Kinetic characterisation of the FAD dependent monooxygenase TropB reveals broad substrate selectivity and biotransformation potential

Amira Abood\textsuperscript{a,b}† Ahmed Al-Fahad,\textsuperscript{a,c}‡ Alan Scott,\textsuperscript{a} Alaa El-Dein M. Hosny,\textsuperscript{d} Amal M. Hashem,\textsuperscript{b} Azza M. A. Fattah,\textsuperscript{b} Paul R. Race,\textsuperscript{e} Thomas J. Simpson\textsuperscript{a} and Russell J. Cox\textsuperscript{a,f}*

a. School of Chemistry, Cantock’s Close, University of Bristol, BS8 1TS, UK.
b. Chemistry of Natural and Microbial Products Department, National Research Centre, Cairo, 12622, Egypt.
c. Chemistry Department, Faculty of Science, Al Baha University, Al Baha, 1988, Saudi Arabia.
d. Microbiology Department, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt.
e. School of Biochemistry, Medical Sciences Building, University of Bristol, BS8 1TD, UK.
f. Institut für Organische Chemie, Leibniz Universität Hannover, Schneiderberg 1B, 30167, Hannover, Germany.
† These authors contributed equally.

Supplementary Information

1. Purification of TropB

Figure S1. SDS-PAGE showing TropB after purification by Ni\textsuperscript{2+} affinity chromatography and S200 size exclusion. Lane 1 shows Mw markers (kDa) and lane 2 shows the purified protein (predicted molecular weight 52 kDa).
2. MALDI analysis of digested TropB

Matched peptides shown in bold red.

1 MGQGLEDQ QEMVQNVSP SIVTLLAS LIVATEGV FQKIVRKEI
21 GREEQAM MEGMEGGR DLEAEPGL VEEVGISGA EADYLYVYE
31 CMHEKGLQ MDGDOLQLE ACMDGFQLE CMTLPREG EQQQQIQTM
41 ERQHTKLK FAPGTPAR OQIVIQDGK SVRGQHNE DSPSHPHS
51 EADSFRLT MLALELHE KSKSTICKVQ GYIRMLHVF VAMENRVA
251 RASQNLTV RKREASLP HHNRHYGEM IEPNDEWA
301 MDTGLGRF PFTQGKGRY GFMARHFP HHNAGGGE DALKVILA
351 HSYLTSHK STVSNLMA AFGSTILVQ TRAQFIFES RRCQDTCQP
401 EADSFRLTV LKREASLP HHNRHYGEM IEPNDEWA

Figure S2. MALDI –TOF results. Detected peptides indicated in red.

3. LCMS analysis of substrate selectivity.

Figure S3. Analytical LC/MS chromatograms and picture of an in vitro assay of TropB with 1-nitroso-2,4-dihydroxy-3,6-dimethylbenzene 23 showing the increase of a new peak at Rt = 5.0 min having a molecular ion mass of m/z 182 [M-H]- and UV absorption λ_{max} 274 nm and the colour change upon the formation of hydroxylation product.
Figure S4. LC-DAD-MS analysis of TropB in vitro assay with 1-nitro-2,4-dihydroxy-3,6-dimethylbenzene.

Figure S5. LCMS analysis of TropB in vitro assay with 1-butanoyl-2,4-dihydroxy-3,6-dimethylbenzene showing the complete consumption of the substrate and formation of hydroxylation product after the addition of NADPH.
Figure S6. LC-DAD-MS analysis of TropB in vitro assay with ± 1-(2-methylbutanoyl)-2,4-dihydroxy-3,6-dimethylbenzene 18, demonstrating the conversion of only 16% of the substrate to oxidation product after 2 hours incubation.
Figure S7. LC-DAD-MS analysis of TropB in vitro assay with 1-acetyl-2,4-dihydroxy-3,6-dimethylbenzene showing the complete consumption of the substrate and the formation of hydroxylation product after 2 hours from the addition of NADPH.
Figure S8. LC-DAD-MS analysis of TropB *in vitro* assay with 1-(6-bromohexanoyl)-2,4-dihydroxy-3,6-dimethylbenzene 19.

Figure S9. LC-DAD-MS analysis of TropB *in vitro* assay with 1-propionyl-2,4-dihydroxy-3,6-dimethylbenzene 16 showing the complete consumption of the substrate and the formation of hydroxylation product after 2 hours from the addition of NADPH.
Figure S10. LC-DAD-MS analysis of TropB *in vitro* assay with 1-benzoyl-2,4-dihydroxy-3,6-dimethylbenzene 20 showing the formation of a trace of the hydroxylation product after 2 hours from the addition of NADPH.

Figure S11. LC-DAD-MS analysis of TropB *in vitro* assay with 1-(2,4-dimethoxybenzoyl)-2,4-dihydroxy-3,6-dimethylbenzene 21 showing the a trace of the hydroxylation product after 2 hours from the addition of NADPH.
4. Kinetic Data

**Figure S12.** Michaelis-Menten plot for 3-methylorcinaldehyde 1 with TropB.

**Figure S13.** Michaelis-Menten plot for 1-acetyl-2,4-dihydroxy-3,6-dimethylnitrene 15 with TropB.
**Figure S14.** Michaelis-Menten plot for 1-propionyl-2,4-dihydroxy-3,6-dimethylbenzene 16.

**Figure S15.** Michaelis-Menten plot for 1-butanoyl-2,4-dihydroxy-3,6-dimethylbenzene 17 with TropB
Figure S16. Michaelis-Menten plot for 1-nitro-2,4-dihydroxy-3,6-dimethylbenzene 22 with TropB.

Figure S17. Michaelis-Menten plot for 1-nitroso-2,4-dihydroxy-3,6-dimethylbenzene 23 with TropB.
Figure S18. $^1$H-$^{13}$C gc2HMBC spectrum of TropB hydroxylated product for 4-Nitroso-2,5-dimethylresorcinol (CD$_3$CN, 500 MHz).