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SDHI: Concealed effects on untargeted targets

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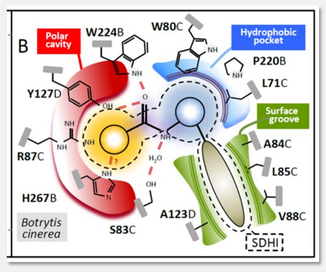
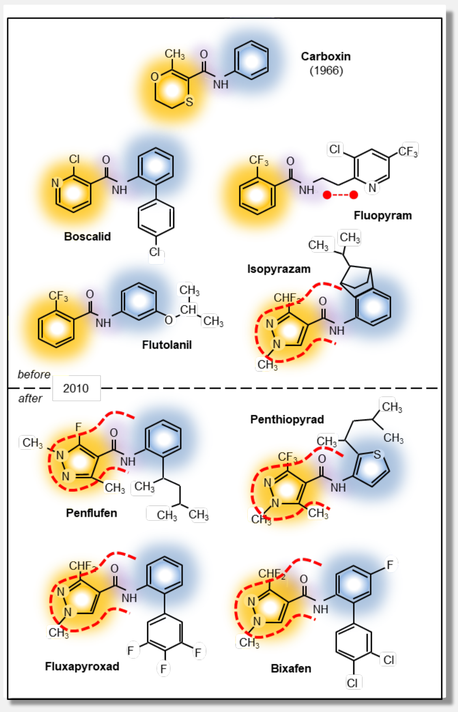
Abstract Succinate dehydrogenase (SDH) inhibitors (SDHIs) are used worldwide to limit the proliferation of molds on plants and plant products. However, as SDH, also known as respiratory chain (RC) complex II, is a universal component of mitochondria from living organisms, highly conserved through evolution, the specificity of these inhibitors toward fungi warrants investigation. We first establish that the human, honeybee, earthworm and fungal SDHs are all sensitive to the eight SDHIs tested, albeit with varying IC_{50} values, generally in the micromolar range. In addition to SDH, we observed that five of the SDHIs, mostly from the latest generation, inhibit the activity of RC complex III. Finally, we show that the provision of glucose *ad libitum* in the cell culture medium, while simultaneously providing sufficient ATP and reducing power for antioxidant enzymes through glycolysis, allows the growth of RC-deficient cells, fully masking the deleterious effect of SDHIs. As a result, when glutamine is the major carbon source, the presence of SDHIs leads to time-dependent cell death. This process is significantly accelerated in fibroblasts derived from patients with neurological or neurodegenerative diseases due to RC impairment (encephalopathy originating from a partial SDH defect) and/or hypersensitivity to oxidative insults (Friedreich ataxia, familial Alzheimer's disease).

