Metabolism of fluoroorganic compounds in microorganisms: impacts for the environment and the production of fine chemicals

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Abstract

Incorporation of fluorine into an organic compound can favourably alter its physicochemical properties with respect to biological activity, stability and lipophilicity. Accordingly, this element is found in many pharmaceutical and industrial chemicals. Organofluorine compounds are accepted as substrates by many enzymes, and the interactions of microorganisms with these compounds are of relevance to the environment and the fine chemicals industry. One the one hand the microbial transformation of fluorinated compounds can lead to the generation of toxic compounds that are of environmental concern, yet similar biotransformations can yield difficult-to-synthesise products and intermediates, in particular derivatives of biologically active secondary metabolites. In this paper we review the historical and recent developments of organofluorine biotransformation in microorganisms, and highlight the possibility of using microbes as models of fluorinated drug metabolism in mammals.

Introduction

Although fluorine is the 13th most abundant element in the earth's crust, and the most abundant halogen, it plays a minor role in biology. One of the main reasons for this is that most of the fluorine in the environment is present in an insoluble form (e.g. as calcium fluoride), thus is biologically unavailable. Nevertheless, microorganisms encounter fluorinated compounds as a result of the myriad industrial, agrochemical and pharmaceutical applications that these compounds have (Lewandowski et al. 2006). Substitution of hydrogen for fluorine in an organic compound can affect the compound's stability, lipophilicity and electronic properties, with minimal steric consequences. Although the general perception of fluoroorganic compounds is that they are biologically inert, some biotransformation can occur because of fluorine's similar size to hydrogen and hydroxyl. There is concern over the fate of fluorinated compounds in the environment, most notably perfluorinated compounds, which have been detected in wildlife and humans (Paul et al. 2009). Studying the microbial metabolism of organofluorines has a dual importance: identifying the fluorinated compounds present in the environment as the result of human activities, and in the development of biotechnological processes for the incorporation of fluorine into industrially or pharmaceutically important organic compounds. In this review, both aspects of organofluorine metabolism in microorganisms will be addressed.

Environmental impact: Biodegradation of organofluorine compounds

Fluoroaromatics

Fluorinated aromatic compounds are used in agriculture as pesticides, herbicides and insecticides (e.g. diflubenzuron and tefluthrin), thus microorganisms in soils will encounter them. Understanding the fate of these compounds in the environment is important, since

transformation products might be highly toxic. A common feature of fluorinated agrochemicals is the trifluoromethyl group, in addition to other functional groups (e.g. hydroxyl, nitro), thus the degradation of these compounds in soils is complex (Key et al. 1997). In pure culture, investigations on fluoroaromatic catabolism have predominantly focussed on less chemically complicated compounds such as fluorobenzoates and fluorophenols (Ribbons et al. 1987). Aerobic bacteria can degrade fluoroaromatic compounds, such as fluorobenzene (Carvalho et al. 2006; Iwai et al. 2009), fluorobenzoates (Boersma et al. 2004) and fluorophenols (Boersma et al. 2001), along the well-established aromatic hydrocarbon-degrading pathways. Figure 1 is an overview of the microbial transformations of mono-fluorinated aromatic compounds. Most commonly, oxygenase attack on the fluorinated substrate yields 3- or 4-fluorocatechol, which subsequently undergoes intra-diol cleavage to form 2- or 3-fluoromuconic acid. 3-Fluoromuconic acid can be further catabolised via 3-fluoromuconolactone, to 3-oxoadipate with concomitant fluoride release, whereas 2-fluoromuconic acid is a dead-end metabolite. The position of the fluorine atom on the aromatic ring determines its biodegradability. Benzoate-1,2-dioxygenase attack on 4-fluorobenzoate will yield only 4-fluorocatechol, and subsequently 3-fluoromuconic acid. On the other hand, benzoate-1,2-dioxygenase action on 3-fluorobenzoate can result in 3- and 4-fluorocatechol being formed, leading to 2- and 3-fluoromuconic acid. Dioxygenase attack on 2-fluorobenzoate can result in spontaneous elimination of fluoride ion, if hydroxylation occurs at C-1 and C-2, or the ultimate production of 2-fluoromuconic acid, if the hydroxylation is at C-1 and C-6. Fluorophenols are converted to fluorocatechols by hydroxylases (Bondar et al. 1998; Reinscheid et al. 1996), and to insoluble polymeric substances, via fluoroquinone, by tyrosinase (Battaini et al., 2002). Most recently, Ferreira (2008) discovered an *Arthrobacter* strain that degraded fluorophenol via a monoxygenase to benzoquinone, which is immediately reduced to hydroquinone, a heretofore unknown

pathway. Anaerobic degradation of fluoroaromatic compounds with stoichiometric fluoride release has also been observed (Schennen et al. 1985; Vargas et al. 2000), and very recently Mouttaki et al. (2009) reported the reductive dehalogenation of 3-fluorobenzoate in *Syntrophus aciditrophicus*. These researchers were also able to detect a novel 3-fluorocyclohexadiene intermediate by GC-MS and ^{19}F NMR analyses of culture supernatants. A small number of reports describe investigations on the bacterial transformation of benzoate and catechol substituted with a trifluoromethyl group, which the most widely used fluorinated moiety in pharmaceuticals and agrochemicals (Engesser et al. 1988; Engesser et al. 1990). No strain has yet been shown to grow on these compounds, but co-metabolism is possible via ortho- and meta-cleavage pathways.

Fungal biodegradation of fluorotoluenes, yielding 3-fluorobenzoate, 3-fluoro-4-hydroxybenzoate and 3-fluoroprotocatechuate, and of difluorophenols to corresponding catechols has been demonstrated (Prenafeta-Boldu et al. 2001; Wunderwald et al. 1997). Furthermore, transformation of fluorophenols via fungal chloroperoxidase yields 1,4-benzoquinone (Osborne et al. 2006) and other fluorinated products arising from the fluorophenoxy radical (Murphy 2007b). Extracellular degradation of 2-fluorophenol yielding 3-fluorocatechol, catechol and fluoride ion has been observed in cultures of *Geophyllum striatum* when cultured in medium containing iron, probably as a result of hydroxyl radicals generated by the Fenton reaction (Kramer et al. 2004).

