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Review Article

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REVIEW ARTICLE

The role of fluorine in medicinal chemistry

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Abstract
The small and highly electronegative fluorine atom can play a remarkable role in medicinal chemistry. Selective installation of fluorine into a therapeutic or diagnostic small molecule candidate can enhance a number of pharmacokinetic and physicochemical properties such as improved metabolic stability and enhanced membrane permeation. Increased binding affinity of fluorinated drug candidates to target protein has also been documented in a number of cases. A further emerging application of the fluorine atom is the use of ^{18}F as a radiolabel tracer atom in the exquisitely sensitive technique of Positron Emission Tomography (PET) imaging. This short review aims to bring together these various aspects of the use of fluorine in medicinal chemistry applications, citing selected examples from across a variety of therapeutic and diagnostic settings. The increasingly routine incorporation of fluorine atom(s) into drug candidates suggests a bright future for fluorine in drug discovery and development. A major challenge moving forward will be how and where to install fluorine in a rational sense to best optimise molecular properties.

Keywords: *Fluorine, metabolic stability, binding affinity, positron emission tomography*

Introduction

Fluorine is both small (van der Waals radius 1.47 Å) and the most highly electronegative element in the Periodic Table (3.98 Pauling scale). This makes fluorine a unique atom that in turn has a profound effect when bound to carbon in small organic molecules. An examination of the role of fluorine in medicinal chemistry reveals that substitution of an organic compound with even a single fluorine atom or trifluoromethyl group located in a key position of a biologically active molecule can result in a profound pharmacological effect.

Traditional medicinal chemistry based on the use of natural compounds or closely related derivatives, where there is a low abundance of compounds containing fluorine, meant that fluorinated compounds were rare in medicinal chemistry until the 1970s. Early work by Fried [1] on 9α-fluorohydrocortisone acetate revealed how introduction of fluorine into an existing biologically active compound imparted beneficial properties to that compound. Currently, many fluorinated compounds are synthesised routinely in pharmaceutical research and are widely used in the treatment of diseases. These include: anti-cancer agents [2], antidepressants, anti-inflammatory agents, anaesthetics and central nervous system drugs [3], and a number of review articles on different therapeutic classes of fluorine-containing agents have been published in recent years [2–6].

Fluorine substitution has been extensively investigated in drug research as a means of enhancing biological activity and increasing chemical or metabolic stability. Important factors to be considered when synthesising fluorine-containing compounds are:

- the relatively small size of the fluorine atom (van der Waals radius of 1.47 Å), comparable to hydrogen (van der Waals radius of 1.20 Å).
- the highly electron withdrawing property of fluorine.

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- greater stability of the C–F bond compared to the C–H bond.
- greater lipophilicity of fluorine compared to hydrogen.

Despite the fact that fluorine is slightly larger than hydrogen, several studies have demonstrated that it is a reasonable hydrogen mimic and is expected to cause minimal steric perturbations with respect to the compound's mode of binding to a receptor or enzyme [7]. At the same time however, the introduction of a fluorine atom significantly alters the physicochemical properties of the compound due to its high electronegativity. Therefore this type of modification can induce altered biological responses of the molecule.

The current strategies for introducing fluorine atoms into molecules are centred on the following topics:

1. *Improved metabolic stability*

   Metabolic stability can determine the bioavailability of compounds and substitution with fluorine at the site of metabolic attack can prevent oxidative metabolism based on the fact that the C–F bond is more resistant to attack than the C–H bond. Also, substitution at adjacent or distal sites to the site of metabolic attack can also affect drug metabolism due to inductive/resonance effects or conformational and electrostatic effects [7].

2. *Altered physicochemical properties*

   As fluorine is the most electronegative element, its introduction into a molecule can alter electron distribution which can impact on the pK_a, dipole moment and even the chemical reactivity and stability of neighbouring functional groups. Fluorine can reduce the basicity of compounds which can improve bioavailability as a result of better membrane permeation of the compound.

3. *Increased binding affinity*

   Fluorine substitution is being increasingly used to enhance the binding affinity of a compound to a target protein. This can occur by direct interaction of fluorine with the protein or indirectly by influencing the polarity of other groups of the compound which interact with the protein.

This paper will discuss these current strategies in further detail, with examples of drugs that have been developed for the above purposes. The selection of drugs mentioned will not be comprehensive but should illustrate some of the wide applications that fluorine can have. In addition to its role in therapeutic agents, fluorine also has biomedical applications, such as ^{18}F in positron emission tomography (PET). PET has been used to study biochemical transformations, drug pharmacokinetics, pharmacodynamics and as a powerful and superior non-invasive diagnostic and scanning technique to survey living tissue in humans.

It is apparent that fluorine plays a huge role in the current areas of pharmaceutical chemistry with many of the most important new drugs containing propitiously placed fluorine groups. The use of fluorine is expanding beyond its incorporation into drugs to its use in investigative agents in the increasingly important field of PET.

Improved metabolic stability

One of the challenges faced in drug discovery is that of low metabolic stability. Lipophilic compounds are susceptible to oxidation by liver enzymes, namely cytochrome P450. This can be overcome by increasing the polarity of the compound or alternatively introducing a fluorine atom into the compound to alter the rate, route or extent of drug metabolism. The latter can be achieved by fluorine substitution at the metabolically labile site or at adjacent or distal sites to the site of metabolic attack provided that this does not compromise binding to the target protein. Substitution at adjacent sites may increase or decrease biotransformation depending on:

(a) if metabolic attack is electrophilic or nucleophilic, and
(b) if inductive or resonance effects of fluorine predominate in the reaction.

