Isoflucypram, the first representative of a new succinate dehydrogenase inhibitor fungicide subclass: Its chemical discovery and unusual binding mode

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Abstract

Succinate dehydrogenase inhibitors (SDHIs) have played a crucial role in disease control to protect cereals as well as fruit and vegetables for more than a decade. Isoflucypram, the first representative of a newly installed subclass of SDHIs inside the Fungicide Resistance Action Committee (FRAC) family of complex II inhibitors, offers unparalleled long-lasting efficacy against major foliar diseases in cereals. Herein we report the chemical optimization from early discovery towards isoflucypram and the first hypothesis of its altered binding mode in the ubiquinone binding site of succinate dehydrogenase.

Keywords: isoflucypram; ISY; N-cyclopropyl-N-benzyl-pyrazole carboxamide; SDHI; ubiquinone binding site

1 INTRODUCTION

Succinate dehydrogenase inhibitors (SDHIs) are a central cornerstone for disease control in cereals. Many important pathogens, such as Zymoseptoria tritici (leaf blotch), Puccinia recondita (brown rust) or Rhynchosporium secalis (leaf scald), are very effectively controlled by SDHIs. Left untreated such diseases could not only reduce the yield by an average of around 20%, but also lead to very low-quality produce. Isoflucypram is the latest innovation in the area of SDHIs from Bayer to help farmers around the globe grow healthy cereals in a sustainable way. This article covers the chemical discovery of isoflucypram with its unusual substitution pattern and its postulated binding mode at the ubiquinone binding site of fungal succinate dehydrogenase enzymes.

2 THE DISCOVERY OF ISOFLUCYPRAM

The discovery of isoflucypram can be traced back to sulfonyl carboxamides of common structure A (Fig. 1) as the early lead class with a narrow focus on fungal diseases like powdery mildews, brown rust, net blotch or leaf spots. This lead class was identified with a chemistry-based library design, incorporating herbicidal structures A1, known from Rohm & Haas, and insecticidal motifs known from flonicamid (A2), with our original intention to discover new herbicides or insecticides. In the frame of variations of propargylamine in A1 or cyanomethylamine in A3, further small amines were introduced, for example allylamine, isopropylamine and cyclopropylamine, the latter being prominently used in Bayer’s fluoroquinoline antibiotic, ciprofloxacin (A4). The 4-(trifluoromethyl)pyridine-3-carboxamide part of the library was extended to five-membered heterocycles, particularly pyrazoles, well known as the key motif in fungicidal carboxamide structures like penflufen (A5).

The sulfonyl carboxamide of formula A6 evolved to be the first hit compound showing fungicidal activity in the greenhouse with an unknown mode of action. A6 served as the starting point for a systematic variation of the arylsulfonic acid, the amine and the pyrazole acid (Fig. 2). It turned out that 3-substituted pyrazole carboxylic acids (A7a–c), particularly 5-fluorine-pyrazol-4-yl carboxylic acids (A7a), were essential for fungicidal efficacy, directing towards SDH as underlying the mode of action. Interestingly, the cyclopropylamine substituent was necessary to maintain activity, and other amine variations were less active or not tolerated. The variation in the lipophilic sulfonic acid part led to two subfamilies with enhanced activity: ortho-chlorine-substituted phenyls (A8) and para-phenoxycarbonyl-substituted phenyls (A9).

Focusing on several combinations of chloro-containing lipophilic sulfonic acids (A8) and 3-substituted pyrazole carboxylic acids (A7a–c), eventually compounds A10 and A11 (Fig. 3) were...
identified with improved fungicidal profile in the greenhouse. A11 was selected to be tested under field conditions in cereals but showed only weak efficacy despite high use rates.

Even though the *in vitro* activity on *Ustilago* SDH was only moderate, A10 proved to be a potent inhibitor of SDH from *Botrytis*, *Zymoseptoria* and *Agaricus*. The strong *in vitro* efficacy was a clear indicator that the cyclopropyl substituent does not serve as pro-drug but is an integral part of the binding mode of A10. Nevertheless, the promising *in vitro* efficacy could not be transferred to the *in planta* environment, as fast degradation of A10 and A11 was observed. Encouraged by these findings, more stable variations of the sulfonyl carboxamides were investigated.

In order to address the poor activity translation of compound A11 from greenhouse to field, some deeper modifications of the sulfonamide moiety were undertaken. If the replacement of the sulfonyl group by a carbonyl group or a sulfur atom proved to yield inactive derivatives, the replacement of the sulfonyl group by a CH2 linker had a profound impact on the *in-vitro* and

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**Figure 1** Chemistry-based discovery of fungicidal sulfonyl carboxamides.

**Figure 2** Fungicidal sulfonyl carboxamides: evolution from hit to lead.

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fungicidal greenhouse activities. The incorporation of the lipophilic side-chain of fluopicolide A12 onto the N-cyclopropyl amide moiety of compound A11 gave compound A13 as prototype of a new chemical class still maintaining a strong level of inhibition of SDH of several fungal pathogens (see Fig. 4). It was rapidly shown that the pyridine ring of compound A13 could be replaced by a phenyl ring and that the dihedral angle between the benzylic moiety and the N-cyclopropyl substituent had to be well controlled. This control could be achieved by a rather bulky substituent in the ortho position and/or a benzylic substituent.

