DISCOVERING THE MAJOR METABOLITES OF THE NOVEL FENTANYL ANALOGUES 3-METHYLCROTONYLFENTANYL, FURANYLBENZYLFENTANYL AND 4-FLUOROCYCLOPROPYLBENZYLFENTANYL FOR FORENSIC CASE WORK

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Background/aim: The opioid analgesic fentanyl and its analogues pose a major health concern due to their high potency and increasing number of overdose deaths worldwide. As new fentanyl analogues are continuously emerging much is still unknown considering the metabolism of these compounds. In clinical and forensic toxicology, metabolite profiling can be an important tool for analytical identification. The aim of this study was to identify the main metabolites of 3-methylcrotonylfentanyl(3-MCF), furanyl-benzylfentanyl (FBF) and 4-fluorocyclopropylbenzylfentanyl (4-FCBF) .

Methods: Human liver microsomes and cryopreserved hepatocytes were incubated with 3-MCF, FBF or 4-FCBF for 0, 0.5, 1, 2 and 4 hours. The formed metabolites were separated by UHPLC using an Acquity HSS T3 column (2.1 x 100 mm, 1.8 µm, Waters) employing gradient elution with a mobile phase consisting of 10 mM ammonium formate pH 3.1 and MeOH. Identification of the compounds was performed by quadrupole time-of-flight mass spectrometry analysis.

Results: The main metabolites of 3-MCF, FBF and 4-FCBF are described in Table 1 and Figure 1. The major metabolites of 3-MCF were formed by *N*-dealkylation, carboxylation, oxidation or hydroxylation of the 3-methyl-2-butene, and hydroxylation of both the 3-methyl-2-butene and the piperidine ring. FBF was metabolized through *N*-dealkylation, amide hydrolysis with/without subsequent hydroxylation at the *N*-phenyl, and dihydrodiol formation at the furan ring. 4-FCBF metabolism was dominated by *N*-dealkylation and *N*-oxidation at the piperidine ring.

Discussion: The metabolism of 3-MCF and 4-FCBF appears to be similar to that of fentanyl, which is dominated by *N*-dealkylation. FBF metabolism resembles that of furanylfentanyl, which is dominated by amide hydrolysis and dihydrodiol formation at the furanyl ring system. The major metabolites of the three fentanyl analogues differed slightly in human liver microsomes and hepatocytes regarding type of biotransformation and ratio of metabolites formed. However, the two *in vitro* models also formed shared metabolites for each compound which can be employed for accurate detection of the parent compounds.

Conclusion: In the present study we successfully discovered and elucidated the structures of the major metabolites of 3-MCF, FBF and 4-FCBF which could be used as markers to confirm intake of these compounds in forensic case work.

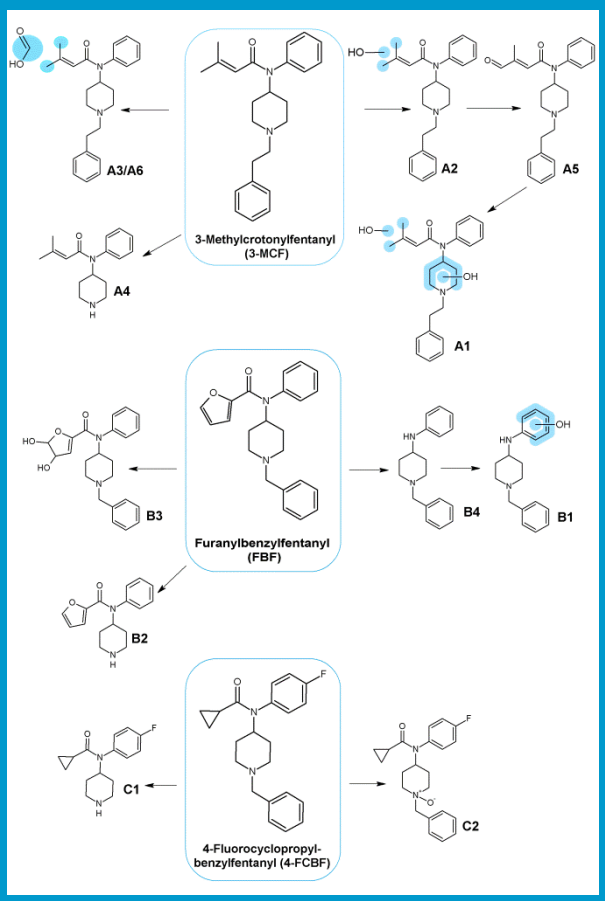

Figure 1: Proposed structures of the major metabolites of 3-MCF (A1-A6), FBF (B1-B4) and 4-FCBF (C1 and C2). Markush bonds in blue color indicate possible locations for a hydroxyl or a carboxyl group.