Comparatively less research has been conducted on the microbial transformation of polycyclic fluorinated compounds. One early study investigated the transformation of 1-fluoronaphthalene by the fungus *Cunninghamella elegans* (Cerniglia et al. 1984), resulting in the formation of *trans*-3,4-dihydroxy-3,4-dihydro-1-fluoronaphthalene and *trans*-5,6-dihydroxy-5,6-dihydro-1-fluoronaphthalene; the fluorine atom blocks epoxidation at the fluoro-substituted double bond. In contrast to polychlorinated biphenyls (PCBs), the

biodegradation of fluorobiphenyl has not been thoroughly investigated. Green et al. (1999) identified hydroxylated fluorobiphenyls upon incubation of 4-fluorobiphenyl with several mycorrhizal fungi, and Murphy et al. (2008) demonstrated that 2- and 4-fluorobiphenyl could be used as carbon and energy sources by the PCB-degrading bacterium *Pseudomonas pseudoalcaligenes* KF707, which degraded the fluorinated analogues via the upper biphenyl pathway (Figure 2). The replacement of PCBs by polyhalogenated diphenyl ethers as flame-retardants prompted investigations on the biodegradation of this class of compound, and *Sphingomonas* spp. SS3 and SS33 degraded 4-fluoro- and 4,4'-difluoro-diphenyl ether via dioxygenase attack to fluorophenol and fluorocatechol, which were subsequently mineralised (Schmidt et al. 1993).

Fluorinated aliphatics

The most common fluorinated aliphatic is fluoroacetate, which is produced by some plants and the bacterium *Streptomyces cattleya*, and is used as a rodenticide in some countries. It is a highly toxic compound owing to the *in vivo* lethal synthesis of (2*R*, 3*R*)-2-fluorocitrate, which is an inhibitor of aconitase (Peters et al. 1953) and citrate transport across mitochondrial membranes (Kirsten et al. 1978). Kelly (1965) made the first observation that bacteria could degrade fluoroacetate, and fluoroacetate dehalogenases have been isolated from *Pseudomonas* spp. (Kawasaki et al. 1981; Donnelly and Murphy 2009), *Burkholderia* sp. (Kurihara et al. 2003) and fungi (Walker and Lien 1981). Liu et al. (1998) studied the mechanism of the fluoroacetate dehalogenase from *Delftia acidovorans* (formerly *Moraxella* sp. B), which shares 18 % amino acid sequence identity with the haloalkane dehalogenase in *Xanthobacter autotrophicus*, by incubating the purified enzyme with fluoroacetate in $H_2{}^{18}O$. Mass spectral analysis of the peptide fragments containing suspected active site residues, revealed that Asp105 was enriched with ^{18}O. Thus Liu et al. (1998) proposed that

defluorination of fluoroacetate occurs by nucleophilic attack on the fluoromethyl group by the side-chain carboxylate of Asp105, displacing fluoride and forming an ester, which is subsequently hydrolysed yielding glycolate (Figure 3), and is analogous to the reaction catalysed by the *X. autotrophicus* dehalogenase. Most recently the fluoroacetate dehalogenase from *Burkholderia* sp. strain FA1 has been crystallised (Jitsumori et al. 2009), and the x-ray structure revealed that the enzyme is a member of the α/β superfamily. It was observed that an active site tryptophan residue (Trp150) binds chloride ion in the crystallisation solution, suggesting that this residue is important in halide ion binding. Furthermore, when this residue was mutated the enzyme no longer dehalogenated fluoroacetate, but retained activity towards chloroacetate, thus it was speculated that hydrogen bonding between Trp150 and fluoroacetate is a requirement to lower the activation energy of defluorination. Gregg et al. (1998) transformed the ruminant bacterium *Butyrivibrio fibrisolvens* with a gene coding for fluoroacetate dehalogenase, which, when introduced to sheep, conferred resistance to fluoroacetate toxicity, thus demonstrating a novel way to protect animals who graze in areas where fluoroacetate-producing plants grow. Heffernan et al. (2009) explored the use of a tubular biofilm of *Pseudomonas fluorescens* to degrade fluoroacetate in wastewater, and determined that fluoroacetate was not as efficiently degraded by biofilm cells compared to suspended cells in a chemostat. The differences observed between planktonic and biofilm cells appear to be at least partially a result of oxygen limitation within the biofilm.

Trifluoroacetic acid is present in the environment as a result of the degradation of hydrofluorocarbons and hydrochlorofluorocarbons in the atmosphere (Franklin 1993). Anaerobic biodegradation of this compound has been observed in mixed cultures, with the detection of di- and mono-fluoroacetic acid, and acetic acid, indicating stepwise reductive

defluorination (Visscher et al. 1994; Kim et al. 2000). However, no enzyme has yet been identified that can specifically cleave C-F bonds in the trifluoromethyl group.

Some bacteria, such as the fluorometabolite producer *Streptomyces cattleya*, can stereospecifically degrade the amino acid L-4-fluoroglutamic acid (Donnelly and Murphy 2007). In cell free extract ammonia and fluoride are produced from 4-fluoroglutamic acid, and although the enzyme(s) involved have not been identified, this reaction appears to be gratuitous, since it occurs in other bacteria that are not known to produce fluorinated secondary metabolites. Dave et al. (2003) highlighted the need to develop methods for the resolution of 4-fluoroglutamate stereoisomers, which can be used as enzyme inhibitors, or as synthons in the preparation of fluorinated pharmaceutical compounds, and the ability of some bacteria to degrade L-4-fluoroglutamic acid is a potential biocatalytic method for generating D-4-fluoroglutamic acid from racemic mixtures.

Perfluorinated surfactants, such as perfluorooctane sulfonate and perfluorooctanoic acid, are particularly recalcitrant, and efforts are being made to design surfactants that are more easily biodegraded. One example is 10-(trifluoromethoxy)decane-1-sulfonate, which can be degraded by non-adapted sewage sludge to trifluoromethanol, via desulfonation and β-oxidation (Peschka et al. 2008). Trifluoromethanol is unstable and spontaneously degrades yielding fluoride ion and CO_2.

