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Hydroxylation of anilides by engineered cytochrome P450_{BM3}Jack A. O'Hanlon,^{a,§} Xinkun Ren,^{b,§} Melloney Morris,^c Luet Lok Wong,^{b,*} and Jeremy Robertson^{a,*}Received 00th January 20xx,
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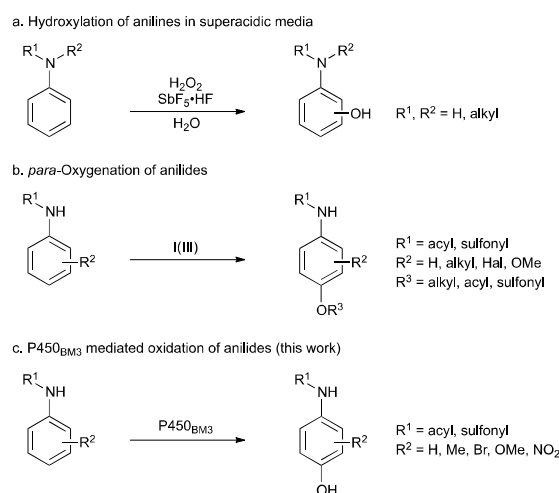
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Biocatalytic direct monohydroxylation of anilides has been achieved on preparative scale using mutant cytochrome P450_{BM3} enzymes. Representative mono- and disubstituted *N*-trifluoromethanesulfonyl anilides are shown to be converted in most cases to the corresponding 4-hydroxy derivatives, with substituent hydroxylation also occurring in two cases. By mutation variation, it is possible to achieve selective hydroxylation of either ring- or side-chain sites.

Introduction

Aminophenols are ubiquitous either in their own right or as synthetic intermediates within natural product chemistry, pharmaceuticals, and materials with specific physical and optical properties. Their synthesis is usually achieved by nitration of phenols or their *O*-alkyl ethers, then reduction either immediately or following elaboration of the molecule elsewhere.¹ Milder equivalent procedures² and transition-metal catalysed *N*-arylation methods have been introduced more recently.³

Examples of the opposite approach to aminophenols—that is, by ring-hydroxylation of aniline derivatives—include: (1) the treatment of free anilines with H₂O₂ in superacidic media to give mainly the *meta*-hydroxylated product (Scheme 1a);⁴ (2) application of peroxydisulfate salts under alkaline conditions to give the *ortho*- and *para*- regioisomers (Boyland–Sims reaction);⁵ and (3) *para*-acetoxylation, etherification, or sulfonyloxylation of anilides with hypervalent iodine reagents (Scheme 1b).⁶ Such procedures, and oxidation chemistries in general, have limited relevance in industrial applications because on production scale energetic reagents may be unacceptably hazardous, require constraining technologies to operate safely, and may create excessive quantities of toxic waste. The development of *catalytic* oxidation systems that harness aerial oxygen as the stoichiometric reagent would lead to safer, potentially sustainable and scaleable methods.⁷ In this context, biocatalysts are likely to become increasingly important because they operate under mild conditions and



Scheme 1 Representative direct oxy-functionalisation of anilines and anilides.

their activity and selectivity is tunable by enzyme engineering.

Cytochrome P450 monooxygenases are able to effect arene hydroxylation in addition to other reaction modes such as aliphatic C–H bond oxidation, alkene epoxidation, and heteroatom dealkylation.⁸ Of these, the P450_{BM3} (CYP102A1) from *Bacillus megaterium* is a catalytically self-sufficient, highly active fatty acid hydroxylase which has been extensively studied for applications in synthesis.⁹ The application of rational design and high-throughput screening has identified mutants with high activity and selectivity towards non-natural substrates.¹⁰ Combinations of mutations at two or more active site residues generate libraries of P450_{BM3} mutants with varied substrate pocket topologies that can be used to explore the scope for oxidation of organic molecules including drugs, fine chemicals, and their precursors. We report here the application of a library of P450_{BM3} mutants for the synthesis of 4-aminophenols by oxidation of anilides (Scheme 1c).¹¹ The library members contain combinations of mutations at the active site residues Val78, Phe81, Ala82, Phe87, Ala184, Ile263, Glu267, Ala328, Ala330, and Leu437 (Fig. 1).