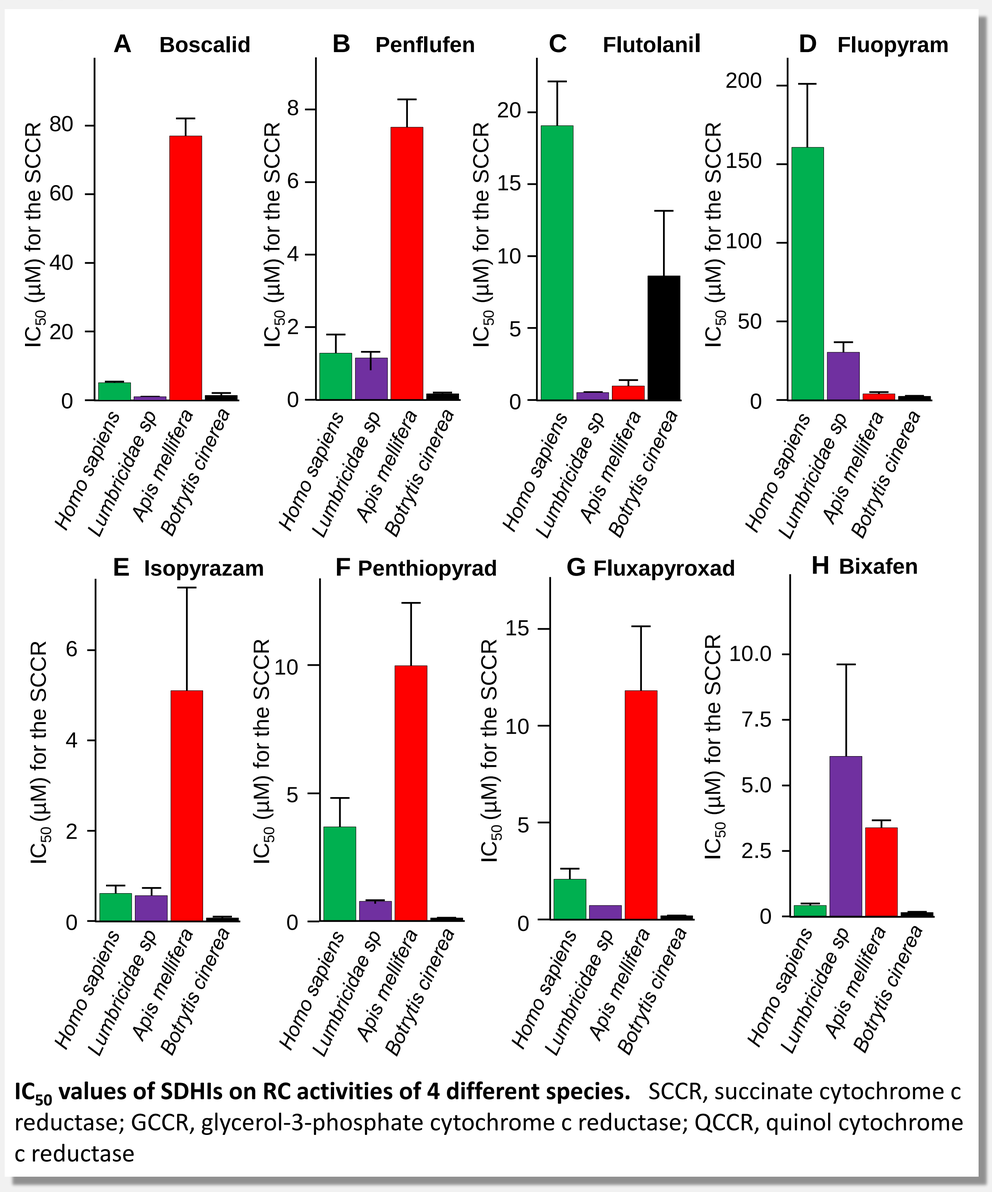
1 SDHI and their mechanism of action

Yellow and blue parts are reminiscent of the carboxin structure. The red dotted lines underline the methyl-pyrazol moiety present in the next generation SDHI. Boscalid 2003 USA by BASF, fluopyram 2010 USA Bayer, flutolanil 1981 USA Nichino America, penflufen 2012 USA Bayer, isopyrazam 2010 GB Syngenta, penthiopyrad 2011 USA Dupont-Fontelis, fluxapyroxad 2011 France BASF, bixafen 2011 GB Bayer. B, UQ-binding site of SDH featuring some of the amino acids that have been said to favor fungal resistance to SDHIs. Encircled by the dotted line, in yellow the part of the SDHI located to the polar cavity of the SDH UQ-binding site ; the hydrophobic pocket is in blue.



