**Abstract**

*Introduction*: Fluorine’s unique physicochemical properties make it a key element for incorporation into pharmacologically active compounds. Its presence in a drug can alter a number of characteristics that affect ADME, which has prompted efforts at improving synthetic fluorination procedures.

*Areas covered*: This review describes the influence of fluorine on attributes such as potency, lipophilicity, metabolic stability and bioavailablility and how the effects observed are related to the physicochemical characteristics of the element. Examples of more recently employed synthetic methods for introduction of fluorine into drug leads are detailed and the potential for using biological systems for fluorinated drug production is discussed.

*Expert opinion*: The synthetic procedures for carbon-fluorine bond formation largely still rely on decades-old technology for the manufacturing scale and new reagents and methods are required to meet the demands for the preparation of structurally more complex drugs. The improvement of in vitro and computational methods should make fluorinated drug design more efficient and place less emphasis on approaches such as fluorine scanning and animal studies. The introduction of new fluorinated drugs, and in particular those that have novel fluorinated functional groups, should be accompanied by rigorous environmental assessment to determine the nature of transformation products that may cause ecological damage.

**Article highlights**

* Approximately 20 % of currently available drugs are fluorinated
* Fluorine has unique physicochemical properties and can be introduced into drug systems in place of H or OH with minor steric, but potentially major electronic, consequences.
* Large-scale synthesis of fluorinated drugs relies on F2 chemistry, but more specific and easier-to-handle reagents have been and continue to be developed to enable bench-scale fluorinations.
* Microorganisms play a significant role in the production of fluorinated compounds and the development of new fluorinated drugs through identification of metabolically labile sites on lead compounds.
* Metabolism of fluorinated drugs can lead to toxic compounds, for example fluoroacetate. The catabolic products of fluorinated drug metabolism are not only relevant for patients, but also the environment.

Keywords: Biotransformation, fluorinating reagent, organofluorine, synthesis

**1. Introduction**

Ever since the observation that a fluoro-corticosteroid possessed significant enhanced biological activity compared to the corresponding non-fluorinated precursor 1, an ever increasing number of commercially important pharmaceuticals that rely on the presence of fluorine atoms within their structures for enhanced drug efficacy have been introduced on to the global marketplace 2. Indeed, it is estimated that around 30% of all new pharmaceuticals have fluorinated sub-units. Some examples of important fluorinated drugs are given in Figure 1 and their application to a wide range of therapeutic areas provides an indication of the extensive role fluorinated systems play in modern drug development programmes.

Analysis of the structures of fluorinated pharmaceuticals approved for use by the FDA since 1950 show that fluorine is most usually part of a fluoro- or trifluoromethyl-aromatic sub-unit 3. However, this situation is changing and more structurally sophisticated fluorinated units, such as the fluorocarbohydrate unit in Sofosbuvir, are becoming increasingly important. Consequently, methods for the synthesis of fluorinated systems is high on the agenda of many synthetic chemists within the life science industries and the metabolism of fluorinated systems is an area of increased study due to regulations that require the establishment of metabolic fates of new drug like chemical entities.

**2.0 The effects of fluorine in drug systems**

Fluorine is present in approximately 20 % of currently available pharmaceuticals, including three of the top 10 selling drugs of 2011, atorvastatin, rosuvastatin and fluticasone 4. The improved pharmacological properties of fluorinated drug molecules compared with their non-fluorinated derivatives are as a consequence of fluorine’s physicochemical characteristics. In particular, fluorine’s small van der Waals radius (1.35 Å), which is between that of hydrogen (1.20 Å) and the oxygen of hydroxyl (1.40 Å), coupled with its electronegativity (4 on the Pauling scale; it is the most electronegative element), means that fluorine can be incorporated into a drug molecule with little steric effect, but considerable electronic consequences 5. The effects of fluorine on drugs and drug-like molecules have been comprehensively reviewed in recent years 6, so only a brief overview is presented below.

2.1 Antimetabolites

One of the earliest marketed fluorinated drugs is 5-fluorouracil **1**, which is an antimetabolite that is widely used in cancer treatment. The drug enters the cell via the uracil transporter and is biotransformed to several metabolites, one of which is fluorodeoxyuridine monophosphate (FdUMP). Fluorine’s small size and high redox potential permits this metabolite forms a stable ternary complex with the enzyme thymidylate synthase and the co-substrate 5,10-methylene tetrahydrofolate, thereby blocking the methylation of the native substrate deoxyuridine monophosphate (dUMP) 7. Consequently, the cell can no longer manufacture deoxythymidine monophosphate and by extension thymine. Cancer cells that multiply faster than healthy cells are disproportionately affected by the drug.

