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Publication date	2013
Publication information	Bright, Tara V., Fay Dalton, Victoria L. Elder, Cormac D. Murphy, Neil K. O'Connor, and Graham Sandford. "A Convenient Chemical-Microbial Method for Developing Fluorinated Pharmaceuticals." Royal Society of Chemistry, 2013. https://doi.org/10.1039/c2ob27140k.
Publisher	Royal Society of Chemistry
Item record/more information	http://hdl.handle.net/10197/5557
Publisher's version (DOI)	10.1039/c2ob27140k

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A Convenient Chemical-Microbial Method for Developing Fluorinated Pharmaceuticals

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Abstract

A significant proportion of pharmaceuticals are fluorinated and selecting the site of fluorine incorporation can be an important beneficial part a drug development process. Here we describe initial experiments aimed at the development of a general method of selecting optimum sites on pro-drug molecules for fluorination, so that metabolic stability may be improved. Several model biphenyl derivatives were transformed by the fungus *Cunninghamella elegans* and the bacterium *Streptomyces griseus*, both of which contain cytochromes P450 that mimic oxidation processes *in vivo*, so that the site of oxidation could be determined. Subsequently, fluorinated biphenyl derivatives were synthesised using appropriate Suzuki-Miyaura coupling reactions, positioning the fluorine atom at the pre-determined site of microbial oxidation; the fluorinated biphenyl derivatives were incubated

with the microorganisms and the degree of oxidation assessed. Biphenyl-4-carboxylic acid was transformed completely to 4'-hydroxybiphenyl-4-carboxylic acid by *C. elegans* but, in contrast, the 4'fluoro- analogue remained untransformed exemplifying the microbial oxidation – chemical fluorination concept. 2'-Fluoro- and 3'-fluoro-biphenyl-4-carboxylic acid were also transformed, but more slowly than the non-fluorinated biphenyl carboxylic acid derivative. Thus, it is possible to design compounds in an iterative fashion with a longer metabolic half-life by identifying the sites that are most easily oxidised by *in vitro* methods and subsequent fluorination without recourse to extensive animal studies.

Introduction

A convenient, rapid *in vitro* chemical-microbial method for establishing the structures of oxidative metabolites of drug-like systems and subsequent improvement of substrate oxidative stability by site selective fluorination at pre-identified, oxidatively sensitive positions is reported here. This strategy may be of potential benefit to the drug development process.

Xenobiotics such as drugs and toxins are commonly metabolised in the liver prior to excretion, and the main class of enzyme involved is the cytochromes P450 (CYP). In humans there are 57 CYP isoforms responsible for oxidative or phase I metabolism of drugs,[1] and one of the key factors in drug design is modulating the rate of CYP-mediated transformation so that optimum efficacy can be achieved. In addition, as part of a drug development programme, the toxicity of metabolites must be assessed to complete regulatory approval.[2] Consequently, the identification, synthesis and isolation of drug metabolites are

key stages in medicinal chemistry campaigns and, in general, *in vivo* studies are used to assess metabolite identity with obvious resource implications.

A simple *in vitro* method that successfully and accurately mimics mammalian metabolic processes would offer an attractive alternative to conventional resource-intensive bio- and medicinal chemistry approaches. Indeed, many chemical methods, such as the use of iron porphyrin systems, have been assessed as potential biomimetic cytochrome P450 mimics *in vitro* with varying degrees of success.[3] In complementary approaches, some microorganisms can transform drugs to mammalian metabolites, since they have CYP enzymes. In particular, the zygomycete fungus *Cunninghamella elegans*, which has been widely studied as a model of mammalian metabolism of xenobiotic compounds[4], offers a non-chemical CYP biomimic.

Over 30% of currently available and commercially valuable pharmaceuticals owe their biological activity to the presence of fluorine atoms within their structures providing an indication of the importance of fluorine incorporation for modifying drug stability, lipophilicity and effective pH range.[5-8] Indeed, a common strategy for increasing the half-life of a drug *in vivo* is to introduce a fluorine atom at the metabolically labile sites.[9] An example of how dramatic this approach can be is observed in the development of the cholesterol uptake inhibitor drug Ezetimibe (SCH 58325), where optimization of the lead compound (SCH4861) included fluorination of the phenyl ring, preventing oxidation to the corresponding phenol derivative, and substitution of a methoxy group with fluorine preventing demethylation (Fig 1). These changes and other structural modifications reduced metabolic degradation to such levels whereby the required dose for activity could be decreased by 55 times, while increasing activity 400-fold.[10, 11] Therefore, in the sphere of fluorinated drug development, it would be very useful to easily determine the site of CYP-catalysed oxidation in a drug candidate *in vitro*, so that the incorporation of a fluorine atom is

then made at the optimal position for increasing oxidative stability as a contributing approach to improving drug efficacy.