In a saturated system, the inductive effect of fluorine should result in decreased susceptibility of adjacent groups to metabolic attack by cytochrome P450. Conversely, studies have shown that a fluorine atom placed *ortho* to a phenolic group can increase its reactivity as a nucleophile in methylation and glucuronidation reactions [7].

Discovery of ezetimibe

Ezetimibe belongs to a class of agents that inhibit cholesterol absorption in the intestine, and was approved in 2002 by the FDA to reduce cholesterol levels in patients with hypercholesterolaemia. It provides a good example of how rational drug design led to the incorporation of fluorine into a compound in order to improve metabolic stability and enhance *in vivo* potency. Ezetimibe was developed from the compound SCH 48461, which is a potent cholesterol absorption inhibitor, (Figure 1). However, this molecule is susceptible to metabolic attack in four primary sites (Figure 1) via demethylation, hydroxylation, and/or oxidation. This data was used together

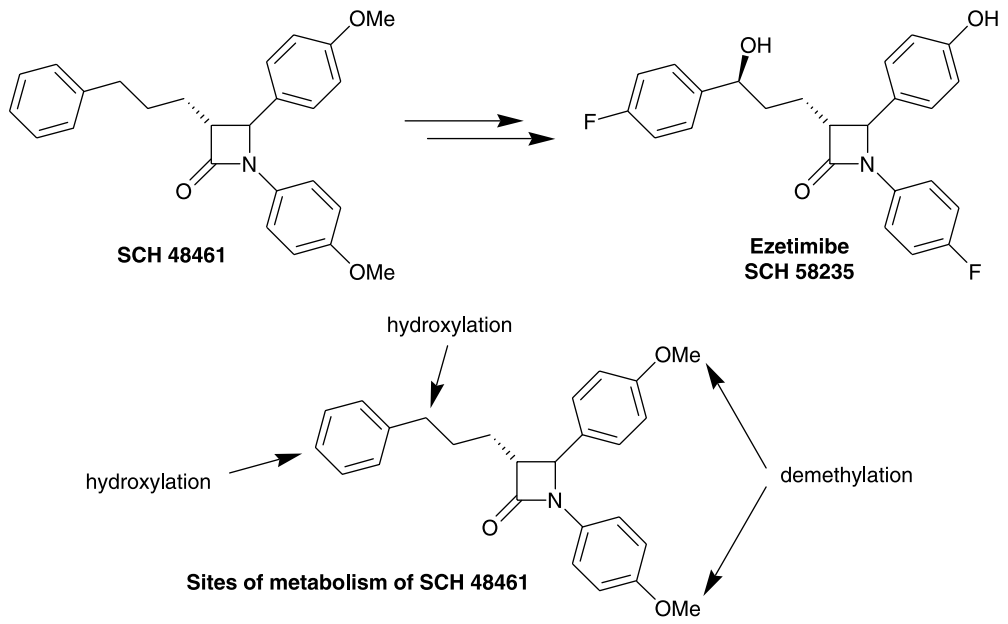


Figure 1. Development of Ezetimibe (SCH 58235) by lead optimisation that involved using fluorine substituents to block two sites of metabolism [4,8].

with structure-activity relationship (SAR) studies [8] to design a metabolically more stable analogue of SCH 48461, without affecting its potency. This eventually led to the development of SCH 58235, now known as Ezetimibe which has shown a 50-fold increase in activity in hamsters ($ED_{50} = 0.04\,mg.kg^{-1}$) compared to SCH 48461 ($ED_{50} = 2.2\,mg.kg^{-1}$) [9].

Fluorinated antitumour benzothiazoles

2-(4-aminophenyl)benzothiazoles have a simple structure and intriguing antitumour properties. Metabolism is thought to play a major role in the mode of action of these drugs since uptake and biotransformation has been observed in sensitive cell lines (e.g. breast MCF-7 and MDA 468 cells) *in vitro* but was negligible in insensitive cell lines (e.g. prostate PC 3 cells) [10]. The initial lead compound (DF 203), however, exhibited a distinctive biphasic dose-response relationship in sensitive breast tumour lines, where cell kill occurs at low nanomolar concentrations, followed by a proliferative response at low micromolar concentrations, known as the second growth phase [11]. This has been postulated to be due to metabolites that inactivate the bioactivating enzyme cytochrome P4501A1 in sensitive cell lines only [12]. 2-(4-amino-3-methylphenyl)-6-hydroxybenzothiazole, 6OH 203, was the major metabolite identified in sensitive cell lines [11]. Various fluorine analogues of DF 230 were synthesised to circumvent this deactivating metabolism in order to eliminate this biphasic dose-response relationship. 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203) was one such fluorine analogue that potently inhibited the growth of MCF-7 cells without the characteristic biphasic dose-response relationship [12,13]. 5F 203 was metabolically stable and had no exportable metabolite in sensitive MCF-7 cells which confirms that oxidative metabolism in the 6-position is essentially completely blocked by 5-fluorination. The water-soluble L-lysine prodrug of 5F 203 have been developed and selected for phase 1 trials [14]. Examination of the patterns of CYP-mediated metabolic oxidation in the potent and selective AhR agonist diaminoflavone series also resulted in a rational fluorination strategy leading to the identification of 5,4′-diamino-6,8,3′-trifluoroflavone (Figure 2) as a preclinical lead compound [15].

A further antitumour benzothiazole structurally related to 5F 203, 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, Figure 2), has recently been reported to possess a potent and selective antitumour profile reminiscent of 5F 203 [16]. Intriguingly in the absence of the fluorine atom, there is no cellular antitumour activity, confirming the crucial role played by fluorine in this series.

