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Understanding organofluorine chemistry. An introduction to the C–F bond

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Fluorine in medicinal chemistry

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It has become evident that fluorinated compounds have a remarkable record in medicinal chemistry and will play a continuing role in providing lead compounds for therapeutic applications. This *tutorial review* provides a sampling of renowned fluorinated drugs and their mode of action with a discussion clarifying the role and impact of fluorine substitution on drug potency.

Introduction

Small molecule natural products have had a significant impact on drug development. The taxoids, the *Vinca* alkaloids, the etoposides or the anthracyclines are illustrative examples of the utility of natural sources in clinically based oncology. Considering that organofluorine compounds are virtually absent as natural products, it is interesting to question why 20–25% of drugs in the pharmaceutical pipeline contain at least one fluorine atom. One of the earliest synthetic fluorinated drugs is the antineoplastic agent 5-fluorouracil, an antimetabolite first synthesised in 1957.[1] It shows high anticancer activity by inhibiting the enzyme thymidylate synthase, thereby preventing the cellular synthesis of thymidine. Since the advent of 5-fluorouracil, fluorine substitution is commonly used in contemporary medicinal chemistry to improve metabolic stability, bioavailability and protein–ligand interactions. Fast progress in this area is fuelled by the development of new fluorinating reagents and fluorination processes increasing the range of synthetic fluorinated building blocks amenable to functional group manipulation. The strategic use of fluorine substitution in drug design has culminated with the production of some of the key drugs available on the market. These include Fluoxetine [antidepressant], Faslodex [anticancer], Flurithromycin [antibacterial] and Efavirenz [antiviral], four drugs that we have selected to illustrate the wide range of disease areas benefiting from fluorine chemistry and, from a molecular point of view, the structural diversity of the fluorinated component.

The antidepressant Fluoxetine (Eli Lilly), sold as the racemate and more commonly known as Prozac®, is a

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Sophie Purser graduated with an MChem (Hons) degree from Jesus College Oxford in 2004, completing her Part II research project with Dr John M. Brown, FRS. She is currently carrying out her DPhil at the University of Oxford under the supervision of Dr Véronique Gouverneur, where her research is focused on the synthesis of enantioenriched allylic fluorides and their application in the synthesis of fluorinated carbohydrate analogues.

Peter R. Moore was born in Torquay in Devon, England. He obtained his BSc in Chemistry from the University of Wales College of Cardiff in 1992 and subsequently completed his PhD studies with Professor Stan Roberts in 1995 at the University of Exeter. He then spent two years as a postdoctoral research fellow with Professor Tim Donohoe at the University of Manchester, working on the development of the osmium tetroxide mediated directed dihydroxylation reaction. After completion of these studies in 1997, Peter took up his current position in the Discovery Chemistry Department at AstraZeneca UK. His current interests remain in the field of organic synthesis and its application to the discovery of new compounds of medicinal importance.

Steve Swallow received his PhD in 1992 from the University of Sheffield under the supervision of Dr D. Neville Jones before taking up a SERC/NATO Postdoctoral Fellowship with Professor Gary A. Molander at the University of Colorado in Boulder. On returning to the UK in 1994 he became a team leader in the medicinal chemistry department at Roche in Welwyn Garden City where he worked on antiviral protease inhibitors. In 2001 he moved to Roche in Palo Alto, California where he continued work on antiviral projects. In 2004 he returned to the UK and joined AstraZeneca at the Alderley Park site where he is currently an Associate Director of medicinal chemistry.

Véronique Gouverneur received her undergraduate degree in chemistry at the Université Catholique de Louvain (Belgium), where she worked with Professor L. Ghosez. She stayed in the group of Professor L. Ghosez for her doctoral studies. In 1992, she moved to a postdoctoral position with Professor R. Lerner at the Scripps Research Institute (USA). She returned to Europe in 1994 where she accepted a position of Maître de Conférence at the University Louis Pasteur in Strasbourg (France). She worked with Dr Charles Mioskowski during this period. She started her independent research career as a member of the chemistry faculty at the University of Oxford in 1998, where her group's research interests are centred on fluorine chemistry. Since her appointment in Oxford, she also holds a tutorial fellowship at Merton College Oxford where she teaches organic and biological chemistry. To date, she has published ~80 papers, patents and book chapters. Véronique's research has recently been recognised by the AstraZeneca Award for organic chemistry 2005. In 2007, she joined the Editorial Board of Organic and Biomolecular Chemistry.

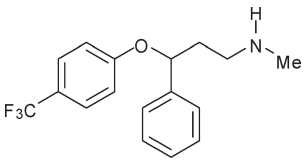


Fig. 1 Prozac®.

molecule featuring a trifluoromethyl group on one of its aryl rings (Fig. 1). It was approved by the Food and Drug Administration (FDA) in December 1987, and grew to become the most prescribed antidepressant drug worldwide, achieving annual sales in the region of one billion US dollars. In 1994, the FDA approved the drug for use in the treatment of both obsessive-compulsive disorder and bulimia. Studies have shown that depression is linked to low levels of the neurotransmitter 5-hydroxytryptamine (5-HT), also known as serotonin. Fluoxetine acts by selectively inhibiting the reuptake of serotonin, allowing the neurotransmitter to activate its specific receptor. Structure–activity relation studies showed that the inclusion of a trifluoromethyl group in the *para*-position of the phenolic ring increased the potency for inhibiting 5-HT uptake by 6-fold, compared to the non-fluorinated parent compound.[2] It is believed that the steric bulk of the trifluoromethyl group at this position allows the phenoxy ring to adopt a conformation which favours binding to the serotonin transporter.[3]

Tamoxifen has been used very successfully since the 1970's in the treatment of hormone dependent breast cancer. It is an oestrogen antagonist in breast tissue, but also acts as an oestrogen agonist in the bones and endometrium. This spares the bones from the full effects of oestrogen deprivation, but has been linked to some undesirable side effects such as an increased risk of endometrial cancer. Faslodex® (AstraZeneca), also known as fulvestrant, is a pentafluorinated 7α-alkylsulfinyl analogue of 17β-oestradiol developed to address the drawbacks of tamoxifen (Fig. 2). It is an oestrogen receptor antagonist, but has no agonist activity. Fulvestrant acts by competitively binding with oestradiol to the oestrogen receptors in breast tissue, reducing proliferation of the tumour cells.[4]

Erythromycin is a macrolide antibiotic which is effective against a wide range of pathogenic bacteria and is used to treat an array of infections including bronchitis and Legionnaire's disease. It is especially important for treatment of patients with penicillin allergies. Erythromycin is unsuitable for the treatment of the *Helicobacter pylori* infection, which causes gastritis, as the drug decomposes under the acidic conditions of the stomach. Flurithromycin (Pharmacia), launched in 1997, is a fluorinated analogue of erythromycin developed with the aim

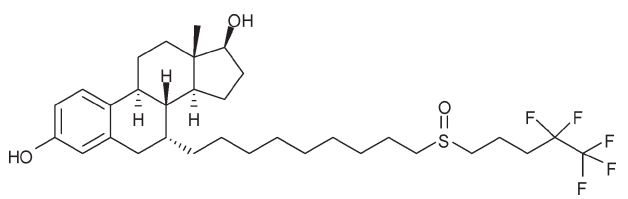


Fig. 2 Faslodex®.