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Electronic Supplementary Information (ESI) available: General experimental details; tables of product distributions during screening; copies of NMR spectra for the hydroxylated products. See DOI: 10.1039/x0xx00000x

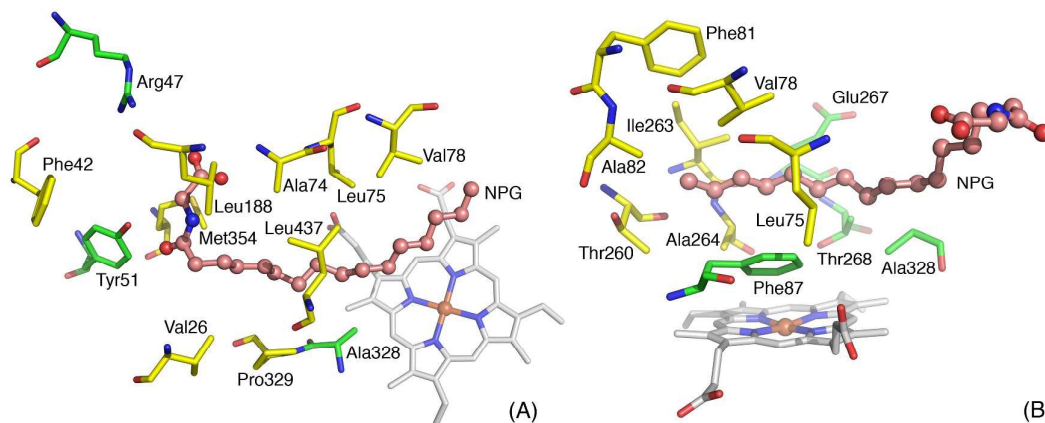


Fig. 1 The active site structure (PDB code: 1JP2) in wild-type P450_{BM3} with *N*-palmitoylglycine (NPG, in pink) bound, viewed from above (A) and from the side of (B) the haem, showing residues that contact the NPG substrate and targeted for site-directed mutagenesis.

Results and discussion

The oxidation of aniline to 4-aminophenol by wild-type (WT) P450_{BM3} has been reported but the conversion was low and 72% of the NADPH consumed did not contribute to product formation (uncoupling).¹² Furthermore, screening of aniline against a 48-mutant P450_{BM3} library (Table, page S5 of Supplementary Information)¹³ showed poor conversion with activity confined to a limited range of mutants. Subsequently, four attenuated aniline derivatives (**1–4**, Table 1) were screened for activity with the 48-mutant library. The *N*-acetyl derivative **1** was a poor substrate for the library, with all but one mutant giving <2% conversion to the 4-hydroxylated product [identified by comparison (GC) with an authentic standard]. Improved results were obtained for the *N*-Boc derivative **2**, but cleavage of the Boc group occurred to varying degrees. Trifluoroacetanilide (**3**) showed good conversion (up to 100%) to the 4-hydroxylated product **7**, along with traces of secondary oxidation products. Excellent conversion to the *para*-hydroxyanilide was also found with the sulfonamide **4**. Reactions of this substrate were cleaner and more selective for the *para*-hydroxylated product than those for the amide **3**; therefore, the trifluoromethanesulfonyl (triflyl) group was chosen for a study of the scope of this transformation.

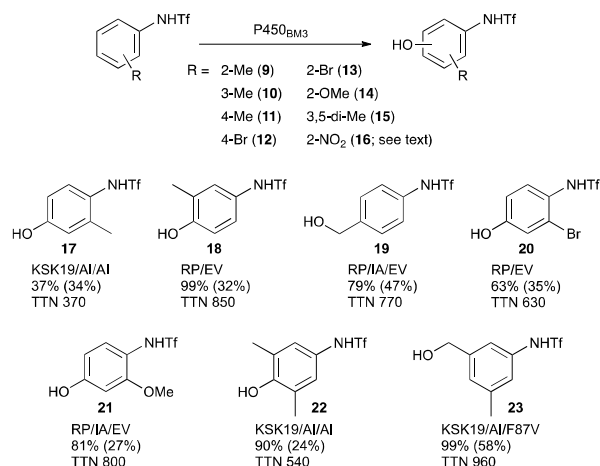
The substrates (**9–16**, Scheme 2) were readily prepared except for *N*-trifluoromethanesulfonyl-2-nitroaniline (**16**) which was found to be unstable so the corresponding *N*-methanesulfonyl derivative was used instead. Screening reactions were carried out as described in the Supplementary Information at ambient temperature for 20 hours. Conversions were assayed by GC analysis of ethyl acetate extracts of each reaction, and the products were identified following scale-up of promising reactions to provide sufficient material for analysis by NMR spectroscopy.

Table 1 Initial screening of *N*-protected anilines

R	Mutant ^a	Conversion ^b	Product
Ac (1)	KSK19/Al/Al	17%	4-OH (5) ^c
Boc (2)	RP/FV/EV/L437LV	66%	Mono-[O] (6)
COCF ₃ (3)	KSK19/Al/Al	100%	4-OH (7)
SO ₂ CF ₃ (4)	RP/FV/EV/L437LV	100%	4-OH (8)

^a 0.1 mol% loading with respect to **1–4**; ^b estimated by GC; ^c compound **5** was generated along with an unidentified product (GC ratio 16:84, respectively).