**Figure 3** Fungicidal sulfonyl carboxamides: lead structure and field compound.

**Figure 4** Fungicidal N-benzyl-N-cyclopropyl carboxamides and first optimization.

**Figure 5** Structure–activity relationships of the acid part.

**Figure 6** Further optimization of fungicidal N-benzyl N-cyclopropyl carboxamides leading to isoflucypram (1).
This effect could be reinforced by an additional substituent in the ortho position leading to two subclasses with compounds A14 and A15 as typical structures.

The acid part was fully investigated by replacing the initial 5-fluoro-1,3-dimethyl-1H-pyrazole-4-carboxylic acid (A7a, R = Me) by more than 200 five- and six-membered rings. The best acids for a broad spectrum were the classical pyrazoles of known SDH inhibitors: acids A7a (R = Me) and A7b. Nevertheless, a clear trend of the key role of the 5-fluoro substituent of the pyrazole ring was noticed, encouraging the assessment of acid A7a (R = CHF₂) originating from the combination of the two best acids: A7a (R = Me) and A7b (see Fig. 5). This resulted in a huge increase in the inhibition of the SDH target by 1 to 2 log units and, accordingly, a decrease in the effective doses (ED50) in planta.

As for the sulfonyl carboxamides, the cyclopropyl substitution was found to be almost the only tolerated substitution of the amide nitrogen apart from the 2-methylcyclopropyl substitution retaining part of the activity. It was again confirmed that the N-cyclopropyl derivatives are not pro-drugs since all NH analogues were found to be mostly inactive. On the contrary, thioamides were found to be true pro-drugs of

| Table 1 | ED50 concentrations (ppm) of derivatives derived from general structure A16 against Zymoseptoria tritici |
| Y/R     | 2-iPr  | 2-CF₃ | 2-I   | 2-TMS |
|---------|--------|-------|-------|-------|
|         | 0.02 (±0.007) | 0.02 (±0.003) | n.t. | 0.16 (±0.023) |
|         | 0.16 (±0.027) | 0.52 (±0.149) | 0.61 (±0.107) | 1.09 (±0.180) |
|         | 0.12 (±0.040) | 0.71 (±0.131) | 0.70 (±0.075) | 6.49 (±0.876) |
|         | 0.20 (±0.018) | 1.01 (±1.01) | 0.98 (±0.0823) | 3.38 (±0.650) |
|         | 0.35 (±0.022) | 3.13 (±0.191) | 2.15 (±0.200) | 3.85 (±0.959) |
|         | 0.79 (±0.049) | n.t. | 2.48 (±0.104) | 2.48 (±0.232) |

n.t., not tested.
ED50 values are the average of eight measurements, standard deviation is shown in parentheses. The ED50 of isoflucypram is 0.05 (±0.008) as reference.
the amides with a similar level of activity in planta but lacking any activity on the target.

The 2-trifluoromethyl substituent of compound A15 (see Fig. 6) could be replaced by halogens (e.g., bromo, iodo), small alkyls (e.g., isopropyl, isobutyl) or cycloalkyls (e.g., cyclopropyl), or even by bigger groups such as trialkyl silanes (e.g., trimethyl silane [TMS], triethyl silane), providing compounds of very high activity on both ascomycetes and basidiomycetes. The structure–activity-relationship (SAR) of the ortho-phenyl substituent was 2-iPr > 2-CF3 ≥ 2-I > 2-TMS. The 2-isopropyl substituted derivatives of A16 consistently delivered the lowest ED50 values in a cell test with Zymoseptoria tritici (see Table 1). The cell test activity translated nicely into greenhouse as well as field trial efficacy.

The combination of the best substitution pattern for phenyl, amide and pyrazole moieties led consequentially to the synthesis of compounds A17. Among those highly active derivatives, isoflucyram (1) was selected, providing the best-balanced efficacy and safety profile (see Fig. 6).

3 AFFINITY DATA AND PROPOSED BINDING MODE FOR N-CYCLOPROPYL-N-BENZYL-CARBOXAMIDES

Succinate-coenzyme Q reductase or SDH is a heterotetrameric enzyme complex located in the inner mitochondrial membrane. Crop protection SDHIs target the ubiquinone binding site constituted by subunits B, C and D located at the inner mitochondrial membrane.

When looking at the FRAC C2–SDHI class of compounds, it becomes obvious that the vast majority of SDHIs contain a secondary carboxamide function. It has been postulated the
carboxamide hydrogen forms a water-mediated hydrogen bond to the Ser83C in the ubiquinone binding site. The corresponding binding mode for bixafen in the ubiquinone binding site is illustrated in Figure 7.