Fluorine substitution and oestrogens

The role of drug metabolism is usually that of detoxification. However, it can result in the formation of toxic metabolites that can cause problems in terms of carcinogenicity, apoptosis, necrosis or hypersensitivity. Therefore fluorine can be introduced into compounds to prevent toxicity and develop safer drugs.

Prolonged treatment of experimental animals with exogenous estrogens has been reported to induce a variety of tumours. This has been proposed to be due to oxidation in the 2- and 4-positions to form metabolites that bind covalently to proteins and

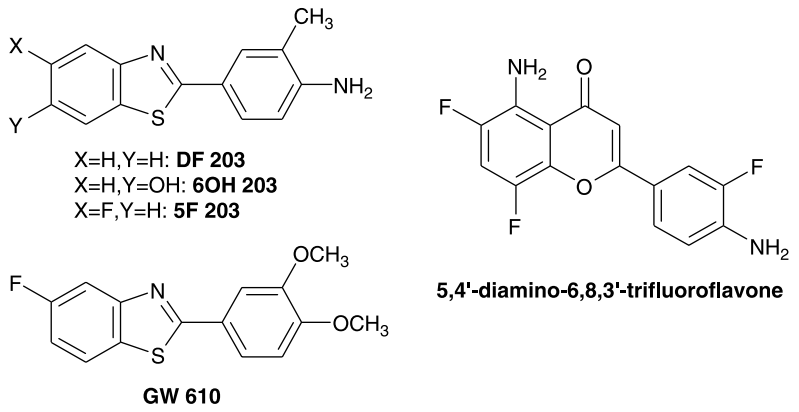


Figure 2. Chemical structures of antitumour agents: 2-(4-amino-3-methylphenyl) benzothiazole (DF 203), its 6-hydroxy metabolite (6OH 203), 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F 203), 5,4′-diamino-6,8,3′-trifluoroflavone and 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (GW 610) [12,15,16].

DNA. Therefore carcinogenesis could be inhibited by interfering with metabolic activation which could be carried out using fluorine as a metabolism-inhibiting substituent. This was undertaken by Liehr [17] who found that 2-fluoroestradiol was highly estrogenic yet non-carcinogenic in an animal model and suggested that this was due to the inability of the oxidising enzymes to cleave the strong C–F bond. Therefore correctly positioned fluorine substituents can have a role in decreasing the risk of carcinogenesis with oral contraceptives, which are taken by many women over a prolonged period of time.

Fluoroestrogen can be used as a radiopharmaceutical but requires selective concentration within the target tissue which can be achieved by blocking or retarding the normally extensive oxidation and conjugation of estradiol *in vivo*, using substituents which do not significantly compromise uptake or binding. 16α-[18F] fluoro substitution of 17β-estradiol allows the C-16 fluorine to function as a radiolabel and metabolism suppressor in a prototype imaging agent [18]. Since it is a positron emitting radiolabel, it can be used to image primary breast tumours, where it accumulates. The use of fluorine in PET will be discussed in further detail later.

Removal of fluorine to develop celecoxib

The importance of fluorine in determining metabolic stability is also demonstrated by the development of the cyclooxgenase-2 (COX-2) inhibitor celecoxib (Figure 3). Penning *et al.* [19] synthesised a series of 1,5-diarylpyrazole derivatives to evaluate their ability to block COX-2 and carried out SAR studies within these to identify potent and selective COX-2 inhibitors. However, the early fluorinated structural lead shown possessed an unacceptably long half-life so pyrazole analogues with potential metabolic sites were evaluated further *in vivo*. The observation that substitution of the fluorine on the benzene ring with a metabolically labile methyl group reduced the half life in the rat from 220 hours to 3.5 hours and led to the development of celecoxib. The role of fluorine is further highlighted by the observation that there is substantial flexibility in the 3-position of the pyrazole and trifluoromethyl and difluoromethyl groups were optimal in terms of potency and selectivity [20].

Fluorine substitution to extend the biological half life

The prostanoids typically have very short half-lives *in vivo* of less than 5 minutes. The platelet-aggregating agent, thromboxane A_2 (TxA_2) (Figure 4) has an

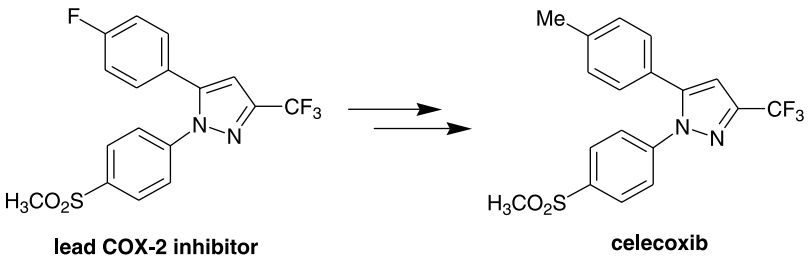


Figure 3. Development of celecoxib [19,4]