Methyl-substituted anilides **9–11** were accepted as substrates, and their reactions on an analytical screening scale proceeded with good conversions. 2-Methyl derivative **9** displayed up to 100% conversion, with total selectivity for the *para*-hydroxylation product **17** achievable with the R47L/Y51F/I401P/I263A/E267V mutant (RP/IA/EV, TTN = 1,000; Table S5). More generally, selectivity with this substrate was good, although de-triflation occurred to varying extents. For the 3-methyl derivative **10**, high activities were observed with seven mutants, most of which contain the RP/EV combination; during screening, RP/EV itself afforded 99% conversion with 85% selectivity for the *para*-phenol **18** (Table S6). Only three mutants showed >50% conversion of the 4-methylanilide **11**, and these gave benzylic oxidation rather than ring hydroxylation, with up to 79% conversion and 98% selectivity for the benzylic alcohol **19** being observed for the RP/IA/EV mutant (Table S7). By contrast, no benzylic oxidation was detected for the 2- and 3-methyl anilides **9** and **10**, respectively.

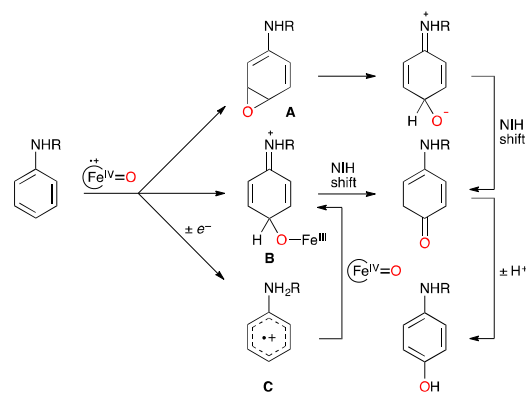


Scheme 2 Results for preparative-scale hydroxylation reactions. For each product (**17–23**) the P450_{BM3} mutant code is followed by the percentage conversion, the isolated yield in parentheses, and the total turnover number (TTN).

Mechanistically, P450-catalysed arene hydroxylation is accepted to occur via an NIH shift¹⁴ within an iminium intermediate to give a dienone that re-aromatises to the phenol (Scheme 3). The iminium ion may be formed by ring-opening of an arene oxide (**A**), or an equivalent Fe^{III}-bound species (**B**) may be formed via either direct electrophilic attack or following a preliminary electron transfer step (\rightarrow **C**), as shown.¹⁵

Within this mechanistic framework, the steric and electronic attributes of the ring substituents influence the product regioselectivity. For example, for the methyl-substituted substrate **9** the two substituents could, in principle, promote the formation of different aminophenol regioisomers. The almost exclusive formation of the 4-phenol (**17**) is consistent with both a stronger directing effect of the nitrogen substituent, and substrate-binding orientations that locate the ring C4 closer to the ferryl oxygen. With substrate **10**, both the nitrogen and the 3-methyl groups activate the 2- and 4-positions cooperatively. The almost exclusive formation of the 4-phenol with the P450 library suggests that the 3-methylanilide **10** is bound in orientations that place the *meta*- and especially *para*-carbons close to the ferryl oxygen. Finally, for the 4-methylanilide **11**, in addition to the benzylic hydroxylation (\rightarrow **19**) observed for all mutants, there is the possibility of oxidation at the 4-position then [1,2]-methyl group migration which would lead to the 3-methyl-4-hydroxy product **18**. The absence of this rearranged product (from **11**) supports a binding orientation in which the 4-methyl substituent is much closer to the ferryl oxygen than the ring C4.

Anilides bearing ring substituents with varied electronic characteristics were also examined as substrates. During screening (Table S8), the 2-bromotriflamide **13** showed poor turnover and selectivity with most mutants; RP/IA/IV was an exception, showing excellent conversion (100%) and some selectivity (44%) towards the 4-hydroxylation product **20**. With bromine incorporated at the 4-position (**12**) no conversion was



Scheme 3 Mechanism of arene hydroxylation by P450 enzymes.

detected, which can be attributed to the bromine atom being sited close to the haem iron (*cf.* binding of substrate **11**, above).

The 2-methoxy derivative **14** afforded three products (Table S9), the major (**21**) arising from *para*-hydroxylation (RP/IA/IV, 99% selectivity at 81% conversion), while *meta*-hydroxylation or further oxidation to the quinone imine were also observed with other mutants. The *N*-methanesulfonyl analogue of 2-nitroanilide **16** was not converted with the majority of mutants (Table S11); although up to 35% conversion was observed with the RT2/AP/IA01M mutant, attempts to scale-up this reaction resulted mainly in recovery of starting material, with none of the minor metabolites isolated for characterisation.

