

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

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Supplementary Information

1. Purification of TropB

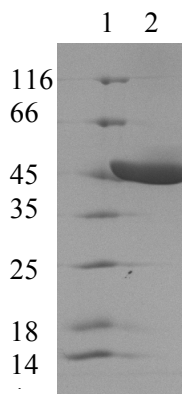


Figure S1. SDS-PAGE showing TropB after purification by Ni²⁺ affinity chromatography and S200 size exclusion. Lane 1 shows Mw markers (kDa) and lane 2 shows the purified protein (predicted molecular weight 52 kDa).

2. MALDI analysis of digested TropB

Matched peptides shown in **bold red**.

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1  MPGLSLIDTRQ  QPLSVGIVGG  GIIGVILAAG  LVRRGIDVKV  FEQARGFREI
51  GAGMPTANA  VRCMELDPA  IVWALRS8GA  VPISIGDHQA  EARDYLRWVD
101 GYHSSKRLY  QLDAGIRGFE  ACREDQFLEA  LVKVLPEGIV  ECQKRLQKIH
151 EKNETEAVTL  EFADGTFAMV  DCVIGADGIR  SRVRQHLFGE  DSPYSHPHY3
201 HKFAPRGLIT  MENAISALGE  DKARTLNGHV  GPNALHIHYP  VANETMWNIA
251 AFVSDPEEWP  DKLSLVGPAT  REEAMGYFAN  WNPGLRAVLG  FMPENIDRWA
301 MFDTYDYFAP  FFSRGKICLV  GDAAHAAVPH  HGAGACIGIE  DALCATVLLA
351 EVFVSTRGKS  SIVNRAIAA  AFGSENAVNR  VRAQWFVDS3  RRVCDLYQQP
401 EWADPQRIK  AENCFFEEKD  RSHKIWHFDY  NSMLQEAIK  YRHMMS
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Figure S2. MALDI –TOF results. Detected peptides indicated in red.

3. LCMS analysis of substrate selectivity.

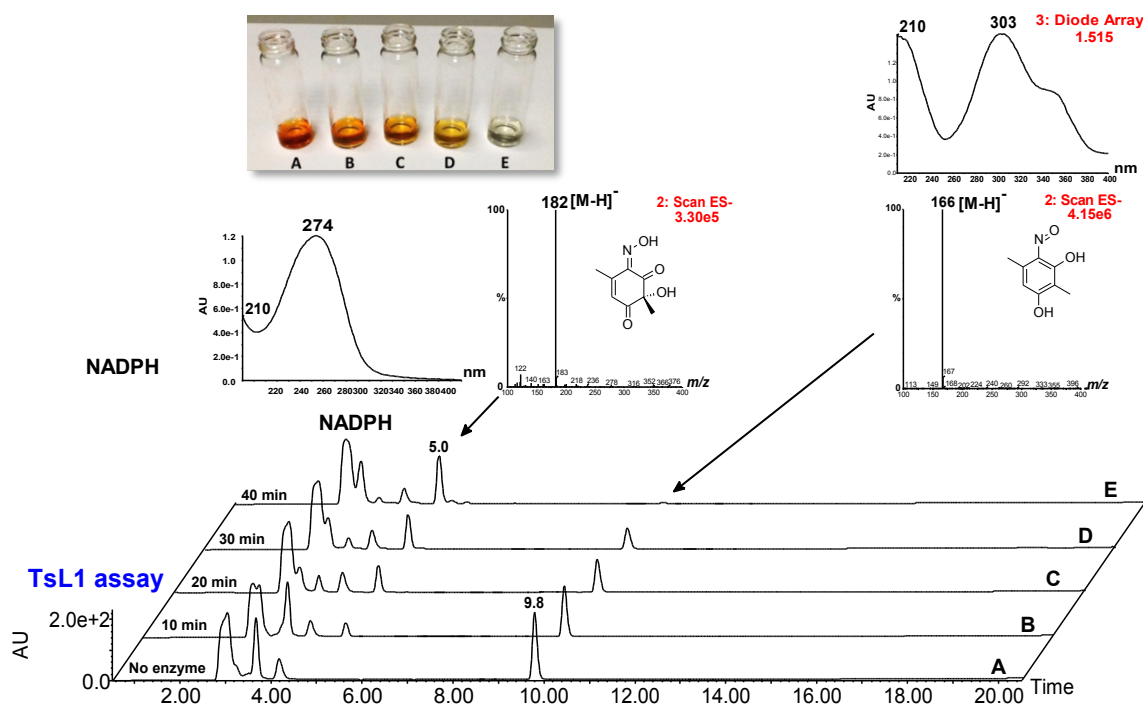


Figure S3. Analytical LC/MS chromatograms and picture of an *in vitro* assay of TropB with 1-nitroso-2,4-dihydroxy-3,6-dimethylbenzene **23** showing the increase of a new peak at $R_t = 5.0$ min having a molecular ion mass of m/z 182 [M-H]⁻ and UV absorption λ_{\max} 274 nm and the colour change upon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