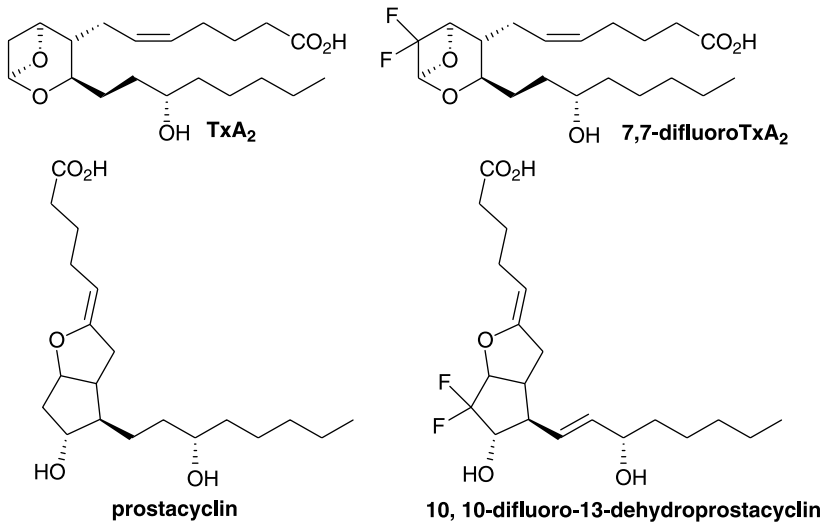


Figure 4. The use of fluorine substitution to extend the biological half-life, illustrated by (a) the platelet aggregating agent thromboxane A_2 and its 7,7-difluoro derivative, and (b) natural prostacyclin and 10,10-difluoro-13-dehydro-prostacyclin.

unusual oxetane acetal structure that undergoes hydrolytic cleavage at pH 7.4 and has a half-life of around 30 seconds. By introducing fluorine into the oxetane ring, the rate of hydrolysis is reduced and the 7,7-difluoro-TxA_2 has been shown to have a 10^8-fold slower rate of hydrolysis than TxA_2 [21].

Prostacyclin, an inhibitor of platelet aggregation contains an acid labile enol-ether group that is responsible for its short half-life. By introducing a fluorine atom α to the enol-ether group, the electron density on the enol-ether group is reduced via its inductive effects, which improves the stability of the molecule to acid hydrolysis. Fried et al. [22] synthesized the 10,10-difluoro-13-dehydro-prostacyclin (Figure 4), and found that it maintained both qualitatively and quantitatively, the powerful biological properties of the natural prostacyclin. This analogue mimics natural PGI_2 in many respects except that it has a half-life 150 times greater than PGI_2 and it is not inactivated by 15-hydroxyprostaglandin dehydrogenase [7]

The fluorine NIH shift

During metabolism, hydroxylation onto an aromatic ring can result in the substituent which was originally present on that aromatic carbon to shift to a carbon adjacent to the site of hydroxylation which leads to the phenomenon of NIH shift [23,24]. This rearrangement occurs via an epoxide or keto intermediate (Figure 5). The NIH shift has been reported for fluorine substituents with the extent to which the substituent shifts dependant on the nature of the substituent and the nature of other substituents present in the molecule. Evidence for this was seen for 1,4-difluorobenzene, which resulted in a significant amount of NIH-shifted 1,2-difluorobenzene metabolite following reaction with activated cytochrome P450. However, the amounts of NIH shifted metabolites of various fluorobenzenes *in vivo* were less than that reported for chlorinated analogues which may suggest that:

(1) the NIH shift is a minor process for fluorinated analogues, or
(2) the intermediate which is formed is lost to an alternative pathway.

The *in vitro* data from liver microsomes showed greater formation of the NIH shifted fluoroaromatic products which demonstrates that this process can be important for fluorinated analogues [25]. This shift can therefore result in cases where the introduction of fluorine into a compound does not prevent oxidation at that site. Dear *et al.* [26] investigated the urinary metabolites of (S)-2-ethyl-7-fluoro-3-oxo-3,4-dihydro-2H-quinoxaline-carboxylic acid isopropylester (GW420867X), and found that fluorine underwent

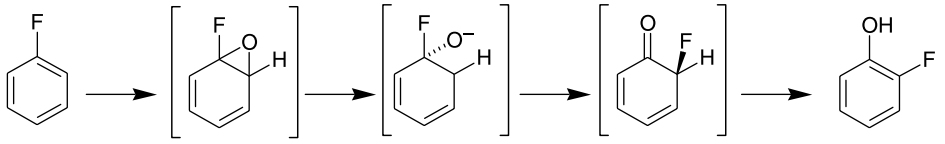


Figure 5. A schematic representation of a proposed reaction pathway to demonstrate the NIH shift of a fluorinated benzene ring via an arene oxide intermediate [23].

an NIH shift in the metabolites isolated and characterised in human urine. The metabolites were detected using NMR and mass spectroscopy and the presence of fluorine provides a useful tool which can be readily used to detect the presence of drug-related molecules and quantify the amounts of metabolites. However, defluorinated metabolites need to be accounted for as these would not be detected by ^{19}F-NMR and this can limit the use of such a method.

Effects of fluorine substitution on physicochemical properties

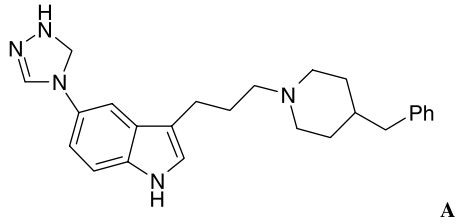
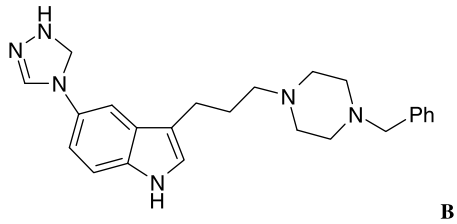
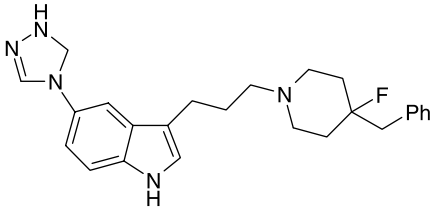
Since fluorine is the most electronegative element, it can strongly affect the acidity or basicity of nearby functional groups. A strongly basic group may be required for binding but this may limit bioavailability due to decreased ability of a strongly basic group to pass through membranes, and so a balance is needed when developing drugs. A high lipophilicity is required for good binding affinity but this can result in decreased solubility as well as other undesirable properties, so again a balance is required between lipophilicity and polarity.