Importance of fluorine in pharmaceutical compounds

The number of fluorinated drugs is continually increasing, and around a fifth of all drugs have at least one fluorinated substituent, including three of the current top 10 selling medicines: Lipitor, Prevacid and Seretide (Isanbor and O'Hagan 2006). .

The fate of drugs in the organism is modulated depending on the passages of adsorption, distribution, metabolism and excretion (ADME). Intuitively, the ideal drug has to

have the right lipophilicity to be adsorbed, distributed in the organism and reach the target organ, and have a good rate of metabolism to form active but not toxic or reactive metabolites that can be finally easily excreted as more polar compounds. With this challenging preface, fluorine is often considered the favourite substituent to modulate unfavourable pharmacokinetic drug properties. Depending on the position where it is incorporated, it can improve metabolic stability, bioavailability and interactions with the biological target (Purser et al. 2008). The peculiar chemical properties that make fluorine such a popular candidate in drug design have been extensively reviewed (Park et al. 2001). Fluorine is the most electronegative element so the acidity or basicity of proximal functional groups is strongly affected. Its small size makes it, of all substituents, the best to mimic hydrogen in the C-H bond, minimising steric challenges. Often it is used to replace hydroxyl groups since its van der Waals radius (1.35 Å) is between that of oxygen (1.47 Å) and hydrogen (1.2 Å). Carbon-fluorine bonds are one of the strongest bonds in nature; thus substitution in a strategic position can make the drug more resistant to enzyme attack. These attractive physicochemical characteristics result in changes in the lipophilicity (and accordingly in bioavailability) of the molecule, which has consequences for penetration of the cellular membrane and interaction with hydrophobic molecules. The trifluoromethyl group, one of the most lipophilic groups known, is widely present as a replacement for methyl groups in fluorinated drugs that act in delicate organs such as the central nervous system (Smart 1999; Park et al. 2001).

Fluorinated natural products

While halogenated metabolites are relatively common in the microbial world, fluorinated natural products are extremely rare, with only a handful of reports in the literature. A number of reasons have been proposed for this scarcity, including the poor nucleophilicity of the hydrated fluoride ion, the low solubility of most fluoride-containing minerals, and the high

oxidation potential of the fluoride ion, which prevents the formation of F^+ species. The occurrence and biosynthesis of fluorinated natural products has been reviewed recently (Deng et al. 2004; Deng and O'Hagan 2008). Structures of fluorinated microbial metabolites are given in figure 4.

The first fluorinated microbial metabolite to be reported was nucleocidin, an antibiotic nucleoside isolated from *Streptomyces calvus*. While the metabolite was first isolated in 1957 (Thomas et al. 1957), the correct structure was not proposed until 12 years later (Shuman et al. 1969), before being confirmed by total synthesis (Jenkins et al. 1976). While the intriguing structure has attracted attention, numerous attempts to re-isolate nucleocidin from *S. calvus* strains have proved unsuccessful (Maguire et al. 1993).

In 1986, a *Streptomyces cattleya* strain was reported to produce fluoroacetate and fluorothreonine (Sanada et al. 1986). The biosynthesis of fluoroacetate and 4-fluorothreonine in *S. cattleya* has been studied, with the key fluorination step occurring through the nucleophilic attack of the fluoride ion on *S*-adenosyl methionine, catalysed by a fluorinase enzyme (Dong et al. 2004; Zhu et al. 2007). The discovery of the fluorinase has opened the door to the possibility of biological production of medicinally important fluorinated compounds *de novo*. One example of this is the generation of fluorine-18 labelled compounds that could be used for positron emission tomography investigations (Deng et al. 2006).

Precursor-Directed Biosynthesis and Mutasynthesis

While fluorinated natural products are very rare, the syntheses of fluorinated natural product derivatives are increasingly common. In many cases, fluorinated natural product derivatives can be formed using synthetic modifications (Begue and Bonnet-Delpon 2006b; Thomas

2006). However, whether due to structural complexity or instability, synthetic modification is often not possible, and as such an alternative strategy is required.

Precursor-directed biosynthesis is a useful strategy for producing natural product derivatives, in which the growth medium of a producing microbial strain is supplemented with an analogue of the natural precursor, forming corresponding derivatives of the natural product. Often, these derivatives may be inaccessible by synthetic methods. In a wild type strain, the modified precursor competes with the natural precursor, and thus a mixture of products is formed. However, in mutasynthetic studies, a mutant microbial strain is used in which a key biosynthetic gene has been inactivated or modified, which can result in the exclusive production of the modified metabolites. Several general reviews on both precursor-directed biosynthesis and mutasynthesis have been published recently (Weist and Sussmuth 2005; Kennedy 2008). As the fluorine atom is almost isosteric with hydrogen, fluorinated precursors have proved particularly effective and are often accepted as precursors by producing microbial strains. Table 1 lists reported instances of the successful incorporation of fluorinated precursors in precursor-directed biosynthesis and mutasynthesis studies, while Figure 5 displays the structures of selected metabolites.

An early example of a fluorinated precursor being used in such a study was in 1968, when Gorman and co-workers reported the metabolism of 6-fluorotryptophan by a *Pseudomonas aureofaciens* strain, yielding 5′-fluoro-pyrrolnitrin (Gorman et al. 1968). Following up on this initial study, the same group produced additional analogues using 5-fluoro-, 7-fluoro-, and 6-trifluoromethyl-tryptophans as precursors (Hamill et al. 1970). Interestingly, the presence of the trifluoromethyl group prevented both chlorination at C-3′ and oxidation of the C-2′ amino group of pyrrolnitrin. 5′-Fluoro-pyrrolnitrin possessed slightly elevated antifungal activity relative to the parent compound, while the remaining derivatives were less active.