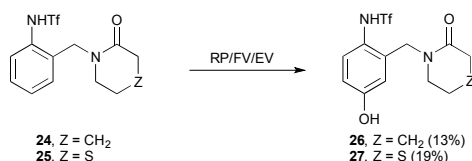
Reactions of the 3,5-dimethyl derivative **15** provide some insight into the activity traits of the P450_{BM3} mutants. The mutant library gave good selectivity and high conversion towards either *para*-hydroxylation (**22**, 100% conversion, 85% selectivity with RP/IA/IV) or benzylic oxidation (**23**, 99% conversion, 96% selectivity with KSK19/Al/F87V). Interestingly, the WT enzyme showed some activity (7% conversion) and gave a 29:71 ratio of *para*- vs. benzylic hydroxylation products (Table S10). Some variants, lacking mutations in the close vicinity of the haem (e.g. RP, RT2/IP, and RP/IV), achieved higher conversions; however, deviations from the WT product distribution were minor except for the RT2 mutant which gave an increased selectivity (89%) for benzylic oxidation. The increase in selectivity for benzylic hydroxylation from 44% to 96% upon changing the F87A mutation (in RLYF/KSK19/Al) for F87V (in KSK19/Al/F87V) exemplifies the importance of mutations at residues close to the haem centre. *para*-Hydroxylation is favoured by the mutation A330P, which constricts space above the haem,^{10c} and by the I263A mutation, which creates space further away from the haem (RP/IV: 29% *para*-hydroxylation vs. 89% for RP/IA/IV).

Compounds **24** and **25** (Scheme 4) are herbicides first patented by Nissan Chemical Industries¹⁶ that share the motif of a trifluoromethanesulfonanilide linked to a lactam *via* a 2-methylene bridge. *N*-(Hydroxybenzyl)piperidinone derivative **26** has been postulated to be an active form, with **24** being a pro-herbicide. P450-mediated oxidation of substrates **24** and

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25 with the mutant RP/FV/EV gave fair conversion to a major *para*-hydroxylated product in each case (37% and 83%, respectively). Reaction of the thiomorpholinone substrate **25** was selective for *para*-hydroxylation, with formation of the sulfoxide also observed (75:25 hydroxylation/*S*-oxidation).



Scheme 4 Preparative-scale hydroxylation reactions of herbicidal *N*-sulfonyl-anilides **24** and **25**.

The direct formation of *para*-hydroxylation products **26** and **27** provided analytical standard samples of these potential biological metabolites without the need to conduct *de novo* syntheses, and it was shown that compound **26** was inactive.

Conclusions and prospects

For the preparation of pharmaceuticals, agrochemicals, and their metabolites, late-stage P450-mediated biocatalytic hydroxylation has the significant advantage that the enzymes can be tuned to achieve specified oxidation outcomes, offering access to a variety of analogues and derivatives from a single advanced intermediate. Furthermore, since reactive hydroxyl functionality is not present until towards the end of the synthesis, OH protecting group regimes are dispensed with and alternative synthetic strategies may be considered.

In this work, hydroxylation of anilines has been demonstrated on both screening- and preparative scales in synthetically useful yield following deactivation of the aniline nitrogen by trifluoromethanesulfonylation. The need to protect the nitrogen in this way is at first sight a drawback but, projecting to wider applications, the *N*-sulfonyl substituent facilitates *N*-alkylation and may be readily removed if the free aniline is required.¹⁷

The production of aminophenols by hydroxylation of anilides exemplifies our groups' joint development of streamlined syntheses of target molecules of interest, and results with terpenoid and heterocyclic substrates will be reported in due course.

Experimental

N-(4-Hydroxyphenyl)-2,2,2-trifluoroacetamide **7**¹⁸

To a solution of anilide **3** (0.79 mL of a 100 mM solution in isopropanol, 0.079 mmol), glucose (4.0 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 4.0 mmol), GDH (198 μ L of a 2.0 U/ μ L solution in 100 mM phosphate buffer, pH 7.5, 396 U), and P450_{BM3} mutant KSK19/AI/AI (1.5 mL of a 50 μ M solution in phosphate buffer, 0.075 μ mol) in 100 mM phosphate buffer (pH 7.5, 30 mL) was added NADP⁺ monosodium salt (198 μ L of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.79 μ mol). The solution was stirred open to air for 19 h. Ethyl acetate was

added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo* to a brown solid. The crude product was purified by flash chromatography (30% ether/petrol to 50% ether/petrol) to afford the title compound (**7**) as a colourless oil (8.0 mg, 51%). *R*_f 0.60 (70% ethyl acetate/petrol); ν_{max} (film)/cm⁻¹ 3315 (br, m), 1693 (s), 1355 (m), 1515 (m), 1188 (s), 834 (m), 698 (w); ¹H NMR (500 MHz, (CD₃)₂CO) δ = 10.05 (1H, br s, NH), 8.48 (1H, s, OH), 7.55–7.52 (2H, m, H1, H5), 6.88–6.85 (2H, m, H2, H4); ¹³C NMR (126 MHz, (CD₃)₂CO) δ = 156.3 (C3), 155.4 (q, *J* 36.5 Hz, C(O)CF₃), 129.3 (C6), 123.5 (C1, C5), 117.2 (q, *J* 287.5 Hz, CF₃), 116.4 (C2, C4); LRMS (ES⁻) *m/z* = 204.0 [M–H]⁻.