Fluorine substitution to alter pK$_a$

Substitution with fluorine to reduce the pK$_a$ of molecules has been investigated by van Niel et al. [27] by fluorination of 3-(3-(piperidin-1-yl)propyl)indoles and 3-(3-(piperizin-1-yl)propyl)indoles to give selective human 5HT$_{1D}$ receptor ligands which have improved pharmacokinetic profiles, following the discovery of the migraine drug Sumatriptan. The piperazine was found to be rapidly absorbed and orally bioavailable but the corresponding piperidine analogue lacked good absorption and bioavailability and one explanation for this was the reduced pK$_a$ of the piperazine nitrogen. Therefore fluorine was introduced into the C-4 position of the piperidine ring. Molecular modelling studies indicate that piperazine could be replaced by 4-fluoropiperidine as the two are similar in terms of basicity and electron density distribution (see Table I). The electron density of the 4-fluoro substituent is similar to the lone pair on the second piperazine nitrogen, although it does extend over a larger region of space. It was also speculated that altering the basicity may also be advantageous to absorption and bioavailability profiles. Table I shows the pK$_a$ for the piperidine, **A**, the piperazine, **B**, and the 4-fluoropiperidine, **C**.

The 4-fluoropiperidine was significantly less basic than the piperidine, and had a pK$_a$ closer to that of the piperazine. Selectivity of 5HT agonists for the 5HT$_{1D}$ receptors is required to reduce coronary side effects possibly thought to occur due to coronary vasoconstriction via activation of 5HT$_{1B}$ receptors [28]. The 4-fluoropiperidine had reduced 5HT$_{1D/1B}$ selectivity compared to the piperidine and piperazine, but it had the advantage of being effectively absorbed and retained measurable systemic levels after 2 hours. Therefore the reduction in pK$_a$ following fluorine substitution enhances oral absorption and this can lead to improved bioavailability.

Table I. pK$_a$ of the piperidine **A**, the piperazine **B**, and the 4-fluoropiperidine **C**.

Structure	pK$_a$
 **A**	9.7
 **B**	8.3
 **C**	8.8

Fluorine can also be used to increase the acidity of groups, as is the case with methotrexate, which can lead to decreased toxicity. Methotrexate, (Figure 6),

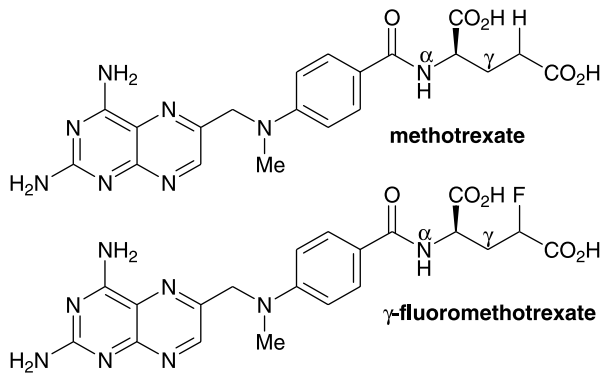


Figure 6. Chemical structure of methotrexate and γ-fluoromethotrexate that has lower toxicity due to the strongly electronegative fluorine atom.

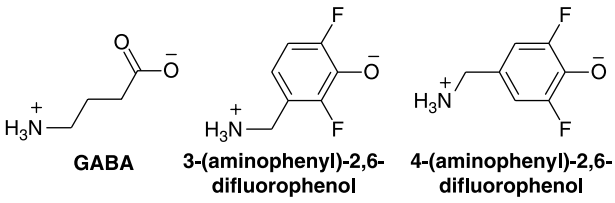


Figure 7. The chemical structures of GABA, 3-(aminomethyl)-2,6-difluorophenol and 4-(aminomethyl)-2,6-difluorophenol in their ionised forms.

can be used in the treatment of rheumatoid arthritis but can cause adverse effects, related to the accumulation of poly-γ-glutamate metabolites. γ-Fluoromethotrexate has lower toxicity than methotrexate, which can be attributed to the presence of a strongly electronegative fluorine atom which can increase the acidity of the neighbouring γ-carboxyl group and reduce polyglutamylation [29].

Fluorine substitution of γ-aminobutyric acid (GABA)

The concentration of the inhibitory neurotransmitter GABA (Figure 7) is regulated by the enzyme that synthesises it, L-glutamate decarboxylase, and the enzyme that degrades it, GABA aminotransferase (GABA-AT). A fall in GABA levels can cause convulsions in the brain which can be blocked by injecting GABA. However this is not practical and since GABA is a small, polar and hydrophilic molecule, it cannot cross the blood brain barrier so cannot be used as an anticonvulsant (Figure 7). Qui *et al.* [30] synthesised GABA mimics using 2,6-difluorophenol since the pK_a of phenol (9.81) decreases to 7.12 following 2,6-difluoro substitution [31]. This occurs at pH 8.5 which is the optimal pH for GABA-AT so this would favour the zwitterionic form and mimic GABA. The 3-(aminomethyl)-2,6-difluorophenol and 4-(aminomethyl)-2,6-difluorophenol (Figure 7) were tested as substrates for GABA-AT from pig brain and both were found to be poor substrates, but were competitive inhibitors of GABA-AT. This suggests that the 2,6-difluorophenols were able to mimic the carboxylic acid functionality and therefore act as lipophilic bioisosteres. The increased lipophilicity of this moiety should facilitate crossing of the blood brain barrier and the development of novel anticonvulsant drugs.

