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METABOLISM OF FLUORINE-CONTAINING DRUGS

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■ **Abstract** This article reviews current knowledge of the metabolism of drugs that contain fluorine. The strategic value of fluorine substitution in drug design is discussed in terms of chemical structure and basic concepts in drug metabolism and drug toxicity.

INTRODUCTION

Fluorine substitution can alter the chemical properties, disposition, and biological activity of drugs (1). Many fluorinated compounds are currently widely used in the treatment of disease. These include antidepressants, antiinflammatory agents, antimalarial drugs, antipsychotics, antiviral agents, steroids, and general anaesthetics (2). The chemistry and medicinal chemistry of fluoro-organic compounds and drugs have been reviewed (1, 3–5). The development of new fluorinating agents has vastly increased the potential for synthesis of novel fluorinated drugs. In addition, the development of sophisticated noninvasive analytical techniques based on fluorine nuclear magnetic resonance (NMR) and positron emission topography has transformed the study of fluorinated drugs in man and animals (6–8).

The inclusion of a fluorine atom in a drug molecule can influence both the disposition of the drug and the interaction of the drug with its pharmacological target (Figure 1). For example, the effects of fluorine substitution on the inter- and intramolecular forces that affect binding of ligands, and thus introduce receptor subtype selectivity, at cholinergic and adrenergic receptors are now well understood (9–11). Fluorine substitution can also have a profound effect on drug disposition, in terms of distribution, drug clearance, route(s), and extent of drug metabolism (12). Such changes can be used constructively by medicinal chemists to improve both the safety and the efficacy of a drug. Therefore, the purpose of this review is twofold. First, to outline the chemical basis of changes in drug disposition that can be achieved by the introduction of fluorine. Second, to consider the pharmacological and toxicological implications of such changes with respect to drug response.

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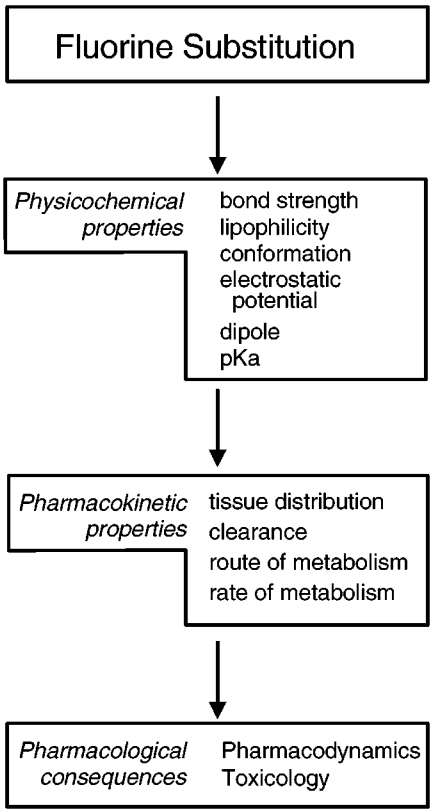


Figure 1 Flow diagram illustrating the effect of fluorine substitution on drug response.

PHYSICOCHEMICAL PROPERTIES OF FLUORINATED DRUGS

The replacement of a hydrogen atom or hydroxyl group by a fluorine atom is a strategy widely used in drug development to alter biological function. Although it is generally thought that fluorine for hydrogen substitution causes minimal steric effects at receptor sites, the actual van der Waals radius of fluorine (1.47 Å) lies between that of oxygen (1.57 Å) and hydrogen (1.2 Å) (Table 1). Despite the fact that fluorine has a greater size than hydrogen, several studies have demonstrated that it is a reasonable hydrogen mimic and exerts only a minor steric demand at receptor sites, at least for monofunctional analogues (3).

In contrast to their slight differences in size, hydrogen and fluorine have quite different electronic properties. Fluorine is the most electronegative element in the periodic table (Table 1). The resulting change in the electron distribution in a molecule, following the replacement of a hydrogen atom for fluorine, can alter the pK_a, the dipole moments, and even the chemical reactivity and stability of neighboring functional groups. The magnitude of the change in these electronic

TABLE 1 Physiochemical properties of the carbon-fluorine bond

Element	Electro-negativity	Bond length (CH_2X, Å)	Van der Waals radius (Å)	Bond energy (kcal/mol)
H	2.1	1.09	1.20	99
F	4.0	1.39	1.35	116
O(OH)	3.5	1.43	1.40	85

properties is often determined by the bonding between the fluorine atom and the functional group. Thus, the presence of a fluorine atom *ortho* to a phenolic group is associated with a reduced pK_a of 1.2, whereas *meta* and *para* fluoro substitutions have much less effect. The incorporation of two fluorine atoms at the 2- and 6-positions of phenol leads to a reduction of pK_a of 2.7 U. Based on this effect, the 2,6-difluorophenol group has been used as an isostere of a carboxylic acid in a series of GABA aminotransferase inhibitors (13). These compounds were shown to inhibit the aminotransferase enzyme demonstrating the potential of this bioisosteric replacement.

The presence of a single fluorine, adjacent to a carboxylic acid function in aliphatic systems, can also have pronounced effect on the pK_a. This fact was used to rationalize the decrease in toxicity of new monofluorinated analogues of methotrexate. The incorporation of a fluorine atom adjacent to the glutamic carboxyl acid function results in an increase in the acidity. As a result, these new analogues are less toxic than methotrexate because they do not form polyglutamates, metabolites associated with undesirable prolonged cellular retention (14).

Fluorine forms a strong bond with carbon (bond energy C-F = 116 kcal/mol), which has an increased oxidative and thermal stability compared with the carbon-hydrogen bond (C-H = 99 kcal/mol). The carbon-fluorine bond is one of the strongest known in organic chemistry. In addition to the formation of covalent bonds, a fluorine atom present in a molecule can also form reversible, electrostatic bonds with certain functional groups.

The isosteric replacement of the hydroxyl group is a commonly used strategy in medicinal chemistry. This substitution is usually based on the premise that the fluorine can hydrogen bond accept in a manner similar to the oxygen of a hydroxyl function. However, the higher electronegativity and lower polarizability of fluorine over oxygen has a major influence on the ability of fluorine to mimic a hydroxyl group (15, 16). Recent calculations have measured the strength of an optimum F...H bond (1.9 Å) to be 2.38 kcal mol^{-1} in an adduct between fluoromethane and water. Therefore, the F...H bond is clearly much weaker than the corresponding O...H (conservatively estimated to be ca 5 kcal mol^{-1}). The carbon-fluorine bond also has a strong dipole, and this may interact, either positively or negatively, with other dipoles. For example, it is thought that in fluorinated derivatives of noradrenaline, interactions between a ring carbon-fluorine bond and the hydroxyl group on the beta-carbon in the side chain determine the conformation of the molecule, and hence, the

position of the fluorine atom in the aromatic ring can determine receptor selectivity (17). Fluorine also has a number of specific stereoelectronic effects, such as the fluorine anomeric effect in carbohydrates, the Anh-Eisenstein stabilization effect in lipase-mediated kinetic resolutions, and the *cis* effect in difluorinated alkanes (3).