Table 1: Peak heights of proposed main metabolites of 3-MCF(A1-A6) , FBF(B1-B4) and 4-FCBF(C1 and C2) in hepatocytes and microsomes with retention time (T_R), molecular formula and accurate mass of protonated molecule.

DRUG	ID	BIOTRANSFORMATION	MOLECULAR FORMULA	T_R (min)	$[M+H]^+$	HEPATOCYTES				MICROSOMES			
						0.5 h	1 h	2 h	4 h	0.5 h	1 h	2 h	4 h
3-MCF	A1	Dihydroxylation	$C_{24}H_{30}N_2O_3$	6.6	395.2326	N.D	N.D	N.D	N.D	$9.6x10^4$	$8.8x10^4$	$6.0x10^4$	$5.2 x10^4$
	A2	Hydroxylation	$C_{24}H_{30}N_2O_2$	7.3	379.2380	$3.7x10^4$	$4.2x10^4$	$4.4x10^4$	$8.7 x10^4$	$1.1x10^6$	$1.9x10^5$	$2.9x10^4$	$1.5 x10^4$
	A3	Carboxylation	$C_{24}H_{28}N_2O_3$	7.3	393.2175	N.D	N.D	N.D	N.D	$3.5x10^5$	$9.8x10^5$	$1.1x10^6$	$1.1x10^6$
	A4	*N*-dealkylation	$C_{16}H_{22}N_2O$	7.6	259.1807	$1.0x10^5$	$1.2x10^5$	$7.9x10^4$	$1.9 x10^5$	$1.0x10^5$	$4.6x10^3$	$4.1x10^3$	$7.8 x10^3$
	A5	Oxidation	$C_{24}H_{28}N_2O_2$	7.8	377.2228	N.D	N.D	N.D	N.D	$1.2x10^5$	$2.8x10^4$	$4.5x10^3$	$2.5 x10^3$
	A6	Carboxylation	$C_{24}H_{28}N_2O_3$	7.8	393.2164	N.D	N.D	N.D	N.D	$2.6x10^4$	$3.5x10^4$	$3.1x10^4$	$4.0 x10^4$
FBF	B1	Amide hydrolysis+hydroxylation	$C_{18}H_{22}N_2O$	4.0	283.1809	$5.9x10^3$	$1.7x10^4$	$4.2x10^4$	$3.6 x10^4$	$1.1x10^6$	$1.2x10^6$	$1.3x10^4$	$1.4 x10^6$
	B2	*N*-dealkylation	$C_{16}H_{18}N_2O_2$	5.8	271.1441	$4.8x10^4$	$6.2x10^4$	$6.8x10^4$	$1.9 x10^4$	$3.3x10^5$	$3.8x10^5$	$3.5x10^5$	4.0 x10
	B3	Alkene to dihydrodiol	$C_{23}H_{26}N_2O_4$	6.4	395.1964	$1.0x10^5$	$1.9x10^5$	$2.9x10^5$	$2.0 x10^5$	$2.3x10^4$	$3.2x10^4$	$3.9x10^4$	$5.1 x10^4$
	B4	Amide hydrolysis	$C_{18}H_{22}N_2$	7.2	267.1856	$4.7x10^4$	$4.1x10^4$	$3.6x10^4$	$8.5 x10^3$	N.D	N.D	N.D	N.D
4-FCBF	C1	*N*-dealkylation	$C_{15}H_{19}FN_2O$	6.7	263.1556	$1.2x10^5$	$2.0x10^5$	$2.6x10^5$	$2.9x10^5$	$1.7x10^4$	$1.8x10^4$	$2.0x10^4$	$2.2 x10^4$
	C2	*N*-oxidation	$C_{22}H_{25}FN_2O_2$	9.5	369.1972	$2.2x10^4$	$2.6x10^4$	$4.4x10^4$	$4.3 x10^4$	$7.1x10^3$	$1.0x10^4$	$1.1x10^4$	$1.2 x10^4$

N.D: Not detected

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