5FU is cardiotoxic and the possible reason for this is as a consequence of the metabolism of the drug to a toxic metabolite. Malet-Martino et al. 8 analysed the urine of patients treated with 5FU by fluorine-19 nuclear magnetic resonance spectroscopy (F-19 NMR) and determined the presence, among other metabolites, of fluoroacetate **2**. This is a known toxin and is produced naturally (Section 4.2). *In vivo*, fluoroacetate, as fluoroacetyl coenzyme A, is converted to (2*R*, 3*R*)-2-fluorocitrate **3** via the actions of citrate synthase, a process which was termed ‘lethal synthesis’ by its discoverers 9. Fluorocitrate is an inhibitor of the citric acid cycle enzyme aconitase 10, and an irreversible inhibitor of the mitochondrial citrate transport protein 11.

2.2 Intermolecular effects

Owing to its electronegativity, fluorine substituents on ligands prefer electropositive regions in proteins such as peptide bonds and side chain amides. C-F⋅⋅⋅⋅H-N, C-F⋅⋅⋅⋅C=O, C-F⋅⋅⋅⋅H-Cα interactions have been observed and implicated in increased binding of fluorinated compounds to receptors and enzymes. For example, atorvastatin inhibits the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase involved in cholesterol biosynthesis and comparison of the 4-fluorophenyl derivative was determined to be the most potent of several tested (hydroxyl, hydrogen, methoxy) owing to a favourable polar interaction between fluorine and the guanidinium side chain of Arg590 12. A comprehensive review of fluorinated ligand-protein interactions was presented by Muller et al. 13.

Trifluoromethylketone (TFMK) substrate analogues are effective inhibitors of metal-dependent hydrolases such as carboxypeptidase A and histone deacetylases (HDACs). In these analogues, owing to the electrophilicity of the CF3, the ketone is hydrated in aqueous solution forming the *gem*-diol and mimics the tetrahedral transition state of the hydrolytic reaction. The TFMK (**4**), which is 70-90 % hydrated in solution is a strong inhibitor of HDAC4cd (protein IC50= 367 nM) and structural studies 14 demonstrated that the catalytic zinc is co-ordinated by the two oxygens of the hemiketal.

2.3 Lipophilicity

Introducing fluorine into an aromatic compound increases lipophilicity 15, for instance, the log P value of fluorobenzene (2.27) is greater than that for benzene (2.13); trifluoromethyl substitution results in an even greater increase in lipophilicity. Many drugs have fluorophenyl and trifluoromethyl phenyl groups in their structures and the increased lipophilicity may enhance partitioning into cell membranes thereby increasing the effective intracellular concentration, or lead to improved binding to the hydrophobic binding pocket of the target protein. Gerebtzoff et al. 16 quantified the increase in hydrophobicity upon replacement of hydrogen by trifluoromethyl in promazine, perazine and perphenazine drugs: the free energy of partitioning into the lipid membrane increased by approximately ΔGlw=-4.5 kJ/mol and the permeability coefficient increased by a factor of ~9. Interestingly, fluorine substitution in aliphatic compounds is less predictable, for example, terminal fluorination of alkanes results in a decrease in lipophilicity, whereas trifluoroethanol is more lipophilic than ethanol.

2.4 Acidity

In organofluorine compounds the pKa of proximal functional groups is influenced by fluorine’s electronegativity, thus the pKa of acetic acid and trifluoroacetic acid are 4.76 and 0.52, respectively. In drug molecules, fluorination can affect receptor-ligand or enzyme-inhibitor binding, and bioavailability, which is the proportion of drug reaching the circulatory system. For example, improved oral bioavailability of 2-phenyl-3-piperidylindoles (h5HT2A receptor agonists) was achieved by reducing the pKa of the basic nitrogen by fluorinating the piperidine ring 17. The inductive effect of fluorine in an organic compound will destabilise the formation of a carbocation on an adjacent site, and this property can been exploited in the design of orally active drugs that would otherwise be hydrolysed by stomach acid.