Polyketides are a popular target for precursor-directed biosynthesis studies. In particular, those biosynthesised from aromatic carboxylic acid starter units are good candidates, as substituted aromatic acids are readily available commercially. A common theme in the production of fluorinated polyketide analogues is the use of fluorobenzoic acids in feeding studies. An example of this strategy is the production of fluorinated squalestatin derivatives by two fungal strains. One report describes the incorporation of monofluorinated benzoic acids by an unidentified fungus to yield fluorinated derivatives (Chen et al. 1994). In a second study, using a *Phoma* species, mono- and di-fluorinated benzoic acids, phenylalanines and benzaldehydes were used, yielding mono- and di-fluorinated squalestatins (Cannell et al. 1993). In both cases, the monofluorinated derivatives were reported to possess potent nanomolar activity as inhibitors of squalene synthase, comparable to the parent compound.

Another group of polyketides that have proved fruitful for directed biosynthesis and mutasynthesis studies are the erythromycins. In one study, semi-synthetic erythromycin aglycones were fed to a *Saccharopolyspora erythraea* (*Streptomyces erythraeus*) strain blocked in erythromycin production, leading to the production of 8-fluoroerythromycins (Toscano et al. 1983). These novel analogues possessed comparable antibiotic activity to the parent compounds, but higher levels were present in the serum and tissue (Benoni et al. 1988). A 2005 report described a mutasynthetic study using a mutant strain, in which the natural loading module had been replaced by a wide-specificity module, was used to produce 16-fluoroerythromycin (Goss and Hong 2005). In several recent studies, a two-step process has been used for the production of 15-fluoroerythromycins. Firstly, mutant *Streptomyces coelicolor* strains are used to produce fluorinated erythromycin aglycones using suitable diketide precursors. These aglycones are then converted to the corresponding erythromycin derivatives using mutant *Sa. erythraea* strains with disrupted polyketide synthase systems

(Ward et al. 2007; Desai et al. 2004; Ashley et al. 2006). 15-Fluoroerythromycin A was found to possess similar activity but lower lipophilicity than erythromycin A.

Peptides are another structural class that lend themselves easily to precursor-directed biosynthesis. Many fluorinated amino acid derivatives are commercially available, in particular the aromatic amino acids phenylalanine, tryptophan, and tyrosine. In addition, the biosynthesis of non-ribosomal peptides is relatively well understood, and thus tools are available for mutasynthetic studies. In 1992, Hensens and co-workers (1992) produced a cyclosporine A derivative using fluoroalanine as a precursor: the new metabolite possessed similar *in vitro* immunosuppressant activity to cyclosporin A but was considerably more polar (Patchett et al. 1992).

Fluorinated amino acids may also be incorporated into alkaloids and other nitrogen-containing metabolites. A rebeccamycin-producing strain of *Saccharothrix aerocolonigenes* was reported to produce fluoroindolocarbazoles when the growth medium was supplemented with 5- and 6-fluorotryptophans. The fluorinated metabolites were tested for anti-leukemia activity in mice, revealing 3 to 6-fold greater activity than rebeccamycin itself (Lam et al. 2001). Another notable study utilised an *Aspergillus fumigatus* strain, which when supplemented with fluorinated phenylalanines yielded fluorinated derivatives of synerazol and several other antibiotics. While most of the fluorometabolites possessed similar biological activity to the parent compounds, fluoro-synerazol A was up to ten times as active against selected cancer cell lines (Igarashi et al. 2004).

A particularly interesting example of a mutasynthesis study involving a fluorinated precursor is that of fluorosalinosporamide. Salinosporamide is a chlorinated metabolite from the marine bacterium *Salinospora tropica* that acts as a potent irrreversible proteasome inhibitor (Feling et al. 2003). Intriguingly, the chlorine of salinosporamide A was found to be incorporated via a chlorinase enzyme, SalL, that functioned in an analogous manner to the

fluorinase of *Streptomyces catteleya* (Eustaquio et al. 2008). In a mutasynthetic study, fluorodeoxyadenosine was fed to an *S. tropica* mutant lacking the *SalL* gene, leading to the production of fluorosalinosporamide. Fluorosalinosporamide also acted as a proteasome inhibitor, though unlike salinosporamide A, the binding was reversible (Eustaquio and Moore 2008).

Finally, it should be noted that not only are fluorinated precursors useful for the production of small molecules, but macromolecules as well. Studies have shown that fluorinated fatty acids can be incorporated into the polysaccharide polymer emulsan, produced by *Acinetobacter calcoaceticus*, leading to polymers with improved emulsification properties (Johri et al. 2003). Similarly, fluorinated hydroxylphenoxyalkanoic acids could be used to produce fluorinated polyhydroxyalkanoates (PHAs) in *Pseudomonas putida,* yielding crystalline polymers with higher melting points (Takagi et al. 2004).

In conclusion, precursor-directed biosynthesis of microbial metabolites using fluorinated substrates has proved a highly successful process. Novel derivatives with altered biological and physical properties have been produced, some of which are significantly more active than their parent compounds. Given some of these notable successes, and bearing in mind the increasing availability of fluorinated precursors and development of safer and more selective fluorinating agents, it seems likely that such studies will only become more common in the future.

Fluorinated drug metabolism

The discovery that microorganisms possess cytochrome P450 monooxygenases, the enzymatic systems responsible for xenobiotic detoxification in mammals, initiated studies to investigate the possibility of using microorganisms as models of mammalian metabolism (Smith and Rosazza 1974; Smith and Rosazza 1981). An intensive screening programme has