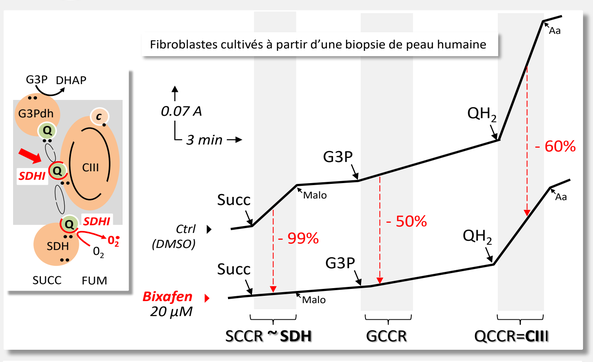


2 man, worm, bee or mushroom → no species specificity



IC_{50} values of SDHIs on RC activities of 4 different species. SCCR, succinate cytochrome c reductase; GCCR, glycerol-3-phosphate cytochrome c reductase; QCCR, quinol cytochrome c reductase

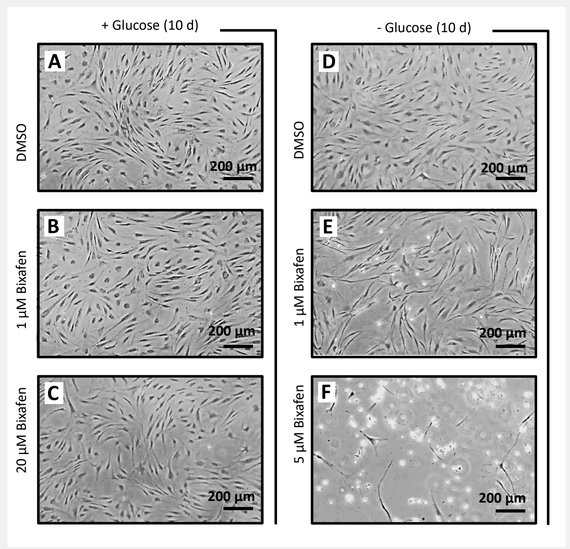
3 → no cell target specificity



The inhibitory effect of bixafen on the respiratory chain of human fibroblasts. Activities were measured according to Benit et al (Clin Chim Acta. 2006 374,81-6) using a pseudo-double wavelenght spectrophotometer measuring the reduction of added cytochrome c.

	Homo sapiens	*Apis mellifera*	*Botrytis cinerea*
Penthiopyrad	SCCR 3.7±1.1 µM GCCR 211±15 µM QCCR 349±72 µM	SCCR 10±2 µM GCCR 251±75 µM QCCR 268±44 µM	SCCR 0.045±0.023 µM GCCR 93.5±47 µM QCCR 105±7.1 µM
Isopyrazam	SCCR 0.63±0.18 µM GCCR 51.3±5.8 µM QCCR 125±35 µM	SCCR 5.1±2.3 µM GCCR 97±9 µM QCCR 87±18 µM	SCCR 0.023±0.004 µM GCCR 27.9±10.6 µM QCCR 14.2±5.6 µM
Bixafen	SCCR 0.34±0.12 µM GCCR 21.1±9.3 µM QCCR 15.5±13.4 µM	SCCR 3.3±0.33 µM GCCR 24.1±11.4 µM QCCR 5.7±5.3 µM	SCCR 0.07±0.06 µM GCCR 22.5±3.5 µM QCCR 22.2±14.4 µM