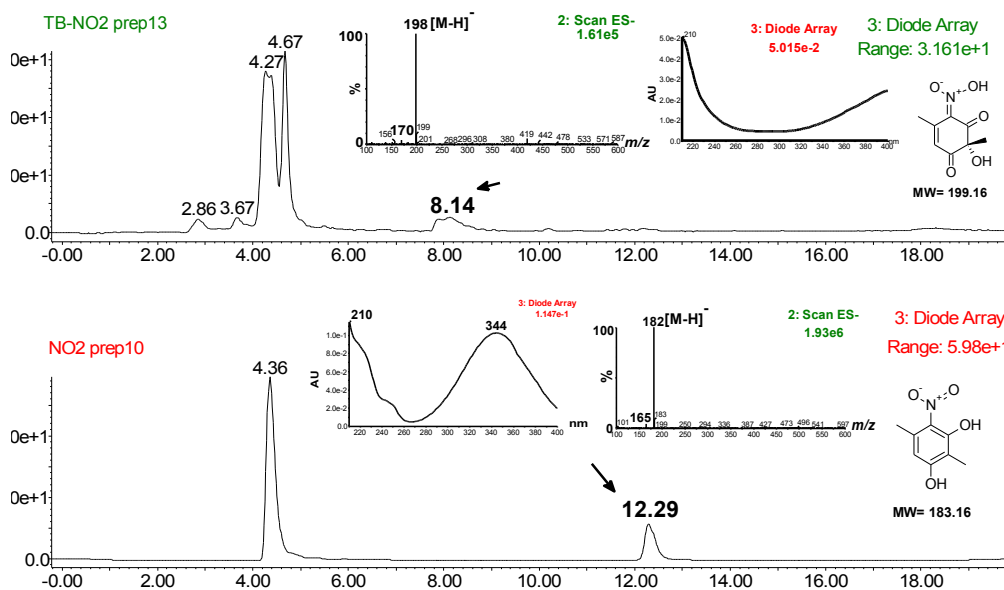


Figure S4. LC-DAD-MS analysis of TropB *in vitro* assay with 1-nitro-2,4-dihydroxy-3,6-dimethylbenzene **22**.

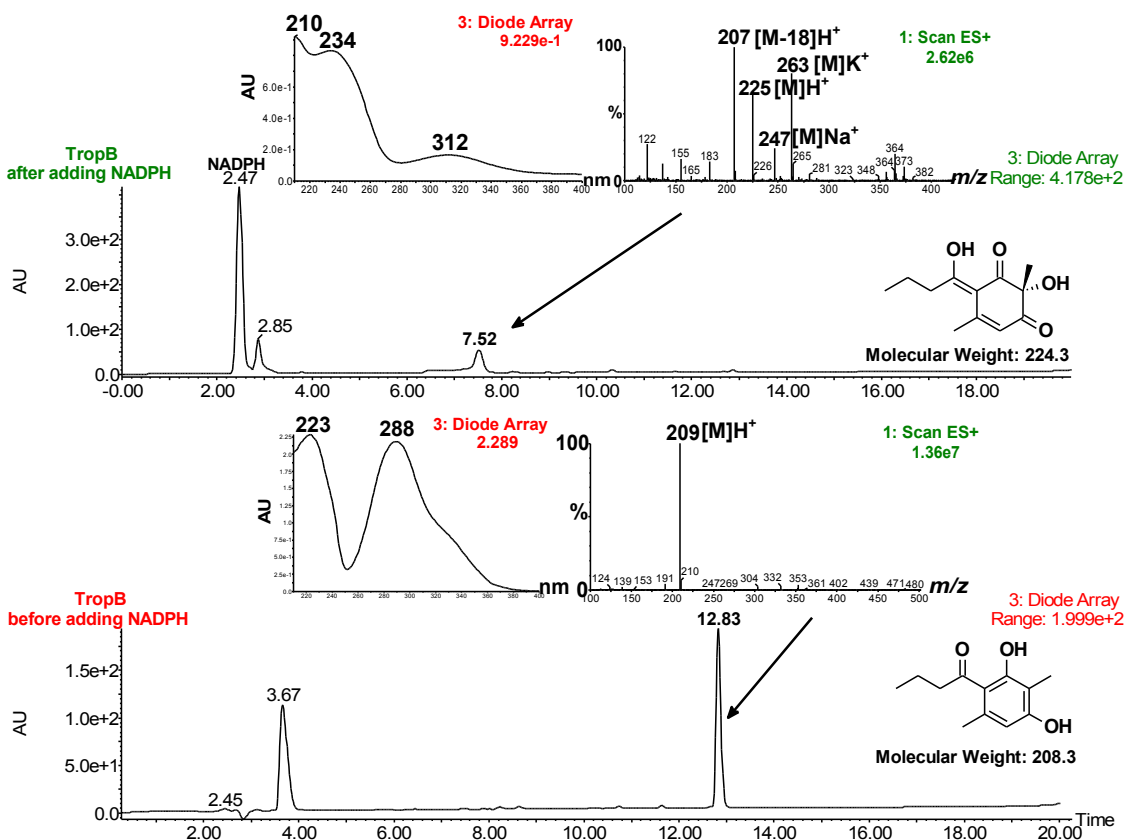


Figure S5. LCMS analysis of TropB *in vitro* assay with 1-butanoyl-2,4-dihydroxy-3,6-dimethylbenzene **17** showing the complete consumption of the substrate and formation of hydroxylation product after the addition of NADPH.