2.5 Conformational effects

Fluorine incorporation into drug compounds can stabilise the most active conformations thus improving potency. Manoharan et al. 18 prepared small interfering RNA (siRNA) to inhibit expression of factor VII, in which the pyrimidine bases contained 2’-fluoro substituents. Compared with the unmodified siRNA the fluorinated form was more stable in serum, did not elicit an immune response in vitro and was twice as active in vivo. The electronegativity of fluorine ensures the sugar is in the RNA-compatible *endo* conformation; however, it is important to stress that the beneficial effects of fluorine are probably a consequence of several features, including small size, ensuring no steric interference when binding to the RISC (RNA-induced silencing complex) protein, and absence of water in the minor groove, which may make binding more thermodynamically favourable.

2.6 Metabolic stability

The C-F bond is highly stable, for example, the bond dissociation energy of the C-F bond in fluoromethane is 115 kcal mol-1, which is greater than C-H in methane (105 kcal mol-1) 19. Consequently, the purpose of fluorine incorporation into drug molecules in place of hydrogen is often to slow or prevent metabolic attack, by increasing resistance to cytochrome P450 (CYP)-mediated oxidation, thereby prolonging the pharmacological effects of the drug. A classic example of this is the development of the cholesterol transport inhibitor Ezetimibe **5**, in which sites that were oxidatively attacked, resulting in reduced efficacy, were selectively fluorinated 20. Blocking metabolism of particular sites by fluorine can also improve drug safety, for example, in the evolution of safer flurane anaesthetics. The previously employed methoxyflurane **6** is readily metabolised and is nephrotoxic, whereas more the more highly fluorinated desflurane **7** is barely metabolised and has no toxic side effects 21.

In drug development, identifying the sites of oxidative attack can be complicated and labour-intensive, particularly if in vivo studies are employed. Increasingly in vitro (e.g. microsomes) and in silico (e.g. MetaSite) methods are used to determine the metabolically-labile sites of a drug candidate and such methods have been shown to yield equivalent results 22. Bright et al. 23 developed a convenient chemical-microbiological method for identifying metabolically labile sites on pharmaceutically important compounds (Figure 2). Initial experiments with non-fluorinated biphenyl carboxylic acids as model drug leads that had been incubated with the fungus *Cunninghamella elegans*, which is a mimic of mammalian drug metabolism owing to the presence of CYPs, revealed the site of oxidative attack. This information enabled rational synthesis of fluorinated biphenyl carboxylic acid via Suzuki–Miyaura coupling reactions, which could then be added to *C. elegans* cultures to assess its metabolic stability. Hydroxylation in the 4’ position was observed in the initial experiments, thus this site was blocked by fluorination in the synthetic step; subsequent incubation with the fungus revealed that the 4’-fluoro derivative was metabolically stable. The method was then applied to the drug flurbiprofen, for which the site of oxidative attack had already been established; 4’fluoro-flurbiprofen was synthesised and incubation with *C. elegans* demonstrated that this was completely stabilised.

**2.7 Metabolic defluorination**

Fluoride is beneficial for dental health in low concentrations (0.8-1.2 mg/l in drinking water), but at higher concentrations (>1.5 mg/l), fluoride can be toxic and excess fluoride intake over a prolonged period my result in fluorosis 24. Whilst the main source is drinking water, fluorinated drugs are also a potential source of fluoride, particularly those that are used to treat chronic conditions. Although the metabolic stabilisation of a drug through the introduction of fluorine is well established, there are circumstances where CYP-catalysed defluorination have been observed. For example, when the pentafluorophenylethylamine derivative **8**, designed as a dipeptidyl peptidase IV inhibitor, was incubated with rat liver hepatocytes and liver microsomes, metabolically activated glutathione (GSH) conjugates were detected by LC-MS 25. Inclusion of the CYP3A inhibitor troleandomycin confirmed the role of CYP in the formation of the metabolites. It was suggested that the degree of fluorination resulted in reduced electron density in the aromatic ring making it susceptible to direct nucleophilic attack by GSH. CYP-catalysed oxidation of the pentafluorophenyl ring, which could potentially occur before or after conjugation with GSH, was proposed to proceed via arene oxide, quinone and/or quinoneimine intermediates.

Elevated fluoride levels were measured in the plasma of transplant patients who were receiving voriconasole for at least 6 months post-transplant; the elevated fluoride levels were directly responsible for periostitis 26. Inter-individual CYP activity is thought to play a role in the observed defluorination, in a similar fashion to the relationship between CYP2E1 activity and elevated plasma fluoride concentrations in patients receiving the highly fluorinated anaesthetic sevoflurane 27, 28.