Strength of the C–F bond

The higher energy of the C–F bond (116 kcal/mol) relative to that of C–H (99 kcal/mol) is another important property that results in increased metabolic inertness of some fluorine containing compounds [4]. An example of this is the antitumour drug 5-fluorouracil that acts by conversion to an active metabolite which inhibits the enzyme thymidylate synthase and leads to decreased thymidine formation and hence reduced DNA synthesis. This inhibition is caused by the unreactive fluorine at the C-5 position, which, due to its small size and the high strength of the C–F bond, enables the molecule to enter the enzyme active site without metabolism [7].

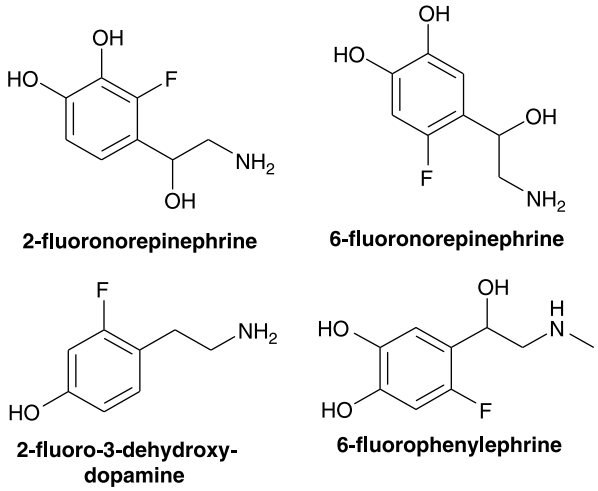


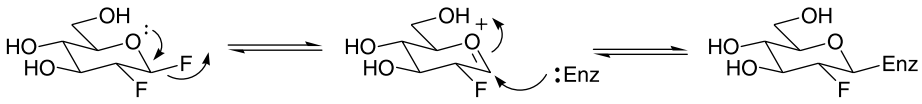
Figure 8. Chemical structures of 2-fluoronorepinephrine, 6-fluoro-norepineephrine, 2-fluorodopamine and 6-fluorophenylephrine.

Effect of strong dipole

The C–F bond has a strong dipole that may interact with other dipoles. DeBernardis *et al.* [32] found that adrenergic specificity of 2-fluoronorepinephrine and 6-fluoronorepinephrine (Figure 8) may result from conformational bias caused by electrostatic repulsion between the side chain β-hydroxyl group and the fluorine attached to the aromatic ring. Kirk *et al.* [33] suggested that similar interactions and conformational changes in fluorinated amines might be responsible for the increased agonist specificities of phenylephrine. This is consistent with the observation that ring fluorinated dopamines, such as 2-fluoro-3-dehydroxydopamine, which lack a phenyl 3-hydroxyl group do not exhibit selective adrenergic specificities. 6-Fluorophenylephrine has increased potency at the α_1 and α_2 adrenoceptors and reduced interaction with β-adrenoceptors and so can be used pharmacologically as a specific α adrenergic agonist.

Irreversible enzyme inhibition

When the fluorine atom is attached to a reactive centre, it can readily undergo elimination of fluoride, which is a good leaving group. The resulting electrophilic species can irreversibly inhibit enzymatic activity through the formation of a covalent bond to the enzyme [34,35]. The glycosylase inhibitor 2-deoxy-2-fluoro-β-D-glucosyl fluoride undergoes rapid elimination of the glycosylic fluoride, resulting in an oxocarbonium ion that can react rapidly with the



Scheme 1. Elimination of the glycosidic fluorine from 2-deoxy-2-fluoro-®-D-glucosyl fluoride to give an oxocarbonium ion, which reacts with the enzyme to give a 2-deoxyfluoro-®-D-glucosyl-enzyme complex.

enzyme. The 2-deoxy-2-fluoro-glycosyl-enzyme complex formed is then stabilised by the C-2 fluorine substituent (Scheme 1). This results in enzyme inactivation via the generation of a more stable intermediate which traps the enzyme and prevents the final transformation. These inhibitors can have therapeutic uses in controlling blood glucose release from the gut by specific inhibition of gut α-glucosidases in type 2 diabetes mellitus. They have greater therapeutic potential than other similar agents since the by-product is a relatively non-toxic fluoride species [36].

Replacement with difluoromethylene

Different properties are conferred on molecules in which a hydrogen atom is substituted with a fluorine atom. Similarly, replacement of a methylene moiety with a difluoromethylene moiety (i.e. CF_2 for CH_2) can have a significant effect on physical properties as well as on conformation. The difluoromethylene moiety has been used as an electronic and steric mimic of labile oxygen atoms in phosphate esters ($R\text{-}CF_2\text{-}PO_3^{2-}$ vs $R\text{-}O\text{-}PO_3^{2-}$). This group has been used to design inhibitors of enzymes that hydrolyse or bind phosphate esters [37]. Protein tyrosine phosphatases (PTP) catalyse the dephosphorylation of the phosphotyrosine residue in proteins and are involved in the regulation of an array of cellular processes including cell proliferation and differentiation. PTPs are involved in a variety of disease states and so PTP inhibitors can have a role as potential therapeutic agents [38]. PTP B1 is an intracellular non-receptor PTP which plays an essential role in regulating the insulin signalling pathway via dephosphorylation of the activated insulin receptor. PTP B1 knockout mice display enhanced insulin sensitivity and resistance to diet induced obesity. Therefore novel PTP 1B inhibitors could be used to treat type 2 diabetes and obesity [39,40]. Fluorinated derivatives, benzylic α,α-difluorophosphonates, α,α-difluorosulfonates and α,α-fluorocarboxylates, have been found to be potent inhibitors of PTP1B compared to non-fluorinated equivalents. This effect is considered to be due to fluorine atoms interacting with the active site rather than due to pK_a shifts, as was previously thought [4,41].

