# 2-Oxabicyclo[2.1.1]hexane as saturated bioisostere of Fluxapyroxad: synthesis and antifungal activity

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## Introduction and Aim

Fluxapyroxad is a marketed broad-spectrum fungicide, developed by BASF, has been approved for use in the United States and the European Union. The development of new active fungicides is currently relevant due to the emergence of new strains of fungiresistant to existing fungicides. In this study, we incorporated the new bioisostere, 2-oxabicyclo[2.1.1]hexane scaffold, into the Fluxapyroxad: structure instead of the monosubstituted phenyl ring.

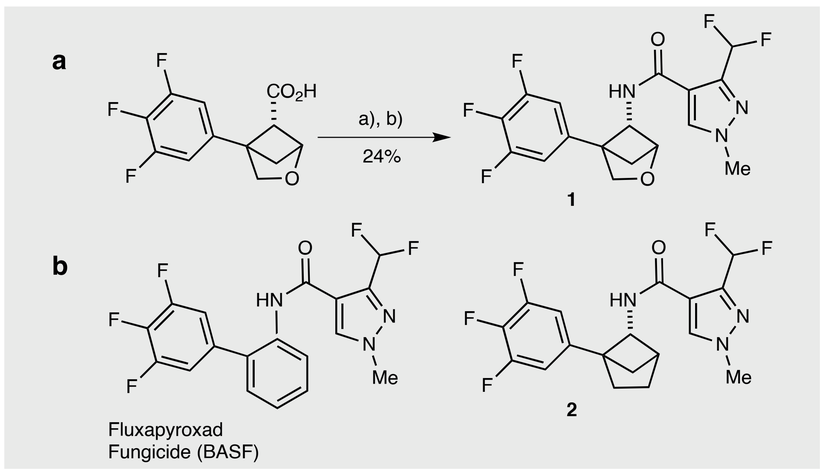
## Methods

Kinetic solubility (2% final DMSO) and $LogD_{7.4}$ of all three compounds were assessed by the shake-flask method. Metabolic stability was tested in human liver microsomes (HLM).

The bioactivity of Fluxapyroxad and its analogs, **1** and **2**, was tested using the agar well diffusion method against *Fusarium oxysporum* and *Fusarium verticillioides*. A minimal inhibitory concentration (MIC) of all compounds was assessed by microdilution method.

The genotoxicity of compounds was studied by Ames test (Liquid Microplate Format) and Mammalian Cell Micronucleus test (High Content Imaging assay).

Physicochemical studies showed that analogue **1** is more soluble at pH 7.4 and has lower $LogD_{7.4}$ compared to Fluxapyroxad. All three compounds can be metabolised by human liver microsomes.




**Scheme 1.** a. Synthesis of compound 1 – a saturated analogue of Fluxapyroxad (BASF). Reaction conditions: **a)** $(COCl)_2$, $NaN_3$, toluene, Δ. **b)** $(COCl)_2$, Het-$CO_2H$, rt. b. Structure of Fluxapyroxad (BASF), and its saturated analogue 2.

**Table 1. ADME properties of Fluxapyroxad and analogs**

| Compound | Solubility in PBS (pH=7.4), uM | LogD, pH=7.4 | Clint, ul/min/mg HLM | T1/2, min HLM | kel, min-1 HLM |
|---|---|---|---|---|---|
| **Fluxapyroxad** | 15±0.6 | 3.37±0.12 | 39 | 43,1 | 0,016 |
| **Analog 1** | 155±3.2 | 2.77±0.02 | 23 | 73,4 | 0,009 |
| **Analog 2** | 34±0.4 | 4.32±0.195 | 35 | 47,4 | 0.015 |

*kinetic solubility, 2% final DMSO

## Results

First, we measured the antifungal activity of all compounds using the agar well diffusion method. Fluxapyroxad, and its saturated analogues **1** and **2**, showed a similar trend in activity at the inhibition of fungi growth. The 2-oxabicyclo[2.1.1]hexane analogue **1** was active, but less potent compared to the original fungicide. Compound 1 and Fluxapyroxad almost identically inhibited the growth of *Fusarium oxysporum* at high concentrations; however, at low concentrations, analogue **1** showed lower activity. Similarly, analogue **1** and Fluxapyroxad effectively inhibited the growth of *Fusarium verticillioides* at high concentrations; however, at low concentrations, only Fluxapyroxad remained active, while analogue **1** did not **(Figure 1)**

Fluxapyroxad, and its saturated analogues **1** and **2** did not show any genotoxicity neither in Ames test, nor in Mammalian Cell Micronucleus test **(Figure 2)**.