**3.0 Synthesis of fluorinated pharmaceuticals**

Since there are only a handful of naturally occurring fluorinated organic systems available, the synthesis of carbon-fluorine bonds and fluorinated functional groups has been a continuously active field of research since the 1920’s. Now, a wide range of fluoroaromatic derivatives are commercially available for both drug discovery and manufacturing programmes and, in general, are prepared on a large scale using anhydrous hydrogen fluoride (aHF) by well-established Balz-Schiemann chemistry 29followed by appropriate functional group aromatic chemistry (Figure 3). Similarly, many trifluoromethyl-aromatic building blocks are synthesised on a very large scale by halogen exchange reactions using aHF as the fluorinating agent 30and, again, many trifluoromethylated arene derivatives are available.

Whilst aHF is routinely used by industry for the synthesis of fluoro and trifluoromethyl-aromatic derivatives, numerous fluorinating agents have been developed for the discovery scale including SelectfluorTM, an electrophilic fluorinating agent of the N-F class which, despite its relative cost is used in the production of various anti-inflammatory steroid drugs. However, there remains a requirement for inexpensive production of novel fluorinated building blocks for incorporation into drug systems. Fluorine gas, long considered to be too reactive for useful preparative synthesis, is an inexpensive reagent that is used in the manufacture of 5-fluorouracil (anti-cancer) and Voriconazole (Pfizer, anti-fungal) on the multi-tonne scale 31. Recently, fluorine has been used for the synthesis of various newly available aryl sulfur pentafluoride building blocks 32, providing an example of how medicinal chemistry programmes are exploring the effects of novel fluorinated functionalities on drug efficacy and the use of previously unused fluorinating reagents (Figure 4). The pentafluorosulfanyl group has physicochemical properties (chemical/thermal stability, electronegativity, lipophilicity) similar to the trifluoromethyl group, although the steric demand of these two groups is different: -CF3 is an isostere of ethyl, whereas -SF5 is similar to tert-butyl 33. The synthetic methods for the production of aryl-SF5 methods have improved, in particular, access to SF5-nitrobenzene as a key synthon 34, which has enabled the incorporation of the functional group into drug molecules. More recently, an economical two step method for the production of aryl-SF5 compounds was developed 35 starting with diaryl disulfides or aryl thiols treated with chlorine in the presence of potassium fluoride. The resulting arylsulfur chlorotetrafluoride is then treated with a fluoride source such as ZnF2. Welch and Lim 36 synthesised the pentafluoroanalogue of the SSRI fluoxetine **9** and measured the inhibition of 5-hydroxytrypamine (5HT) receptor binding, demonstrating that 10 µM resulted in over 70 % inhibition of 5HT 1e, 2a, 2b and 2c. The same researchers also synthesised the SF5 analogue of the appetite suppressant fenfluramine **10** and observed increased 5HT receptor binding compared with the parent drug. Wipf and colleagues 37, 38, 39 synthesised 6-, 7- and 8-SF5 analogues of the antimalarial drug mefloquine **11** that is used to treat strains of *Plasmodium falciparum* that are resistant to chloroquine. Mefloquine has severe side-effects, which are attributed to the drug’s ability to cross the blood-brain barrier and accumulate in the central nervous system, thus derivatives of the drug that do not cross the blood brain barrier to the same extent would be very useful.

The development of new fluorinating reagents, for instance, 4-*tert*-butyl-2,6-dimethyl phenylsulfur trifluoride (Fluolead TM), which is a deoxofluorinating reagent that is more thermally stable than DAST and displays versatile fluorination capabilities 40, is an active area of research. In essence, the use of non-conventional fluorinating agents, allow the preparation of many fluorinated architectures where fluorine atoms are located in increasingly ‘difficult’ sites and novel fluorinated functional groups which continue to drive and meet demand from medicinal chemistry and manufacturing campaigns.

**4.0 Biological production of fluorinated drugs**

4.1 Precursor-directed biosynthesis

Naturally produced compounds, that are themselves used as drugs or are valuable lead compounds, are challenging substrates for synthetic fluorination with the currently available reagents. Nevertheless, it is possible to employ fluorinated analogues of natural product building blocks, which are added to the culture medium. Fluorine’s small size enables the unnatural precursors to be transformed by the enzymes in the biosynthetic pathway, resulting in the production of a fluorinated natural product. This approach has been demonstrated with the lipopeptide antibiotics iturin, surfactin and fengycin that are produced by *Bacillus* spp.; 3-fluoro-L-tyrosine is incorporated into iturin and fengycin in place of tyrosine 41, 42, whereas trifluorovaline can be incorporated in place of L-valine in surfactin biosynthesis 43 (Figure 5).