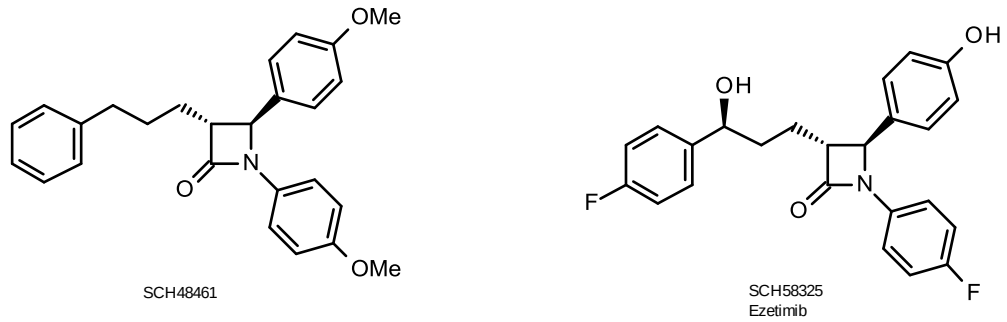


Figure 1. Structures of lead compound SCH48461 and its fluorinated analogue, the cholesterol inhibitor Ezetimibe.

A common motif in several drug classes is the biphenyl system,[12] and various valuable pharmaceuticals bearing this structural unit are given in Fig. 2. Previously, it has been demonstrated that biphenyl and fluorinated biphenyl [13, 14] derivatives are metabolised by *C. elegans* in a similar fashion to mammals.

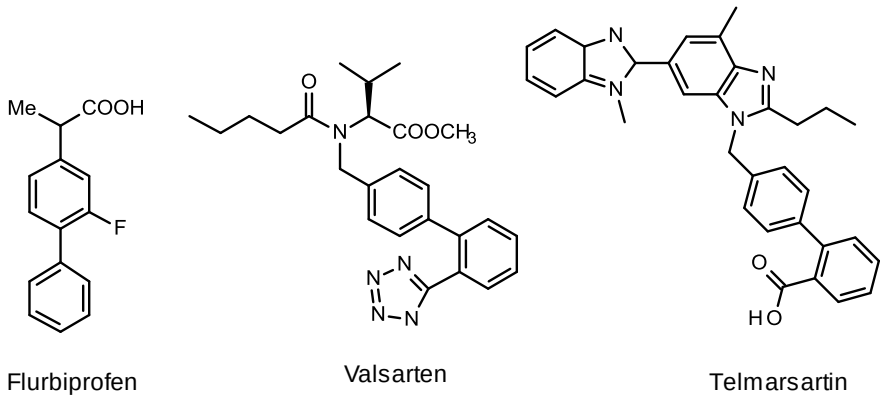
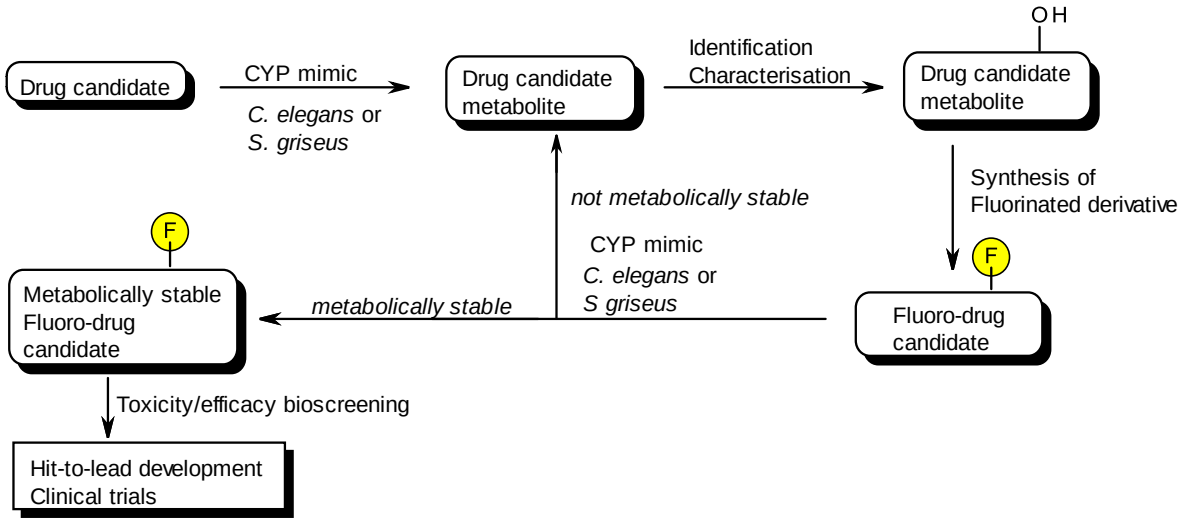


Figure 2. Examples of biphenyl-containing pharmaceuticals

In this paper, we describe proof-of-concept studies concerning a microbial-chemical method of identifying the most oxidatively sensitive sites in a range of model biphenyl

substrates bearing a variety of functional groups by employing *C. elegans* and the bacterium *Streptomyces griseus*, which is known to also mimic human drug metabolism,[15, 16] to determine the sites of CYP-catalysed oxidation. The effect of strategic fluorination on metabolism was measured by further incubation of the appropriately fluorinated biphenyl system with the microorganisms in an overall strategy to develop systems that are oxidatively stable to CYP. This iterative process is shown in Scheme 1 where, ultimately, a fluorinated lead compound with appropriate metabolic stability can be identified in an early stage of the drug discovery process.