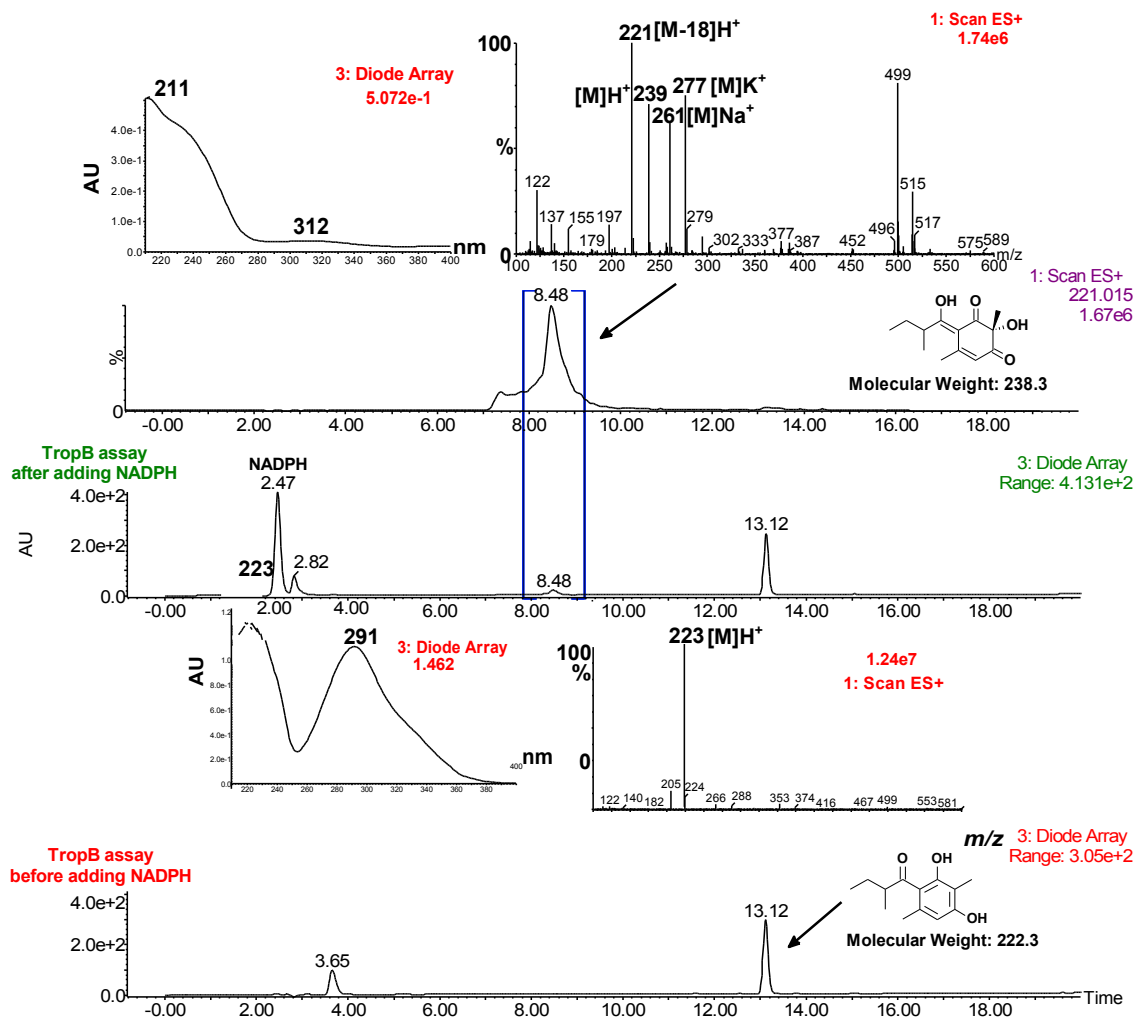


Figure S6. LC-DAD-MS analysis of TropB *in vitro* assay with \pm 1-(2-methylbutanoyl)-2,4-dihydroxy-3,6-dimethylbenzene **18**, demonstrating the conversion of only 16% of the substrate to oxidation product after 2 hours incubation.

