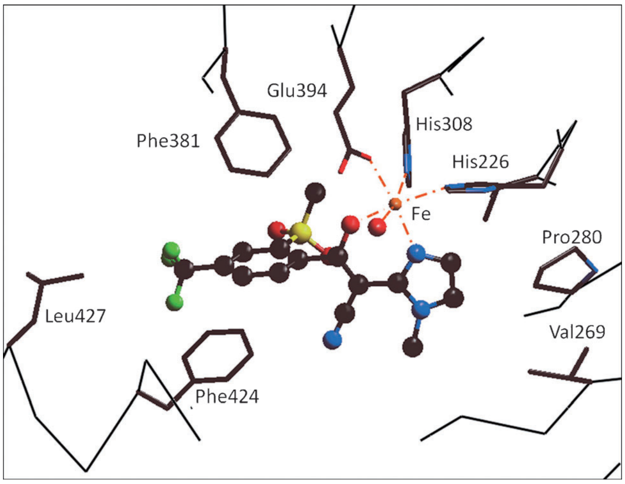


Figure 15. Crystal structure of HPPD from *A. thaliana* in complex with a new HPPD inhibitor derived from the fragment approach. The structure shows that this novel chelator interacts with the HPPD from *A. thaliana* in a very similar way to tembotrione.

In parallel to the discovery of new HPPD-inhibiting compound classes, the research into new safeners is also continuing. However, as an alternative approach to protect crops from the herbicidal effect of HPPD inhibitors, research and development of herbicide-tolerant genetically modified crops has also started.[42]