Scheme 1. Chemical-microbial oxidation-fluorination process for drug development

Results and discussion

Initially, we incubated a series of commercially available model biphenyl compounds with *C. elegans* and *S. griseus,* and ethyl acetate extracts from the cultures were analysed by GC-MS to identify the products formed (Table 1) from these small scale experiments.

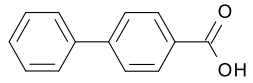
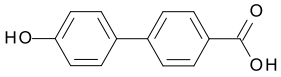
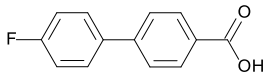
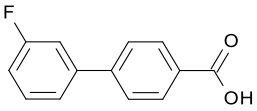
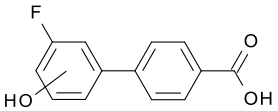
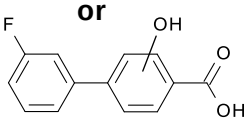
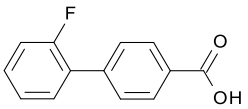
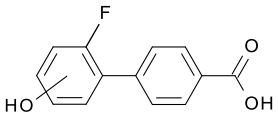
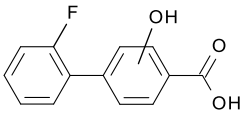
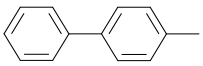
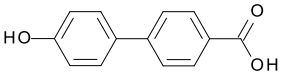
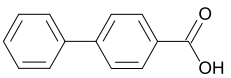
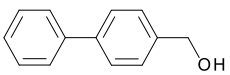
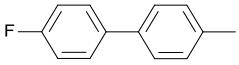
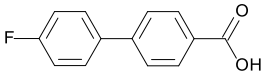
Biphenyl-4-carboxylic acids

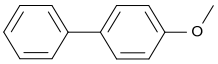
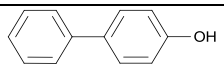
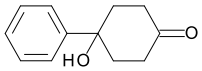
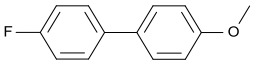
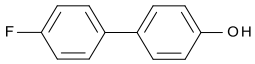
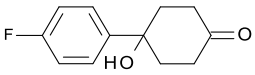
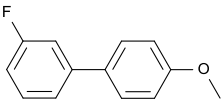
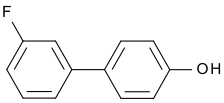
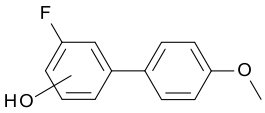
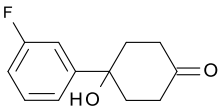
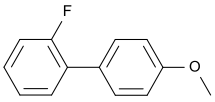
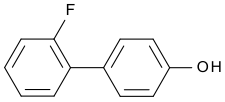
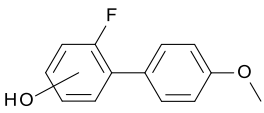
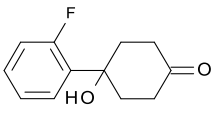
Biphenyl-4-carboxylic acid **1a** was completely transformed by *C. elegans*, but remained untransformed by *S. griseus*. By GC-MS, one product was detected in the *C. elegans* extract, which had the expected mass spectrum of 4'-hydroxy-biphenyl-4-carboxylic acid **2a**, and this was confirmed by ^{1}H NMR of the purified compound, which showed two diagnostic AX resonances centred at 7.53 and 6.86 ppm attributed to the two 1,4-disubstituted aromatic ring systems. Furthermore, this is the expected site of mammalian CYP-catalysed oxidation, based on previous observations.[17]

Consequently, following the strategy outlined in Scheme 1, the corresponding 4'-fluoro-biphenyl carboxylic acid **1b** was synthesised by Suzuki-Miyaura coupling of 4-fluorobenzene boronic acid with ethyl 4-bromobenzoate, followed by acid catalysed hydrolysis (Table 1, Supporting Information) following usual procedures and incubated with the microorganisms (Table 1).

We observed that the 4'fluoro biphenyl carboxylic acid **1b** is not transformed, demonstrating, in this case, that fluorine substitution in a position that favoured hydroxylation by CYP will block further oxidation thus exemplifying our strategy described in Scheme 1.