Salinosporamide A **12** is a polyketide produced by the marine bacterium *Salinospora tropica*, and is currently in stage III trials as an anticancer compound. Knockout mutants of *S. tropica* were able to produce fluorosalinosporamide A **13** from the fluorinated precursor 5’fluoro-5’-deoxyadenosine **14**, which is an analogue of the natural 5’-chloro intermediate **15** 44. In vitro assay revealed that the new fluorinated derivative was not as potent a proteasome inhibitor as salinsosporamide A. Compared with chlorine, fluorine is a relatively poor leaving group, and the crystal structures of bound salinosporamide A and fluorosalinsporamide revealed that the duration of inhibition of the 20S proteasome was determined by the rate of elimination of chlorine/fluorine to form a stable cyclic ether 45, 46. This is a good example of the potential of fluorine incorporation to ‘tune’ the biological activity of a drug.

4.2 Enzymatic C-F bond formation

In contrast to the number of synthetic bioactives that are fluorinated, there are very few natural products that incorporate the element. The reasons for this are a consequence of the unique properties of fluorine: i) the small fluoride ion is heavily solvated in water, thus is a weak nucleophile, ii) the high redox potential of fluoride ion makes the enzymatic oxidation of fluoride via haloperoxidase or FADH2-dependent halogenase thermodynamically unfeasible and iii) despite being highly abundant, most fluorine is biologically unavailable as it is locked into insoluble minerals such as fluorspar (CaF2). Nevertheless, fluorinated natural products are known, and the most common is the toxic compound fluoroacetate **2**, which is produced by plants such as *Dichapetalum chymosum* and a small number of bacteria. The fluorinase enzyme, responsible for C-F bond formation, was first identified in *Streptomyces cattleya* 47, and catalyses a nucleophilic displacement of methionine by fluoride on the substrate *S*-adenosyl methionine. The improved access to bacterial genome sequences has enabled the discovery of other fluorinase homologues 48, 49. Whilst synthetic methods of fluorination require harsh conditions, the fluorinase functions in aqueous solutions under mild conditions of temperature, pressure and pH. However, one drawback of the fluorinase is its narrow substrate specificity, which limits its application in fluorinated drug development. Nevertheless, it is possible to generate clinically important compounds using the enzyme, for example, it has been employed in the production of F-18 labelled compounds for positron emission tomography (PET), obviating the need for ion exchange chromatography to obtain dry [18F] fluoride from [18O] water. Furthermore, access to the fluorinase gene opens the possibility of biological production of fluorinated analogues of antibiotics and other natural products from fluoride ion 50, 51.

4.3 Semi-synthetic incorporation of fluorine

A combination of enzymatic (cytochrome P450) hydroxylation and deoxofluorination with DAST was employed by Rentmeister et al. 52 to generate regioselectively fluorinated derivatives of known natural products and drug compounds. Mono- and di-fluorinated derivatives of Ibuprofen methyl ester were generated by sequential hydroxylation/deoxofluorination reactions on the isobutyl side chain (Figure 6). The fluorinated had a marked effect on the membrane permeability of the molecules compared to the non-fluorinated parent compound.

**5. Conclusion**

Fluorine is a significant element in medicinal chemistry and drug design owing to its unique physicochemical properties that result in major electronic effects with minor steric penalty when it is incorporated into organic compounds in place of hydrogen or hydroxyl. The presence of fluorine in a drug can alter aspects such as receptor- and active-site binding, lipophilicity, bioavailability and metabolic stability. Improvement in a drug’s activity is often a combination of these factors, for example, the C6-fluoro in fluoroquinolones is responsible for enhanced activity compared with the non-fluorinated analogues and the effect is a result of both increased DNA gyrase inhibition and cell penetration 53.

The synthesis of carbon-fluorine bonds has become more amenable as new fluorinating reagents, such as Selectfluor™ and DAST along with safe methods for handling fluorine gas, are developed, enabling researchers to make organofluorine compounds of increased complexity. The relatively recent discovery of fluorinase enzymes has opened the door to biocatalytic production of carbon-fluorine bonds. Furthermore, the ability of enzymes to employ fluorinated analogues as substrates can be exploited to make complex fluorinated natural products with enhanced medicinal properties.

