



## Limited applicability of 7-methoxy-4-trifluoromethylcoumarin as a CYP2C9-selective substrate

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### Abstract:

Fluorometric substrates selective for various cytochrome P450 isoforms (P450s) have great advantages in *in vitro* enzyme inhibition and induction studies because they are highly sensitive and suitable for rapid screening. 7-Methoxy-4-trifluoromethylcoumarin (MFC) has been reported as a CYP2C9-selective substrate. The present study investigated the relative catalytic selectivity of several human P450s in the *O*-demethylation of MFC and the applicability of MFC as a probe substrate for CYP2C9. The CYP2C9-selectivity in liver microsomes was not supported by the correlation analysis within a series of microsomes from individual donors or by studies using chemical inhibitors. MFC *O*-demethylation of microsomes did not correlate with tolbutamide 4-hydroxylation, the classical CYP2C9-marker activity, suggesting the possible participation of some of the other P450s. Results of inhibition studies using model P450 inhibitors also brought the CYP2C9-selectivity of MFC *O*-demethylation into question. In microsomes containing cDNA-expressed individual P450s, CYP2B6 and CYP2E1 seemed to be the most active in the *O*-demethylation of MFC. Our results support the participation of several P450 enzymes (CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2E1 and CYP3A4) in MFC *O*-dealkylation. Therefore, MFC cannot be considered a suitable probe substrate in models that express several P450s, such as liver microsomes or primary hepatocytes. Moreover, MFC is a more potent fluorogenic substrate for CYP2B6 and CYP2E1 than for CYP2C9 in microsomes containing cDNA-expressed P450s.

### Key words:

7-methoxy-4-trifluoromethylcoumarin *O*-demethylation, CYP2C9-selectivity, fluorogenic substrate, high-throughput screening, drug-drug interaction

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