Table 1. Products formed upon incubation of substrate with *C. elegans* and *S. griseus*

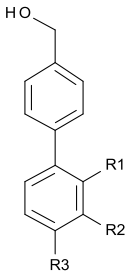
Substrate	Products after 48 h incubation	Yield (%) *C. elegans*	Yield (%) *S. griseus*
 **1a**	 **2a**	100	-
 **1b**	No products detected		
 **1c**	 **or**  **2c**	100	-
 **1d**	 **or**  **2b**	100	-
 **1e**	 **2d**  **1a**  **2e**	95 - -	- 69 21
 **1f**	 **1b**	99	100

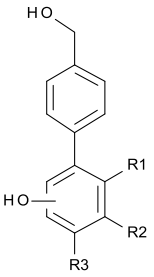
 **1g**	 **2f**  **2g**	22 -	16.5 22.5
 **1h**	 **2h**  **2i**	91 -	40 15
 **1i**	 **2j**  **2k**  **2l**	30 42 -	25 2 1
 **1j**	 **2m**  **2n**  **2p**	29 61 -	30 1 22

To further examine the effect of fluorine substitution on the oxidation of biphenyl carboxylic acid derivatives, incubation of 2'- or 3'-fluoro-biphenyl-4-carboxylic acid **1c** and **1d** with *C. elegans* was carried out, resulting in 100 % conversion (by GC-MS) to hydroxylated products **2b** and **2c**, respectively, whose structure could not be identified with certainty. Nevertheless, the experiment demonstrated that the 4'-position must bear a fluorine atom to prevent oxidative transformation.

The observation that 2'- and 3'-fluoro-biphenyl-4-carboxylic acid **1c** and **1d** were completely transformed prompted experiments to determine if there was any difference in the rate of oxidation at the 4'-positions compared with the non-fluorinated biphenyl **1a**. Biphenyl-4-carboxylic acid **1a** was completely transformed to the hydroxylated product after 48 h incubation, whereas approximately 30 % of the 2'- and 3'-fluorobiphenyl carboxylic acid **1c** and **1d** were converted to the corresponding fluoro-hydroxy-biphenyl-4-carboxylic acids **2b** and **2c** in the same time frame, indicating that the presence of fluorine on the aryl ring slowed the transformation, reflecting the lower oxidation potential of the aromatic ring upon fluorine incorporation. Interestingly, GC-MS analysis showed that the composition of the product mixture changed over the course of the experiment; biphenyl-4-carboxylic acid **1a** giving 4-biphenylmethanol, which was detected after 24 h, but was not present in the product mixture 48 h incubation (Figure 3). Correspondingly, in both the fluorobiphenyl-4-carboxylic acid cultures **1c** and **1d**, fluorinated biphenyl methanol and/or fluorinated hydroxylated biphenyl methanol were detected in the extracts, after 24 h and 48 h incubation, but were not present after 72 h, leaving the final hydroxylated carboxylic acid (Table 3).

Table 3. Proportion of products formed upon incubation of fluorinated biphenyl-4-carboxylic acid with *C. elegans* for 48 h, as determined by GC-MS.





3a, R_1 = F; R_2 = R_3 = H
3b, R_2 = F; R_1 = R_3 = H
3c, R_3 = F; R_1 = R_2 = H

4a, R_1 = F; R_2 = R_3 = H
4b, R_2 = F; R_1 = R_3 = H
4c, R_3 = F; R_1 = R_2 = H

Fluorine position	% Product					
	Biphenyl methanol		OH-biphenyl methanol		OH-Carboxylic acid	
	Incubation (h)		Incubation (h)		Incubation (h)	
	24	48	24	48	24	48
1d, 2’	**3a**, 80	**3a**, 53	**4a**, 4	**4a**, 3	-	**2b**, 29
1c, 3’	-	- [a]	**4b**, 65	**4b**, 63	**2c**, 30	**2c**, 34
1b, 4’	**3c**, 69	**3c**, 93	**4c**, 2	**4c**, 3	-	-
-	**2e**, 39	-	-	-	**2d**, 16	**2d**, 100