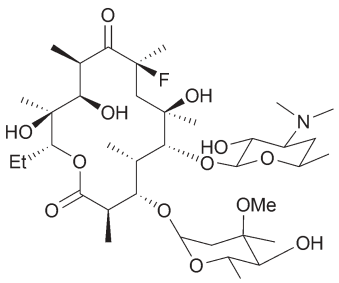


Fig. 3 Flurithromycin.

of improving stability under acid conditions (Fig. 3). In the treatment of gastritis, a condition which can lead to peptic ulcers, flurithromycin has a longer biological half live, better bioavailability and reaches higher tissue concentrations than erythromycin *in vivo*.[5,6]

Efavirenz (Bristol-Myers Squibb, trade names Sustiva® and Stocrin®) is a non-nucleoside reverse transcriptase inhibitor used in the treatment of patients with HIV (Fig. 4). In the absence of a cure for the HIV infection, current strategy is to aim to suppress the replication of the virus for as long as possible. Efavirenz, a molecule with a trifluoromethyl group attached to a tertiary stereogenic centre in a heteroaliphatic ring, acts by binding to the reverse transcriptase enzyme, remote from the active site, altering its conformation, and hence inhibiting the enzyme. In order to minimise the development of drug resistance, current treatment guidelines recommend the use of a combination of two nucleoside reverse transcriptase inhibitors with either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor. In 2001, the Efavirenz-based combination therapies were found to be the most active against the retrovirus, and were better tolerated by patients.[7,8] Structure–activity relationship studies showed that the presence of the trifluoromethyl group improved drug potency by lowering the pK_a of the cyclic carbamate, which makes a key hydrogen bonding interaction with the protein.[9]

So how does fluorine contribute to the efficiency of a drug? Several key features are important for drugs to be effective. In the case of an orally administered drug, it must be able to withstand physiological pH in the stomach long enough to cross into the blood stream and to be transported in sufficient quantity to the site of action. It must then perform its desired task efficiently, and finally be metabolised at an appropriate rate into non-toxic materials. In this tutorial review, we have opted for a case study approach to explain how fluorine substitution is used to improve drug potency by addressing one or several of the criteria listed above.

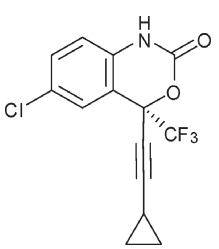


Fig. 4 Efavirenz.

Table 1 The effect of fluorine substitution on pK_a and pK_b values

Carboxylic acid	pK_a	Alcohol	pK_a	Amine	pK_b
CH_3CO_2H	4.76	CH_3CH_2OH	15.9	$CH_3CH_2NH_2$	10.6
CH_2FCO_2H	2.59	CF_3CH_2OH	12.4	$CF_3CH_2NH_2$	5.7
CHF_2CO_2H	1.34	$(CH_3)_3COH$	19.2	$C_6H_5NH_2$	4.6
CF_3CO_2H	0.52	$(CF_3)_3COH$	5.1	$C_6F_5NH_2$	−0.36

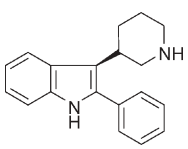
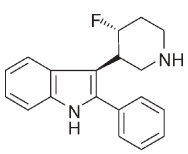
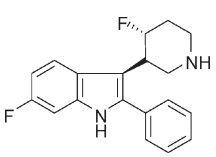
The effect of fluorine on physicochemical and conformational properties

Perturbation of pK_a

The perturbation of pK_a can strongly modify the binding affinity and the pharmacokinetic properties of a pharmaceutical agent. Modulation of pK_a may impact on bioavailability (% of the dose reaching the circulatory system and denoted F) by affecting the absorption process.[10] In contrast to intravenously administered drugs (100% bioavailability), the bioavailability of drugs orally administered decreases due to either poor absorption or first-pass metabolism. Fluorine is the most electronegative element [χ_P (Pauling) 4.0] and its inclusion in a molecule has a very strong effect on the acidity or basicity of proximal functional groups (Table 1).[11,12]

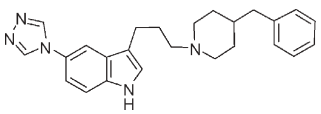
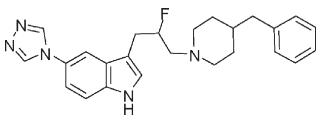
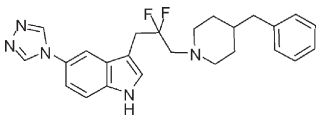
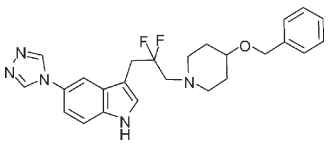
In a series of 3-piperidinylindole antipsychotic drugs, it was found that fluorination decreased the basicity of the amine, thereby improving bioavailability (Table 2).[13] It has been suggested that antipsychotics with a greater affinity for serotonin 5-HT_2 receptors than for dopamine D_2 receptors display a reduction in undesirable side effects, such as delusions and hallucinations. γ-Fluorination of the amine reduced the pK_a by more than one unit to give a compound with moderate bioavailability in rats (F 18%) and good pharmacokinetics in dogs (F 37%, $T_{1/2}$ 7.4 h). The compound also had good selectivity for the 5-HT_{2A} receptors over the D_2 receptors. As the compound was metabolised primarily by hydroxylation at the 6-position on the indole ring, this position was blocked by further fluorine substitution. This structural modification led to a greater improvement in bioavailability and increased binding affinity by an order of magnitude.

Table 2 Basicity and bioavailability in a series of 3-piperidinylindole antipsychotics

Indole	5-HT_{2A}[a]	pK_a[b]	F (%)
	0.99	10.4	Poor
	0.43	8.5	18
	0.06	—	80

[a] Affinities at human cloned 5-HT_{2A} receptor (nM). [b] Bioavailability calculated from dosing at 0.5–2 mg per Kg iv and po.