In contrast to the single replacement of a hydrogen for fluorine, replacement of a methylene function with a difluoromethylene function (CF_2 for CH_2) can have a significant effect on both conformation and physical properties (3). The difluoromethylene moiety has in fact been used as an electronic mimic of labile oxygen atoms in phosphate esters (R-CF_2-PO_3^{2-} vs R-OPO_3^{2-}). This functional group has found extensive use in the design of inhibitors of enzymes that hydrolyze or bind phosphate esters (18). The CF_2 has been proposed as a reasonable isosteric and isopolar replacement for the hydroxyl group because of their size, electron distribution, and ability to act as a hydrogen bond acceptor (19–21). The CF_2H group is particularly favored because of its ability to act as a hydrogen donor (22), potentially allowing interaction with solvent and biological molecules. Further introduction of fluorine causes even greater steric restrictions. The frequently used trifluoromethyl group (-CF_3) is closer in size to an isopropyl group (23). Indeed, several workers have suggested that the CF_3 group can exert an effect comparable to a phenyl ring or even a *tert*-butyl function (24).

The presence of a fluorine atom can influence the lipophilicity of a molecule and hence affect the partitioning of the drug into membranes, and also facilitate hydrophobic interactions of the drug molecule with specific binding sites, on either receptors or enzymes. The replacement of a single aromatic hydrogen atom usually results only in a modest increase in lipophilicity, whereas the CF_3 group is among the most lipophilic of all substituents.

The fluoride ion is a good leaving group, being the conjugate base of a strong acid. Therefore, the fluoride ion can be lost, in both displacement and elimination reactions, and this aspect of fluorine chemistry can be utilized in the design of drugs or chemical agents that form stable covalent bonds with target receptors or enzymes as part of their pharmacological response. This is the basis of the "lethal synthesis" concept (25). More recently, fluorinated inhibitors of GABA aminotransferase have been synthesized as potential mechanism-based inhibitors (13).

The presence of fluorine can alter the oxidation potential of an aromatic system, and thus alter the rate of autoxidation and formation of quinones and quinoneimines. Sequential introduction of fluorine atoms into the nucleus of paracetamol produced an increase in the oxidation potential of the molecule, as measured by cyclic voltammetry (26).

Before embarking on fluorination as a synthetic strategy to alter drug disposition, it is imperative to determine whether the changes in the physicochemical properties will diminish the inherent pharmacological activity of the drug. Molecular modeling techniques can, in theory, be used to examine (*a*) the importance of the group to be replaced in the drug-receptor interaction and (*b*) whether the resulting C-F bond can provide the same chemical interaction with the receptor. However, it must be stressed that modeling of the C-F bond in drug-receptor interactions

is not a trivial task, even for very simple molecules in an aqueous environment (27).

STRATEGIES FOR THE USE OF FLUORINE SUBSTITUTION TO ALTER DRUG DISPOSITION

The introduction of fluorine into a molecule can be used to alter the rate, route, and extent of drug metabolism. Fluorine substitution can also be used to dissociate the pharmacological and toxicological properties of a drug when a toxic metabolite has been identified.

Such alterations are most commonly achieved by fluorine substitution at the site of metabolic attack, based on the premise that the carbon-fluorine bond is much more resistant to direct chemical attack by cytochrome P450, in comparison to the carbon-hydrogen bond. In addition, substitution at sites adjacent to and, in some instances, distal to the site of metabolic attack can also affect drug metabolism, by either inductive/resonance (through bond) effects or conformational and electrostatic (through space) effects. The presence of a fluorine atom adjacent to a site of metabolic attack could, in theory, either increase or decrease the rate of biotransformation, depending on (*a*) whether the metabolic attack is nucleophilic or electrophilic in nature and (*b*) inductive or resonance effects of the fluorine atom predominate in the reaction. For example, in a simple saturated system, the inductive effect of fluorine should reduce the susceptibility of adjacent groups to attack by P450 enzymes. In contrast, it might be anticipated that the presence of fluorine *ortho* to a phenolic group might increase its reactivity as a nucleophile in methylation and glucuronidation reactions, and there is some evidence to support this hypothesis (28, 29).

Fluorine substitution can therefore have complex effects on drug metabolism. The framework outlined in Figure 1 is used to consider the importance of the role of fluorinated subgroups on various aspects of drug disposition, and the consequent impact on drug efficacy and drug toxicity.

THE EFFECT OF FLUORINE SUBSTITUTION ON DRUG DISTRIBUTION

The inclusion of fluorine in a molecule has two benefits with respect to drug distribution. First, certain fluorine-containing functional groups enhance lipophilicity and therefore passive diffusion of drug across membranes. Second, noninvasive techniques can be used to assess penetration of the drug to the site of action, whether brain, tumor, or site of infection (30–32).

Centrally acting drugs must pass through the blood brain barrier in sufficient concentration to elicit their pharmacological effect. For example, there are three categories of neuroleptics that act by blocking dopamine receptors in the central nervous system (CNS): tricyclics, butyrophenones, and diarylbutylamines. Many of these drugs contain either a CF_3 group or a fluoro-phenyl group, which

contribute to the overall pharmacological activity of the compounds by enhancing CNS penetration and retarding metabolic degradation. Several clinically useful phenothiazines containing fluorine have been introduced (4, 5). Trifluoropromazine, trifluperazine, and fluphenazine are all more active than chlorpromazine (5-, 50-, and 100-fold, respectively).

The most widely used butyrophenone is haloperidol. It has been established that the *para*-fluorophenyl group is optimal for neuroleptic activity of the butyrophenones. In the search for more potent neuroleptics, it was found that the diarylbutylamine, pimozide, which contains two fluorophenyl groups, was longer acting than haloperidol (12).

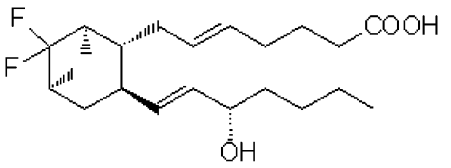
Another important class of centrally acting drugs containing fluorine that have gained clinical prominence over the past few years is the selective serotonin uptake inhibitors. Of the most widely used agents in this class (fluoxetine, fluvoxamine, paroxetine sertraline, and citalopram), only sertraline does not contain a fluorine atom in its structure. Sertraline has the shortest half-life of the drugs mentioned (26 h), is the most slowly absorbed, and undergoes almost total metabolic conversion, principally through presystemic elimination: all properties that may be associated with the lack of fluorine (2).

Quinolone antibacterial agents act by inhibition of bacterial DNA synthesis. Fluoroquinolones were first introduced in the early 1980s (norfloxacin) and include ofloxacin, enoxacin, ciprofloxacin, tosufloxacin, sparfloxacin, grepafloxacin, levofloxacin, and, most recently, trovafloxacin. This class of drugs is active against a wide range of gram-positive and gram-negative pathogens, possesses improved oral absorption and systemic distribution, and therefore has extended clinical applications that include urinary tract infections, respiratory tract infections, skin infections, and soft tissue infections. The structural features, which determine tissue distribution and cellular uptake, are complicated by specific efflux mechanisms (33). Nevertheless, it has been proposed that the 5-fluoro group is important for both cell penetration and gyrase affinity (34).

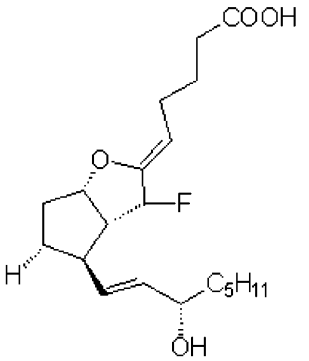
THE EFFECT OF FLUORINE SUBSTITUTION ON THE RATE OF DRUG METABOLISM

Fluorine substitution has been used to extend the biological half-life of endogenous compounds and synthetic compounds. Fluorinated analogues are not only useful tools for physiological investigations (Figure 2), they may also be potential therapeutic agents. For example, the prostanoids, prostacyclin and thromboxane, play an essential physiological role in the regulation of the cardiovascular system and platelet function. Both compounds have short ($<$5 min) half-lives in vivo. Consequently, there has been a concerted effort to synthesize analogues with enhanced stability and, thus, extended activity.