[a] 3’-fluorobiphenylmethanol **3b** could only be detected after silylation

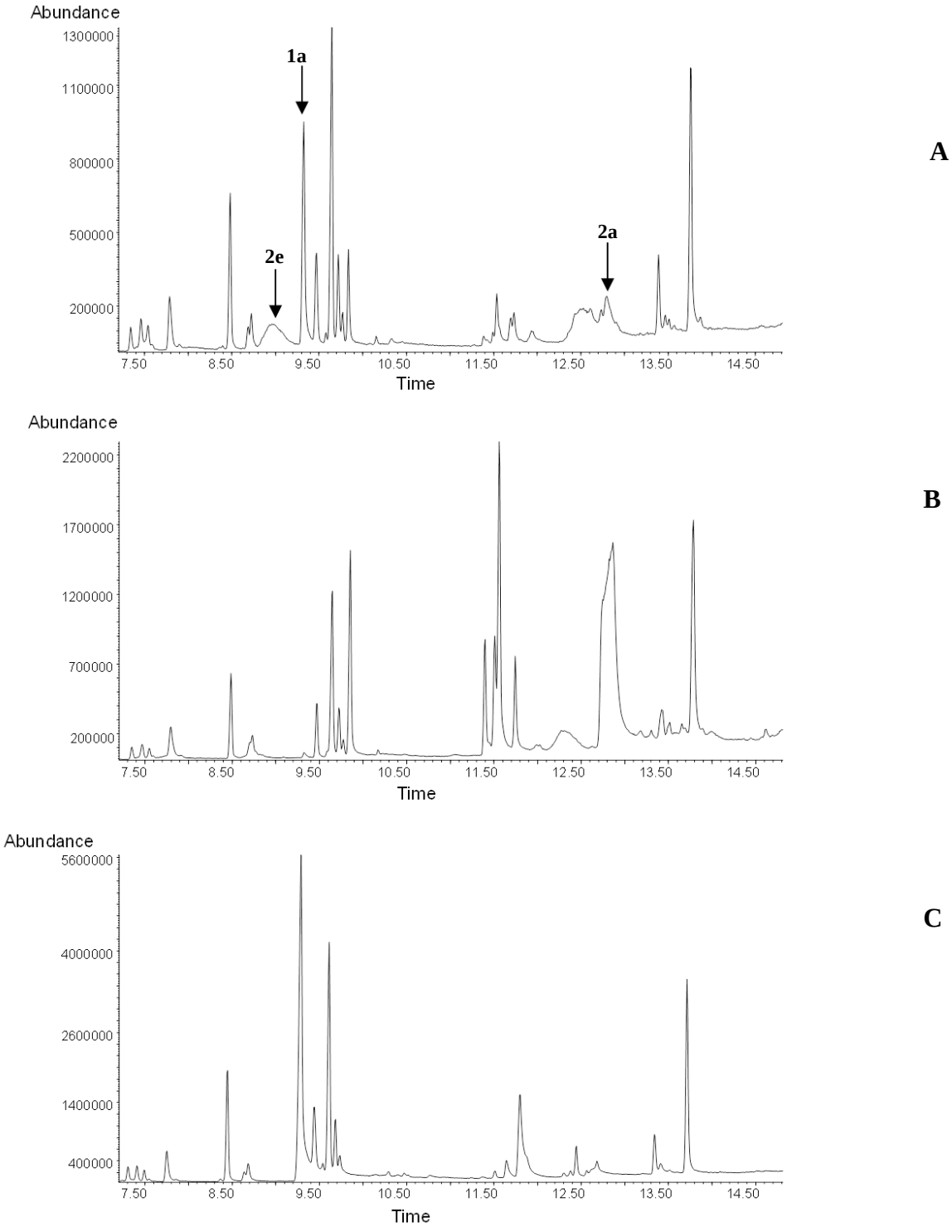
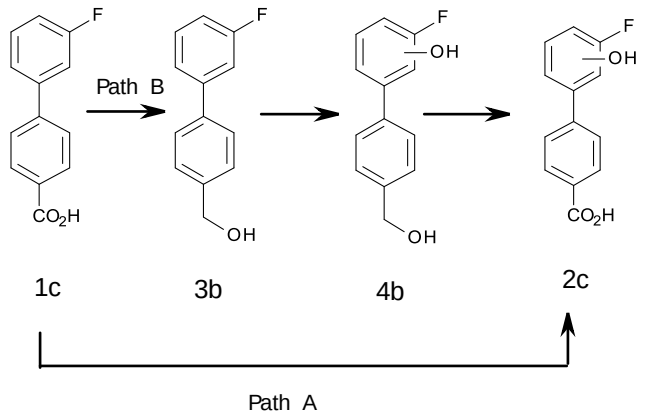


Figure 3. Gas chromatograms showing the appearance of 4-biphenylmethanol **2e** (retention time, 9 min) after 24 h incubation of biphenyl-4-carboxylic acid **1a** (retention time, 9.3 min) with *C. elegans* (A), and its subsequent disappearance after 48 h with the formation of 4'-hydroxy-biphenyl-4-carboxylic acid **2a** (retention time 12.8 min) (B). Panel C shows the chromatogram from an uninoculated control flask.

Thus, the carboxylic acid group is reduced initially by the culture and then re-oxidised, possibly reflecting the reducing power available in the cell at the various time points of the experiment. It is also possible that the reduction promotes hydroxylation, of 2'- and 3'-fluorobiphenyl-4-carboxylic acid on the second ring, as the $-CH_2OH$ group is electron donating, activating the ring towards oxidation processes. Interestingly, 4'-fluoro-biphenyl-4-carboxylic acid **2a** was almost completely converted to the biphenyl methanol **3a** after 48 h, before being re-oxidised back to the acid **2a**. The sequences of these transformations are shown in Scheme 2 and two possible pathways, **A** and **B**, can be envisaged.