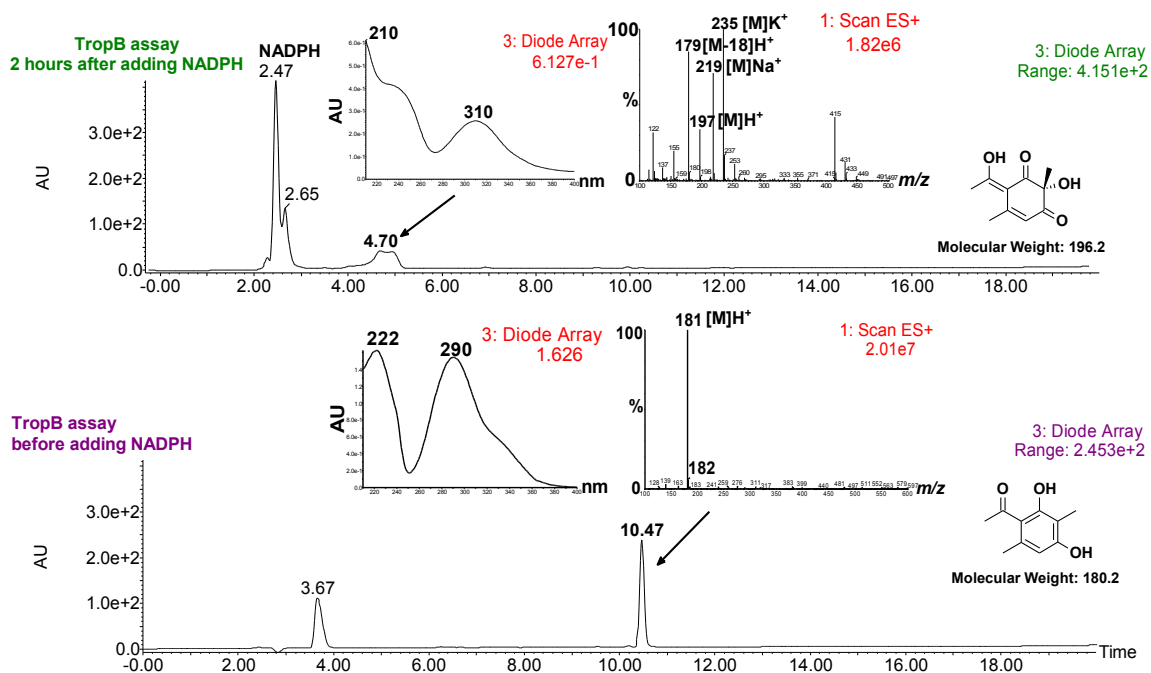


Figure S7. LC-DAD-MS analysis of TropB *in vitro* assay with 1-acetyl-2,4-dihydroxy-3,6-dimethylbenzene **15** showing the complete consumption of the substrate and the formation of hydroxylation product after 2 hours from the addition of NADPH.

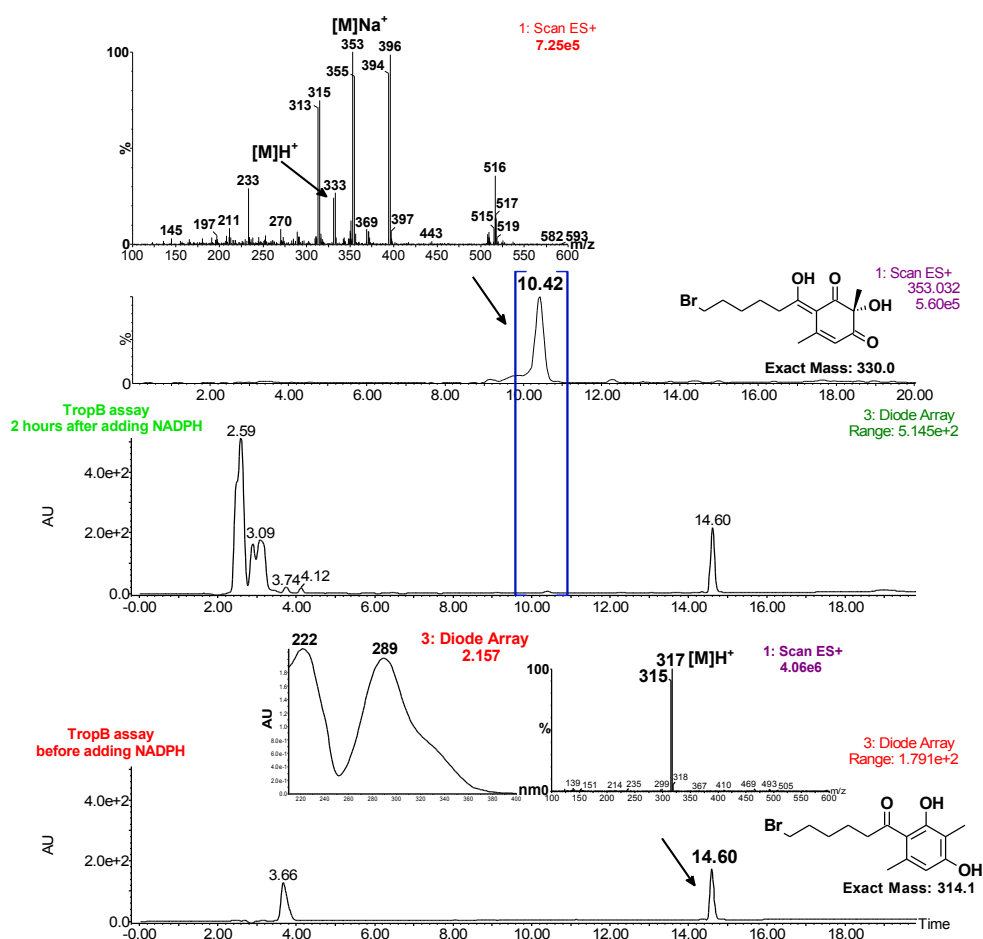


Figure S8. LC-DAD-MS analysis of TropB *in vitro* assay with 1-(6-bromohexanoyl)-2,4-dihydroxy-3,6-dimethylbenzene **19**.