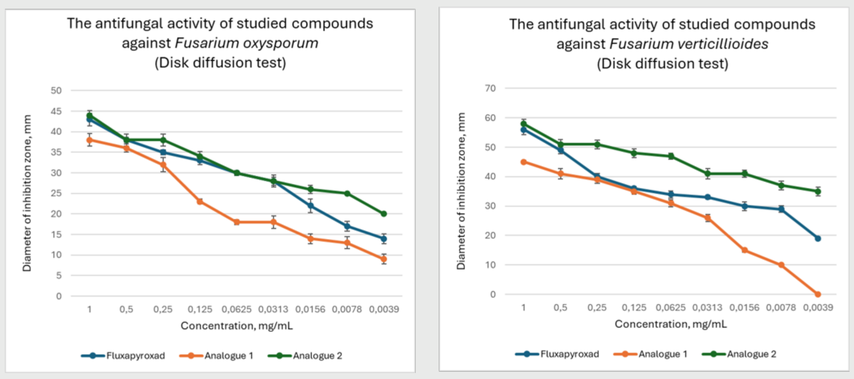



**Figure 1.** The antifungal activity of Fluxapyroxad and its saturated analogs 1 and 2 against *Fusarium oxysporum* and *Fusarium verticillioides* in the agar well diffusion method

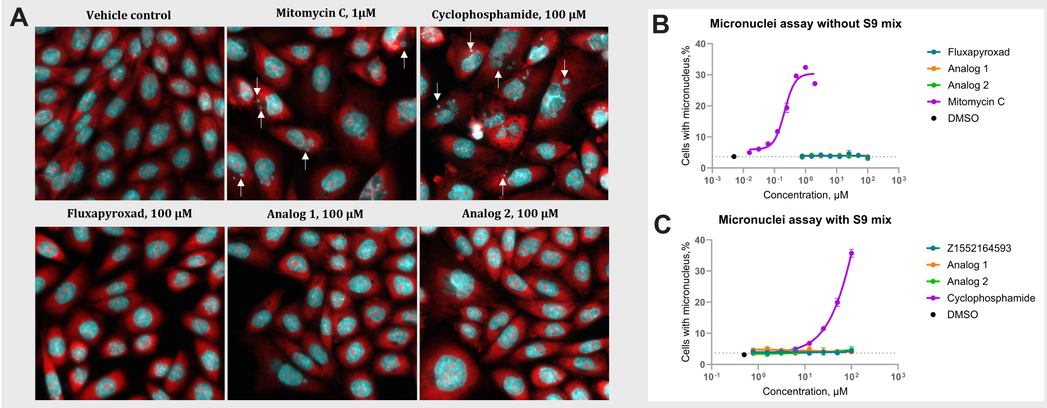



**Figure 2. A)** Representative confocal images of CHO-K1 cells treated with vehicle, Mitomycin C, Cyclophosphamide, Fluxapyroxad, Analogs 1 and 2 for 48 hours. Nuclear dye Hoechst 33345 (cyan) stains nuclei and micronuclei (white arrows). Cytoplasm is labeled with Pyronin Y (red). **B)** Micronuclei assay without S9 metabolizing system. **C)** Micronuclei assay with S9 metabolizing system.

Additionally, we measured a minimal inhibitory concentration (MIC) of all compounds by microdilution method **(Table 2)**. Interestingly, Fluxapyroxad and its 2-oxabicyclo[2.1.1]hexane analogue 1 exhibited equal MIC values of 0.250 mg ml–1 at the inhibition of the growth of *F. verticillioides.* at the same time, analogue 2 was more effective than analogue 1 and the original fungicide: it inhibited the growth of *F. oxysporum* and *F. verticillioides* at a concentration of 0.125 mg ml–1.

**Table 2.** The Minimal Inhibitory Concentrations (MIC) values of Fluxapyroxad, and its saturated analogs against *Fusarium oxysporum*, and *Fusarium verticillioides*

| Antifungal compounds | MIC, mg/ml | |
|---|---|---|
| | F. oxysporum | F. verticillioides |
| **Fluxapyroxad** | -* | 0.250 |
| **Analog 1** | 0.125 | 0.125 |
| **Analog 2** | -* | 0.250 |

## Conclusions

These primary data suggested that Fluxapyroxad and both its analogs, **1** and **2**, could have similar antifungal effects. These results also show that 2- oxabicyclo[2.1.1]hexane core could indeed mimic the phenyl ring in bioactive compounds. Thus, 2-oxabicyclo[2.1.1]hexane can be added to the set of available saturated bioisosteres of (hetero)aromatic rings for use in discovery projects.

## Contact

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## References

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