Table 3 Basicity and bioavailability in a series of piperidinyl and piperazinyl indoles

Amine	pK_a	IC_{50} 5-HT_{1D}	Bioavailability
	9.7	0.3	Poor
	8.7	0.9	Good
	6.7	78	—
	5.9	8.5	Good

A similar effect of the pK_a on bioavailability can be seen in a series of piperidinyl and piperazinyl indoles, synthesised with the aim of improving the treatment of migraine. Vasoconstriction in the cranial blood vessels, and/or inhibition of neurogenic inflammation are responsible for pain relief associated with migraine drugs. It is important for the drugs to be selective for 5-HT_{1D} receptors over 5-HT_{1B} receptors, as 5-HT_{1B} receptors are implicated in coronary vasoconstriction. The basicity of the amines was reduced by fluorination, improving dramatically the oral bioavailability. Although beneficial, in a series of structurally related compounds, the effect of fluorine substitution on oral bioavailability could not always be accurately predicted (Table 3).[14]

Modulation of lipophilicity

There are two routes for an orally administered drug to be absorbed and distributed: active transport (a mediated process requiring energy from ATP) and passive transport (a process not requiring energy). Passive transport is the more common process and is dependent on the permeability of the cell membrane. For a drug molecule to pass through a cell membrane its lipophilicity must be such that it can pass into the lipid core but not become trapped in it. Lipophilicity is expressed as a partition coefficient (log *P*) between octanol and water, with the most lipophilic compounds being partitioned in the octanol layer, and the least lipophilic compounds being partitioned in the water layer. A distribution coefficient (log *D*) is also used to quantify lipophilicity when charge states need to be considered, and is the logarithmic coefficient of the distribution of a molecule between water and octanol at a given pH, typically 7.4. The Lipinski "rule-of-5", a set of

Table 4 The effect of fluorine substitution on lipophilicity (log *P*)

	Log *P* (octanol–water)
CH_3CH_3	1.81
CH_3CHF_2	0.75
$CH_3(CH_2)_3CH_3$	3.11
$CH_3(CH_2)_3CH_2F$	2.33

empirical rules used as a guide to predicting good drug candidates, states that a log $P > 5$ will probably give poor absorption.[15] Excess lipophilicity is a common cause of poor solubility, leading to erratic and incomplete absorption following oral administration. A common misconception is to assume that fluorination always increases lipophilicity. Monofluorination or trifluoromethylation of saturated alkyl groups usually decreases lipophilicity due the strong electron withdrawing capabilities of the fluorine (Table 4).[16]

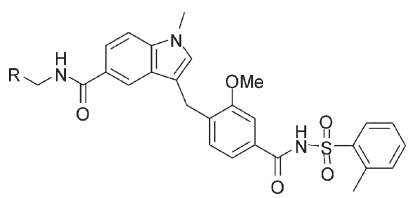
In contrast, aromatic fluorination, per/polyfluorination and fluorination adjacent to atoms with π-bonds (with the exception of some α-fluorinated carbonyl compounds) increases lipophilicity.[16] It is the excellent overlap between the fluorine 2s or 2p orbitals with the corresponding orbitals on carbon, making the C–F bond highly non-polarizable, thereby contributing to increased lipophilicity. The impact of fluorine substitution on lipophilicity has been explored for the development of leukotriene receptor inhibitors. These compounds reduce bronchioconstriction in asthma sufferers and were found to be more potent drug candidates when featuring amide substituents of increased lipophilicity. Increasing the chain length to greater than six carbon atoms improved lipophilicity but this structural modification could not be exploited fully as longer chains resulted in a loss of affinity for the receptor. The introduction of fluorine substituents addressed this problem and resulted in, on average, a 10-fold increase in potency *in vivo* compared to the non-fluorinated analogues. Measurement of the log *P* values showed that each fluorinated amide was more lipophilic than its non-fluorinated counterpart (Table 5).[17]

Conformational changes

Substitution of a hydrogen or hydroxyl group for a fluorine in biologically active molecules is commonly tolerated, as the fluorine van der Waals radius r_v (1.47 Å) lies between that of oxygen (1.57 Å) and hydrogen (1.20 Å). Fluorine substitution therefore exerts only a minor steric demand at receptor sites. The introduction of a trifluoromethyl group within a molecule may impose a more drastic steric change as its van der Waals volume is estimated to be close to that of an ethyl group although of significantly different shape.[18] These steric variations combined with the high electronegativity of the fluorine atom, can lead to changes in preferred molecular conformation upon fluorine substitution. Methoxybenzene and trifluoromethoxybenzene do not adopt similar ground state conformations.

Although methoxybenzene adopts a planar conformation, trifluoromethoxybenzene has its OCF_3 group out of the plane (dihedral angle for C–C–O–C up to 90°). This difference in conformational preference has been exploited to design superior inhibitors of the cholesteryl ester transfer protein (Fig. 5). The cholesteryl ester protein is implicated in coronary heart disease, mediating the transfer of cholesteryl ester from high-density lipoprotein to low-density lipoprotein. When the R group was changed from a tetrafluoroethyl group to a non-fluorinated ethoxy group, an 8-fold loss in potency was observed. Molecular modelling (*ab initio*) experiments revealed that the trifluoroethyl substituent prefers an out-of-plane orientation with respect to the phenyl ring, resulting in more efficient binding to the target protein.[19]

Table 5 The effect of fluorine substitution on lipophilicity (log *P*)



R	Log *P*
$CH_3CH_2CH(CH_3)$	5.85
$CF_3CH_2CH(CH_3)$	6.18
$(CH_3CH_2)_2CHCH_2$	6.29
$CF_3CH_2CH(CH_3CH_2)CH_2$	6.45
$CF_3CH(CH_3)CH_2CH_2$	6.30
$CF_3CH(CH_3)CH_2$	5.89

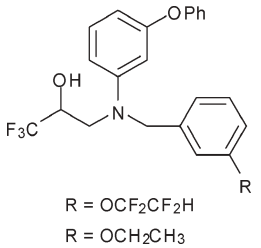


Fig. 5 Cholesteryl ester transfer protein inhibitor.

Strong electronic modification may also influence conformation. This is best illustrated with 1,2-difluoroethane, a compound known to adopt preferentially a *gauche* rather than *anti* conformation. In the preferential *gauche* conformation of 1,2-difluoroethane, both the electronegative fluorine atoms are antiperiplanar to a C–H bond, a conformation benefiting from double stabilising hyperconjugative ($\sigma \rightarrow \sigma^*$) interactions (Fig. 6). The presence of a fluorine substituent on a stereogenic centre positioned vicinal to another electronegative group may therefore significantly influence conformation and has important consequences in lead-optimisation programmes.

A conformational study was conducted on fluorinated analogues of the HIV-1 protease inhibitor Indinavir (Merck).