**6. Expert opinion**

The methods of regioselectively incorporating fluorine into pharmaceutically important compounds are steadily improving, but further reagents are required to be developed to meet the needs of increasingly precise strategic drug design. Most recently, Halperin et al. 54 developed a method for direct fluorination of unactivated C-H bonds with a decatungstate photocatalyst, which abstracts hydrogen, plus the fluorinating reagent *N*-fluorobenzenesulfonimide, thereby providing relatively easy access to previously difficult-to-synthesise acyl fluorides and fluorinated amino acids. The continued development of protocols for reactions with fluorine gas, such as continuous flow reactors 55, will further enable direct fluorination of a wider range of suitable substrates on the manufacturing scale and subsequent reaction without the need to purify potentially unstable intermediates. Re-evaluation of the conditions under which previously discovered fluorinating reagents are used, e.g. Langlois reagent 56, may also provide methods for convenient, large-scale fluorinations. Additionally, the combination of chemistry and biology has led to significant developments in fluorinated drug development, from identification of sites for strategic fluorination to enabling biocatalytic production of pharmaceutically relevant organofluorine compounds. This association is highly likely to yield further methodological improvements resulting in more efficient production of drugs in which fluorine is selectively incorporated. To ensure the continued innovation in this area one of the most important factors is the high level training of fluorine chemists and biochemists, which is the central aim of the FLUOR21 Marie Curie Network, funded by the European Commission.

The CYP-catalysed metabolism of fluorinated drugs can be inhibited by the presence of the fluorine atom and the strength of the C-F bond. However, fluorine can be displaced, e.g. by nucleophilic attack via an active site thiol, releasing fluoride ion, which can be toxic at high concentrations. Owing to fluorine’s size, fluorinated drugs may be metabolised via intracellular catabolic pathways leading to toxic metabolites, such as fluoroacetate. In addition these reactions may occur in the environment leading to ecological damage. The widespread use of fluorinated drugs will inevitably lead to the contamination of waste-water streams, for example, Kosjek et al. 57 detected 5-fluorouracil in municipal and hospital wastewater, and determined six photodegradation products. The development of drugs with new fluorine-containing functional groups, such as SF5, should be accompanied by a rigorous assessment of the likely environmental transformations that might occur, and the potential downstream consequences of these. SF5-containing drugs should be seen as emerging pollutants, since they are likely to be increasingly common as the methods for synthesis are improved and the building blocks become more accessible. There are so far only two reports on the transformation of aryl-SF5 compounds 58, 59, revealing a dearth of information on the environmental impact of these compounds. Significantly, there is a risk of fluorinated drugs suffering from a similar problem as GM crops in relation to the public’s perception of the risks associated with them, as opposition to fluoridated public water supplies also draws attention to other sources of fluorine in the environment, including pharmaceuticals. Therefore, prudent foresight in relation to the potential environmental consequences of releasing new fluorine-containing drugs into the environment via waste-streams will ensure that progress in this field is not hampered by resources being diverted to engage in public relation campaigns.

The approaches to strategically identifying ideal sites for fluorination in a drug molecule are limited, for example, the ‘fluorine scan’, whereby different sites in a molecule are fluorinated and each derivative is assessed, is a common approach, but involves many redundant syntheses. Identifying metabolically labile sites can require animal studies, for example, in the development of Ezitimibe **5** animal studies with radiolabelled non-fluorinated lead compounds were conducted in order to identify the optimum sites for fluorination 60. Whilst it is not likely that animal studies can be removed from the drug development workflow, the principle of the 3Rs (replacement, reduction and refinement) should be applied as much as possible when developing fluorinated drugs, for example, by employing in vitro methods to complement sophisticated in silico approaches to identify the site of CYP-catalysed oxidations in lead compounds that can be subsequently fluorinated.

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Figure legends

**Figure 1**. Examples of commercially important fluorinated pharmaceuticals

**Figure 2**. Chemical-microbiological method for designing fluorinated drugs that block metabolism

**Figure 3**. Large synthesis of fluoro-and trifluoromethyl aromatic systems

**Figure 4**. Uses of fluorine gas in chemical intermediate manufacture

**Figure 5**. Precursor-directed biosynthesis of fluorinated iturin A and surfactin in *Bacillus* sp. CS93

**Figure 6**. Semi-synthesis of difluorinated derivatives of ibuprofen methylester.

Figure 1



Figure 2



Figure 3



Figure 4



Figure 5



Figure 6



Numbered structures









Numbered structures (continued)