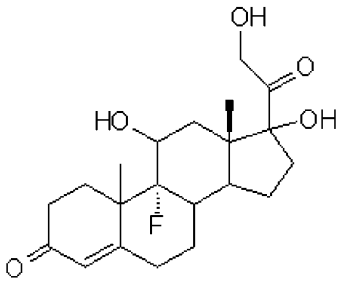
The potent platelet aggregating agent thromboxane A_2 has a half-life of only 32 s under physiological conditions. Incorporation of fluorine into the oxetane ring



7,7-Difluoro Thromboxane A_2



7-Fluorodehydroprostacyclin



9-α-Fluorocortisol

Figure 2 Examples of how fluorination of endogenous compounds can enhance metabolic stability.

alpha to the acetalic linkage reduces the rate of carbonium ion formation and acid hydrolysis. Thus, model compounds related to 7,7-difluoro-TxA_2 have a rate of hydrolysis that is 10^8-fold slower than that of TXA_2 (35), whereas 10,10-difluoro-TxA_2 is also a stable analogue and retains thromboxane-like activity (36).

Prostacyclin, which is an inhibitor of platelet aggregation, contains an acid-labile enol-ether group, which is responsible for its short biological half-life. Electrophilic attack of a hydroxonium ion at the enol-ether double bond is the rate-limiting step in the metabolic degradation of the compound. Fluorination alpha to the labile group reduces the electron density on the enol-ether group and, thus, improves the stability of the molecule toward acid hydrolysis. Thus 10,10-difluoro-13-dehydro-prostacyclin exhibits a half-life 150 times greater than

that of PGI_2 and has equal potency to the natural compound (37). Similarly, the introduction of a 7-fluoro group into PGI_2 stabilized the enol by reducing the electron density of the double bond at C-5 through the inductive effects of fluorine (38).

Fluorination of natural hormones can lead to molecules with enhanced efficacy, and also to qualitative differences in activity. The changes in biological activity can be complex (pharmacodynamic and pharmacokinetic) and involve several chemical factors, including conformational changes in the steroid nucleus and altered receptor binding.

The introduction of fluorine into the 9α-position, of corticosteroids has a dramatic reduction in some of the major routes of metabolism of cortisol. The most striking finding is the lack of oxidation of the 11β-hydroxyl group to a ketone, which occurs rapidly with cortisol and leads to a loss of biological activity. Hence, in vivo, there is a shift in the enzymatic equilibrium between the biologically inactive 11-oxo compounds and the active reduced 11β-hydroxy form (39). In vitro studies have shown that although cortisone and A-ring–reduced metabolites are the major products of cortisol metabolism in liver, 9α-fluorocortisol undergoes preferential 6β-hydroxylation and 20β-reduction (40).

We have investigated how introduction of fluorine may alter the balance between metabolism of the A- and D-rings of estrogens (Figure 3). Introduction of fluorine into the 2-position can block 2-hydroxylation and bioactivation to a quinone (41, 42). Introduction of fluorine into the 16-position blocked not only C-16 hydroxylation but also dehydrogenation of the C-17 hydroxyl group (43). The restriction on D-ring metabolism was partly compensated for by enhancement of glucuronylation and A-ring hydroxylation.

The 4-fluorophenyl group is usually resistant to aromatic hydroxylation, especially at the 4-position, although aromatic hydroxylation at the unsubstituted positions does occur. Defluorination of the fluoromethyl group is extremely rare, although 5-trifluoromethyluracil is converted into 5-carboxyuracil in man with concomitant excretion of inorganic fluoride (44). Metabolism by cytochrome P450 enzymes does occur at the *ortho* and *para* positions. In molecules such as fluphenazine, which contain two rings that are chemically equivalent apart from the one being substituted with a CF_3 group, hydroxylation occurs in the unsubstituted ring because of the deactivating effect of the CF_3 group (45).

The presence of the fluorine group in aromatic systems serves two purposes. First, the presence of fluorine per se can block oxidation at a specific position. Second, fluorine decreases the rate of reaction of the π-system of the benzene ring with activated cytochrome P450$(FeO)^{3+}$ (46). Nevertheless, cytochrome P450 enzymes can attack at positions adjacent to the carbon-fluorine bond. Direct evidence for such a process comes from the demonstration that 1,4-difluorobenzene forms a significant amount of the NIH-shift product 2,4-difluorophenol (Figure 4). However, molecular orbital calculations show that increasing the extent of fluorine substitution further decreases nucleophilicity and, thus, reduces further the rate of reaction of di-, tri-, and tetrafluorobenzenes (46, 47). Evidence for such a process occurring in man is the detection of an NIH-shift metabolite of the novel

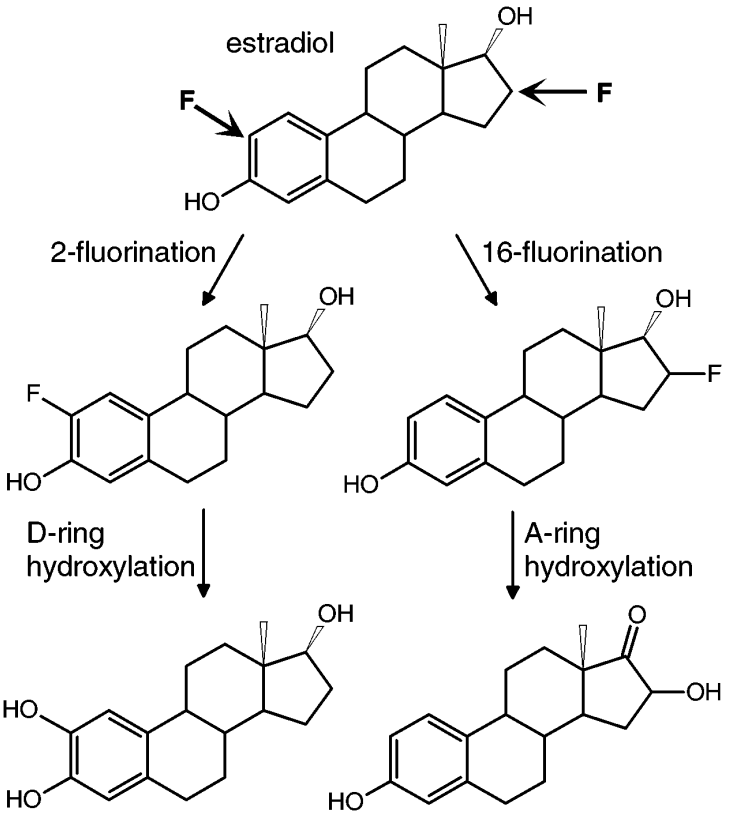


Figure 3 Differential effects of A- and D-ring fluorination on the regioselective metabolism of estradiol (29, 41).

quinoxazoline reverse trancriptase inhibitor GW420867X in human urine, using NMR and tandem mass spectrometry (48).

The blockade of aromatic hydroxylation by the CF_3 group may have undesirable consequences. Endoperoxides are a new class of antimalarials that destroy parasites by selective bioactivation of the peroxide group, to carbon-centered radicals, within the parasite. In contrast, bioactivation in mammalian systems is extremely limited (49). Arteflene is a synthetic derivative of the natural product, which has a promising biological and pharmacokinetic profile. However, blockade of aromatic hydroxylation by fluorination promotes extensive bioactivation of the peroxide system to potentially toxic metabolites in mammalian systems (50).