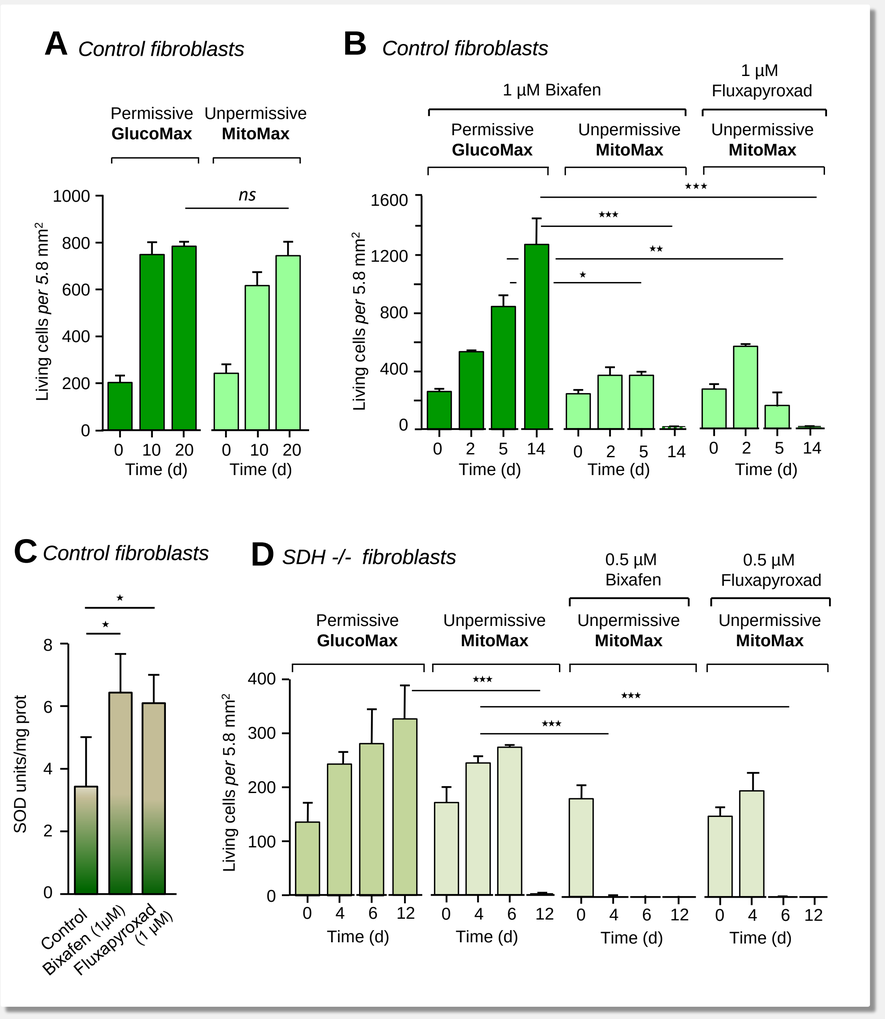
IC_{50} values of 4 SDHIs on SCCR, succinate cytochrome c reductase; GCCR, glycerol-3-phosphate cytochrome c reductase; QCCR, quinol cytochrome c reductase



4 SDHI readily kill human fibroblasts in culture.

A, D, Ctrl cells (10 d) *plus* (**A**) or *minus* (**D**) glucose (glutamine, source of carbon). **B, E,** Cells (10 d) without changing the medium *plus* 1 µM bixafen. Note the presence of numerous white spots (dead cells) in the absence of glucose (**E**). **C, F,** Despite the presence of 20 µM bixafen, after 10 d, no sign of cellular suffering in the presence of glucose (**C**). In the absence of glucose (F) a massive cell death occurs with 5 µM bixafen. In each figure, the black bar represents 200 µm. The culture medium was not changed during the experiment.