N-(4-Hydroxyphenyl)-1,1,1-trifluoromethanesulfonamide **8**¹⁹

To a solution of anilide **4** (670 μ L of a 100 mM solution in methanol, 0.067 mmol), glucose (3.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 3.3 mmol), GDH (165 μ L of a 2.0 U/ μ L solution in 100 mM phosphate buffer, pH 7.5, 330 U) and NADP⁺ monosodium salt (165 μ L of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.66 μ mol), in 100 mM phosphate buffer (pH 7.5, 25 mL) was added P450_{BM3} mutant RP/FV/EV/L437LV (3.3 mL of a 20 μ M solution in phosphate buffer, 0.066 μ mol). The solution was stirred open to air for 20 h. Methanol (5.0 mL) was added and the solution was centrifuged at 9,500 g and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (30% ether/petrol to 60% ether/petrol) to afford the title compound (**8**) as a colourless oil (9.0 mg, 56% at 100% conversion of **4**). *R*_f 0.20 (30% ethyl acetate/petrol); ν_{max} (film)/cm⁻¹ 3528 (br, m), 3283 (br, m), 1495 (m), 1409 (m), 1361 (w), 1205 (s), 1136 (s), 1011 (w), 950 (w), 711 (w), 630 (s); ¹H NMR (400 MHz, CDCl₃) δ = 7.20–7.16 (2H, m, H1, H5), 6.85–6.82 (2H, m, H2, H4); ¹³C NMR (101 MHz, CDCl₃) δ = 155.7 (C3), 127.7 (C1, C5), 126.1 (C6), 116.5 (C2, C4) (CF₃ resonance not observed); LRMS (ES⁻) *m/z* = 240.0 [M–H]⁻.

N-(4-Hydroxy-2-methylphenyl)-1,1,1-trifluoromethanesulfonamide **17**²⁰

To a solution of anilide **9** (1.0 mL of a 100 mM solution in isopropanol, 0.1 mmol), glucose (1.7 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.7 mmol), GDH (260 μ L of a 2.0 U/ μ L solution in phosphate buffer, pH 7.5, 520 U), and P450_{BM3} mutant KSK19/AI/AI (5.2 mL of a 20 μ M solution in phosphate buffer, pH 7.5, 0.104 μ mol) in 100 mM phosphate buffer (pH 7.5, 43 mL) was added NADP⁺ monosodium salt (260 μ L of a 4.0 mM solution in phosphate buffer, pH 7.5, 1.04 μ mol). The solution was stirred open to air for 18 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether/petrol to 40% ether/petrol) to

afford the title compound (**17**) as a colourless oil [9.0 mg, 34% (= 92% based on 37% conversion of **9**)]. R_f 0.25 (30% ethyl acetate/petrol); ν_{\max} (film)/ cm^{-1} 3488 (m, br), 3288 (m, br), 1591 (w), 1417 (w), 1359 (w), 1197 (s), 1140 (s), 931 (w), 623 (m); ^1H NMR (400 MHz, CDCl_3) δ = 7.20 (1H, d, J 8.5 Hz, H2), 6.73 (1H, d, J 3.0 Hz, H5), 6.67 (1H, dd, J 8.5, 3.0 Hz, H3), 2.33 (3H, s, H7); ^{13}C NMR (101 MHz, CDCl_3) δ = 155.9 (C4), 137.7 (C1), 129.6 (C2), 124.6 (C6), 119.9 (q, J 322.5 Hz, CF_3), 117.9 (C5), 114.0 (C3), 18.3 (C7); HRMS (ES^-) Found: 254.0105, $\text{C}_8\text{H}_7\text{F}_3\text{NO}_3\text{S}$ [$\text{M}-\text{H}$] $^-$ requires 254.0104.

***N*-(4-Hydroxy-3-methylphenyl)-1,1,1-trifluoromethanesulfonamide **18**²⁰**

To a solution of anilide **10** (0.63 mL of a 100 mM solution in isopropanol, 0.063 mmol), glucose (3.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 3.1 mmol), GDH (155 μL of a 2.0 U/ μL solution in 100 mM phosphate buffer, pH 7.5, 310 U), and P450_{BM3} mutant RP/EA (3.1 mL of a 20 μM solution in phosphate buffer, 0.062 μmol) in 100 mM phosphate buffer (pH 7.5, 23 mL) was added NADP^+ monosodium salt (155 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.62 μmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether/petrol to 40% ether/petrol) to afford the title compound (**18**) as a colourless oil (5.0 mg, 32% at 99% conversion of **10**). R_f 0.55 (50% ethyl acetate/petrol); ν_{\max} (film)/ cm^{-1} 3524 (br, m), 3286 (br, m), 1511 (w), 1411 (m), 1349 (m), 1201 (s), 1140 (m), 971 (w), 631 (w); ^1H NMR (500 MHz, CDCl_3) δ = 7.07 (1H, d, J 2.5 Hz, H6), 7.01 (1H, dd, J 8.5, 2.5 Hz, H2), 6.76 (1H, d, J 8.5 Hz, H3), 2.25 (3H, s, H7); ^{13}C NMR (126 MHz, CDCl_3) δ = 154.0 (C4), 128.8 (C6), 125.7 (C5), 125.5 (C1), 124.9 (C2), 120.0 (q, J 320.5 Hz, CF_3), 115.7 (C3), 15.9 (C7); LRMS (ES^-) m/z = 254.0 [$\text{M}-\text{H}$] $^-$.