The strategic value of fluorine substitution in rational drug design is neatly illustrated by the development of the orally active inhibitor of cholesterol absorption SCH58235 from SCH48461 (51). Fluorine was introduced to block undesirable metabolic transformations and produce a lead compound with 50-fold greater potency in vivo (Figure 5).

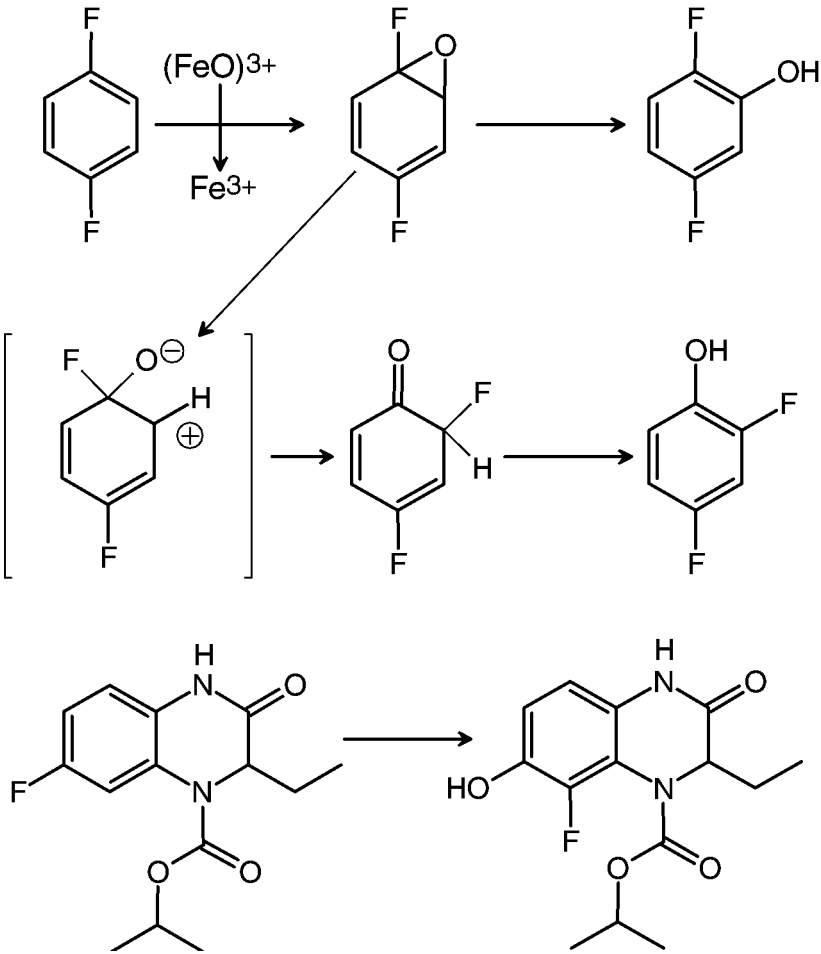


Figure 4 Mechanism of aromatic hydroxylation of fluorobenzenes. The NIH shift of fluorine (47, 48).

THE USE OF FLUORINE SUBSTITUTION TO PREVENT METABOLIC BIOACTIVATION

The physiological role of drug metabolism is that of detoxication. However, certain biotransformations, and in particular those catalyzed by cytochrome P450, can produce chemically reactive metabolites. Normally enzymes such as epoxide hydrolase and glutathione transferase rapidly detoxify such metabolites. However, if such reactive metabolites interact with cellular macromolecules, they may cause carcinogenicity, apoptosis, necrosis, or hypersensitivity (52). Fluorine substitution has been used as a tool to investigate mechanisms of chemical toxicity and in the development of safer drugs.

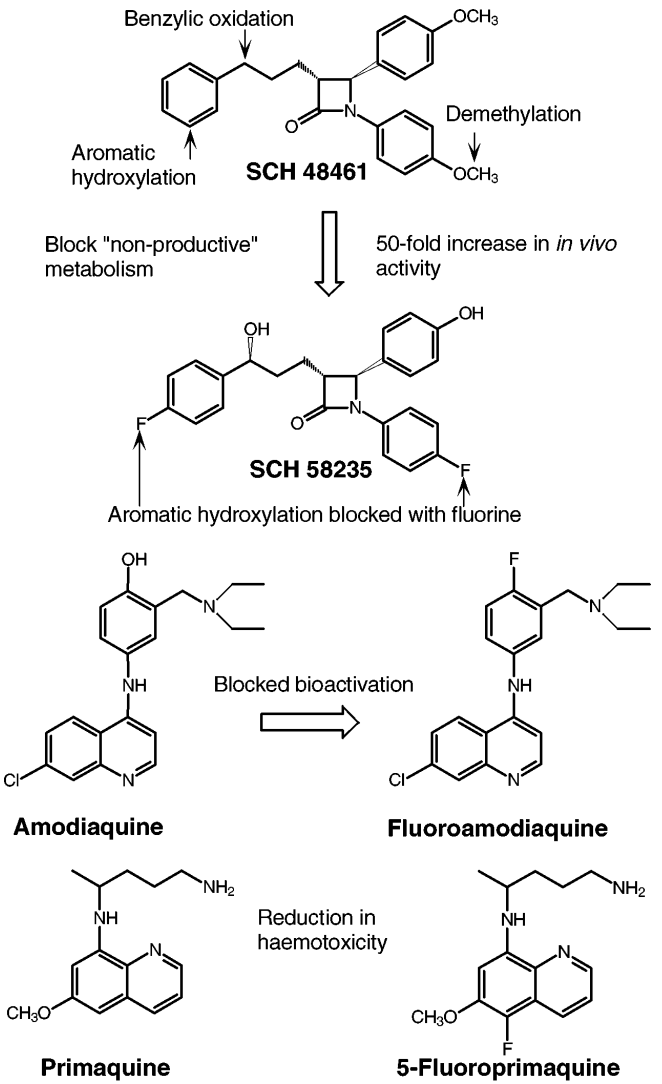
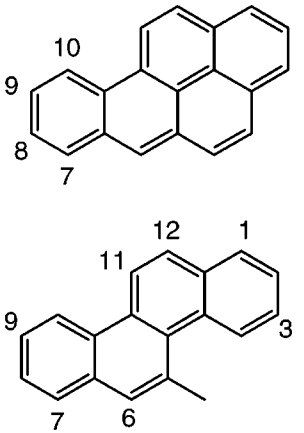


Figure 5 Examples of fluorine substitution in rational drug design (51, 64, 65, 67).

Polycyclic aromatic hydrocarbons undergo metabolic bioactivation to arene oxides, which bind covalently to DNA and thus initiate carcinogenesis. Metabolic activation by the cytochrome P450 system is generally blocked at the carbon to which fluorine is attached. Miller & Miller (53) used fluorine substitution as a tool in the early mechanistic investigations of carcinogenicity and found that substitution of fluorine in the 3-position of 10-methyl-1,2-benzanthracene virtually abolished the carcinogenic activity of this hydrocarbon toward mouse skin. This technique has been widely used to define the chemistry of carcinogen activation (Figure 6).

Figure 6 Use of fluorine substitution to define the role of metabolic activation in chemical carcinogenicity (54, 55).



Mouse skin carcinogenicity:
1-F, 3-F, inactive as complete carcinogens;
2-F, significantly less carcinogenic

Benzpyrene was the first member of the polycyclic aromatic hydrocarbon class of compounds for which the structures of both proximate and ultimate carcinogens were defined. The metabolic pathway is catalyzed by CYP1A enzymes and epoxide hydrolysis leading to the ultimate carcinogen, 7.8-dihydroxy-9,10-epoxy-tetra-hydrobenzpyrene. Accordingly, it was found that 7-, 8-, 9-, and 10-fluorobenz (a)pyrenes were not tumorigenic in mice and that fluorine substitution, at each position, had blocked formation of the respective diol-epoxides (54).