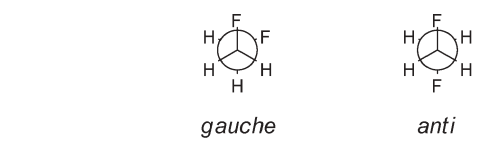


Fig. 6 *Gauche* effect of 1,2-difluoroethane.

The most active *syn*, *syn* fluorinated analogue has an inhibition constant (K_i) of 2.0 nM indicating that this compound is as effective as the parent compound (K_i = 1.9 nM). The *gauche* effect stabilises the fully extended conformation of the carbon backbone for the *syn*, *syn* fluorhydrin allowing for optimal binding.[20] It was found that the *syn*, *anti* fluorinated analogue was 8-fold more potent than the corresponding non-fluorinated parent compound C_{17}-*epi*-Indinavir, with K_i's of 20 nM and 160 nM respectively (Fig. 7). Notably, each of the fluorinated inhibitors was found to be slightly more lipophilic (<0.28 log units) than Indinavir, with the exception of the stereoisomer *anti*, *anti*. These data suggest that fluorination of carbons adjacent to heteroatom substituents results in increased lipophilicity, a result consistent with literature data.[21]

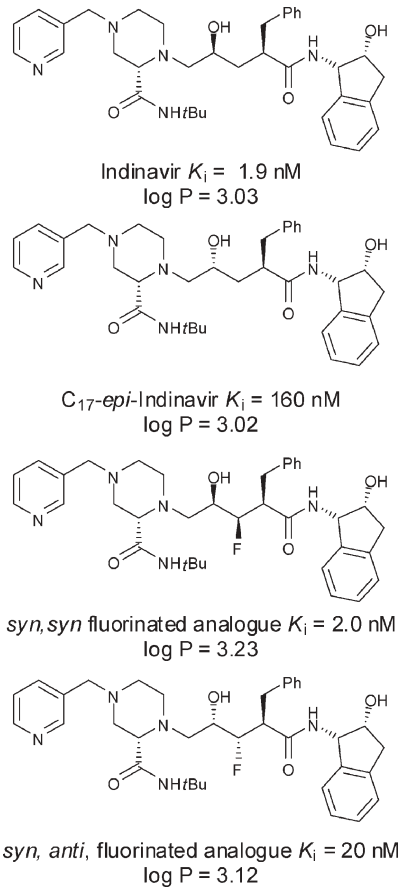


Fig. 7 Indinavir and its analogues.

Hydrogen bonding and electrostatic interactions

The importance of the carbon–fluorine bond in hydrogen bonding is still in contention. The C–F bond is highly non-polarizable and can participate in weak hydrogen bonding only. An example of the involvement of a C–F bond in hydrogen bonding can be seen in the differing modes of action of the two isomers of fluoronorepinephrine (F-NE). The 2F-isomer is an α-adrenergic agonist, whilst the 6F-isomer is a β-adrenergic agonist. This difference has been attributed to two distinct preferred conformations, both stabilised by hydrogen bonding (Fig. 8).[22]

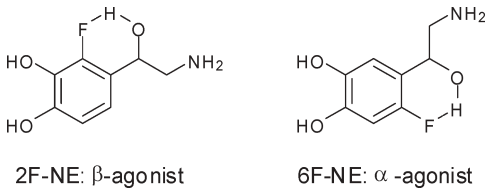


Fig. 8 Hydrogen bonding in two structural isomers of fluoronorepinephrine.

In contrast to hydrogen bonding, the participation of fluorine in electrostatic interactions is widely accepted and may contribute to the enhanced binding affinity of organofluorine compounds for the enzyme's active site. The effect of aromatic fluorine substitution was investigated in a series of thrombin inhibitors. Several fluorinated and chlorinated inhibitors were tested and were found to have inhibitory constants similar to the parent molecule. Notably, only the 4-fluorophenyl derivative was found to be significantly more active (Fig. 9). Measurement of log *D* values showed that an increase in lipophilicity could not explain the increased binding potency of the 4-F derivative. X-Ray crystal structure analysis of the *para*-fluorinated analogue bound to thrombin revealed that the C–F bond came into close contact with a positively polarized carbon atom of a C=O unit and an H–C_α unit of Asn98 in the D-pocket (Fig. 9). These dipolar C–F···H–C_α and C–F···C=O interactions are believed to be the major determinant in the observed increase in potency.[23]

Metabolic stability

Oxidative metabolism

Following drug administration, the physiological response is to detoxify and eliminate the drug. Drugs can be eliminated unchanged, but more commonly are metabolised prior to elimination. The most important group of enzymes metabolising drugs are the Cytochrome P450 monooxygenases, a superfamily of heme-thiolate proteins found mainly in the liver. Upon oxidation, their lipophilicity is decreased, allowing for more rapid clearance. Low metabolic stability due to oxidation processes mediated by P450 enzymes is a common problem in drug discovery, but can be circumvented by blocking metabolically labile sites with fluorine substitution. This is exemplified in the lead optimisation of the cholesterol inhibitor Ezetimib (Schering-Plough) (Fig. 10).[24]

Radiolabelling studies of the first generation drug indicated a complex metabolic mixture, in the bile of rats, which proved to be more potent than the drug itself. The primary metabolism pathways were found to be dealkylation of the N1- and C4-methoxyphenyl groups, *para* hydroxylation of the pendent C3-side chain phenyl group, benzylic oxidation and opening of the azetidinone ring.[25] Structure–activity relationship studies showed that the incorporation of "productive" functional groups was beneficial. The benzylic (*S*)-hydroxy group and the C4 hydroxy group were introduced, leading to an increase in potency. "Non-productive" metabolism was blocked by the incorporation of fluorine at the *para* positions of the phenyl rings to minimize oxidation by the P450 enzymes. The electron withdrawing nature of the fluorine substituent

Aromatic Substitution	K_i [μM]
H	0.31
2-F	0.50
3-F	0.36
4-F	0.057
2,3-F_2	0.49
2,6-F_2	0.61
3,4-F_2	0.26
3,5-F_2	0.59
1,2,3,4,5-F_5	0.27
4-Cl	0.19

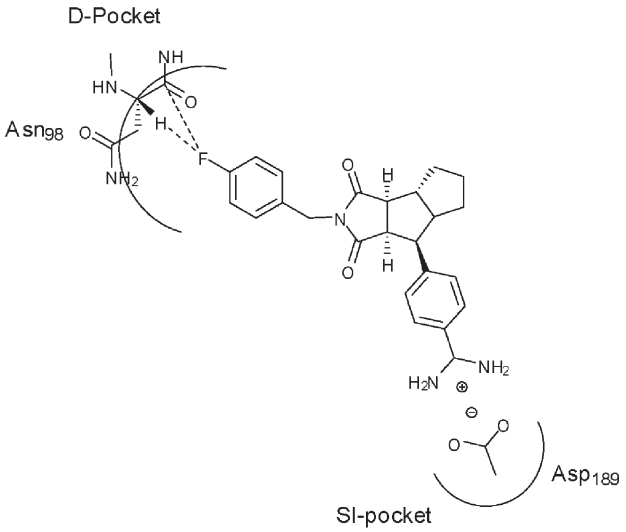


Fig. 9 Fluorine in electrostatic interactions in a series of thrombin inhibitors.

deactivates the aromatic ring towards metabolic pathways. These structural modifications led to a second generation drug which was found to be 400 times more potent, requiring lower doses due to improved metabolic stability *in vivo*.