N*-(4-(Hydroxymethyl)phenyl)-1,1,1-trifluoromethanesulfonamide **19*

To a solution of anilide **11** (0.63 mL of a 100 mM solution in isopropanol, 0.063 mmol), glucose (3.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 3.1 mmol), GDH (155 μL of a 2.0 U/ μL solution in 100 mM phosphate buffer, pH 7.5, 310 U), and P450_{BM3} mutant RP/IA/EA (1.5 mL of a 50 μM solution in phosphate buffer, 0.075 μmol) in 100 mM phosphate buffer (pH 7.5, 23 mL) was added NADP^+ monosodium salt (155 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.62 μmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (15% ether/petrol to 50% ether/petrol) to afford the title compound (**19**) as a colourless oil [8.0 mg, 47%

(= 59.5% based on 79% conversion of **11**)]. R_f 0.25 (50% ethyl acetate/petrol); ν_{\max} (film)/ cm^{-1} 3279 (br, w), 2921 (w), 1514 (w), 1417 (w), 1374 (m), 1212 (s), 1142 (s), 958 (w); ^1H NMR (500 MHz, CDCl_3) δ = 7.39–7.37 (2H, m, H3, H5), 7.25–7.23 (2H, m, H2, H6), 7.14 (1H, br s, NH), 4.71 (2H, s, H7), 1.87 (1H, br s, OH); ^{13}C NMR (126 MHz, CDCl_3) δ = 140.6 (C4), 133.0 (C1), 128.3 (C3, C5), 124.2 (C2, C6), 119.9 (q, J 320.5 Hz, CF_3), 64.6 (C7); HRMS (ES^-) Found: 254.0104, $\text{C}_8\text{H}_7\text{F}_3\text{NO}_3\text{S}$ [$\text{M}-\text{H}$] $^-$ requires 254.0104.

N*-(2-Bromo-4-hydroxyphenyl)-1,1,1-trifluoromethanesulfonamide **20*

To a solution of anilide **12** (0.49 mL of a 100 mM solution in isopropanol, 0.049 mmol), glucose (0.8 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 0.80 mmol), GDH (125 μL of a 2.0 U/ μL solution in 100 mM phosphate buffer, pH 7.5, 250 U), and P450_{BM3} mutant RPEV (4.5 mL of a 20 μM solution in phosphate buffer, 0.090 μmol) in 100 mM phosphate buffer (pH 7.5, 20 mL) was added NADP^+ monosodium salt (125 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.50 μmol). The solution was stirred open to air for 19 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (10% ether/petrol to 80% ether/petrol) to afford the title compound (**20**) as a colourless oil [6.0 mg, 35% (= 55.5% based on 63% conversion of **12**)]. R_f 0.45 (50% ethyl acetate/petrol); ν_{\max} (film)/ cm^{-1} 3488 (br, w), 3282 (br, w), 1606 (w), 1495 (m), 1419 (w), 1365 (w), 1231 (m), 1203 (s), 1139 (m), 954 (w), 886 (w), 623 (m); ^1H NMR (500 MHz, CDCl_3) δ = 7.44 (1H, d, J 9.0 Hz, H2), 7.13 (1H, d, J 3.0 Hz, H5), 6.82 (1H, dd, J 9.0, 3.0 Hz, H3); ^{13}C NMR (126 MHz, CDCl_3) δ = 155.6 (C4), 127.2 (C2), 125.5 (C1), 120.0 (C5), 119.8 (q, J 322.0 Hz, CF_3), 119.6 (C6), 116.0 (C3); HRMS (ES^-) Found: 317.9054, $\text{C}_7\text{H}_4^{79}\text{BrF}_3\text{NO}_3\text{S}$ [$\text{M}-\text{H}$] $^-$ requires 317.9053.

N*-(4-Hydroxy-2-methoxyphenyl)-1,1,1-trifluoromethanesulfonamide **21*

To a solution of anilide **14** (0.78 mL of a 100 mM solution in isopropanol, 0.078 mmol), glucose (1.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.30 mmol), GDH (195 μL of a 2.0 U/ μL solution in phosphate buffer, pH 7.5, 390 U), and P450_{BM3} mutant RP/IA/EA (1.1 mL of a 71 μM solution in phosphate buffer, pH 7.5, 0.078 μmol) in 100 mM phosphate buffer (pH 7.5, 31 mL) was added NADP^+ monosodium salt (195 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.78 μmol). The solution was stirred open to air for 24 h and aqueous HCl (3.0 M) was added. The solution was centrifuged at 9,500 g, and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (20% ether/petrol to 30% ether/petrol) to afford the title compound (**21**) as a colourless oil [6.0 mg, 27% (= 33.5% based on 81% conversion of **14**)]. R_f