The metabolic activation of 5-methylchrysene has been of interest because this carcinogen is typical of the class of methylated polynuclear aromatic hydrocarbons and is the most carcinogenic of all the methylchrysene isomers. Hecht et al (55) investigated the comparative carcinogenicity of seven derivatives fluorinated at the 1-, 3-, 6-, 7-, 9-, 11-, and 12-positions, respectively. Investigation of mouse skin tumor formation showed that the 6-, 7-, 9-, and 11-fluorinated derivatives were as carcinogenic as 5-methylchrysene, 12-fluoro was significantly less potent, and both 1-F and 3-F were inactive as complete carcinogens. The results are entirely consistent with the characterization of 5-methylchrysene-1,2-dihydrodiol-3,4-epoxide-DNA adducts (56, 57).

Synthetic and natural estrogens have been associated in humans with a variety of vaginal, breast, hepatic, and cervical cancers. It has been suggested that both hormonal potency and oxidative metabolism may play a role in the carcinogenicity of steroid estrogens. The natural estrogens, estrone and estradiol, undergo extensive oxidation in the 2- and 4-positions by cytochrome P450 (CYP) enzymes. The resulting catechols are readily oxidized to chemically reactive quinones

and semiquinones, which form covalent bonds with proteins and DNA. Liehr (58) found that 2-fluoro-estradiol was as estrogenic as estradiol but noncarcinogenic in the Syrian hamster. In vivo studies (42, 43) showed that the presence of a 2-fluoro substituent diverted the metabolism of the steroid from 2-hydroxylation to glucuronidation, thus providing direct evidence for the role of bioactivation in the model of oestrogen carcinogenicity used by Liehr (58).

The widely used analgesic paracetamol serves as a paradigm for the role of metabolic bioactivation in drug toxicity. Studies in knockout mice have clearly shown that formation of a reactive metabolite, N-acetylbenzoquinoneimine, is responsible for the hepatic necrosis seen when the drug is taken in overdose (59, 60). Introduction of fluorine into the paracetamol molecule alters the oxidation potential in a manner dependent on the number and position of the fluorine atoms (26). The presence of fluorine at the 2- and 6-positions increased the oxidation potential of paracetamol sufficiently to reduce the propensity of the molecule to undergo oxidative bioactivation in vivo and thereby reduced hepatotoxicity (61). Introduction of fluorine did not affect the important detoxication pathways of glucuronidation or sulphation. It is interesting that 2,6-fluorine substitution also resulted in a loss of analgesic activity, indicating a role for oxidation in the analgesic activity of the drug (61).

The 4-aminoquinoline antimalarial amodiaquine also contains the 4-aminophenol function. It is thought that the hemotoxicity and hepatotoxicity of this drug may be a result of oxidative metabolism to a chemically reactive quinoneimine metabolite (62, 63). The effect of systematic fluorine substitution of the aminophenol ring was investigated with respect to (*a*) increasing the oxidative stability of the ring system and (*b*) reducing in vivo oxidation to potentially toxic quinoneimine metabolites. As was the case for paracetamol, 6-difluorination of the aminophenol ring of amodiaquine produced an analogue with significantly raised oxidation potential and reduced bioactivation in vivo. Isosteric replacement of the hydroxyl function with fluorine provided an analogue, fluoroamodiaquine, which had similar antimalarial potency to the parent drug but was not bioactivated to toxic metabolites in vivo. In contrast to the reduction in biological activity observed in the fluorinated paracetamol series, fluorinated analogues of amodiaquine retained pharmacological activity, which suggested that oxidation to quinonimine metabolites is not required for antimalarial activity (64). Fluorinated 4-aminoquinolines and 8-aminoquinolines are being assessed as lead compounds for drug development (65, 66) (Figure 5).

The effect of fluorine substitution on the metabolism and toxicity of drugs and use of such knowledge for the development of safer therapeutic agents is neatly illustrated by the flurane group of anaesthetics (68) (Figure 7). Methoxyflurane was widely used in clinical anaesthesia during the 1960s, until it was discovered that there was an association with nephrotoxicity. A high urine output syndrome leading to dehydration, and in some cases fatal renal failure, was related to the extensive (40%) metabolism of methoxyflurane and high serum concentrations of

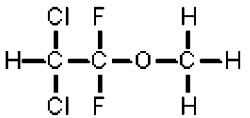
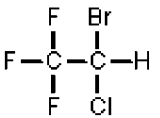
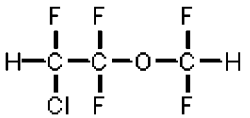
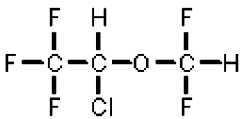
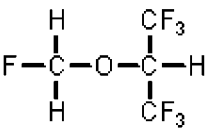
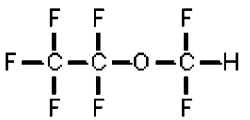
	Metabolism (% dose)	Toxicity
 methoxyflurane	50%	nephrotoxicity
 halothane	20-50%	reversible and irreversibe hepatotoxicity
 enflurane	3%	rare cases of hepatotoxicity
 isoflurane	<1%	rare cases of hepatotoxicity
 sevoflurane	3%	rare cases of hepatotoxicity
 desflurane	<1%	none reported

Figure 7 The toxicity and metabolism of general anesthetics.

inorganic fluoride. This theory has been questioned in recent years, since the introduction of sevoflurane, which also produces high serum fluoride but is not reported to cause nephrotoxicity (69). Methoxyflurane has been shown to be metabolized in man and animals to oxalic acid and free fluoride (70). Studies to date have shown only modest inorganic fluoride levels with enflurane and isoflurane, and there are only occasional reports of nephrotoxicity for enflurane and none at all for isoflurane (68, 71, 72). Increasing fluorine substitution results in a reduction in overall metabolism, and in specifically biotransformations leading to defluorination.

In man, enflurane is metabolized to the fluoride ion, difluoromethoxydifluoroacetic acid, and an unidentified acid metabolite. Metabolism is catalyzed by CYP2E1 and is stereoselective (73). The predicted products of oxidation of the difluoromethyl group, chlorofluoroacetic acid and oxalic acid, have not been detected (74). Inorganic fluoride and trifluoroacetic acid have been identified as end products of isoflurane metabolism (75). These products are thought to arise by a sequence that begins with insertion of an active oxygen atom into the bond connecting hydrogen to the ethyl α-carbon and which is catalyzed by CYP2E1 in man (76, 77). Desflurane, in which the chlorine atom in isoflurane is replaced by a further fluorine atom, is excreted by the lungs and appears resistant to biotransformation. The oil gas partition coefficient for desflurane (18.7) is considerably less than that of isoflurane (91), which explains the more rapid rates of onset and offset of anesthesia of the former (78, 79).