Scheme 2. Transformation of 3'-fluoro-biphenyl carboxylic acid **1c** in *C. elegans*.

Whilst transformation of the acid directly to the hydroxylated acid (Path **A**) cannot be ruled out, the observation of the methanol and hydroxyl methanol derivatives within the cultures suggest a step-wise process (Path **B**) where the reduction of the acid group precedes hydroxylation. In the case of the 2'-fluoro derivatives, observation of significant (80%) quantities of 2'-fluorobiphenyl methanol **3a** shows that the hydroxylation of the aromatic ring is a slower process than oxidation of the CH_2OH group whereas for the 3'-fluoro derivative, the methanol system is not observed indicating rapid hydroxylation, reflecting an increased electron donating effect of the CH_2OH group on the 3'-fluoro system. Consequently, these *in*

vitro studies provide more information regarding possible metabolite intermediates that may be generated compared to *in vivo* studies in which only the final metabolite is typically observed upon excretion.

The study was expanded by synthesising a range of fluorobiphenyl derivatives with varying functional groups by Suzuki-Miyaura coupling reactions (Table 2, Supporting Information) and incubating these with the microorganisms as described above (Table 1).

4-Methyl biphenyl **1e**

Incubation of 4-methyl biphenyl **1e** with *C. elegans* resulted in almost complete transformation to 4'-hydroxybiphenyl-4-carboxylic acid **2a**, indicating both oxidation of the methyl group and CYP-mediated hydroxylation. In *S. griseus*, two products were detected, 4-biphenyl methanol and biphenyl-4-carboxylic acid **1a**, demonstrating that the oxidation of the substrate most likely occurs in a stepwise fashion; however, no hydroxylation of the rings occurs, which is consistent with our observations described above. When 4'-fluoro-4-methyl biphenyl **1f** was incubated with the microorganisms, the transformation stopped at the corresponding carboxylic acid **1b** with no ring oxidation, again consistent with results described above.

4-Methoxybiphenyl **1g**

Incomplete demethylation of 4-methoxybiphenyl **1g** to **2f** was observed in *C. elegans* and *S. griseus*; however, in the bacterium, a highly unusual metabolite, 4-hydroxy-4-phenyl cyclohexane **2g**, was also detected by GC-MS and the structure was identified by comparison with data in the NIST MS database. A sequence of intermediates for this unusual transformation is suggested in Scheme 4 and is consistent with a related pathway described

by Gopishetty et al. [18] in which *S. griseus* transforms naphthalene to 4-hydroxy-1-tetralone, via 1-naphthol (Figure 4).

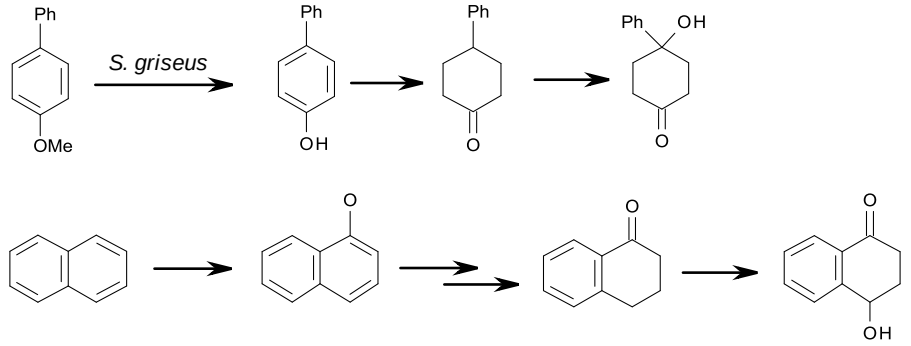


Figure 4. Proposed CYP-catalysed transformation of naphthalene and methoxy biphenyl in *S. griseus*

Incubation of fluorinated 4-methoxybiphenyl **1h** also resulted in demethylation to **2h** in both microorganisms, and in *C. elegans* hydroxylated 2'- and 3'-fluoro-4-methoxybiphenyl derivatives **2k** and **2n** were detected, but no hydroxylated product was detected from 4'fluoro-4-methoxybiphenyl and is consistent with the previous experiments with 4'-fluoro-substituted biphenyls. Interestingly, in the *S. griseus* cultures, a metabolite with the expected mass spectrum of fluorinated 4-hydroxy-4-phenyl cyclohexane was detected from 2', 3' and 4'-fluoro-4-methoxybiphenyl.

Nitrobiphenyl

No transformation of nitrobiphenyl was observed, probably as a consequence of the very strong electron withdrawing effect of the nitro group which reduces the oxidation potential of the biphenyl system.

