Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Expression of Recombinant Human Flavin Monooxygenase and Moclobemide N-oxide Synthesis on Multi-mg Scale

Steven P. Hanlon, Andrea Camattari, Sandra Abad, Anton Glieder, Matthias Kittelmann, Stephan Lütz, Beat Wirz, Margit Winkler*

Cultivation and pH optimization conditions page 2

HPLC chromatogram of Mocolobemide-N-oxide page 3

^{1}H NMR spectrum of Mocolobemide-N-oxide page 4

Standard cultivation protocol in shake flasks

10 mL pre- and a 500 mL LB-media culture incubated at 37°C and 90 rpm. Induction was performed at 30°C and was started after addition of IPTG (1 mM) as soon as the optical density at 600 nm (OD_{600}) of ~0.8 was reached. After 24 h of induction, the cells were harvested by centrifugation.

pH Influence on biooxidation of moclobemide

The influence of the pH on biooxidation of moclobemide was tested on the 10 mL scale. The reactions were carried out in 100 ml baffled flasks containing a total volume of 10 ml phosphate buffer (100 mM) supplemented with citrate/$NADP^+$ and moclobemide (100 mg/L) at 27°C and 200 rpm. At pH 8.5, 81% of moclobemide was converted to the *N*-oxide within 27 h reaction time (Fig. S1)

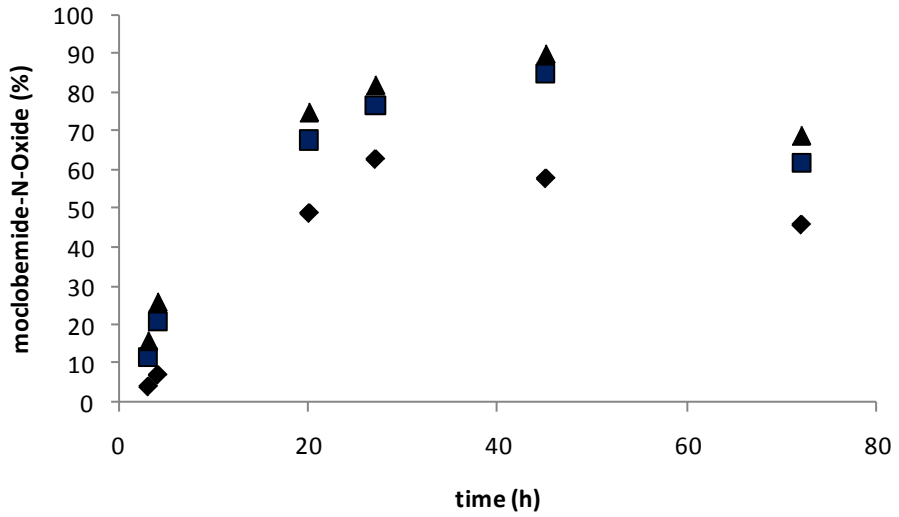
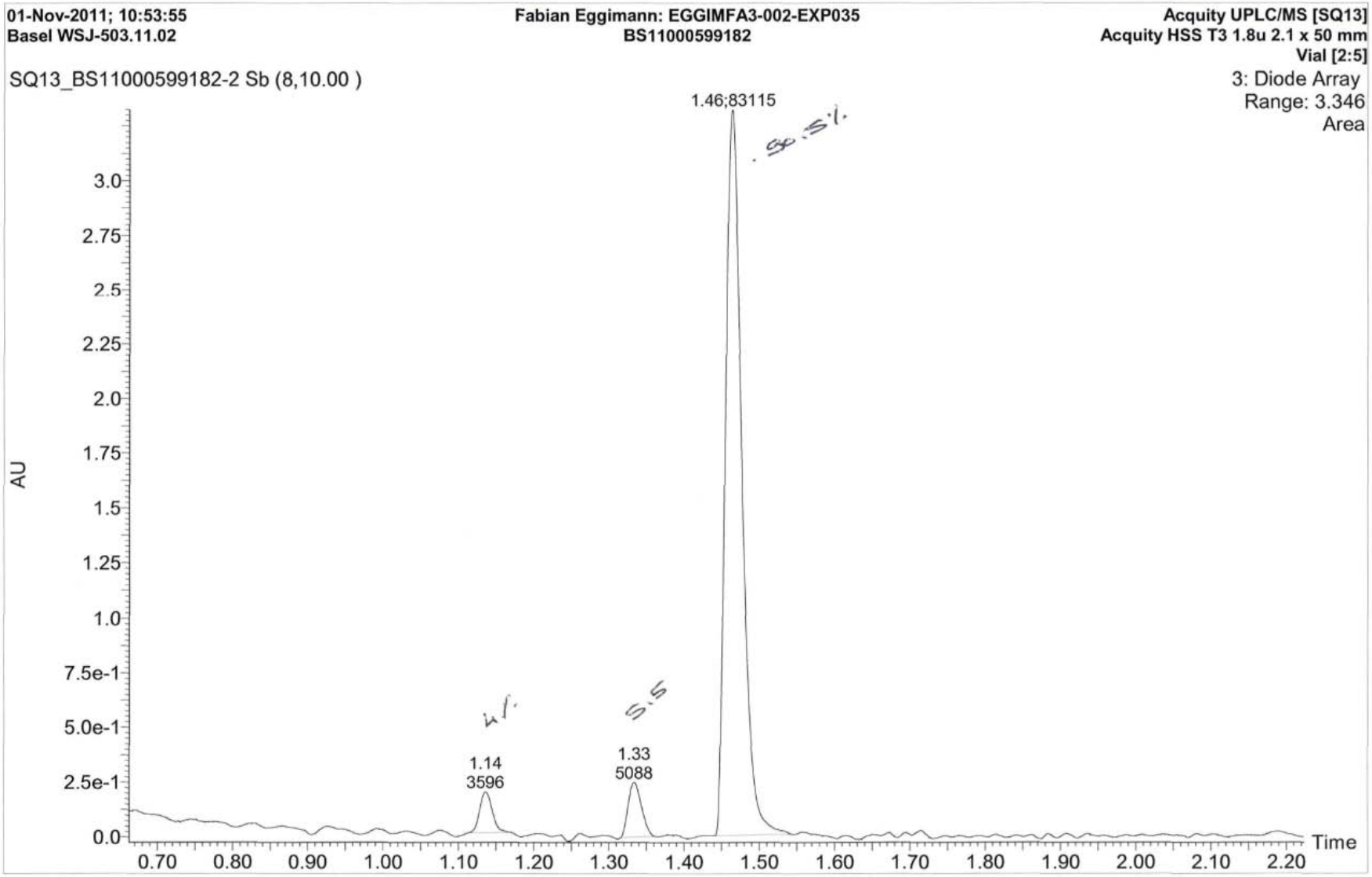