More on **SDHI**, Endsdhi.com



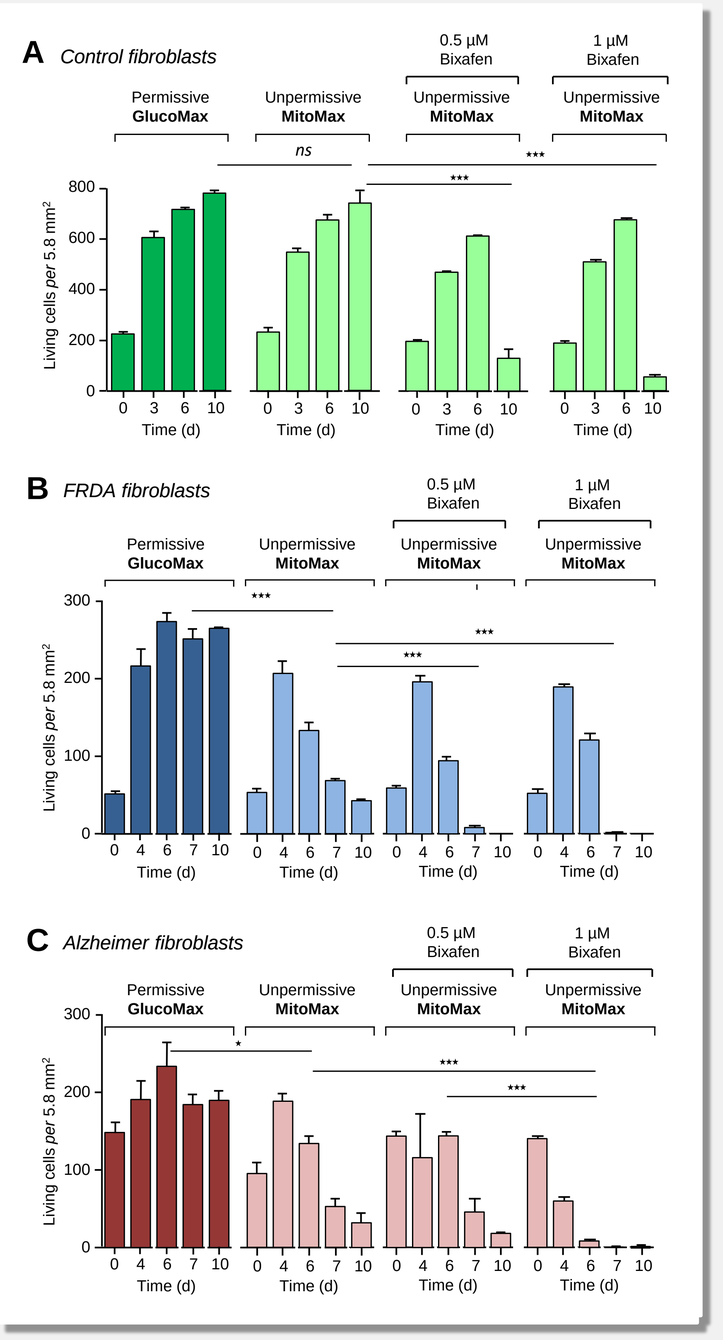
5 "There are none so blind as those who will not watch"… or will add glucose !

A, Control cells (n = 3); **B**, SDHIs (1 µM) were tested (n = 3) in permissive and nonpermissive media. Both bixafen and fluxapyroxad resulted in massive cell death after 14 days of cultivation under nonpermissive conditions. **C**, Superoxide dismutase activity of ctrl fibroblasts (n=4; permissive conditions minus (control DMSO) or plus 1 µM bixafen or 1 µM fluxapyroxad. **D**, Effect of bixafen and fluxapyroxad on patient fibroblasts with 60% residual SDH activity. In nonpermissive medium, massive cell death was observed at 4 days of cultivation in the presence of 0.5 µM bixafen and 6 days for the same concentration of fluxapyroxad. Culture media were not changed for the duration of the experiment (n = 3).

6 → better not to be sick!

Death of control, FRDA and FAD patient cells induced by bixafen

A, Ctrl cells were allowed to grow (n = 3) in either GlucoMax or MitoMax culture medium, respectively, in the absence or presence of 0.5 µM or 1 µM bixafen. A similar experiment on fibroblasts from a Friedreich ataxia patient (FRDA fibroblasts) (**B**) and from a patient suffering from familial Alzheimer's Disease (**C**). Culture media were not changed for the duration of the experiment (n = 3).



7 Conclusion

1. SDH: evolutionary conserved → SDHI: no species specificity
+
2. Last-generation SDHI: CIII inhibitors → Cell target specificity: none
+
3. Inadequate regulatory tests
Non-existent animal models
Epigenetic absent from tests
→ Predictability of safety inadmissible
+
4. Pesticides blocking the RC → Recognized toxic after… 25 years
+
5. A no-future strategy: microorganisms become more and more (multi-) resistant → Next, cross-infection between species

Apply the precautionary principle
get rid of the SDHI