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0.30 (40% ethyl acetate/petrol); ν_{max} (film)/ cm^{-1} 3470 (br, m), 3262 (br, m), 2924 (w), 1610 (w), 1512 (m), 1369 (m), 1231 (m), 1200 (s), 1140 (m), 949 (w), 836 (w), 640 (w); ^1H NMR (500 MHz, CDCl_3) δ = 7.30 (1H, d, J 8.5 Hz, H2), 6.73 (1H, br s, NH), 6.46 (1H, d, J 2.5 Hz, H5), 6.39 (1H, dd, J 8.5, 2.5 Hz, H3), 5.01 (1H, br s, OH), 3.86 (3H, s, OCH_3); ^{13}C NMR (101 MHz, CDCl_3) δ = 155.9 (C4), 153.1 (C6), 125.6 (C2), 120.0 (q, J 322.5 Hz, CF_3), 116.1 (C1), 107.4 (C3), 99.5 (C5), 56.1 (OCH_3); HRMS (ES^+) Found: 270.0051, $\text{C}_8\text{H}_7\text{F}_3\text{NO}_4\text{S}$ [$\text{M}-\text{H}$] $^-$ requires 270.0053.

N-(4-Hydroxy-3,5-dimethylphenyl)-1,1,1-trifluoromethanesulfonamide **22**²⁰

To a solution of anilide **15** (0.79 mL of a 100 mM solution in isopropanol, 0.079 mmol), glucose (1.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.30 mmol), GDH (195 μL of a 2.0 U/ μL solution in phosphate buffer, pH 7.5, 390 U), and P450_{BM3} mutant KSK19/Al/Al (1.6 mL of a 50 μM solution in phosphate buffer, pH 7.5, 0.080 μmol) in 100 mM phosphate buffer (pH 7.5, 35 mL) was added NADP $^+$ monosodium salt (195 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.78 μmol). The solution was stirred open to air for 17 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (5% ether/petrol) to afford the title compound (**22**) as a colourless oil (5.0 mg, 24% at 90% conversion of **15**). R_f 0.55 (40% ethyl acetate/petrol); ν_{max} (film)/ cm^{-1} 3544 (br, w), 3282 (br, w), 2925 (w), 1682 (w), 1489 (m), 1413 (w), 1361 (w), 1195 (s), 1139 (m), 1032 (w), 915 (w), 612 (m); ^1H NMR (500 MHz, CDCl_3) δ = 6.92 (2H, s, H2, H6), 2.24 (6H, s, H7, H8); ^{13}C NMR (126 MHz, CDCl_3) δ = 151.2 (C4), 125.0 (C2, C6), 123.9 (C1), 123.2 (C3, C5), 118.8 (q, J 322.0 Hz, CF_3), 14.9 (C7, C8); LRMS (ES^-) m/z = 268.0 [$\text{M}-\text{H}$] $^-$.

N-[3-(Hydroxymethyl)-5-methylphenyl]-1,1,1-trifluoromethanesulfonamide **23**

To a solution of anilide **15** (0.79 mL of a 100 mM solution in isopropanol, 0.079 mmol), glucose (1.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 1.30 mmol), GDH (195 μL of a 2.0 U/ μL solution in phosphate buffer, pH 7.5, 390 U), and P450_{BM3} mutant KSK19/Al/FV (1.0 mL of a 78 μM solution in phosphate buffer, pH 7.5, 0.078 μmol) in 100 mM phosphate buffer (pH 7.5, 35 mL) was added NADP $^+$ monosodium salt (195 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.78 μmol). The solution was stirred open to air for 21 h. Ethyl acetate was added, the solution was centrifuged at 9,500 g, and the layers were separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (40% ethyl acetate/petrol) to afford the title compound (**23**) as a colourless oil (12 mg, 58% at 99% conversion of **15**). R_f 0.45 (40% ethyl acetate/petrol); ν_{max} (film)/ cm^{-1} 3566 (br, m), 3288 (br, m), 2980 (m), 1603 (w), 1372 (m), 1229 (s), 1195 (s), 1141 (s); ^1H NMR (400 MHz,

CDCl_3) δ = 7.07 (1H, s, H4), 7.01 (1H, s, H2), 7.00 (1H, s, H6), 4.65 (2H, s, H8), 2.34 (3H, s, H7); ^{13}C NMR (101 MHz, CDCl_3) δ = 142.1 (C5), 140.3 (C1), 134.2 (C3), 126.8 (C4), 123.4 (C6), 119.9 (q, J 320.0 Hz, CF_3), 119.2 (C2), 64.7 (C8), 21.4 (C7); HRMS (ES^-) Found: 268.0260, $\text{C}_9\text{H}_9\text{F}_3\text{NO}_3\text{S}$ [$\text{M}-\text{H}$] $^-$ requires 268.0261.

