Supplementary Information

Towards understanding transfluthrin efficacy in a pyrethroid-resistant strain of the malaria vector *Anopheles funestus* with special reference to cytochrome P450-mediated detoxification

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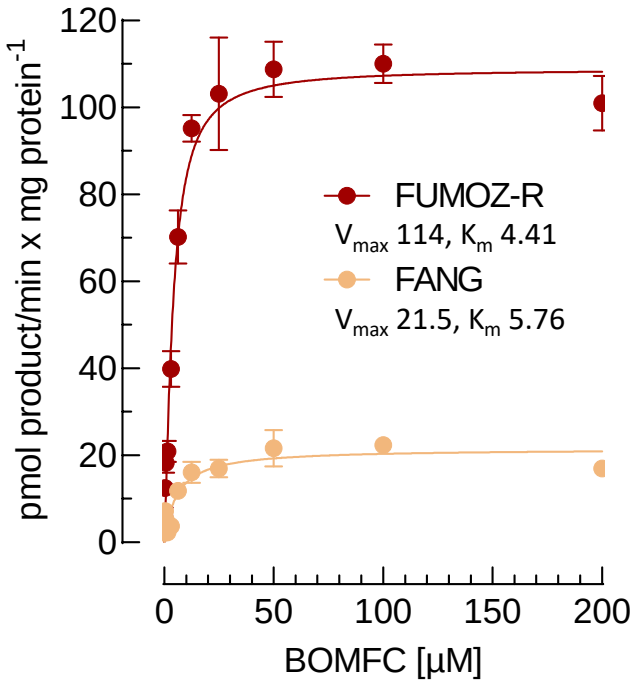
Supplementary Figure S1. Steady-state kinetics of BOMFC O-debenzylation leading to 7-hydroxy-4-(trifluoromethyl)coumarin (HC) by cytochrome P450s of microsomal preparations of *Anopheles funestus* strain FANG und FUMOZ-R. Data points are mean values ± standard deviation (SD) (n = 3). The calculated K_m- and V_{max}-values for BOMFC are apparent values based on general microsomal monooxygenase activity.



Supplementary Table S1. Primer sequences and GenBank numbers for target and reference genes used in qPCR.

Gene name	Forward 5' to 3'	Reverse 5' to 3'	Amplicon (bp)
Ribosomal Protein S7	GTGTTCGGTTCCAAGGTGAT	TCCGAGTTCATTTCCAGCTC	111
Actin 5c	TTAAACCCAAAAGCCAATCG	ACCGGATGCATACAGTGACA	98
CYP6P9A	AACTTGCGGCACAGGCATTC	TCTCCTCGATTGCTTGGTTG	144
CYP6P9B	CGATCCACTATCAGGACATC	AAGTGCCACATCCCATAGTG	130

Gene name	Vector Base code	GenBank Acc.	UniProt
Ribosomal Protein S7	AFUN007153	EF450776.1	A0A182RLN5
Actin 5c	AFUN006819-RA	not available	A0A182RKQ1
CYP6P9A	AFUN015792	KR866022.1	A0A0S1S5U9
CYP6P9B	AFUN015889	KR866046.1	A0A096XPX9

Supplementary Table S2. EC_{50}-values (µM) of common pyrethroids and transfluthrin derivatives measured on functionally expressed house fly voltage-gated sodium channels (VGSC) using a cell-based membrane potential cation influx assay. Data were taken from Figs. 2A and 2B.

	EC_{50} (µM)	95% CI	-LogEC_{50} (M)	95% CI
Deltamethrin	0.00529	0.00443–0.0063	8.28	8.20–8.35
Cypermethrin	0.0389	0.0342–0.0443	7.41	7.35–7.47
Permethrin	0.721	0.591–0.882	6.14	6.05–6.27
Transfluthrin	1.77	1.62–1.94	5.75	5.71–5.79
TF-0	1920*	1109–3748	2.72	2.43–2.95
TF-1	315*	207–525	3.50	3.28–3.68
TF-3	4.89	4.23–5.66	5.31	5.25–5.37
TF-5	0.736	0.65–0.834	6.13	6.08–6.19

* Extrapolated value

Supplementary Table S3. LC_{50}-values (mg/m²) of different pyrethroids against *Anopheles funestus* strains FANG and FUMOZ-R in glazed tile contact bioassays. Synergists were applied prior to insecticide exposure.