Fluorine substitution has also been utilised to block metabolism of aromatic methoxy groups, for example in the synthesis of a series of second generation cyclic nucleotide phosphodiesterase inhibitors. The first generation of compounds, developed for treatment of asthma, entered into clinical trials, and were found to be extensively metabolised *in vitro*, with a concomitant short half-life *in vivo*. The reactive quinone metabolites were thought to be implicated in toxic events such as acute toxicity and idiosyncratic reactions. Replacement of the metabolically labile methoxy group with a difluoromethoxy group increased half-life and prevented the formation of the reactive quinone intermediates (Fig. 11).[26]

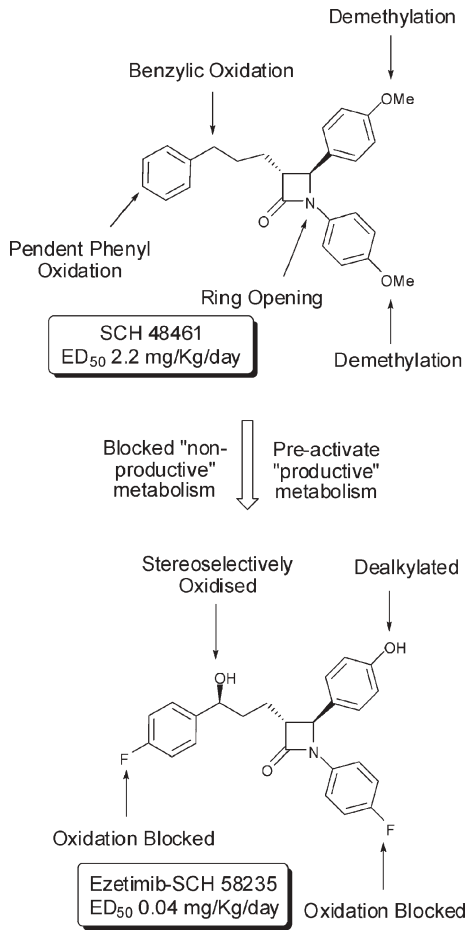


Fig. 10 Lead optimisation of Ezetimib.

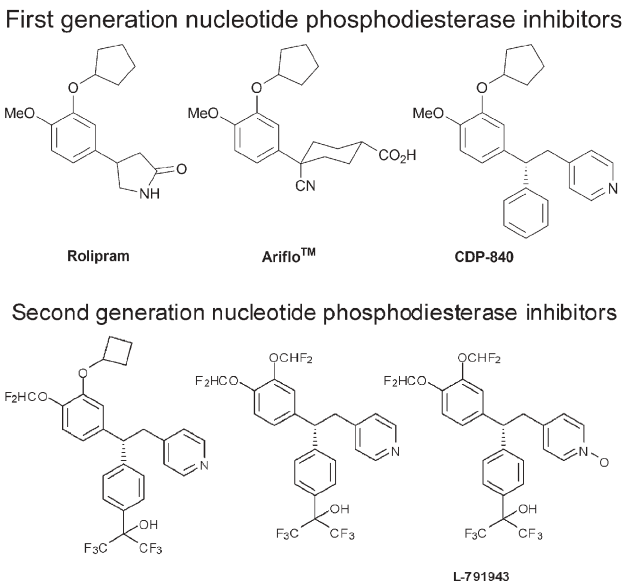
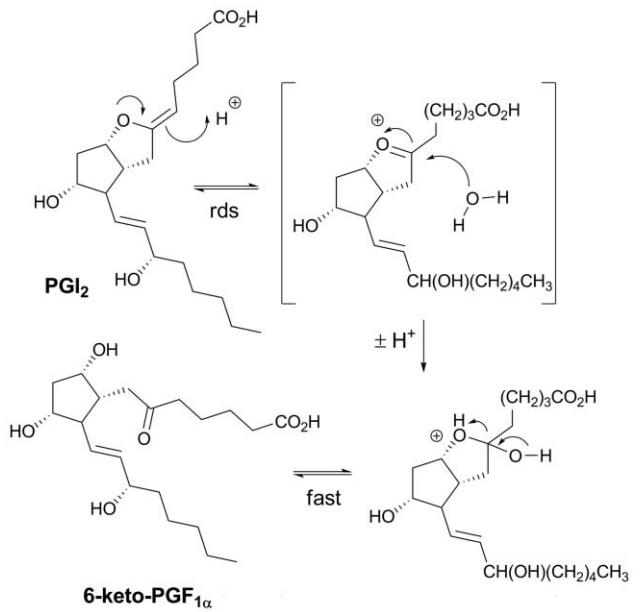


Fig. 11 Nucleotide phosphodiesterase inhibitors.



Scheme 1 *In vivo* hydrolysis of prostacyclin.

Hydrolytic metabolism

Prostacyclin (PGI_2) is a powerful vasodilator and a potent inhibitor of platelet aggregation and hence an attractive drug candidate for use in the treatment of vascular disease, for example thrombosis. Its clinical application is severely limited as the compound is easily hydrolysed *in vivo* to afford the inactive 6-oxo-$PGF_{1\alpha}$ derivative, in both acidic and neutral media. The origin of this intrinsic hydrolytic instability is the acid labile enol ether moiety (Scheme 1).[27]

Hydrolytic stability can be greatly enhanced by fluorination. The highly electronegative fluorine acts by withdrawing electron density from the enol ether, consequently decreasing its rate of hydrolysis. The chemical half life of the mono-substituted 7-F-PGI_2 analogue was found to be greater than one month, compared to only 10 minutes for prostacyclin itself.[28] The difluorinated analogue AFP-07 shows an even larger increase in chemical stability, exhibiting a half life of 90 days[29] (Fig. 12).