Conclusions

The incorporation of fluorine into drugs can be an important aspect of hit-to-lead development as incorporation of fluorine atoms can have dramatic effects on bioactivity, stability and lipophilicity. In this paper our initial efforts to develop a method for selectively fluorinating drugs to improve metabolic stability, by first identifying the most likely site of oxidation using microbial transformation, then selectively fluorinating that position has been presented (Scheme 1). The effectiveness of this approach is most clearly demonstrated with a model system, biphenyl 4-carboxylic acid, which was completely oxidised to 4'-hydroxy-biphenyl-4-carboxylic acid by *C. elegans*, yet the corresponding system 4'-fluoro-biphenyl-4-carboxylic acid remained unoxidised. Furthermore, the importance of the regioselectivity of fluorine incorporation was illustrated by the incubation of 2'- and 3'-fluoro-biphenyl-4-carboxylic acid with *C. elegans*, which resulted in the production of hydroxylated metabolites.

The study also revealed the formation of intermediate/transient metabolites arising from metabolism of the fluorinated biphenyl-4-carboxylic acids (biphenyl methanol). Such metabolites are also likely to occur in other species, including mammals, in vivo, and given the significance of the relatively new 'Metabolites in Safety Testing' (MIST) guidelines from the US Food and Drug Administration, [19] microorganisms can provide a method for relatively easy access to biologically active compounds for toxicity testing. Thus we have developed initial insights into a straightforward microbiological-chemical method for determining the optimum site for fluorination and for subsequent evaluation of the metabolism. Employing microorganisms, which are relatively easy to handle, will reduce the costs of using either animal models, or mammalian cell cultures.

Experimental

Synthesis of fluorobiphenyl derivatives - General procedure

Aryl boronic acid, potassium carbonate and $Pd(PPh_3)_4$ were placed in a round bottomed flask filled with an argon atmosphere. A mixture of toluene (30 mL) and water (1 mL), which had been degassed via the freeze-thaw method, and haloarene were added to the flask and the mixture was heated at reflux temperature overnight. The reaction mixture was cooled and extracted with water (15 mL) and toluene (15 mL) and separated. The organic extracts were combined and washed with water and brine before being dried ($MgSO_4$). The solution was filtered and solvents evaporated to give crude fluorobiphenyl product which was purified by column chromatography on silica gel or recrystallization.

Ethyl 4'-fluorobiphenyl-4-carboxylate

4-Fluorobenzene boronic acid (1.5 g, 10.7 mmol), potassium carbonate (2.67 g, 19.3 mmol), $Pd(PPh_3)_4$ (0.37 g, 3.22 mmol), ethyl-4-bromobenzoate (1.6 ml, 9.65 mmol), toluene (30 mL) and water (1 mL), after column chromatography on silica gel using ethyl acetate:hexane(1:3) as elutant, gave *ethyl 4'-fluorobiphenyl-4-carboxylate* (1.473 g, 62 %) as a white solid; mp 64.5-65.5 °C (lit. [20], 63.5 – 64 °C); (Found: C, 73.6; H, 5.4. $C_{15}H_{13}FO_2$ requires: C, 73.8; H, 5.3%); ν_{max} (cm^{-1}) 2991, 1713 (C=O), 1282; δ_H1.40 (3H, t, *J*7.1, CH_3), 4.39 (2H, q, *J* 7.1, CH_2),7.54-7.58 (2H, m, ArH),7.58-7.60 (4H, m, ArH),8.08-8.11 (2H, m, ArH); δ_F -114.72 (m);δ_C14.33 (CH_3), 60.97 (CH_2),115.82 (d, $^2J_{CF}$21.5, C-3'), 126.79 (C-3),128.87 (d, $^3J_{CF}$ 8.1, C-2'),130.08 (C-2),136.14 (d, $^4J_{CF}$ 3.3, C-1'),144.43 (C-4),146.23 (C-1), 162.89 (d, $^1J_{CF}$ 247.8, C-4'), 166.37 (C=O); m/z (ASAP$^+$) 244 ($[M]^+$, 100%).

***4'-Fluorobiphenyl-4-carboxylic Acid* 1b**

A solution of 4'-fluorobiphenyl -4-carboxylic acid ethyl ester (0.71 g, 2.91 mmol) in THF (8 mL) and MeOH (8 mL) was treated with 2M NaOH (5mL) at room temperature overnight. The organic solvent was evaporated leaving the aqueous layer which was acidified by the addition of 2M HCl. The solution was extracted using ethyl acetate and the organic layers combined and dried ($MgSO_4$). The solution was filtered and solvent evaporated to give *4'-fluorobiphenyl-4-carboxylic acid* **1b** [21] (0.41 g, 66 %) as a white solid; mp 242.2-244.9 °C; (Found: C, 71.9; H, 4.4. $C_{13}H_9FO_2$ requires: C, 72.2; H, 4.2%); ν_{max} (cm^{-1}) 2828, 2554, 1678 (C=O), 1290; δ_H(DMSO-d_6)7.24-7.32 (2H, m, ArH), 7.70-7.77 (4H, m, ArH),7.95-8.02 (2H, m, ArH); δ_F(DMSO-d_6) -114.66 (s); δ_C(DMSO-d_6) 116.31 (d, $^2J_{CF}$ 21.4, C-3'), 127.15 (C-2),129.46 (d, $^3J_{CF}$ 8.3, C-2'),130.03 (C-3),130.38 (C-4),135.91 (d, $^4J_{CF}$3.1, C-1'),143.64 (C-1),162.75 (d, $^1J_{CF}$ 245.6, C-4'), 167.53 (CO_2H); *m/z*($ASAP^+$) 216 ($[M]^+$, 100%).