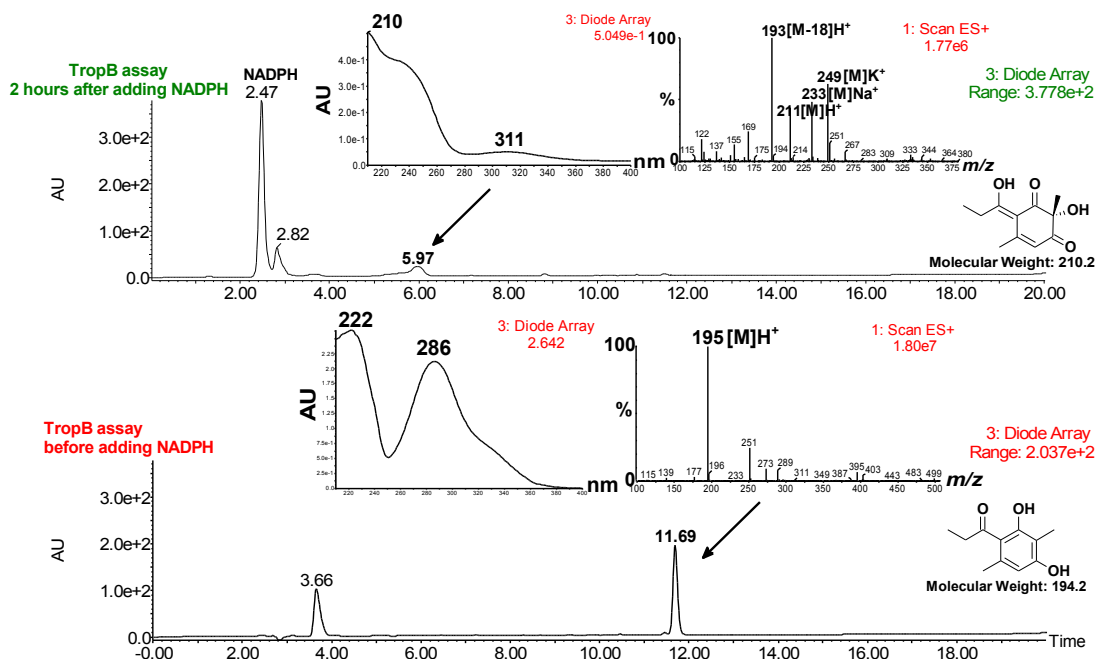


Figure S9. LC-DAD-MS analysis of TropB *in vitro* assay with 1-propionyl-2,4-dihydroxy-3,6-dimethylbenzene **16** showing the complete consumption of the substrate and the formation of hydroxylation product after 2 hours from the addition of NADPH.

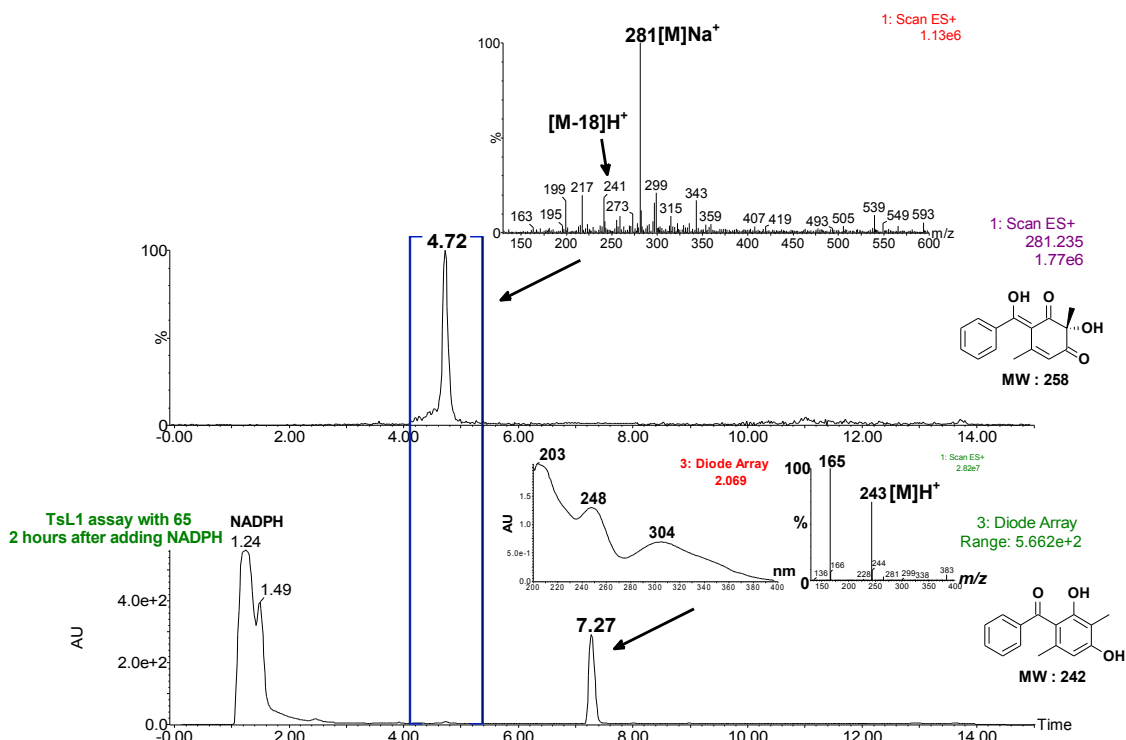


Figure S10. LC-DAD-MS analysis of TropB *in vitro* assay with 1-benzoyl-2,4-dihydroxy-3,6-dimethylbenzene **20** showing the formation of a trace of the hydroxylation product after 2 hours from the addition of NADPH.