Fig S1. pH Optimization and time course of Moclobemide oxidation by recombinant hFMO3 on 10 mL scale. ♦: pH 7.5, ■: pH 8.0, ▲ pH 8.5



11/4/2011 10:59:50 AM

Acquisition Time (sec)	2.7329						
Comment	LABOR F.EGGIMANN SAMPLE EGGIMFA3-002-EXP035 LABJID EGGIMFA3-002-EXP035 ORDER BS11000617363 h_1h.aut DMSO u lochmth1 36						
Date	04 Nov 2011 05:43:56	*Date Stamp*	04 Nov 2011 05:43:56	*File Name*	C:\TEMP\03947267.DX		
Frequency (MHz)	600.13	*Nucleus*	1H	*Number of Transients*	16	*Origin*	Bruker BioSpin GmbH
Original Points Count	32768	*Owner*	sag	*Points Count*	32768	*SW(cyclical) (Hz)*	11990.04
Solvent	DMSO-d6	*Spectrum Offset (Hz)*	4793.6831	*Sweep Width (Hz)*	11989.68	*Temperature (degree C)*	29.000

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm 3.27 (d, *J*=11.71 Hz, 2 H) 3.50 (t, *J*=10.25 Hz, 2 H) 3.62 (t, *J*=4.80 Hz, 2 H) 3.76 (d, *J*=12.07 Hz, 2 H) 3.79 (br. s., 2 H) 4.10 (t, *J*=11.53 Hz, 2 H) 7.55 (d, *J*=8.30 Hz, 2 H) 7.82 (d, *J*=8.30 Hz, 2 H) 9.86 (br. s., 1 H)