N-4-Hydroxy-2-[(2-oxopiperidin-1-yl)methyl]phenyl-1,1,1-trifluoromethanesulfonamide **26**

To a solution of anilide **24** (450 μL of a 0.1 M solution in ethanol, 0.045 mmol), glucose (2.3 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 2.30 mmol), GDH (225 μL of a 2.0 U/ μL solution in phosphate buffer, pH 7.5, 450 U), NADP $^+$ monosodium salt (225 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.90 mmol), and catalase (from *Micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution, 34,500 U) in 100 mM phosphate buffer (pH 7.5, 34 mL) was added P450_{BM3} mutant RP/FV/EV (4.5 mL of a 20 μM solution in phosphate buffer, pH 7.5, 0.09 μmol). The solution was stirred open to air for 23 h. The solution was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a brown oil. The crude product was purified by flash chromatography (30% ethyl acetate/petrol to 70% ethyl acetate/petrol) to afford the title compound (**26**) as a colourless oil (2.0 mg, 13% (20% brsm)). R_f 0.25 (80% ethyl acetate/petrol); ν_{max} (film)/ cm^{-1} 3200 (br, w), 2919 (w), 1584 (w), 1504 (w), 1448 (w), 1376 (m), 1120 (s), 1145 (s), 908 (m), 732 (s); ^1H NMR (500 MHz, CD_3OD) δ = 7.15 (1H, d, J 8.5 Hz, H2), 6.70 (1H, dd, J 8.5, 3.0 Hz, H3), 6.67 (1H, d, J 3.0 Hz, H5), 4.62 (2H, s, H7), 3.34–3.32 (2H, m, H8), 2.46–2.43 (2H, m, H11), 1.85–1.82 (4H, m, H9, H10); ^{13}C NMR (126 MHz, CD_3OD) δ = 173.2 (C12), 158.0 (C4), 135.7 (C1), 134.1 (C6), 129.7 (C2), 121.9 (q, J 320.0 Hz, CF_3), 116.0 and 115.9 (C3 and C5), 49.2 (C8, from HSQC), 47.9 (C7), 32.9 (C11), 23.9 and 22.0 (C9 and C10); HRMS (ES^+) Found: 353.0763, $\text{C}_{13}\text{H}_{16}\text{F}_3\text{N}_2\text{O}_4\text{S}$ [$\text{M}+\text{H}$] $^+$ requires 353.0777.

N-4-Hydroxy-2-[(3-oxothiomorpholino)methyl]phenyl-1,1,1-trifluoromethanesulfonamide **27**

To a solution of anilide **25** (420 μL of a 0.1 M solution in ethanol, 0.042 mmol), glucose (2.1 mL of a 1.0 M solution in phosphate buffer, pH 7.5, 2.10 mmol), GDH (210 μL of a 2.0 U/ μL solution in phosphate buffer, pH 7.5, 420 U), NADP $^+$ monosodium salt (210 μL of a 4.0 mM solution in phosphate buffer, pH 7.5, 0.84 mmol), and catalase (from *Micrococcus lysodeikticus*, 3.0 mL of a 11,500 U/mL solution, 34,500) in 100 mM phosphate buffer (pH 7.5, 32 mL) was added P450_{BM3} mutant RP/FV/EV (4.2 mL of a 20 μM solution in phosphate buffer, pH 7.5, 0.084 μmol). The solution was stirred open to air for 23 h. The solution was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to a yellow oil. The crude product was purified by flash chromatography (30% ethyl acetate/petrol to 100% ethyl acetate) to afford the title compound as a colourless oil (3.0 mg, 19%). R_f 0.60 (100% ethyl acetate); ν_{max} (film)/ cm^{-1} 3230 (br, w), 2918 (w), 1604 (m), 1498 (m), 1373 (m), 1206 (s), 1143 (m); ^1H NMR (500 MHz, CDCl_3) δ = 7.44 (1H, d, J 9.0 Hz, H2), 6.83 (1H, dd, J 9.0, 3.0 Hz,

H3), 6.78 (1H, d, J 3.0 Hz, H5), 4.48 (2H, s, H7), 3.79 (2H, t, J 6.0 Hz, H8), 3.37 (2H, s, H10), 2.86 (2H, t, J 6.0 Hz, H9); ^{13}C NMR (126 MHz, CDCl_3) δ = 168.4 (C11), 154.1 (C4), 130.4 (C6), 127.3 (C1), 126.6 (C2), 120.2 (q, J 323.4 Hz, CF_3), 117.6 (C5), 116.9 (C3), 50.7 (C8), 49.9 (C7), 30.7 (C10), 26.1 (C9); HRMS (ES^+) Found: 393.0153, $\text{C}_{12}\text{H}_{13}\text{F}_3\text{N}_2\text{NaO}_4\text{S}_2$ $[\text{M}+\text{Na}]^+$ requires 393.0161.

Conflicts of interest

The authors confirm that there are no conflicts of interest to declare.

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