Increased binding affinity

The substitution of fluorine atoms for hydrogen atoms is a common method employed in medicinal chemistry to enhance ligand binding to proteins. The similarity of the van der Waals nucleus of fluorine and hydrogen and the similar C–F and C–H bond lengths can allow selective substitution of the C–F group for the C–H group in an organic molecule without significant increase in the overall size of the molecule [42]. Therefore a fluorinated compound should be able to bind to a protein in generally the same location as a non-fluorinated compound but the chemical properties of the C–F bond may affect protein-ligand affinity and selectivity. The small size of the fluorine atom and its ability to possibly act as a hydrogen bond acceptor allow fluorodeoxy analogues to be processed by enzymes that would normally act on corresponding hydroxyl groups, thus enabling fluorine to be substituted for the hydroxyl group [43].

Carbonic anhydrase inhibitors

Carbonic anhydrase II (CAII) is a zinc metalloenzyme which catalyses the hydration of CO_2 to yield bicarbonate and a proton. The aliphatic sulphonamides e.g. $CH_3SO_2NH_2$ are weak inhibitors of carbonic anhydrase ($K_i \sim 1 \times 0^{-4}$M) and very weak acids ($pK_a = 10.5$). $CF_3SO_2NH_2$ is a more potent inhibitor of carbonic anhydrase ($K_i = 2 \times 10^{-9}$ M) and a stronger acid ($pK_a = 5.8$), due to the strong electron withdrawing effect of the CF_3 moiety. At neutral pH $CF_3SO_2NH_2$ is dissociated and binds to the enzyme more strongly than $CH_3SO_2NH_2$. Maren & Conroy [44] synthesised a series of fluorinated aliphatic sulphonamides and found a direct relationship between pK_a and the binding constant K_i although this may only hold for this series as other factors such as lipophilicity may influence activity [4].

Fluorine can also interact directly with the protein, and this is also seen with CAII. Work by Kim et al. [42], using x-ray crystallography, on the CAII inhibitor, N-(4-sulfamylbenzoyl)benzylamine (SBB) (Figure 9) demonstrated that this tight binding inhibitor ($K_d = 2.1$ nM) could be improved by substituting fluorine for hydrogen at strategic locations to enhance

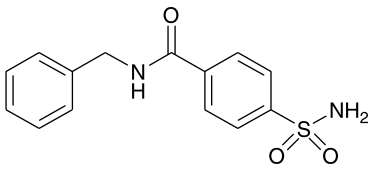


Figure 9. Chemical structure of the CAII inhibitor, N-(4-sulfonamidobenzoyl)benzylamine.

weakly polar interactions with adjacent enzyme residues. The changes in affinity are due to a combination of dipole-induced dipole, dipole-quadrupole and quadrupole-quadrupole interactions between fluoroaromatic inhibitors and two residues in the CAII active site: Pro202 and Phe131. Further work [45] was carried on substitution of fluorine atoms in the benzoyl group of SBB to modify edge-to-face interactions (an example of a quadrupole-quadrupole interaction) between Phe131 and the benzoyl group of SBB to influence affinity for CAII. Fluorine substitution on the 2,3,5 and 6 positions of the lower (face) benzene interacts favourably with the H_0 hydrogens on the upper (edge) benzene due to attraction between the partially negative charge on the fluorine and the partially positive charge on the hydrogen. This stabilizes the dimer compared to the system where the fluorine atoms are placed on the 1 or 4 positions. A similar pattern is seen for difluoro, trifluoro and tetrafluoro substituted compounds. Work by Abbate *et al.* [46], who synthesised fluorinated analogues of the CAII inhibitor, methazolamide found that the fluorinated analogues bound almost 10 times more strongly ($K_i = 1.5$nM) compared to methazolamide ($K_i =$ 14nM). X-ray crystallography demonstrated that a stacking interaction occurred between the fluorinated ring of the inhibitor and the Phe131 aromatic ring [4].

Inhibition of human CAII is of clinical importance and can be useful for treating various diseases, including glaucoma, epilepsy and gastric ulcers. Inhibiting CAII in the eye lowers intraocular pressure in glaucoma patients and so novel and tight-binding CAII inhibitors, with strategically placed fluorine atoms are potentially useful for glaucoma therapy.

