Biotransformation of fluorophenyl pyridine carboxylic acids by the model fungus Cunninghamella elegans

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Introduction:

A strategy for increasing the metabolic half-life of a drug is to introduce a fluorine atom at metabolically labile sites\textsuperscript{1}. A chemical motif that has become more commonly used in pharmaceuticals is the fluorophenyl pyridine system, exhibited by Fig.1.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{Structures of the recently FDA-approved fluorinated drugs Sivestro\textsuperscript{a} and Zentivity\textsuperscript{a}}
\end{figure}

Previous investigations have demonstrated that microbial biotransformation of drug candidates can reveal sites vulnerable to oxidative attack\textsuperscript{2}. To study the motif shown in Fig.1, we examined the biotransformation of various fluorophenyl pyridine carboxylic acids (Fig. 2) as model compounds.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2.png}
\caption{Structures of the fluorophenyl pyridine carboxylic acids}
\end{figure}

Biotransformation of fluorophenyl pyridine carboxylic acids:

The fungal microorganism Cunninghamella elegans, widely studied as a model for mammalian drug metabolism\textsuperscript{2}, was selected for the study. The compounds were incubated with \textit{C. elegans}, with subsequent metabolites extracted for further analysis.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3.png}
\caption{Hydroxylated biotransformation products 6-11 formed upon incubation of pyridine carboxylic acids 1, 2, 3 and 5.}
\end{figure}

With exception of 4, \textit{C. elegans} effectively transformed all of the (fluoro-) phenyl pyridine carboxylic acids (Fig.3). GC-MS and NMR identified alcohols and hydroxylated carboxylic acids as metabolites.

Metabolic stability:

A quantitative method using \textsuperscript{19}F NMR was developed to examine the degree of transformation (Table 1) for each compound. A 24-hour biotransformation study was performed using 2 mg of starting material.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{Substrate} & \textbf{Fluorometabolite(s)} & \textbf{Compound} & \textbf{Yield} \\
\hline
1 & -112.5 & -135.6 & 8 & 95\% \\
2 & -118.6 & -116.9 & 9 & 22\% \\
3 & -113.2 & N.D. & \\
4 & -116.7 & -114.0 & -117.5 & 10 & 11 & 36\%, 59\% \\
\hline
\end{tabular}
\caption{\textsuperscript{19}F NMR analysis of the biotransformation products 8-11 from compounds 2-5 in \textit{C. elegans} (N.D. Not detected).}
\end{table}

Orbital density and atomic charge calculations:

Calculated frontier orbital density distributions (Table 2) confirm the regioselectivity for compounds 1-3 and 5, where the highest HOMO densities are found at the 4' carbon sites. Interestingly, the calculated atomic charges for C4' of all the substrates reveal that compound 2 has the highest negative charge (Table 2), which might account for this compound being more readily hydroxylated than compound 3.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{C2} & \textbf{C3} & \textbf{C4} & \textbf{C5} & \textbf{C1'} & \textbf{C2'} & \textbf{C3'} & \textbf{C4'} & \textbf{C5'} \\
\hline
\textbf{LUMO} & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 \\
\textbf{HOMO} & 8.0 & 8.0 & 8.0 & 8.0 & 8.0 & 8.0 & 8.0 & 8.0 & 8.0 \\
\textbf{Charge} & 0.016 & 0.046 & -0.168 & 0.059 & 0.013 & 0.091 & -0.250 & 0.358 & -0.103 & -0.155 & 0.170 \\
\hline
\textbf{Compound} & \textbf{C2} & \textbf{C3} & \textbf{C4} & \textbf{C5} & \textbf{C1'} & \textbf{C2'} & \textbf{C3'} & \textbf{C4'} & \textbf{C5'} \\
\hline
\textbf{LUMO} & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 & 1.0 \\
\textbf{Charge} & 0.017 & 0.046 & -0.166 & 0.070 & 0.022 & 0.014 & 0.028 & 0.134 & -0.127 & -0.132 & 0.176 \\
\hline
\end{tabular}
\caption{Calculated frontier orbital densities (%) and atomic charges (a.u.) on aromatic carbons of 2 and 3}
\end{table}

Mammalian biotransformation:

- An \textit{in vitro} method using rat liver microsomes was developed for comparison.
- No biotransformation products were detected by GC-MS.
- As a positive control, flurbiprofen was incubated with various concentrations of 2 (Fig.4).
- Fluorophenyl pyridine carboxylic acids are most likely inhibitors of microsomal preparations.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig4.png}
\caption{Total ion chromatograms of the microsomal biotransformation product of flurbiprofen formed in the presence of various concentrations of 2.}
\end{figure}

Conclusion:

- \textit{Cunninghamella elegans} is able to produce hydroxylated metabolites of fluorophenyl pyridine carboxylic acids and can serve as an effective alternative for \textit{in vitro} assessments of xenobiotic biotransformation.
- Extent and regioselectivity of hydroxylation was dependent on the position of the fluorine substituent.

References: