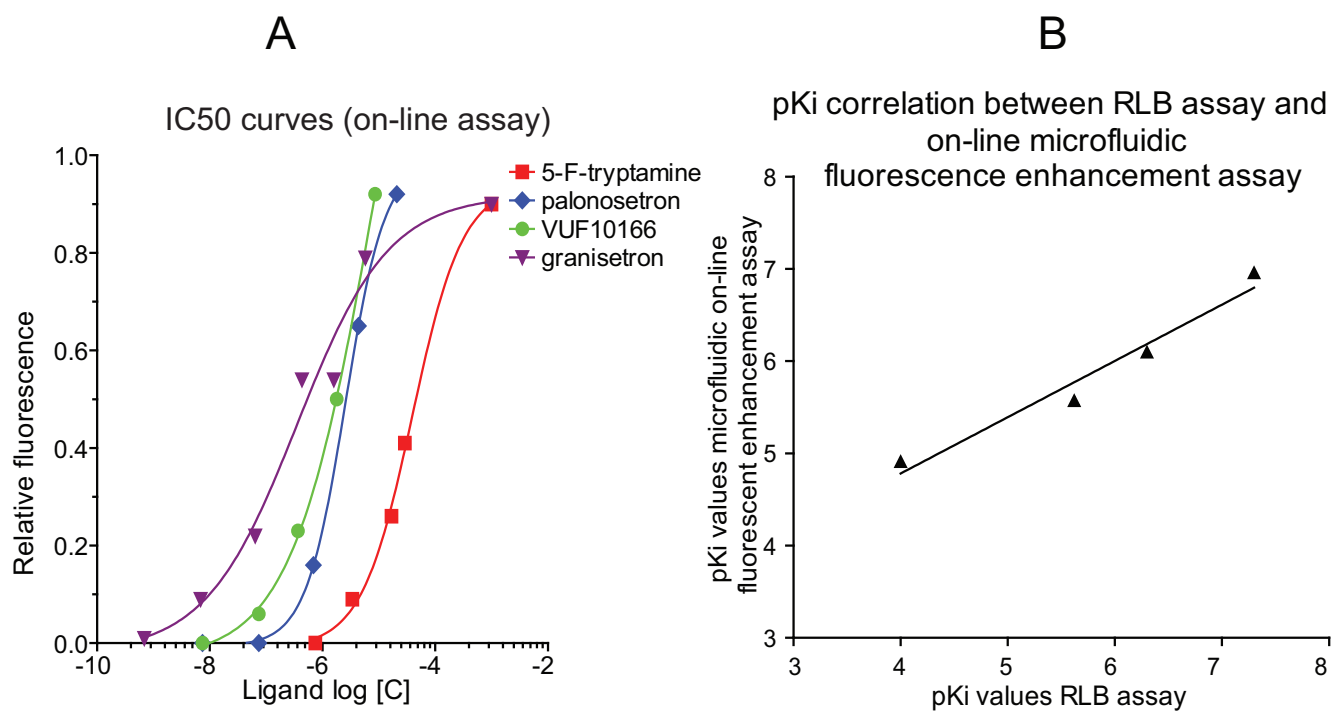


## Supplementary Information



**Figure S1.** (A) Concentration-response of four 5HT3 receptor ligands obtained with the microfluidic on-line system. Due to dilution effects in the nano-LC and on-line assay, the actual concentration in the assay is lower than the injected concentration. The dilution factor of ligands injected was calculated as described by Falck *et al.* (Falck, de Vlieger *et al.* 2010), and then used to estimate the assay concentration of the analyzed ligand; (B) Correlation between the RLB assay and the microfluidic on-line fluorescence enhancement assay ( $r^2 = 0.9598$ ).

Mascot score: 832

Short neurotoxin 3 OS=Pseudonaja textilis GN=SNTX3 PE=2 SV=1 (Uniprot number: Q9W7K0)

Sequence coverage: 67%

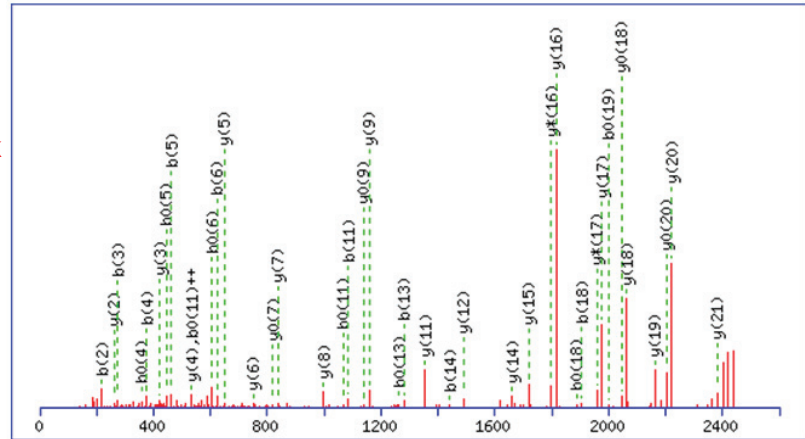
1 MKTLLLTLMV TIMCLDLGY TLTCYKGYHD TVVCKPHETI CYRYLVPATH  
51 GNAIPARGCG TSCPGGNHPV CCSTDLCNK

Sequence coverage without the signal peptide (MKTLLLTLMV TIMCLDLGYT): 93 %

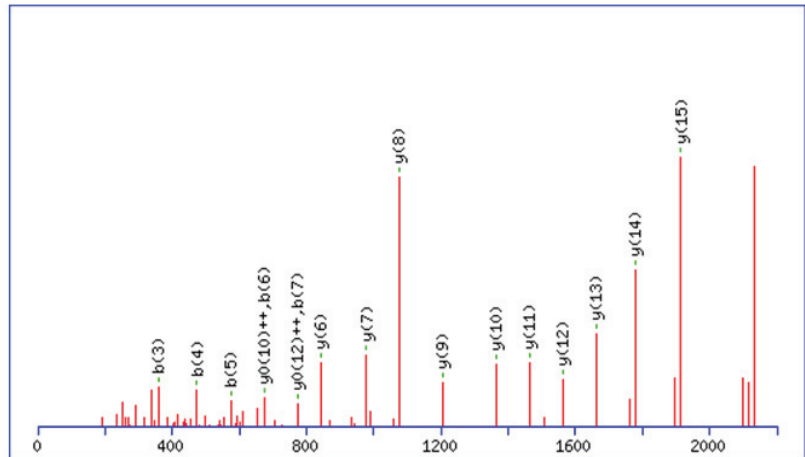
1 LTCYKGYHD TVVCKPHETI CYRYLVPATH GNAIPARGCG TSCPGGNHPV CCSTDLCNK

MS/MS fragmentation:

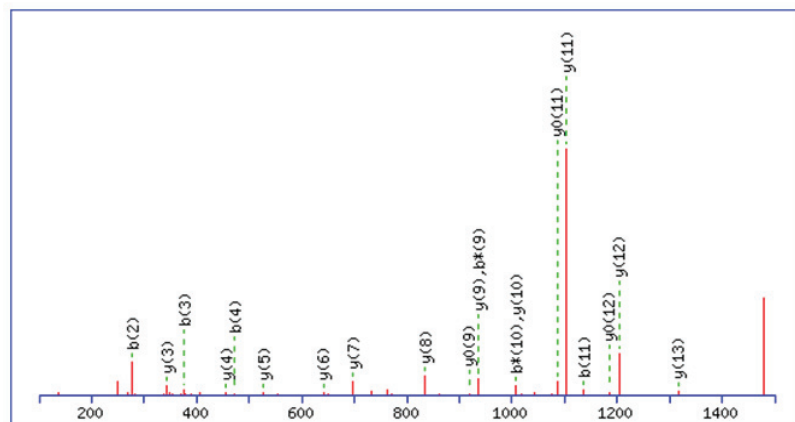
CGGTSCPGGNHPVCCSTDLCNK



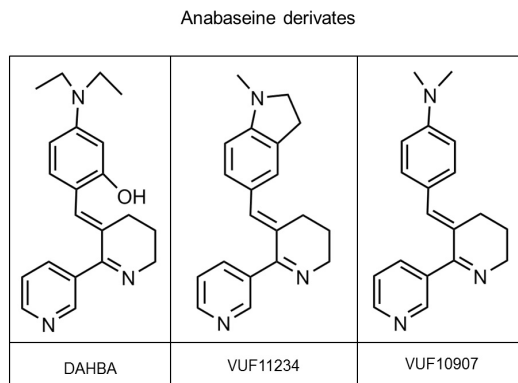
GYHDTVVCKPHETICYR



YLVPATHGNAIPAR

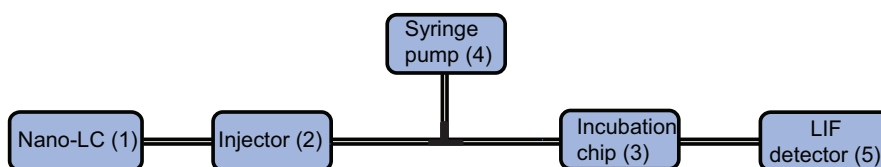


**Figure S2.** Mascot results from the tryptic digestion of the bioactive with  $m/z$  value of 1244.78 from *Pseudonaja affinis*.

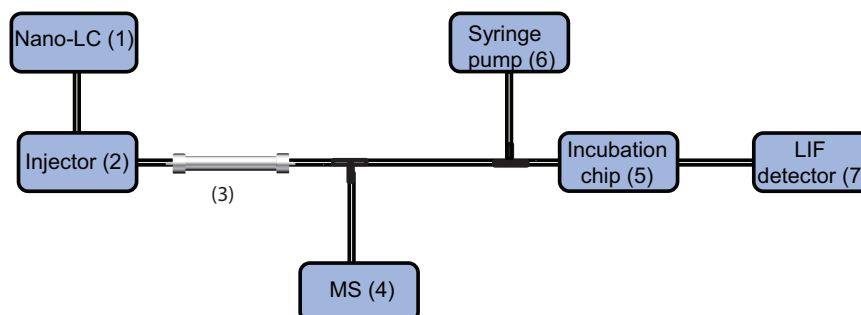


**Figure S3.** Structure of the three anabaseine derivatives tested in the study.

**A** Microfluidic on-line assay in nano-LC flow-injection mode



**B** Microfluidic on-line HRS



**Figure S4.** (A) Schematic view of the microfluidic on-line assay in nano-LC flow-injection mode. With a nano-LC system (1) 500 nL of samples are injected (2). The eluent flow is directed into a 4  $\mu$ l microfluidic incubation chip (3) where the sample is mixed with the bioassay mixture, infused in the chip by a syringe pump (4). The fluorescence signal was detected by an in-house built LED-induced fluorescence detector (5); (B) Schematic view of the microfluidic on-line HRS setup. 500 nL of samples are injected (2) and separated with nano-LC (1). After separation by the capillary column (3) the effluent flow was split in 1:1 ratio. One part of the flow was directed to a high resolution MS (4), and the other part of the flow was directed into a 4  $\mu$ l microfluidic incubation chip (5) where it was mixed with the bioassay mixture infused by a syringe pump (6). After incubation the fluorescence signal was detected by an in-house built LED-induced fluorescence detector (7).

**Table S1.** Comparison of Ki values measured for 14 5HT-3 ligands using the fluorescence enhancement plate reader assay and the radioligand binding assay.

Ligands	Ki fluorescence enhancement (A1B2D1R 5HTBP mutant) ( $\mu\text{M}$ )	Ki RLB (A1B2D1R 5HTBP mutant) ( $\mu\text{M}$ )
granisetron	0.24 $\pm$ 0.14	0.05 $\pm$ 0.01
serotonine	103.03 $\pm$ 32.01	218.27 $\pm$ 24.98
tropisetron	0.04 $\pm$ 0.72	0.01 $\pm$ 0
quipazine	72.98 $\pm$ 32.22	53.84 $\pm$ 13.74
VUF10166	11.54 $\pm$ 3.58	2.39 $\pm$ 0.58
RS56812	3.57 $\pm$ 0.91	1.6 $\pm$ 0.34
mirtazapine	12.82 $\pm$ 3.99	9.29 $\pm$ 1.87
SR57227	85.03 $\pm$ 7.62	39.74 $\pm$ 15
zacopride	4.05 $\pm$ 1.14	1.62 $\pm$ 0.57
iodophenpropit	1.44 $\pm$ 0.2	0.23 $\pm$ 0.06
B-HT920	3.85 $\pm$ 0.76	0.93 $\pm$ 0.24
RS 16566	3 $\pm$ 0.02	0.38 $\pm$ 0.09
5-fluorotryptamine HCL	110.35 $\pm$ 14.03	100.95 $\pm$ 50.19
palonosetron	2.2 $\pm$ 0.63	0.51 $\pm$ 0.11

**Table S2.** Mass of bioactives binding to 5THBP found in snake venoms.

Species	Most abundant $m/z$	Charge state	~Nominal mass (Da)
<i>Pseudonaja affinis</i>	1244.779	5	6218.86
<i>Pseudonaja affinis</i>	1303.993	5	6514.93
<i>Pseudonaja affinis</i>	918.745	4	3670.95
<i>Pseudonaja inframescula</i>	1244.779	5	6218.86
<i>Pseudonaja inframescula</i>	1260.397	5	6296.95
<i>Pseudonaja inframescula</i>	1306.287	6	7831.68
<i>Pseudonaja inframescula</i>	1338.006	5	6684.99
<i>Dendroapsis polylepis</i>	639.271	1	638.26
<i>Dendroapsis polylepis</i>	483.255	1	482.25
<i>Dendroapsis polylepis</i>	1312.864	5	6559.28
<i>Dendroapsis polylepis</i>	1362.613	5	6808.03
<i>Dendroapsis polylepis</i>	1202.608	6	7209.60