In vivo racemisation

(±)-Thalidomide was released onto the market in 1956 as a sedative hypnotic used for the treatment of morning sickness (Fig. 13). After unexpected serious birth defects were seen in babies whose mothers had been prescribed the drug, racemic thalidomide was withdrawn from the market in 1962. This was one of the most notorious medical disasters of the 20th century. In 1965, it was discovered that the drug had a dramatic beneficial effect in the treatment of leprosy. More recent studies have shown its clinical utility in the treatment of various haematologic malignancies and solid tumours as well as a variety of inflammatory diseases such as rheumatoid arthritis, Crohn's disease, asthma and even AIDS.

It has been suggested that whilst the (*R*)-enantiomer causes the clinically effective sedative hypnotic effects, the (*S*)-enantiomer is responsible for the teratogenic side effects.

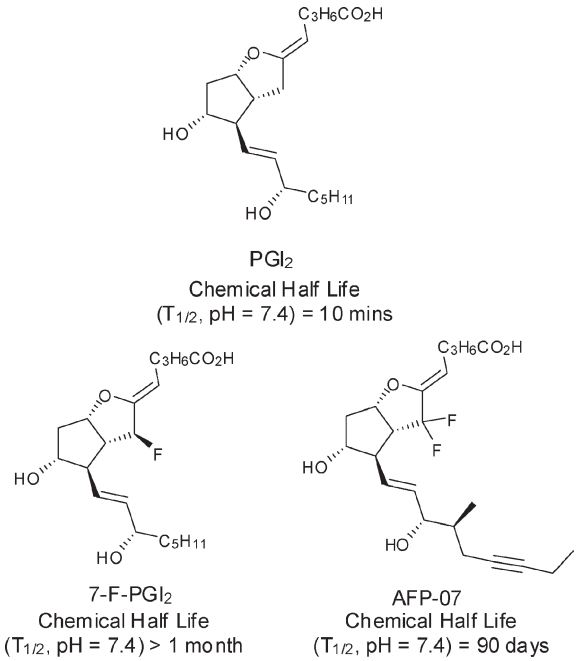


Fig. 12 Chemical half lives of PGI_2 analogues.

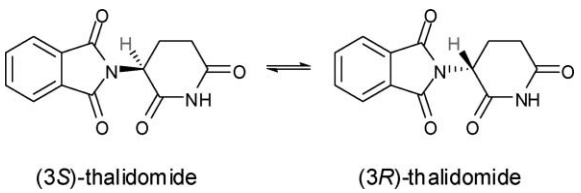


Fig. 13 *In vivo* epimerisation of thalidomide.

Thalidomide rapidly epimerises under physiological conditions due to the presence of the acidic hydrogen atom on the stereogenic centre situated adjacent to a carbonyl group. This undesirable racemisation renders any bioassay of the individual enantiomers very difficult.[30] Replacement of this acidic hydrogen located on the stereogenic centre with fluorine prevents the *in vivo* epimerisation process, allowing the synthesis and evaluation of both enantiomers (Fig. 14). The (3*S*)-fluorothalidomide analogue was found to be a more active inhibitor of tumour necrosis factor-α (TNF-α) which is implicated in inflammatory processes, than the (3*R*)-fluorothalidomide or the racemic parent thalidomide.[31]

Mechanism based inhibitors

Due to its small size, the incorporation of a single fluorine atom into a molecule causes minimal steric perturbation.

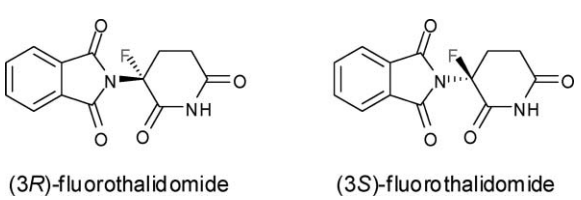


Fig. 14 Fluorothalidomide enantiomers.

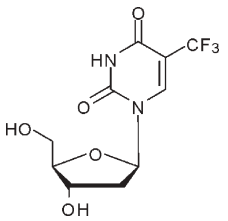


Fig. 15 Trifluridine.

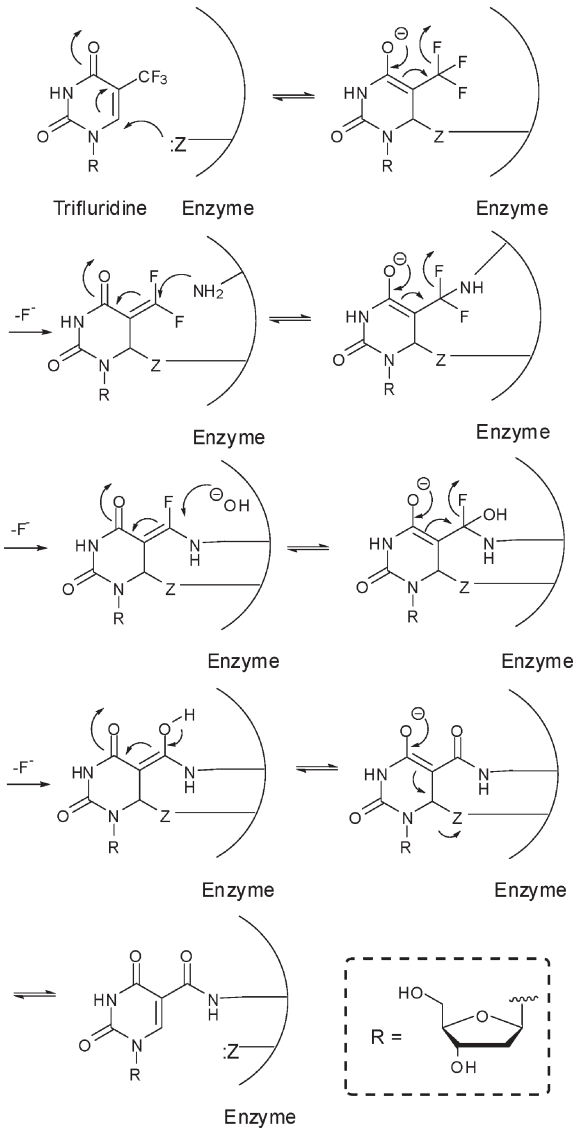
However the high electronegativity of fluorine may result in unusual metabolic pathways, leading to inhibition of the target enzyme. Trifluridine (Viroptic®) (Fig. 15) is an antiviral drug used for the treatment of eye infections caused by viral herpes. It is a mechanism-based inhibitor, causing irreversible inhibition of thymidylate synthase (TS), an enzyme mediating the methylation of deoxyuridine monophosphate (dUMP) into thymidine monophosphate (TMP), a key step in DNA biosynthesis. Inhibition of this enzyme causes apoptotic cell death, which affects rapidly dividing cells such as viral or cancer cells.