The first of the modern fluorinated anesthetics was halothane; however, its clinical use is now limited because of metabolism-associated liver toxicity. The National Halothane Study (80) identified two distinct forms of hepatotoxicity: The first, a mild form of transaminitis, occurred in 20% of cases but was reversible. In contrast, a small group (approximately 1 in 35,000) suffered severe hepatitis, characterized by massive liver necrosis, which could be fatal. The incidence of hepatitis increased on reexposure (to 1 in 3,700), indicative of an immunological aetiology. Two pathways of halothane metabolism occur (Figure 8): a reductive pathway, mediated by CYP3A4 and CYP2A6 (81), and oxidation, principally involving CYP2E1 (82). The reductive pathway generates free radicals, which, following further reduction and fluorine elimination, gives rise to $CF_2 = CHCl$. It is this route that is thought to cause the mild transaminitis. Oxidation produces trifluoroacetyl (TFA) chloride, an electrophilic reactive intermediate capable of covalently binding to proteins. This species can either form trifluoroactic acid, and be excreted in urine, or result in the formation of TFA adducts, which are established antigens in halothane-induced hepatitis. Binding occurs to an array of intracellular proteins; however, it has also been noted recently that expression of TFA-CYP2E1 adducts occurs on the surface of hepatocytes, providing a possible mechanism for presentation of the antigen to cells of the immune system (83).

TFA adducts are not unique to halothane. Hydrochlorofluorocarbons (HCFCs) were developed as a potential replacement for chlorofluorocarbons (CFCs)

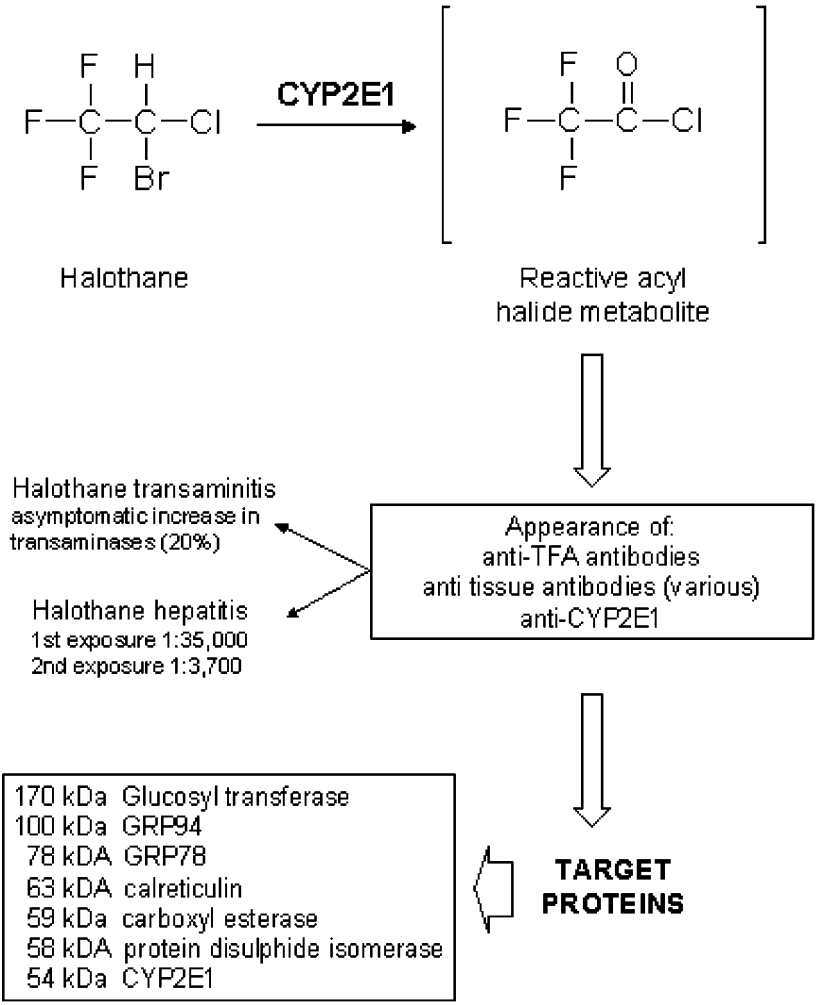


Figure 8 The role of metabolism in halothane hepatotoxicity (83).

following concerns over the ability of CFCs to deplete stratospheric ozone. Because of similarities in the structures of HCFCs and halothane, researchers have looked for TFA adducts in animals exposed to HCFCs and found levels equivalent to those after halothane exposure (84, 85). In addition, both oxidative and reductive pathways of HCFC metabolism occur because urinary excretion of both trifluoroacetic acid and fluoride increase after exposure (86). A major advance in the design of safer chemicals of this class is the development of computer models that predict in vivo rates of drug metabolism (87).

METABOLIC DEFLUORINATION

Defluorination can readily occur during biotransformation, which favors formation of the stable fluoride ion, despite the strength of the carbon-fluorine bond (Figure 9). Defluorination may also occur spontaneously, if a molecule is sufficiently electrophilic to undergo direct reaction with nucleophilic groups present in proteins and amino acids, such as the amino group in lysine, the hydroxyl group in serine, and the sulphydryl group in cysteine. Such compounds can therefore be highly toxic, with the type of toxicity depending on the target macromolecule. Two examples are diisopropyl phosphorofluoridate, which phosphorylates the serine residue at the active site of the enzyme acetylcholinesterase, and dinitrofluorobenzene, a model immunogen. Dinitrofluorobenzene reacts directly with lysine residues in proteins, with direct displacement of the fluoride ion, to form the dinitrophenyl hapten (88). In vivo, approximately 10% of the compound binds to protein, whereas most of the compound undergoes metabolic detoxication by conjugation with glutathione (89).

The release of fluoride from aliphatic compounds is readily achieved by simple hydroxylation at centers adjacent to the carbon-fluorine bond. Thus α-hydroxylation, and subsequent elimination of hydrofluoric acid, will yield a ketone or an acyl halide, as described earlier for the metabolism of methoxyflurane.

In contrast, fluorinated aromatic compounds are generally more resistant to metabolism. Nevertheless, metabolic defluorination catalyzed by cytochrome P450 enzymes does occur. The production of the fluoride ion during biological oxidation of aryl fluorides has been observed in several systems (90); metabolism of 4-fluoro-L-phenylalanine by phenylalanine hydroxylase results in L-tyrosine and fluoride ion (91), and metabolism of 3,5-difluoro-4-hydroxybenzoic acid gives fluorobenzoquinone-5-carboxylic acid and fluoride ion (92).

The enzymes responsible for metabolism of 4-fluoro-N-methylaniline have been investigated in detail, both in vitro and in vivo using ^{19}F-labeled NMR (93). The major enzyme responsible for N-demethylation and aromatic ring hydroxylation was the cytochrome P450 system, whereas the contribution for flavin-containing monoxygenase to formation of defluorinated ring hydroxylated products would be about three to ten times higher than that of cytochromes P450. It was suggested that defluorination, which accounted for 40% of recovered metabolites, could have resulted from N-oxidation rather than metabolic ring hydroxylation. In keeping with this hypothesis, it has been suggested that 4-defluorination of pentafluoraniline proceeds by formation of the fluoride anion and a reactive benzoquinonimine, which undergoes subsequent reduction to an aminophenol (94).