been conducted to select the most appropriate microorganisms, and it is widely recognised that fungal species of *Cunninghamella* and *Beauvearia*, and Actinomycetes bacteria are the most suitable for the investigation of drug metabolism. Parallels between these selected microorganisms and mammals' phase I and phase II metabolism of a wide number of important classes of drugs has been demonstrated (Casillas et al. 1996; Zhang et al. 1996). However, little attention has been given to the categories of drugs containing fluorine, despite the high probability of encountering fluorine in pharmacological treatments (Begue and Bonnet-Delpon 2006a; Kirk 2006). One notable exception is the fluoroquinolones (FQs), which are synthetic fluorinated antibiotics with broad application in both human and veterinary medicine (Fig. 6). FQs have been widely studied with different varieties of fungal strains to assess both mammalian drug metabolism and environmental risks assessment. It has been reported in several investigations that fungal metabolism of enrofloxacin, sarafloxacin, ciprofloxacin and norfloxacin is similar to human and animal metabolism (Parshikov et al. 2000; Parshikov et al. 2001a; Parshikov et al. 2001b). Predominantly, four typical mammalian metabolites have been isolated from fungal cultures; all products show that the transformations occur at the piperazine ring level. From their results, neither the fluorine atom nor the fluorinated ring are affected during metabolism. Since FQs are introduced into the soil directly from manure of treated livestock, and into drinking water from municipal sewage (Al-Ahmad et al. 1999), the study of their environmental degradation path has become a priority. Common soil microbes have been investigated for their ability to degrade FQs directly in the environment, resulting in a large variety of products (Martens et al. 1996; Al-Ahmad et al. 1999; Wetzstein et al. 1999). Most notably basidiomycetes, such as the brown rot fungus *Gloephyllum striatum*, which produces 87 metabolites from enrofloxacin via hydroxyl radical-based degradation (Wetzstein et al. 1997; Karl et al. 2006). Wetzstein et al. (2006) demonstrated that other basidiomycetes, from agricultural soils and animal waste,

which express laccase- and peroxidase-type activity, generate 48 additional products to those identified in *G. striatum* cultures.

The microbial metabolism of a small number of other fluorinated drugs has been investigated (Xie et al. 2005), and the selected microorganisms transform the parent drug but the fluorine-containing groups are not altered. It is expected that investigations into the microbial catabolism of fluorinated drugs will become more common in the future, partly because of the increasing use of fluorine in pharmaceuticals, but also as a result of accessibility of analytical technology, such as fluorine-19 nuclear magnetic resonance spectroscopy (^{19}F NMR). This technique is ideal for assessing the biotransformation of fluorinated compounds, since it allows the detection of μM concentrations without purification of the fluorinated analytes from culture supernatants (Murphy 2007a). One study reported the application of this technique to the rapid screening of microoganisms involved in drug metabolism (Corcoran et al. 2001), and it is increasingly used to monitor the catabolism of fluorinated xenobiotics.

Concluding remarks

Fluorine's role in medicinal and agricultural chemistry shows no sign of diminishing, and increasingly, biological/enzymatic methods are being sought to replace classical synthetic methods of chemical production. Microorganisms can incorporate simple fluorinated building blocks into complex biologically active metabolites, and synthesise fluorinated compounds *de novo*. Therefore, in the manufacture of fluorinated pharmaceuticals and agrochemicals microorganisms could potentially have an important role. Furthermore, microorganisms may also be used to determine the likely intermediates that arise from mammalian metabolism of fluorinated drugs, reducing the need for animal dosing.

There is a mounting awareness that anthropogenic organofluorine compounds are of serious environmental concern, and their disposal must be carefully monitored. A comprehensive understanding of the biotransformations of organofluorines that are present in the environment is needed to evaluate the effects that such compounds have over time. While there is some understanding of the likely catabolism, the research in this area is particularly lacking, in comparison with organochlorines, and requires significantly more attention. Advances in analytical technology, most importantly ^{19}F NMR, will allow for more convenient identification of fluorometabolites and provide a deeper understanding of the environmental fate of organofluorine compounds.

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Table 1: Fluorinated natural product derivatives formed by directed biosynthesis and mutasynthesis.

Organism	Compound class	Precursor	Biological activity	Citation
Micromonospora sp.	diazepinomycins	fluoroindoles, 5-fluoroanthranilate	antibacterial	(Ratnayake et al. 2009)
Bacillus subtilis	iturins	fluorotyrosine	*n/t*	(Moran et al. 2009)
Salinospora tropica	salinosporamides	5'fluoro-deoxyadenosine	proteasome inhibitor	(Eustaquio and Moore 2008)
Actinosynnema pretiosum	ansamytosins	3-amino-4-fluorobenzoate	anticancer	(Taft et al. 2008)
Streptomyces coelicolor	CDA	tryptophans, CF_3-glutamate	*n/t*	(Powell et al. 2007; Amir-Heidari et al. 2008)
Beauvaria bassiana	beauvericins	3-fluorophenylalanines	cytotoxic, antihaptotactic	(Xu et al. 2007)
Penicillium sp.	communesin	6-fluorotryptophan	*n/t*	(Wigley et al. 2006)
Chondromyces crocatus	chondramides	5-fluorotryptophan	*n/t*	(Rachid et al. 2006)
Aspergillus fumigatus	synerazol, gliotoxin	fluorophenylalanines	antimicrobial, cytotoxic	(Igarashi et al. 2004)
Pseudomonas putida	PHA polymer	fluorophenoxyalkanoic acids	*n/t*	(Takagi et al. 2004)
Sorangium cellulosum	soraphens	fluorocinnamate, fluorophenylalanine	*n/t*	(Hill and Thompson 2003)
Acinetobacter calcoaceticus	emulsan	fluorinated fatty acids	*n/t*	(Johri et al. 2003)
S. hygroscopicus, S. maritimus	enterocin, wailupemycin	fluorobenzoic acids	antimicrobial	(Kawashima et al. 1985a; Kalaitzis et al. 2003)
Amycolatopsis mediterranei	balhimycin	fluorohydroxytyrosines	antibiotic	(Weist et al. 2002)
Saccharothrix aerocolonigenes	indolocarbazoles	fluorotryptophans	anticancer	(Lam et al. 2001)
Streptomyces griseoviridis	rhamnopyranosides	3-fluorobenzoic acid	*n/t*	(Grond et al. 2000)
Poronia piltformis	piliformic acid	8-fluorooctanoic acid	*n/t*	(Culceth et al. 1998)
Streptomyces staurosporeus	tryptamines, indoles	fluorotryptamines	*n/t*	(Yang and Cordell 1997a; Yang and Cordell 1997b)
Aspergillus parasiticus	norsolorinic acid	6-fluorohexanoic acid	*n/t*	(McKeown et al. 1996)
Aureobasidium pullulans	aureobasidins	fluorophenylalanines	antifungal	(Takesako et al. 1996)
Streptomyces pactum	pactamycin	3-amino-5-fluorobenzoic acid	antimicrobial, cytox.	(Adams and Rinehart 1994)
Phoma sp., unidentified fungus	squalestatins	fluorobenzoic acids	squalene synthase	(Cannell et al. 1993; Chen et al. 1994)
Tolypocladium inflatum	cyclosporins	fluoroalanine	immunosuppressant	(Hensens et al. 1992)
Pseudomonas aeruginosa	pyochelin	5-fluorosalicylic acid	iron transport	(Ankenbauer et al. 1991)
Aspergillus alliaceus	asperlicin	fluorotryptophan, fluoroleucine	*n/t*	(Houck et al. 1988)
S. griseochromogenes	blasticidin S	5-fluorocytosine	antimicrobial	(Kawashima et al. 1987)
Streptomyces parvullus	actinomycins	fluorotryptophan	anticancer	(Kawashima et al. 1985b)
S. coelicolor, Sa. erythraea	erythromycins	erythronolides, diketides	antimicrobial	(Toscano et al. 1983; Goss and Hong 2005; Ashley et al. 2006; Ward et al. 2007)
Streptomyces cacaoi	polyoxins	5-fluorouracil	antimicrobial	(Isono et al. 1973)
Pseudomonas aureofaciens	pyrrolnitrin	fluorotryptophans	antifungal	(Gorman et al. 1968; Hamill et al. 1970)
Penicillium notatum	penicillins	fluorobenzoic acids	antimicrobial	(Behrens et al. 1948; Clarke et al. 1949)