The binding mode of the N-cyclopropyl-N-benzyl-carboxamides is assumed to be similar to that for classical SDHIs, as shown in Figure 8. The carboxamide oxygen forms a hydrogen bond to the conserved tryptophan NH sidechain, while the pyrazole moiety forms another hydrogen bond with the histidine NH sidechain. The chloro-substituted phenyl ring is likely to bind into a hydrophobic pocket, which is characteristic for fungi and nematodes.

However, in contrast to the classical SDHIs, the N-cyclopropyl group does not support water-mediated hydrogen bonding to Ser83C. Several compounds with different N-substituents (X) were synthesized based on the generic structure and tested in a biochemical assay to determine their inhibitory potency on complex II enzymes from Botrytis cinerea, shown in Table 2.

If the N-cyclopropyl-N-benzyl-carboxamides behaved as the usual SDHIs, it would be expected that the NH derivative 2b is more

| Compound | Substituent (X) | Botrytis cinerea biochemical assay complex II pIC₅₀ (−lg) |
|----------|----------------|-------------------------------------------------------|
| 2a       | Cyclopropyl    | 7.40 (±0.10)                                          |
| 2b       | H              | 5.50 (±0.10)                                          |
| 2c       | Propargyl      | 6.67 (±0.09)                                          |
| 2d       | Allyl          | 4.93 (±0.09)                                          |
| 2e       | Methyl         | 4.90 (±0.00)                                          |
| 2f       | Ethyl          | 5.50 (±0.08)                                          |
| 2g       | isopropyl      | ≤4.20 (n.d.)                                          |
| 2h       | Cyclopropylmethyl | 4.70 (±0.00)                                                   |
| 2i       | Cyclobutyl     | 6.33 (±0.12)                                          |
| 2j       | Cyclopentyl    | ≤4.20 (n.d.)                                          |
| 2k       | 2,2-difluoroethyl | 5.40 (±0.08)                                                   |
| 2l       | 3-oxetanyl     | 4.30 (±0.08)                                          |

*Average pIC₅₀ derived from three measurements, standard deviation in brackets. The pIC₅₀ of isoflucryram is 8.6 (±0.0) as reference.
potent than the N-cyclopropyl derivative 2a. Even though 2a is not capable of forming the above-mentioned water-mediated hydrogen bond, it generates very high pI50 values, clearly outperforming the inhibitory potency of the 2b. The binding mode of the N-cyclopropyl group seems to be very sensitive to a change in the N-substituent. The pI50 drops by one magnitude if the ring size is increased from N-cyclopropyl (2a) to N-cyclobutyl (2b), and by three magnitudes for N-cyclopentyl (2j). Even opening the ring of the cyclopropyl to N-isopropyl (2g) leads to an almost complete loss of in vitro efficacy. The SAR for the N-carboxamide substituent is very sharp overall, with N-cyclopropyl as clear favorite.

The increased pI50 values are probably driven by the expulsion of a water molecule from the binding pocket. The entropic gain is overcompensating by far the lack of the water-mediated hydrogen bonding to the Ser-83C, as proven by the extraordinary high pI50 for complex II enzyme from Botrytis cinerea generated with molecules based on the generic structure

![Image](https://www.soci.org/journal/ps/Pest%20Management%20Science%20published%20by%20John%20Wiley%20&%20Sons%20Ltd%20on%20behalf%20of%20Society%20of%20Chemical%20Industry.)

**Figure 9** Comparison of N-cyclopropyl compounds 3 (C1 linker), 4 (no linker) and isoflucypram (1) as reference. The pI50 values were determined with SDH from NEUSCR (Neurospora crassa).

In contrast to the very sharp in vitro SAR for the N-cyclopropyl group, such a clear SAR was not observed for linker extensions (A) for compounds of substructure 5, as presented in Table 3.

All (un)substituted C1 and C2 linkers lead to high in vitro efficacy in combination with the N-cyclopropyl moiety. However, compounds with an unsubstituted C1 linker showed higher potency in greenhouse and field-testing as well as a broader disease spectrum.

The unprecedented substitution pattern for an SDH inhibitor prompted the FRAC community to create within the C2–succinate dehydrogenase inhibitor class a new subclass for isoflucypram called N-cyclopropyl-N-benzyl-carboxamide. Whether or not the altered binding of isoflucypram 1 has an effect on the cross-resistance pattern in comparison with other SDHIs is the object of ongoing investigations.

### 4 SUMMARY

Isoleucypram-based products are likely to set new standards in the cereal fungicide market, providing excellent long-lasting control of all relevant leaf diseases and enabling optimized yield potential. The discovery of isoflucypram (1) provides a very elegant example for the smart combination of mix and match approaches and chemical optimization driven by the deep knowledge around SDH chemistry. The particular biochemical SAR suggests altered binding of isoflucypram in the SDH ubiquinone binding site. The two unique structural moieties, N-cyclopropyl substituent and the C1 linker between carboxamide and phenyl, support the formation of a discrete subclass for isoflucypram in the FRAC complex II inhibitor family.

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