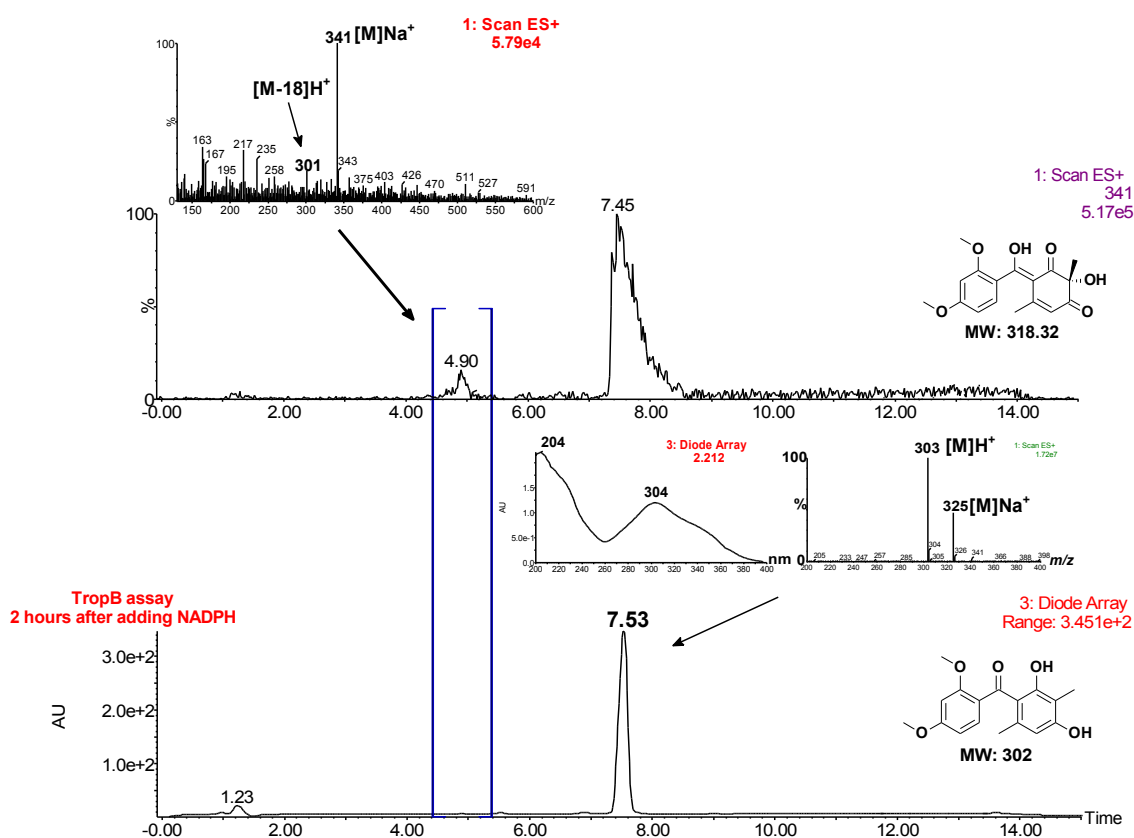


Figure S11. LC-DAD-MS analysis of TropB *in vitro* assay with 1-(2,4-dimethoxybenzoyl)-2,4-dihydroxy-3,6-dimethylbenzene **21** showing the a trace of the hydroxylation product after 2 hours from the addition of NADPH.

4. Kinetic Data

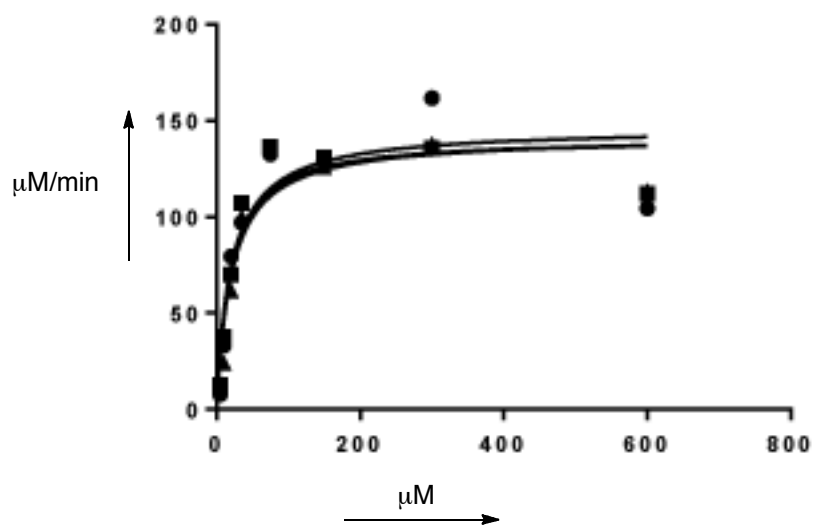


Figure S12. Michaelis-Menten plot for 3-methylorcinaldehyde **1** with TropB.