n/t: not tested

Figure legends

Figure 1. Overview of catabolism of fluorinated aromatic compounds

Figure 2. Biodegradation of 4-fluorobiphenyl in *Pseudomonas pseudoalcaligenes* KF707. Enzymes: BphA, biphenyl-2,3-dioxygenase; BphB, 2,3-dihydro-2,3-dihydroxybiphenyl dehydrogenase; BphC, 2,3-dihydroxybiphenyl 1,2-dioxygenase; BphD, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase

Figure 3. Mechanism of fluoroacatate dehalogenase

Figure 4. Structure of bacterial fluorinated natural products

Figure 5. Fluorinated natural product derivatives formed via precursor-directed biosynthesis and mutasynthesis

Figure 6. The general structure of fluoroquinolone antibiotics.

Figure 1

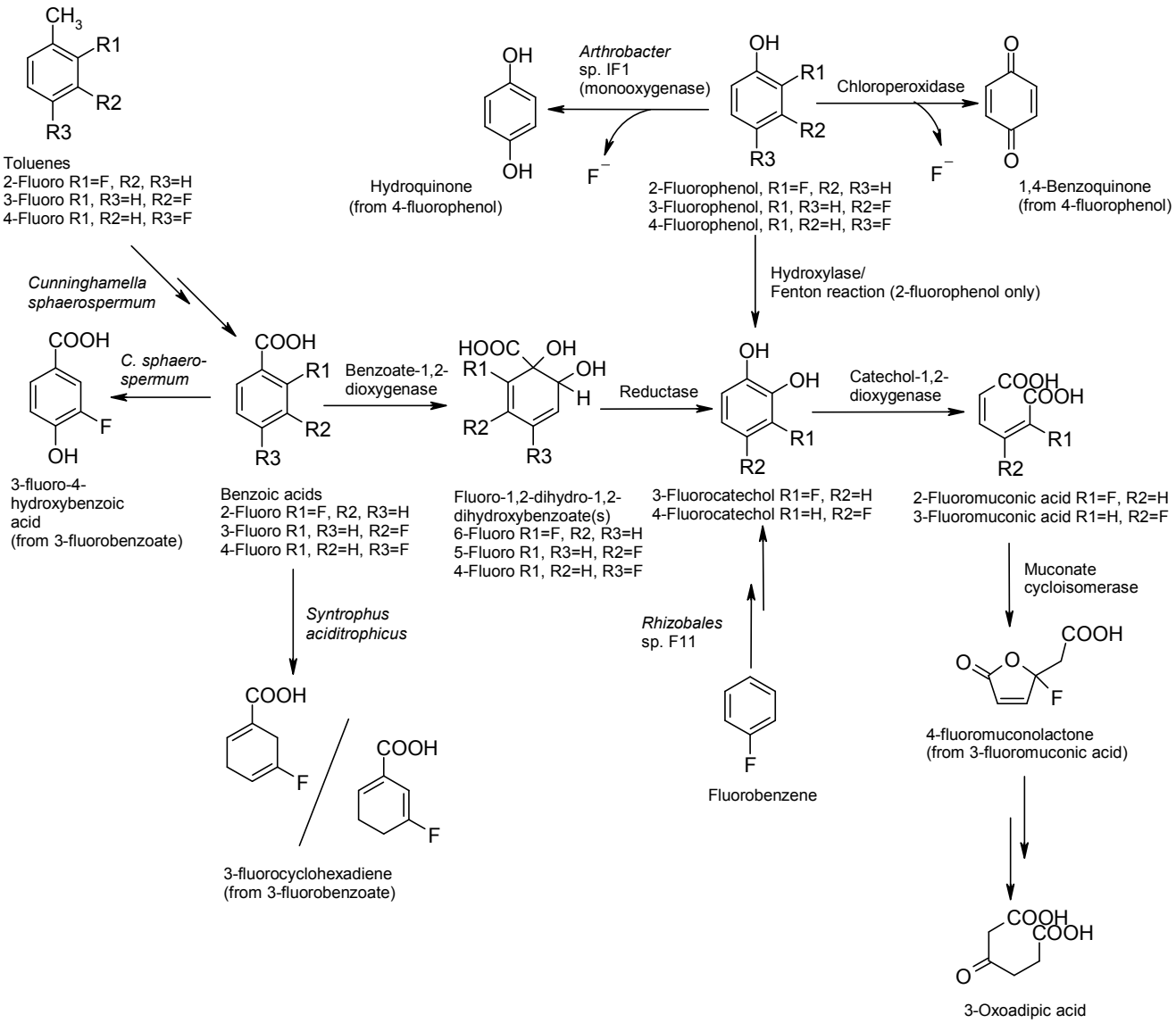

Toluenes
2-Fluoro R1=F, R2, R3=H
3-Fluoro R1, R3=H, R2=F
4-Fluoro R1, R2=H, R3=F
Arthrobacter
sp. IF1
(monooxygenase)
Hydroquinone
(from 4-fluorophenol)
2-Fluorophenol, R1=F, R2, R3=H
3-Fluorophenol, R1, R3=H, R2=F
4-Fluorophenol, R1, R2=H, R3=F
Chloroperoxidase
1,4-Benzoquinone
(from 4-fluorophenol)
Cunninghamella
sphaerospermum
Hydroxylase/
Fenton reaction (2-fluorophenol only)
C. sphaero-
spermum
Benzoate-1,2-
dioxygenase
Reductase
Catechol-1,2-
dioxygenase
3-fluoro-4-
hydroxybenzoic
acid
(from 3-fluorobenzoate)
Benzoic acids
2-Fluoro R1=F, R2, R3=H
3-Fluoro R1, R3=H, R2=F
4-Fluoro R1, R2=H, R3=F
Fluoro-1,2-dihydro-1,2-
dihydroxybenzoate(s)
6-Fluoro R1=F, R2, R3=H
5-Fluoro R1, R3=H, R2=F
4-Fluoro R1, R2=H, R3=F
3-Fluorocatechol R1=F, R2=H
4-Fluorocatechol R1=H, R2=F
2-Fluoromuconic acid R1=F, R2=H
3-Fluoromuconic acid R1=H, R2=F
Muconate
cycloisomerase
Syntrophus
aciditrophicus
Rhizobales
sp. F11
Fluorobenzene
4-fluoromuconolactone
(from 3-fluoromuconic acid)
3-fluorocyclohexadiene
(from 3-fluorobenzoate)
3-Oxoadipic acid