	A. funestus FANG						*A. funestus* FUMOZ-R							
	LC_{50}	Synergistic Ratio (FANG)	95 % CI	Slope ± SE		n	LC_{50}	95 % CI	Slope ± SE		n	Resistance Ratio	Synergistic Ratio (FUMOZ-R)
TF	0.023		0.0155 -0.0324	1.47	0.168	420	0.0576	0.0191 -0.112	1.6	0.218	360	2.51	-
+ PBO	0.0389	0.591	0.0201 -0.0699	4.10	1.48	140	0.00733	0.00389 -0.0148	4.07	1.51	120	0.319	7.86
+ 1-ABT	0.0184	1.25	0.00502 -0.0337	2.38	0.676	140	0.0057	0.000468 -0.00867	3.51	1.62	120	0.248	10.1
+ Triflumizole	0.0187	1.23	0.00999 -0.0279	2.28	0.499	280	0.00465	0.0000688 -0.0167	1.55	0.395	120	0.203	12.4
TF-0	1.19		0.565 -1.79	2.90	0.932	140	4.47	2.89 -6.01	3.17	0.655	360	3.77	-
+ PBO	0.691	1.72	0.223 -1.65	1.59	0.304	140	0.553	0.281 -0.805	3.52	1.13	120	0.47	8.07
+ 1-ABT	1.33	0.895	0.849 -2.05	2.66	0.565	140	1.21	0.601 -3.025	3.63	0.917	120	1.02	3.69
+ Triflumizole	1.16	1.026	0.761 -1.78	3.24	0.823	140	0.609	0.316 -0.944	2.79	0.798	120	0.51	7.34
TF-1	1.42		1.02 -1.85	2.97	0.453	560	1.41	1.15 -1.83	4.78	0.772	350	0.99	-
+ PBO	1.4	1.01	0.872 2.19	3.12	0.813	140	3.77	2.4 -5.93	2.60	0.576	140	2.65	0.376
+ 1-ABT	0.843	1.68	0.434 -1.44	3.43	1.16	140	0.34	0.0645 -0.579	1.72	0.434	420	0.403	4.16
+ Triflumizole	1.62	0.877	1.03 -2.54	3.04	0.688	140	2.42	0.537 -5.42	2.80	0.758	140	1.70	0.584
TF-3	0.0494		0.0350 -0.0664	1.96	0.256	420	0.285	0.225 -0.354	3.32	0.495	360	5.77	-
+ PBO	0.0158	3.13	0.00652 -0.0314	1.41	0.285	140	0.00759	0.00212 -0.0142	1.93	0.513	120	0.154	37.6
+ 1-ABT	0.0183	2.7	0.00795 -0.0257	4.53	1.43	140	0.0304	0.0187 -0.0483	3.70	1.11	120	0.617	9.36
+ Triflumizole	0.0263	1.87	0.0132 -0.0419	2.52	0.688	140	0.012	0.00408 -0.0214	2.94	0.788	120	0.243	23.8
TF-5	0.0237		0.0132 -0.0394	2.03	0.243	420	0.0446	0.0299 -0.0592	2.08	0.316	560	1.88	-
+ PBO	0.012	1.98	0.00686 -0.0258	4.24	1.40	140	0.018	0.00558 -0.0338	2.23	0.574	140	0.758	2.48
+ 1-ABT	0.0224	1.06	0.00174 -0.0468	2.05	0.695	140	0.0141	0.00515 -0.0241	2.36	0.687	140	0.593	3.17
+ Triflumizole	0.0283	0.837	0.0143 -0.0481	1.91	0.414	140	0.0203	0.00372 -0.0384	2.28	0.706	140	0.856	2.20
Permethrin	0.543		0.409 -0.702	2.28	0.294	420	4.21	2.79 -5.88	1.99	0.278	360	7.76	-
+ PBO	0.273	1.99	0.197 -0.471	5.18	1.43	140	0.327	0.128 -0.516	4.13	1.2	120	0.602	12.9
+ 1-ABT	0.0834	6.51	0.0478 -0.14	1.80	0.331	140	0.523	0.147 -0.935	2.48	0.693	120	0.962	8.06
+ Triflumizole	0.329	1.65	0.0884 -0.505	2.69	1.03	140	0.22	0.102 -0.422	2.46	0.496	120	0.404	19.2
Cypermethrin	0.0968		0.0686 -0.126	2.74	0.479	420	7.54	5.24 -10.4	1.86	0.204	540	77.9	-
+ PBO	0.00882	11	0.00277 -0.0178	1.8	0.459	140	0.0451	0.0140 -0.0880	1.73	0.4	140	0.466	167
+ 1-ABT	0.0157	6.17	0.00408 -0.0327	1.34	0.323	140	0.0358	0.0057 -0.146	1.34	0.243	140	0.37	210
+ Triflumizole	0.065	1.49	0.0312 -0.121	1.95	0.371	140	0.493	0.168 -0.95	1.23	0.171	400	5.1	15.3
Deltamethrin	0.0206		0.0153 -0.0273	1.38	0.119	560	4.61	2.73 -7.5	1.07	0.102	540	223	-
+ PBO	0.00074	27.8	0.000224 -0.0016	1.23	0.356	280	0.0103	0.0037 -0.0231	1.88	0.341	140	0.499	448
+ 1-ABT	0.00327	6.29	0.00090 -0.0105	0.741	0.242	140	0.0318	0.00515 -0.0822	1.43	0.337	140	1.54	145
+ Triflumizole	0.00518	3.98	0.00142 -0.0126	1.40	0.263	140	0.0364	0.0239 -0.053	1.34	0.137	460	1.77	127