Amodiaquine is an antimalarial drug that undergoes extensive bioactivation in vivo to a quinone imine metabolite that is excreted in bile as the 5-glutathionyl metabolite. Attempts were made to block this biotransformation by substitution of fluorine at the 5-position (95). However, it was discovered that the major metabolite of 5-fluoro-amodiaquine was again 5-glutathionylamodiaquine. The elimination

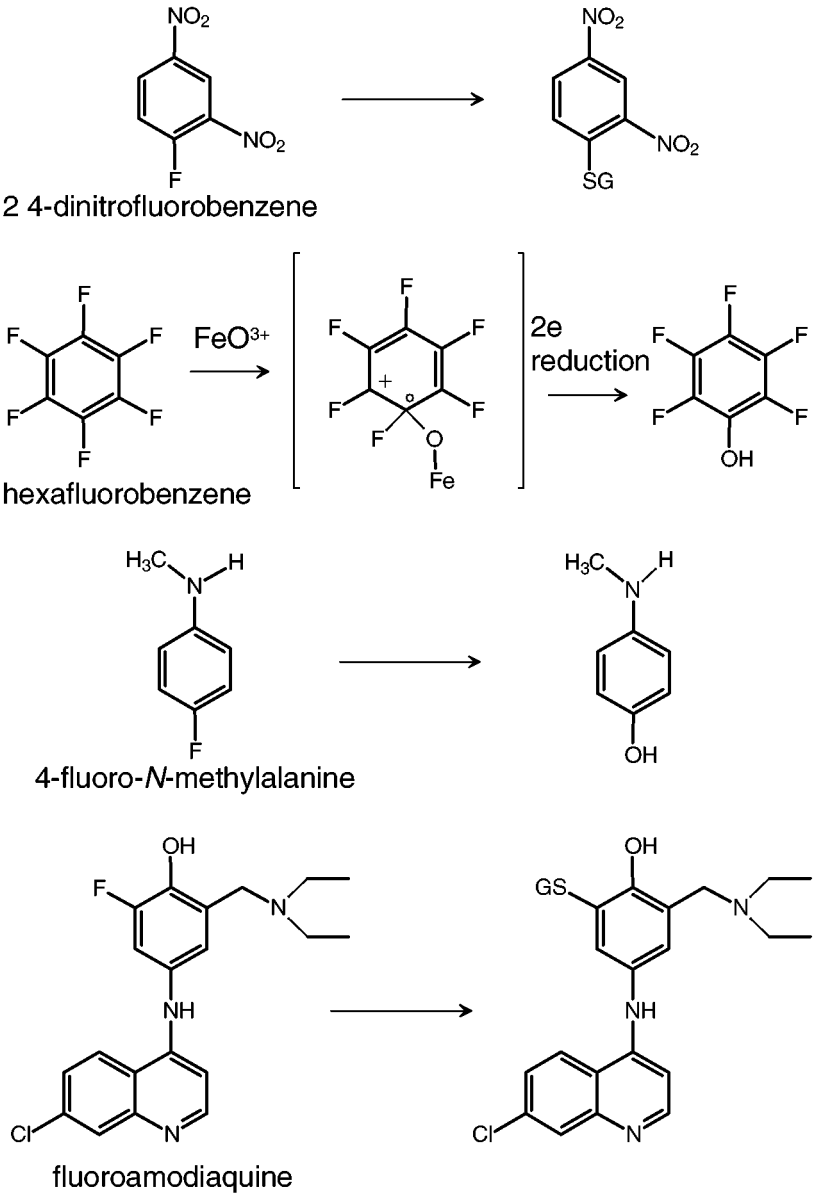


Figure 9 Some examples of metabolic defluorination.

of fluoride is quite logical in a chemical sense and results from oxidation to a quinoneimine, Michael addition of glutathione, and subsequent elimination of hydrofluoric acid to restore the aromatic system.

Similarly, 2-fluoroethynyloestradiol, unlike 2-fluoroestradiol, undergoes partial defluorination in vivo in rats to produce 2-hydroxyethynylestradiol because alternative metabolism of the D-ring is blocked (42). The mechanism is thought to involve epoxidation, rearrangement to a quinone mediated by facile loss of fluoride, and reduction of the intermediate quinone.

The dehalogenation of hexahalobenzenes provides a simple model to explore the fascinating concept of direct interaction of the cytochrome P450 system with a carbon-fluoride bond. The mechanisms of such reactions have been reviewed (96). The precise order of the chemical steps involved in the oxidative dehalogenation of hexahalogenated benzenes is not clear. Nevertheless, it is thought that the initial product is a halophenoxenium cation. Furthermore, a further two-electron reduction is required to allow release of the anion fluoride.

In rare instances, even the CF_3 group may undergo defluorination. Indeed, the presence of a hydroxyl group can lead to spontaneous decomposition to a carboxylic acid function formed from an intermediate quinone methide (97).

The intentional introduction of a fluorine atom at an appropriate position in the molecule, from where it can be eliminated as fluoride ion, provides a mechanism for suicide enzyme inhibition. The use of fluorinated ketones and fluoroamino acids in this respect is well documented. Fluorinated amino acids have been successfully employed as suicide inhibitors. Such inhibitors form a reversible complex with the target enzyme. Although bound to the enzyme, the substrate is transformed in such a way as to activate the latent functionality present in the molecule. The activated suicide inhibitor then undergoes an irreversible reaction with a nucleophilic group on the enzyme and, thus, inactivates the enzyme.

FLUORINE-CONTAINING DRUGS AS ANTIMETABOLITES

Fluorinated nucleosides and nucleotides represent an important group of drugs, which have found use in the treatment of cancer and AIDS. Fluorine has been introduced into both the base and the sugar residue (Figure 10).

Fluorouracil is a major antimetabolite used in the treatment of solid cancers. Fluorouracil is converted into a pharmacologically active metabolite 5-fluorodeoxyuridine monophosphate, which inhibits the enzyme thymidylate synthetase, which then results in reduced formation of thymidine and, hence, DNA (98). The presence of the fluorine atom at C-5 blocks the essential addition of formate (Figure 11). Additionally, fluorouracil is metabolized to ribo- and deoxyribonucleotides, which act as false bases for incorporation into RNA and DNA. Because fluorouracil and its anabolites are concentrated in cancer cells, the enzymatic blockade inhibits tumor growth. The inhibition of thymidylate kinase is ascribed to several properties of fluorine. The size of the fluorine atom and the strength

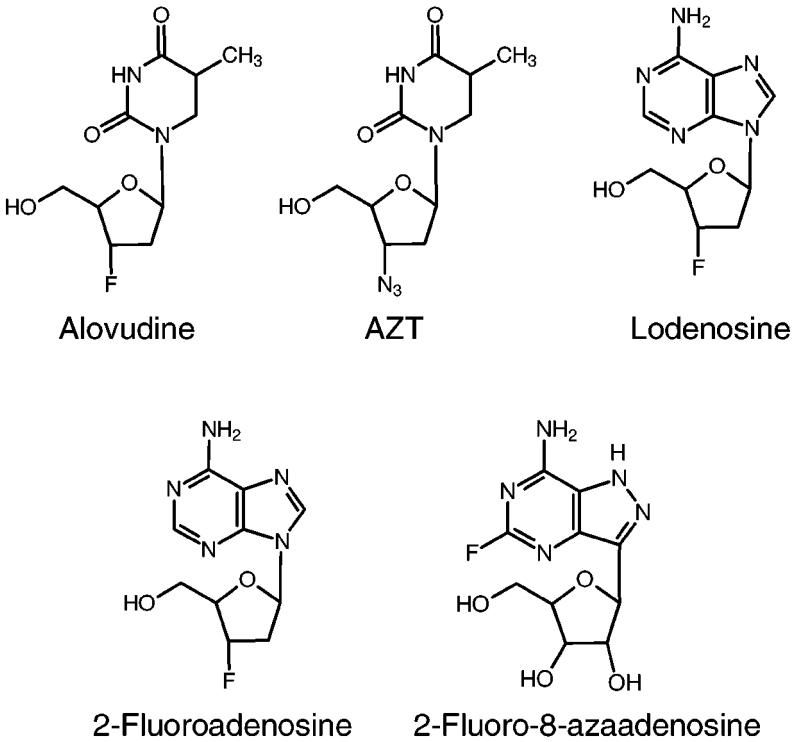


Figure 10 Chemical structures of fluorine-containing antimetabolites.

of the carbon-fluorine bond allow the molecule to enter the active site without metabolism. The affinity of the antimetabolite for the enzyme is 1000-fold greater than the natural substrate.