Figure 2

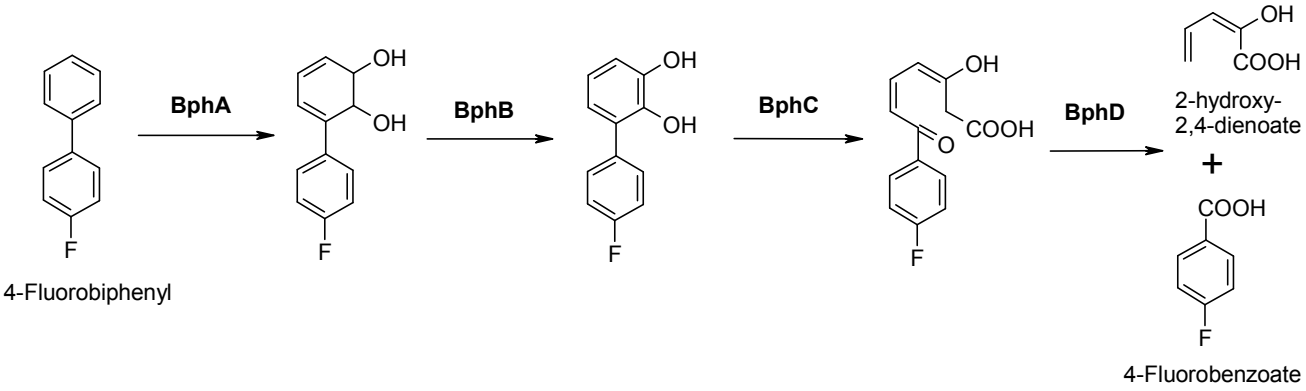


Figure 3

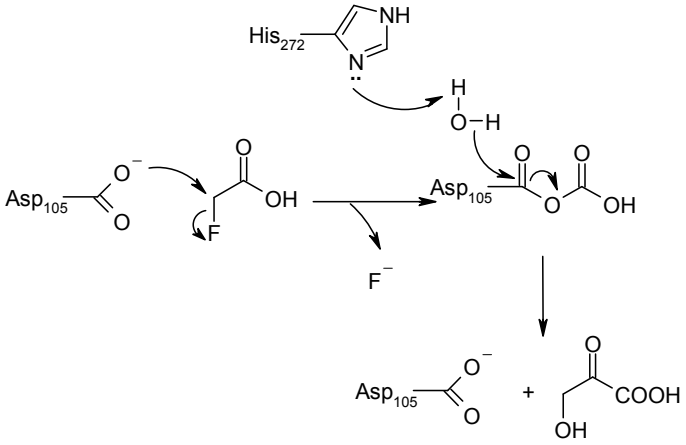


Figure 4

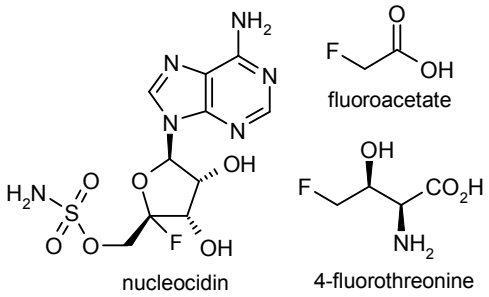


Figure 5

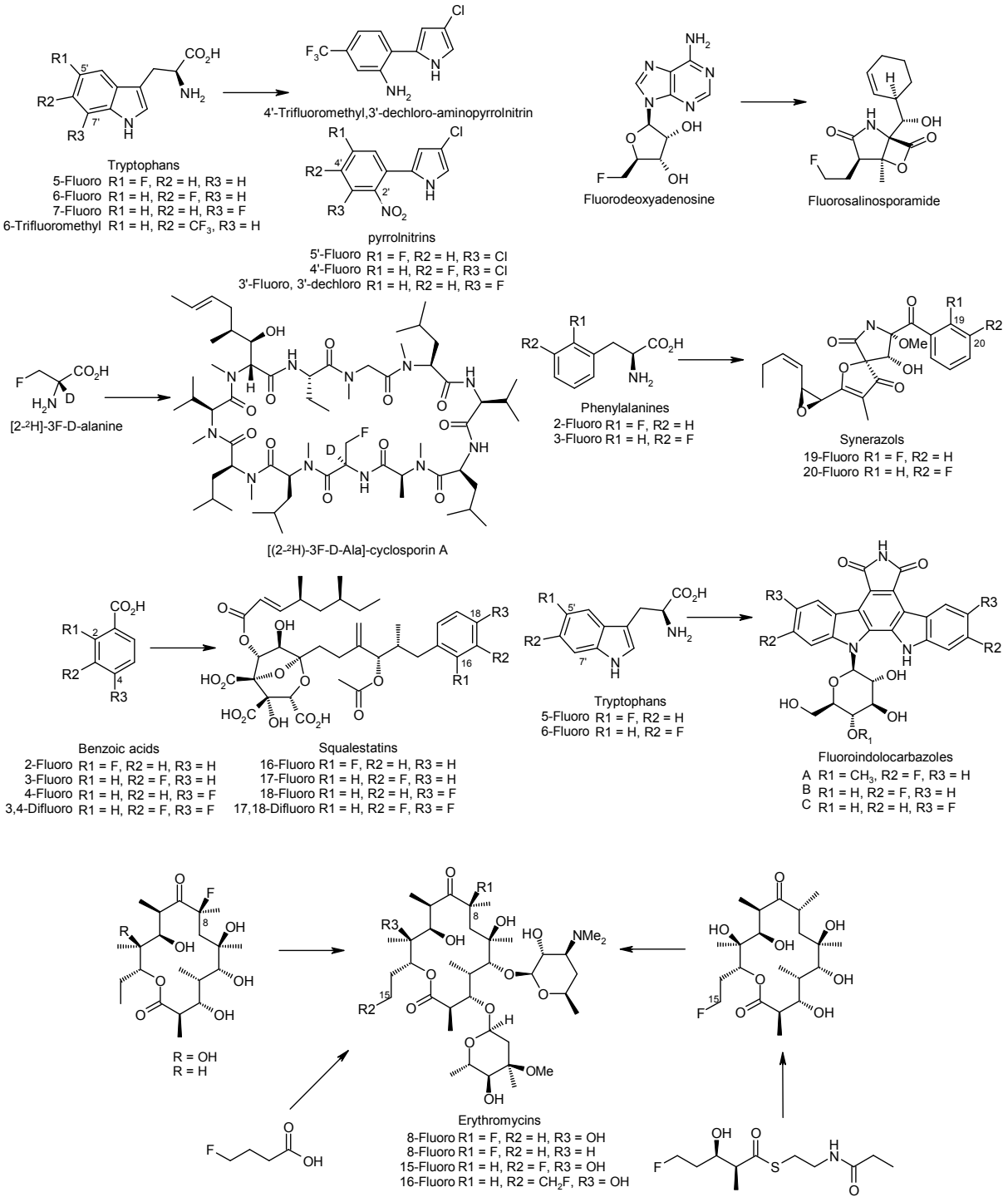
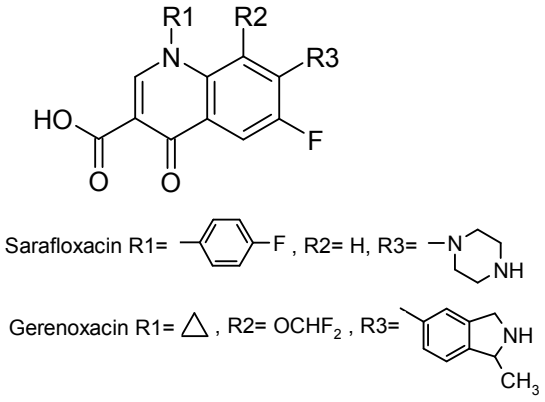

Tryptophans
5-Fluoro R1 = F, R2 = H, R3 = H
6-Fluoro R1 = H, R2 = F, R3 = H
7-Fluoro R1 = H, R2 = H, R3 = F
6-Trifluoromethyl R1 = H, R2 = CF3, R3 = H
4'-Trifluoromethyl,3'-dechloro-aminopyrrolnitrin
pyrrolnitrins
5'-Fluoro R1 = F, R2 = H, R3 = Cl
4'-Fluoro R1 = H, R2 = F, R3 = Cl
3'-Fluoro, 3'-dechloro R1 = H, R2 = H, R3 = F
Fluorodeoxyadenosine
Fluorosalinosporamide
[2-2H]-3F-D-alanine
[(2-2H)-3F-D-Ala]-cyclosporin A
Phenylalanines
2-Fluoro R1 = F, R2 = H
3-Fluoro R1 = H, R2 = F
Synerazols
19-Fluoro R1 = F, R2 = H