Culture conditions

Streptomyces griseus ATCC 13273 was maintained on agar slants containing ISP4 medium. The mycelia were inoculated into 250 mL Erlenmeyer flasks containing 30 mL sterile soya bean media consisting of soya bean meal (5 g/L), glycerol (20 g/L), yeast extract (5 g/L) and K_2HPO_4 (5 g/L), and the pH was adjusted to 7. Cultures were incubated with rotary agitation (200 rpm) at 30 ºC for 72 h. Substituted biphenyl (3 mg) solubilised in 30 µL dimethylformamide was added to the flask, and incubation was then continued for a further 72 h. Cultures were sonicated on ice (Sonicator U200S control, IKA Labortechnik) for 1 min at 50 % amplitude and the sonicate centrifuged (3200 rpm, 15 min)to remove the cell debris. The supernatant was extracted twice with ethyl acetate (30 mL).

Cunninghamella elegans was cultivated on sabouraud dextrose agar for 120 h at 27 °C. Inoculum was prepared by homogenising the mycelium, including the agar in sterile 8 % NaCl (100 mL). The homogenate (5 mL) was then used to inoculate 250 mL Erlenmeyer flasks containing 50 mL sabouraud dextrose broth, which were incubated 72 h with rotary agitation (150 rpm) at 27 °C. Substituted biphenyl (5 mg) solubilised in 50 µL dimethylforamide was added to the flask and incubation was continued for a further 72 h. Cultures were sonicated on ice for 3 min at 100 % amplitude with 30 s intervals every minute. Sonicate was then centrifuged to remove the cell debris and the supernatant was extracted twice with ethyl acetate (50 mL).

Metabolite analysis

Organic extracts were dried and the solid redissolved in ethyl acetate (1 ml), and analysed by gas chromatography-mass spectrometry (GC-MS). Samples (1 µl) were injected in the splitless mode onto a HP5MS column (30m x 0.25 mm x 0.25 µm) and the oven temperature held at 70 °C for 3 min, then raised to 250 °C at 10 °C min^{-1}. For the identification of some metabolites a portion of the extract was dried under a stream of nitrogen and derivatised by heating with *N*-methyl-*N*-(trimethyl-silyl) trifluoroacetamide (50 µL) at 100 ^{0}C for 1 h. The silylated samples were diluted with ethyl acetate (100 µL) and analysed by GC-MS. Extracts from cultures that were incubated with fluorinated biphenyls were also analysed by fluorine-19 nuclear magnetic resonance spectroscopy on a Varian 400 MHz spectrometer, after drying the extract in a stream of nitrogen and redissolving in $CDCl_3$.

4'-Hydroxybiphenyl carboxylic acid was further purified from the organic extracts of *C. elegans* culture supernatants by preparative HPLC using a Varian Prostar system equipped with a ZORBAX SB-C18 5µm (150 x 9.4mm) column. Organic extract (250 µL) from eight flasks of *C. elegans* incubated with biphenyl-4-carboxylic acid was injected onto the column

and the compound was eluted with a gradient of methanol/water (10-90% methanol) over 38 min with a flow rate of 4 mL min^{-1}.GC-MS analysis of the fractions revealed that the fraction containing 4'-hydoxybiphenyl-4-carboxylic acid contained an impurity, which was removed by further HPLC purification with a modified elution gradient: methanol/water (50 %) with a gradient of methanol/water (10/90 %) over 25 min and then to (90/10 %) for 13 min with a flow rate of 4 mL min^{-1}. The purified compound was characterised by ^{1}H NMR (Varian 300 MHz spectrometer); δ_H 6.88 (2H, d, *J* 9 Hz), 7.53 (2H, d, *J* 9 Hz), 7.65 (2H, d, *J* 9 Hz), 8.08 (2H, d, *J* 9 Hz).

Products from transformation of non-fluorinated biphenyl derivatives were identified based on their mass spectra employing the NIST MS library. Fluorinated derivatives could be readily deduced from the change in mass arising from the presence of fluorine (Supplemental Information, Table S1).

Acknowledgements

We thank the Royal Society for an International Joint Project Grant, the Irish Research Council, Durham University M.Chem programme and UCD Research Demonstrator scheme.

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