Trifluridine acts by irreversibly forming a covalent bond with thymidylate synthase. The overall transformation is a substitution at the C5 position with loss of the trifluoromethyl group. The proposed mechanism for this transformation involves Michael addition of an active site nucleophilic group (such as thiohydroxyl, hydroxyl, or amino groups) at the C6 position followed by fluoride elimination, thereby converting the trifluoromethyl group into the highly reactive exocyclic difluoromethylene intermediate. This intermediate can further react with a nucleophilic amino residue of the enzyme to lead, after fluoride elimination and hydrolysis, to an amide bond linking irreversibly and covalently the inhibitor to the enzyme (Scheme 2).[32]

Another mechanism-based inhibitor of thymidylate synthase (TS) is 5-fluorouracil (5-FU) (Fig. 16). 5-FU is used in standard therapy for treatment of a variety of malignancies including gastrointestinal cancers, breast cancer and head and neck cancer. Because of the short biological half-life of the drug and its irregular absorption profile, 5-FU is administered by continuous infusion or by intravenous bolus. Unfortunately the drug also displays neurotoxic and cardiotoxic side effects.

The enzymatic methylation of 2′-deoxyuridine-5′-phosphate (dUMP) involves the formation of a ternary complex between the substrate, the enzyme and the methylene tetrahydrofolic acid (CH_2FAH_4) cofactor. The catalytic event is initiated with a Michael addition of a cysteine residue of the enzyme onto dUMP, followed by covalent linkage with the cofactor. The proton at the C5 position is then abstracted to β-eliminate tetrahydrofolic acid, a process followed by reductive cleavage to release thymidine.[33] The 5-FU reacts with the enzyme and cofactor in the same manner, but the reaction pathway is halted due to the unavailability of the C5 proton (Scheme 3). The enzyme becomes inactive, preventing DNA replication and repair, and programmed cell death pathways ensue.[34]

It is thought that the cytotoxicity of 5-FU comes from a combination of modes of action, one of which is its metabolic degradation into the extremely toxic fluoroacetate, which may be responsible for the cardio- and neurotoxic side effects (Scheme 4).[34,35]



Scheme 2 Mechanism of inhibition of thymidylate synthase by trifluridine.

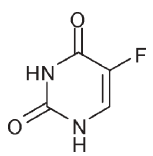
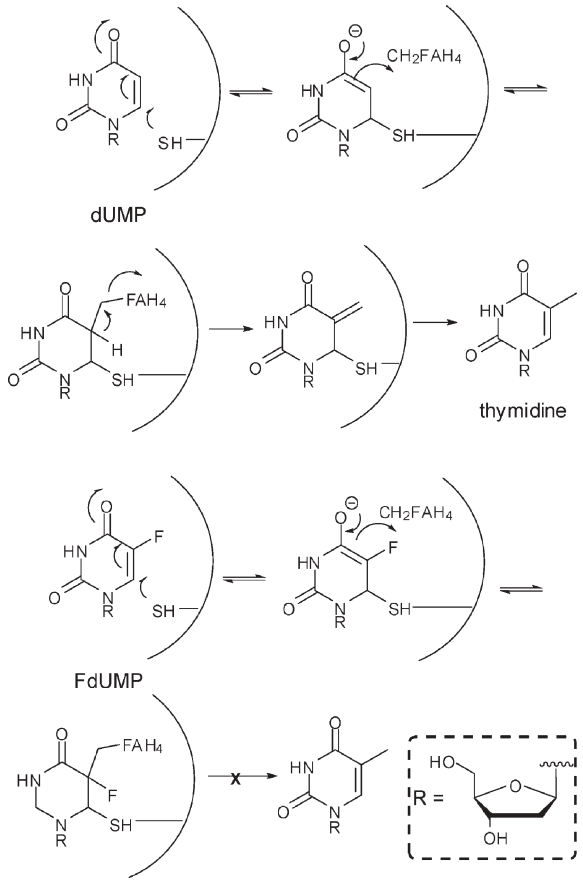
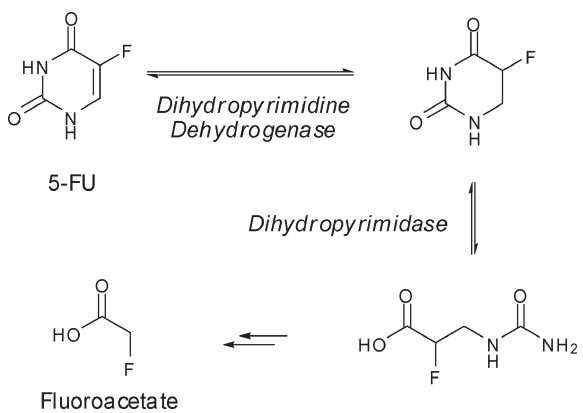


Fig. 16 5-Fluorouracil.

Another important class of fluorinated mechanism based inhibitors are the nucleoside reverse transcriptase inhibitors used in the treatment of HIV and AIDS (Fig. 17). These drugs are structurally similar in size and shape to their non-fluorinated analogues and are hence incorporated into the growing viral DNA strand. They are phosphorylated but the lack of a hydroxyl group in the 3′-position leads to chain termination.[36] It is also known that fluorination on the carbohydrate ring can affect its conformation, in particular the degree of puckering, which in turn can affect enzyme recognition.[37]



Scheme 3 Mode of action of uracil and 5-fluorouracil.



Scheme 4 Metabolic degradation of 5-FU into fluoroacetate.

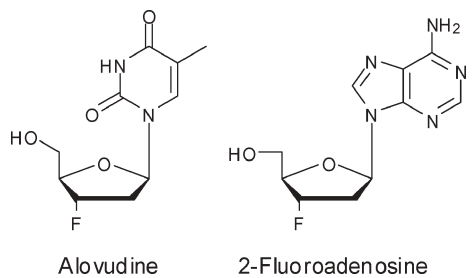


Fig. 17 Nucleoside reverse transcriptase inhibitors.