Received: March 20, 2013

[1] E.-C. Oerke, *J. Agric. Sci.* **2006**, *144*, 31–43.
[2] S. O. Duke, S. B. Powles, *Pest Manage. Sci.* **2008**, *64*, 319–325.
[3] "Herbicide cross resistance and multiple resistance in plants": S. B. Powles, C. Preston, November 16, **2012**, available online on http://www.hracglobal.com/Publications.
[4] A. Edmunds, J. Morris, A. van Almsick in *Modern Crop Protection Compounds, 2nd* ed., *Vol. 1* (Eds.: W. Krämer, U. Schirmer, P. Jeschke, M. Witschel), Wiley-VCH, Weinheim, **2012**, pp. 235–258 and 262–275.
[5] a) T. Konotsune, K. Kawakubo, JP 50126830, DE 2513750, GB 1463473, **1975**; b) N. Nishiyama, F. Kimura, T. Haga, N. Sakashita, T. Nishikawa, GB 2002375, **1979**.
[6] M. H. Schmitt, A. van Almsick, L. Willms, *Pflanzenschutz-Nachr. Bayer* **2008**, *61*, 7–14.
[7] R. Beaudegnies, A. J. F. Edmunds, T. E. M. Fraser, R. G. Hall, T. R. Hawkes, G. Mitchell, J. Schaetzer, S. Wendeborn, J. Wibley, *Bioorg. Med. Chem.* **2009**, *17*, 4134–4152.
[8] D. L. Lee, C. G. Knudsen, W. J. Michaely, H.-L. Chin, N. H. Nguyen, C. G. Carter, T. H. Cromartie, B. H. Lake, J. M. Shribbs, T. Fraser, *Pestic. Sci.* **1998**, *54*, 377–384.
[9] P. A. Cain, S. M. Cramp, G. M. Little, B. M. Luscombe, EP 0527036, **1993**.
[10] E. Beltran, H. Fenet, J. F. Cooper, C. M. Costle, *Pest Manage. Sci.* **2001**, *57*, 366–371.
[11] K. Grossmann, T. Ehrhardt, *Pest Manage. Sci.* **2007**, *63*, 429–439.
[12] A. van Almsick, J. Benet-Buchholz, B. Olenik, L. Willms, *Bayer CropSci. J.* **2009**, *62*, 5–16.
[13] A. van Almsick, L. Willms, T. Auler, H. Bieringer, C. Rosinger, WO 00/21924, **2000**.
[14] C. Raspail, M. Graindorge, Y. Moreau, S. Crouzy, B. Lefèbvre, A. Y. Robin, R. Dumas, M. Matringe, *J. Biol. Chem.* **2011**, *286*, 26061–26070.
[15] a) A. Schulz, O. Ort, P. Beyer, H. Kleinig, *Fed. Eur. Biochem. Soc.* **1993**, *318*, 162–166; b) T. R. Hawkes, *Modern Crop Protection Compounds, 1st* ed., *Vol. 1* (Eds.: W. Krämer, U. Schirmer), Wiley-VCH, Weinheim, **2007**, pp. 211–221.
[16] I. M. Fritze, L. Linden, J. Freigang, G. Auerbach, R. Hubert, S. Steinbacher, *Plant Physiol.* **2004**, *134*, 1388–1400.
[17] J. Freigang, B. Laber, G. Lange, A. Schulz, *Pflanzenschutz-Nachr. Bayer (Engl. Ed.)* **2008**, *61*, 15–28.
[18] O. Hoffmann, *Plant Physiol.* **1953**, *28*, 622–628.
[19] G. Heubach, K. Bauer, H. Bieringer, EP 0174562, **1986**.
[20] A. S. A. S. Shawali, A. Osman, *Tetrahedron* **1971**, *27*, 2517.
[21] H. Bieringer, K. Bauer, E. Hacker, G. Heubach, K. H. Leist, E. Ebbert, *Proc. Brighton Crop Pro. Conf.* **1989**, 77–82.
[22] E. Sohn, R. Handte, H. Mildenberger, H. Bürstell, K. Bauer, H. Bieringer, EP 0269806, **1988**.
[23] E. Sohn, H. Mildenberger, K. Bauer, H. Bieringer, DE 3808896, **1989**.
[24] a) W. Rösch, E. Sohn, K. Bauer, H. Bieringer, WO 199107874, **1991**; b) E. Hacker, H. Bieringer, L. Willms, O. Ort, H. Koecher, H. Kehne, *Proc. Brighton Crop Pro. Conf.* **1999**, 15–22.
[25] G. Schlegel, WO 199912909, **1999**.
[26] C. Rosinger, K. Bartsch, W. Schulte in *Modern Crop Protection Compounds, 2nd* ed., *Vol. 1* (Eds.: W. Krämer, U. Schirmer, P. Jeschke, M. Witschel), Wiley-VCH, Weinheim, **2012**, pp. 371–397.
[27] H.-J. Löher, W. Schwab, K. Bauer, H. Bieringer, WO 199108202, **1991**.
[28] H.-J. Löher, K. Bauer, H. Bieringer, DE 4026018, **1992**.
[29] H.-J. Löher, K. Bauer, H. Bieringer, EP 0509433, **1992**.
[30] L. H. Brannigan, R. J. Brinker, R. J. Kaufman, S. Metz, US4964893, **1990**.
[31] L. Willms, K. Bauer, H. Bieringer, WO 199507897, **1995**.
[32] W. Vaughan, J. L. Spencer, *J. Am. Chem. Soc.* **1960**, *25*, 1160–1164.
[33] a) B. Collins, D. Drexler, M. Merkl, E. Hacker, H. Hagemeister, K. Pallett, C. Effertz, *Proc. Brighton Crop Prot. Conf.* **2001**, pp. 35–42; b) K. Pallett, P. Veerasekaran, M. Crudace, H. Koecher, B. Collins, *Proc. NCWSS Conf.* **2001**, Abstract 77; c) E. Hacker, H. Bieringer, L. Willms, G. Schnabel, H. Koecher, H. Hagemeister, W. Steinheuer, *Z. Pflanz. Pflanzenschutz Sonderheft XVIII* **2002**, pp. 747–756.
[34] U. Burckhardt, R. Soliman, W. Töpfl, H.-R. Wäspe, EP 365484, **1990**.
[35] F. Ziemer, K. Haaf, L. Willms, K. Bauer, H. Bieringer, C. Rosinger, WO 199745016, **1997**.
[36] F. Ziemer, L. Willms, T. Auler, H. Bieringer, C. Rosinger, WO 199916744, **1999**.
[37] S. Pazenok, M. J. Ford, G. Schlegel, EP 1491527, **2004**.
[38] D. Riechers, K. Kreuz, Q. Zhang, *Plant Physiol.* **2010**, *153*, 3–13.
[39] W. Schulte, H. Köcher, *Bayer CropSci. J.* **2009**, *62*, 35–52.
[40] W. Schulte, H. Köcher, *Pflanzenschutz-Nachr. Bayer (Engl. Ed.)* **2008**, *61*, 29–42.
[41] G. Lange, D. Lesuisse, P. Deprez, B. Schoot, P. Loenze, D. Bénard, J.-P. Marquette, P. Broto, E. Sarubbi, E. Mandine, *J. Med. Chem.* **2003**, *46*, 5184–5195.
[42] G. Lange, P. Fabien, B. Laber, F. Jörg, A. Schulz, EP 2453012, **2012**.