The limited efficacy of fluorouracil has been attributed to first pass elimination catalyzed by dihydropyridamine dehydrogenase. Eniluracil is being developed as an inhibitor of this enzyme and has been found to reduce liver uptake and increase the radiotracer half-life in tumors (8). An undesirable consequence of the catabolism of fluorouracil is the diversion into metabolic routes that lead to formation into the highly toxic fluoroacetate, by scission of the pyridimine ring (99). It has been postulated that fluoroacetate is responsible for the cardiotoxicity associated with fluorouracil.

Fluoroacetate enters the tricarboxylic acid cycle in mammalian cells and is converted into fluorocitric acid by "lethal synthesis" (25), which is a potent inhibitor of the enzyme aconitase. The false substrate becomes irreversibly bound to the enzyme, in a process in which fluorine acts as a good leaving group (100).

Fluorine-substituted analogues are of interest in the development of anti-AIDS drugs. Alovudine [3′-fluorothymidine (FLT)] is the direct fluorine analogue of zidovudine [3-azidothymidine (AZT)]. From a metabolic viewpoint, it has been

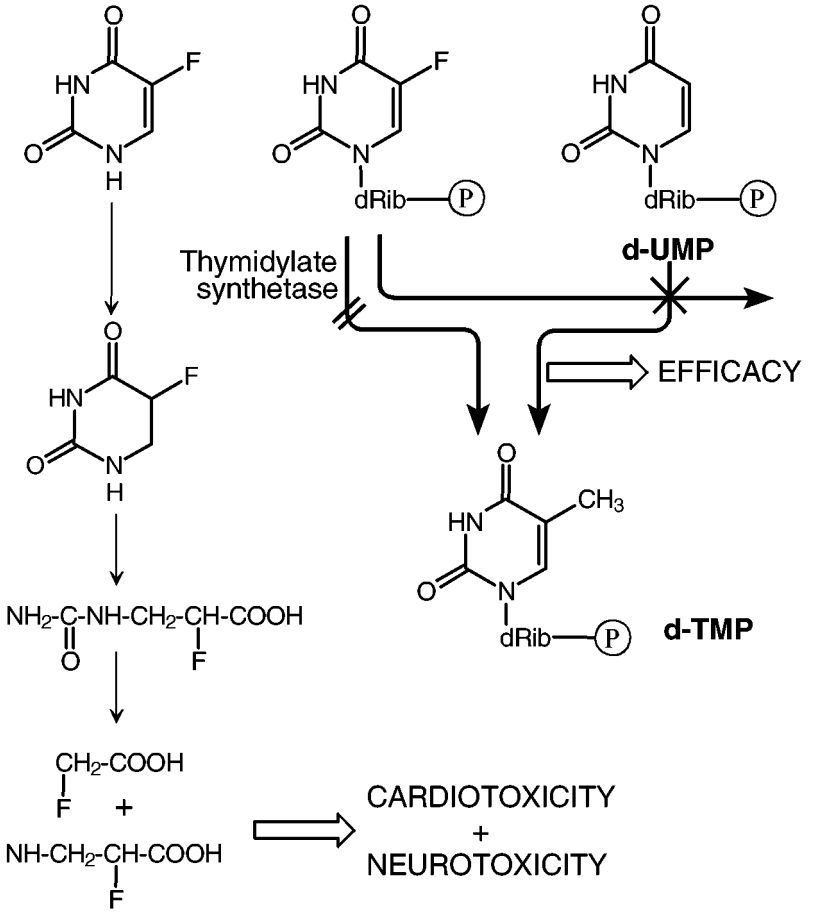


Figure 11 Role of metabolism in the efficacy and toxicity of fluorouracil (98).

shown that FLT is well phosphorylated in relevant cell lines to the 5-triphosphate, which is the active inhibitor of HIV-associated reverse transcriptase (101). In addition, the compound, like AZT, could act as a terminator of DNA synthesis because of the lack of the 3′-hydroxyl group, but unlike AZT it would not form a toxic 3′-amino metabolite. FLT was found to exhibit an anti–HIV-7 potency similar to that of AZT but with slightly better selectivity of effects and with higher intracellular active metabolite levels (102). The elimination half-life of FLT in monkeys is slightly longer than AZT, which may reflect the fact that FLT is excreted in urine as unchanged drug, unlike AZT, which undergoes some glucuronidation. However, alovudine was not developed into a drug.

The dideoxypurine nucleosides 2′,3′-dideoxyadenosine and 2′,3′dideoxyinosine have demonstrable clinical efficacy and in vivo virustatic activity in patients with AIDS. However, because of the extreme acid lability of their glycosidic

bonds, these compounds require administration either with antacids or in an enterically coated tablet to be orally bioavailable. Lodenosine (2′-fluoro-2′,3′-dideoxyadenosine) is a rationally designed nucleoside analogue that was designed to have increased chemical and metabolic stability. The introduction of fluorine has been found to render the molecule acid stable without loss of antiretroviral activity (103). It is also much less susceptible to enzymic deamination in MOLT4 cells but could still form the active antiviral 5′-triphosphate anabolite. Furthermore, after oral administration, the 2′-fluoro analogue has CNS penetration properties equivalent to the parent compound in rhesus monkeys, but the greatly improved acid stability and increased plasma half-life make it an attractive alternative to 2′3′-dideoxyadenosine as an adjunct to HIV therapy (104).

Fluorine substitution can be used to increase the metabolic stability of the purine ring. Thus 2-fluoroformycin, 2-fluoroadenosine, 2-fluoro-8-azadenosine, and 9-arabinofluranosyl-2-fluoroadenine are poor substrates for adenosine deaminase (105–107). In addition, the 2-fluoro substituent does not seriously impair phosphorylation by adenosine kinase.

Emtricitabine is a nucleoside analogue reverse transcriptase inhibitor structurally related to lamivudine, which retains activity against a substantial proportion of lamivudine resistant isolates. It possesses good CNS penetration in animals, and phase I/II trials are underway (108). Unfortunately, recent field trials that involved the drug had to be halted because of five deaths (109).

CONCLUSION

A large number of therapeutic agents contain strategically placed fluorine atoms. The introduction of fluorine into a molecule can alter both the rate and route of drug metabolism in a manner dependent on the site of fluorination in relation to the sites of metabolic attack. Fluorine substitution can also influence the tissue distribution of a drug, and fluorinated drugs have the distinct advantage that their in vivo tissue pharmacokinetics can be monitored noninvasively by ^{19}F-labeled magnetic resonance spectroscopy. Substitution of fluorine for hydrogen at the site of oxidative attack can block metabolism, or deflect metabolism along an alternative route of metabolism. However, oxidative defluorination can occur in both aromatic and aliphatic systems, and therefore formal metabolic studies are always required when using fluorine substitution to determine the role of a particular biotransformation in a physiological or toxicological process.

In terms of drug design, fluorine substitution can be used to alter the rate of drug metabolism and thereby produce a drug with a longer duration of action. Such an approach has already been used successfully for several classes of drugs. In addition, fluorine substitution can be used to reduce toxicity by blocking the formation of toxic metabolites and, in particular, chemically reactive metabolites. This can be achieved by fluorine substitution, at the appropriate site of the molecule, with an alteration in the balance between direct detoxication and metabolic bioactivation, provided the chemical modification does not impair drug efficacy.

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