Fluorine in nuclear medicine

Positron emission tomography (PET) is a nuclear medical imaging modality mapping functional processes *in vivo*. Radionuclides typically used in PET have short half lives: ^{11}C ($t_{1/2}$ = 20 min), ^{13}N ($t_{1/2}$ = 10 min), ^{15}O ($t_{1/2}$ = 2 min), and ^{18}F ($t_{1/2}$ = 110 min). These radionuclides are produced in a cyclotron, and immediately incorporated into the radiotracer before use. ^{18}F tracers are advantageous because of their longer half lives compared to the other commonly used radionuclides. PET imaging using ^{18}F tracers is a very rapidly developing area in medicinal chemistry. Whereas MRI and CT scans show accurate anatomical information, PET scans can show biological processes, giving metabolic information.[38]

The most frequently used radiopharmaceutical for PET is 2-deoxy-2-[^{18}F]fluoro-D-glucose or [^{18}F]FDG (Fig. 18) with applications in oncology, neurology and cardiology. [^{18}F]FDG is a glucose analogue, reflecting glucose metabolism *in vivo*. It is taken up by high-glucose-using cells such as brain, kidney and cancer cells. It is phosphorylated in the same manner as glucose, but due to the absence of a hydroxy group in the C2 position, [^{18}F]FDG cannot undergo glycolysis before it decays, so remains trapped in the tissues under investigation. The ^{18}F decays into ^{18}O, which is non-toxic and non-radioactive, and is excreted in the same manner as the parent glucose. The major clinical application of [^{18}F]FDG is in oncology, based on increased glucose metabolism of tumour cells, where it is exceptionally valuable not only in initial diagnosis, but also treatment planning, response to therapy and detection of recurrence of disease. Whole body scanning allows detection of a broad variety of tumour tissue at an early stage across the entire body, and the localisation of metastatic spread, even if it is distant from the original tumour. Currently, scanners can identify metastases as small as 3 mm in diameter.[39]

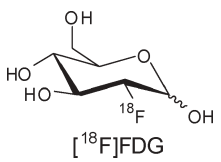


Fig. 18 2-Deoxy-2-[^{18}F]fluoro-D-glucose.

In addition to the use in diagnostic medicine, PET is emerging as an *in vivo* pharmacological imaging tool in drug development, particularly in the areas of biodistribution and drug occupancy studies. The technique is particularly advantageous for evaluation of drugs in very early clinical development, as "microdoses" can be used to enable non-invasive measurements in humans. Structure–activity relationships are used in drug design and discovery in order to identify ligands with optimum affinity for the target receptors. However, in a study conducted by seven UK-owned pharmaceutical companies between 1964 and 1985, it was found that 40% of new drug candidates were withdrawn due to serious shortfalls in their pharmacokinetics, for example poor oral absorption, unfavourable distribution, or extensive first-pass metabolism.[40] A refined approach to drug development combines structure–activity relationship studies with additional

data on the compound's metabolites, its pharmacokinetic and pharmacodynamic properties and toxicological implications, at an early stage of the discovery process.

For biodistribution studies, the drug itself can be radiolabelled. In this context, ^{18}F substitution is becoming a very powerful tool. Due to the increasing number of fluorine containing drugs, PET provides quantitative information on drug delivery and turnover in specific tissues. For example, PET may be used to investigate if a drug crosses the blood brain barrier, which is otherwise notoriously difficult to predict or assess. PET allows for the quantitative estimation of equilibrium partition coefficients between plasma and brain tissue.[41]

Another emerging strategy is the use of PET in drug occupancy studies. This technique can be utilised to determine appropriate dosing levels, and to assess selectivity and affinity of a drug for a specific target, at an early stage in clinical trials. The NK_1 receptor-binding selective tracer [^{18}F]SPA-RQC was used to determine the level of NK_1 receptor occupancy obtained by therapeutically relevant dosing of aprepitant (Merck) (Fig. 19). The drug, used for the treatment of nausea and vomiting in patients undergoing chemotherapy, was administered to healthy volunteer subjects at a variety of dosing levels. After 14 days, the [^{18}F]SPA-RQC tracer was administered, and the volunteers scanned. The images were compared to scans obtained before the drug-trial with no aprepitant present, and the occupancy of the NK_1 receptors were calculated for each dose (Fig. 20).[42]

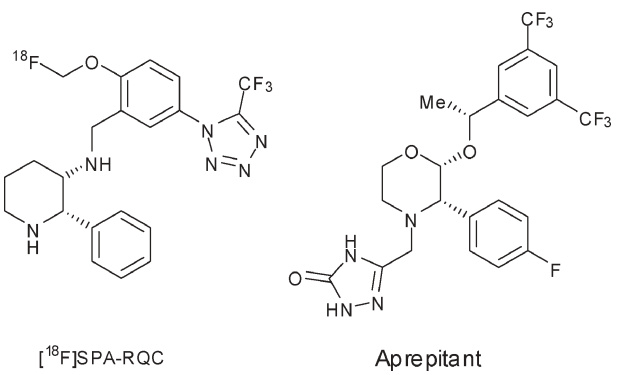


Fig. 19 The use of [^{18}F]SPA-RQC in drug occupancy studies of the drug aprepitant.

Conclusions

Due to the major successes of fluorinated compounds in medicinal chemistry, it may be predicted that the number of fluorine containing drugs on the market will continue to increase. As our understanding of the effects of fluorine substitution expands, further applications in medicinal chemistry will emerge. With the advent of major advances being made in asymmetric fluorination, there is now much further scope for the synthesis of targets featuring a fluorine atom on a stereogenic centre. The wider range of accessible substrates will, in turn, allow for further understanding of the subject. Another major development in the field is the rapid improvement in imaging technology allowing broader availability of PET scanners for researchers, and a wider range of tracers. The onset of PET imaging in both diagnostics and as a pharmacological imaging tool may even alter the decision making processes in drug discovery.

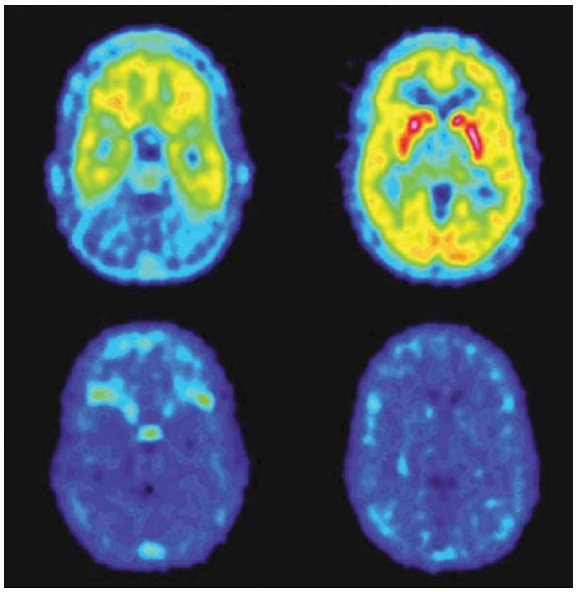


Fig. 20 Positron emission tomography (PET) images from a subject who received aprepitant 100 mg. Predose (top) and postdose (bottom) PET scans at the level of the cerebellum (left) and striatum (right). Subject number 01, estimated occupancy = 94%.[42] Reprinted from ref. 42 with permission from Elsevier.

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