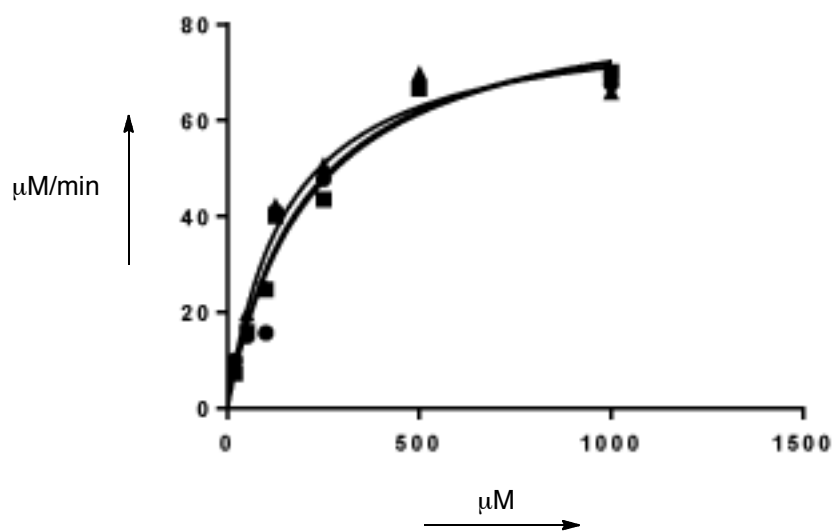


Figure S13. Michaelis-Menten plot for 1-acetyl-2,4-dihydroxy-3,6-dimethylbenzene **15** with TropB.

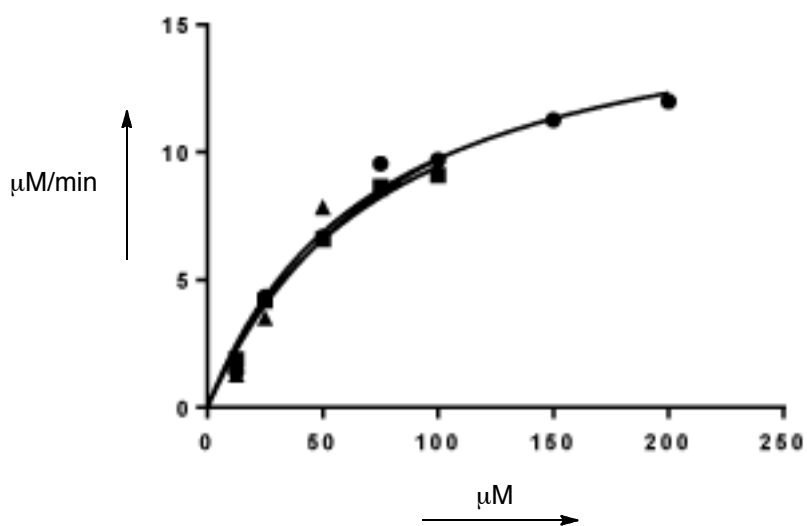


Figure S14. Michaelis-Menten plot for 1-propionyl-2,4-dihydroxy-3,6-dimethylbenzene **16**.

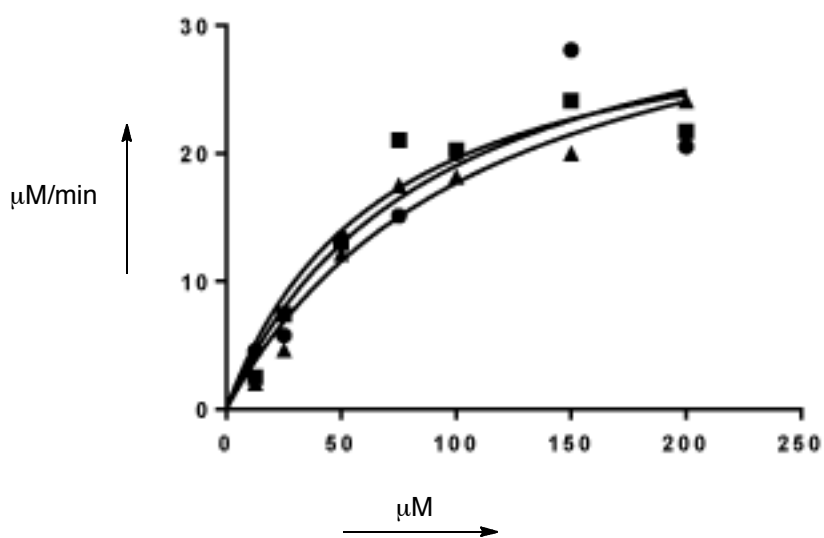


Figure S15. Michaelis-Menten plot for 1-butanoyl-2,4-dihydroxy-3,6-dimethylbenzene **17** with TropB

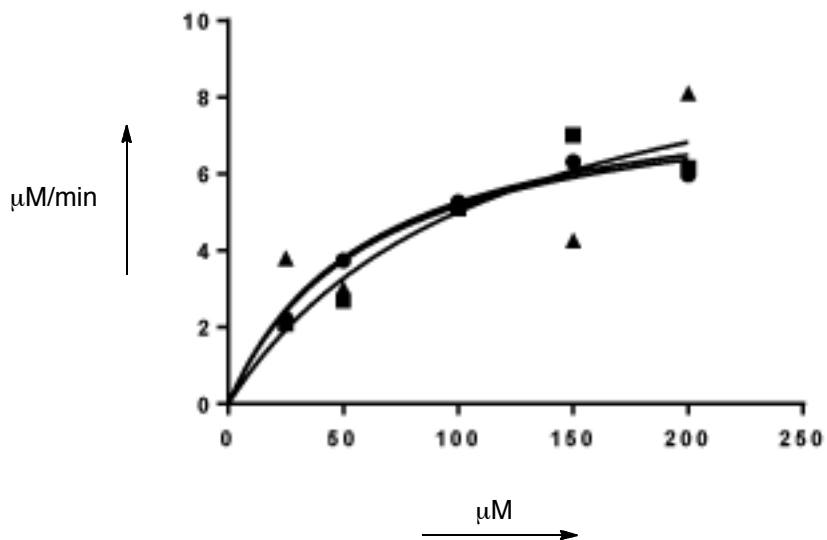


Figure S16. Michaelis-Menten plot for 1-nitro-2,4-dihydroxy-3,6-dimethylbenzene **22** with TropB.