20-Fluoro R1 = H, R2 = F
Benzoic acids
2-Fluoro R1 = F, R2 = H, R3 = H
3-Fluoro R1 = H, R2 = F, R3 = H
4-Fluoro R1 = H, R2 = H, R3 = F
3,4-Difluoro R1 = H, R2 = F, R3 = F
Squalestatins
16-Fluoro R1 = F, R2 = H, R3 = H
17-Fluoro R1 = H, R2 = F, R3 = H
18-Fluoro R1 = H, R2 = H, R3 = F
17,18-Difluoro R1 = H, R2 = F, R3 = F
Tryptophans
5-Fluoro R1 = F, R2 = H
6-Fluoro R1 = H, R2 = F
Fluoroindolocarbazoles
A R1 = CH3, R2 = F, R3 = H
B R1 = H, R2 = F, R3 = H
C R1 = H, R2 = H, R3 = F
R = OH
R = H
Erythromycins
8-Fluoro R1 = F, R2 = H, R3 = OH
8-Fluoro R1 = F, R2 = H, R3 = H
15-Fluoro R1 = H, R2 = F, R3 = OH
16-Fluoro R1 = H, R2 = CH2F, R3 = OH

Figure 6.


R1
R2
N
R3
HO
F
O
O
Sarafloxacin R1=
F , R2= H, R3=
N
NH
Gerenoxacin R1=
, R2= OCHF2 , R3=
NH
CH3