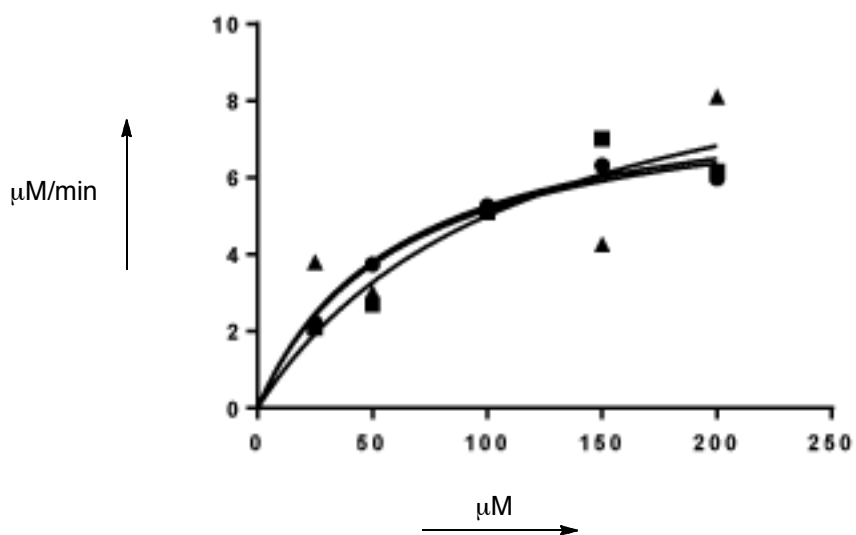


Figure S17. Michaelis-Menten plot for 1-nitroso-2,4-dihydroxy-3,6-dimethylbenzene **23** with TropB.

5. 2D NMR data for 28.

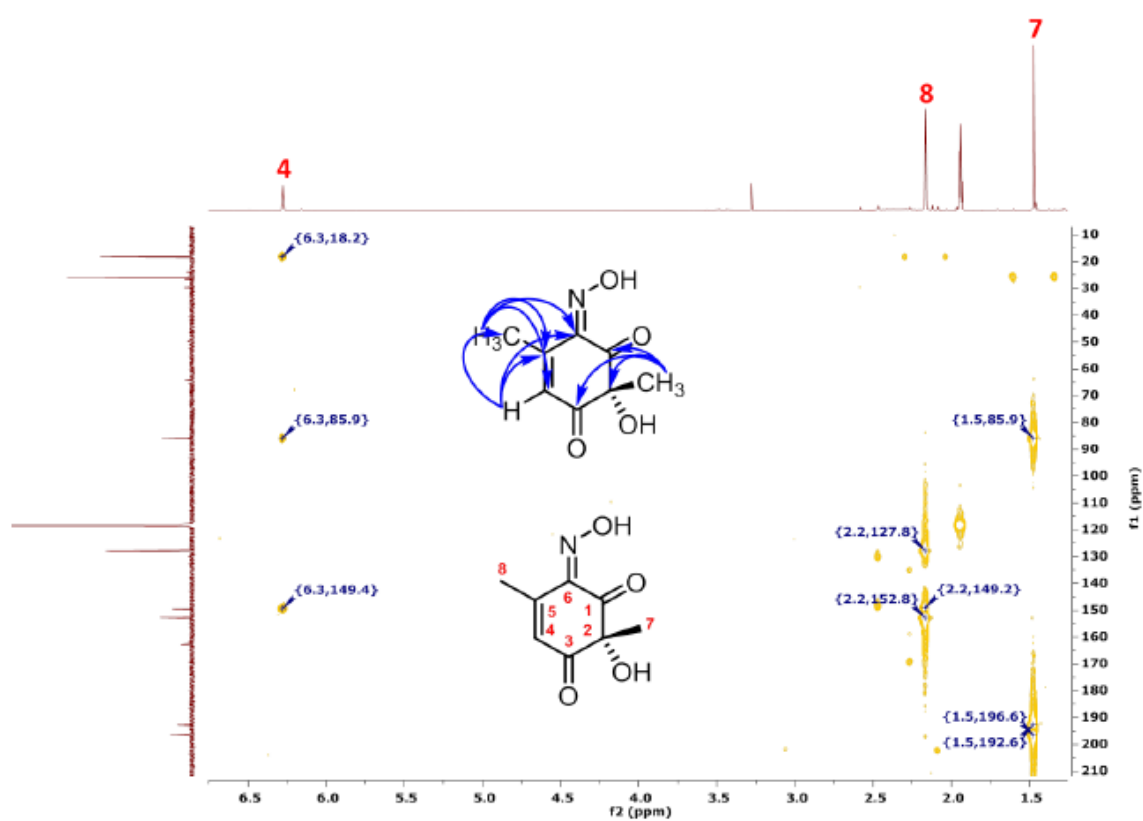


Figure S18. ^1H - ^{13}C gc2HMBC spectrum of TropB hydroxylated product for 4-Nitroso-2,5-dimethylresorcinol (CD_3CN , 500 MHz).