Thrombin inhibitors

Hof et al. [47] explored non-covalent interactions of fluorine with groups found in the active site of thrombin by carrying out a fluorine scan. They found that in the hydrophobic pocket (Figure 10) of this serine protease, the potency of a closely related series of fluorinated inhibitors (Figure 11), was strongly influenced by the position of the fluorine atom. The 4-fluorobenzyl derivative exhibited 5-fold better inhibition ($K_i = 0.057\mu$M) compared to the benzyl derivative ($K_i = 0.27\mu$M). The X-ray structure of the 4-fluorobenzyl derivative-enzyme complex revealed two close contacts between the fluorine atom and the C–H atom and also the carbonyl C carbon of Asn 98. These interactions are seen between other organic fluorine atoms and carbonyl carbon atoms and have characteristic geometry with the fluorine atom residing orthogonally above the pseudotrigonal axis of the carbonyl group. Vargas *et al.* [48] reported that C = O···H–C hydrogen bonds contribute significantly in stabilising the folded structure of proteins. Therefore it can be argued that these

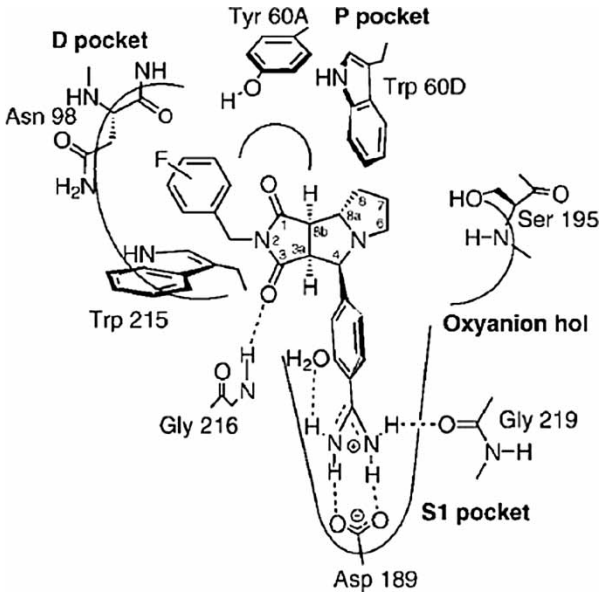


Figure 10. Schematic representation of the binding mode of tricyclic inhibitors in the thrombin active site. The active site can be described in terms of selectivity pocket (S1), small proximal pocket (P), a large hydrophobic distal pocket (D) and an oxyanion hole [47].

C–F···H–C interactions can be classified as weak hydrogen bonds. The enhanced positive polarisation of the hydrogen atom *ortho* to the fluorine substituent is thought to enhance the face-to-edge interaction of the inhibitor with Trp 215 in the hydrophobic D pocket but the interaction of fluorine with Asn 98 is largely responsible for the increase in activity seen with the 4-fluorobenzyl derivative [49].

Intravascular clot formation is an important factor in a variety of cardiovascular diseases such as myocardial infarction, unstable angina, deep vein thrombosis, pulmonary embolism and ischemic stroke. Therefore prevention of blood coagulation has become a major target for new therapeutic agents. This can be achieved using fluorine substitution of inhibitors which have already been identified, to increase their binding affinity. Further model studies, such as those carried out by Hof et al. [47] can help

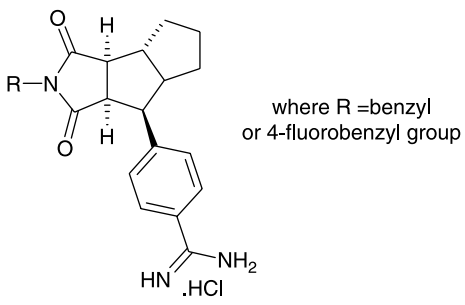


Figure 11. Chemical structure of inhibitors designed to investigate the non-covalent interactions of fluorine with the thrombin active site.

to clarify the role of fluorine atoms in influencing binding affinity and selectivity of enzyme inhibitors.

Fluoroquinolones

The fluoroquinolone antibacterials generated much excitement after the discovery that the fluorine atoms at the C-6 position enhanced anti-bacterial activity. Domagala et al. [50] synthesised a series of quinolones and found that those with fluorine substituents were the best inhibitors of DNA gyrase, a bacterial topoisomerase. Inhibition of this enzyme can lead to the death of bacterial cells. By comparing the potency of fluorinated parent drugs with the non-fluorinated derivatives, it was found that a fluorine atom in the C-6 position improves gyrase-complex binding by 2-17 fold, in various species of bacteria. For example, enoxacin had 15-fold greater gyrase activity than the non-fluorinated derivative, naphthyridine (Figure 12). As well as increasing the binding affinity, it appears that the fluorine atom also increases cell penetration by 1–70 fold, which could be due to the increased lipophilicity of the molecule as a result of fluorine substitution. In addition to this, the introduction of fluorine into the C-6 position has also substantially increased the spectrum of activity of these antibiotics [51]. It was found from structure-activity studies that a second fluorine atom at the C-8 position was desirable for optimum in vivo efficacy but this has been associated with phototoxicity [52,53]. Antibiotics have had a major influence on the reduction of morbidity and mortality from bacterial infections over the past few decades. Newer quinolone antibiotics are continuously being designed and further investigation of the binding site of DNA gyrase and the precise role of fluorine at the active site may aid in the development of newer antibiotics with a broader spectrum of activity, fewer side effects and possibly to overcome bacterial resistance mechanisms.

Use of fluorine in positron emission tomography (PET) imaging

In addition to PET, fluorine can also be used for non-invasive monitoring of therapeutic agents by ^{19}F magnetic resonance imaging (MRI), where the negligible natural fluorine background and high sensitivity of ^{19}F NMR spectroscopy has made it possible to study the *in vivo* action and metabolic pathways of fluorine containing drugs [54]. However, this review will focus on the use of fluorine as an imaging tool in PET, which is a current area of great interest and development.

Positron emission tomography (PET)

PET is a nuclear medicine imaging tool which allows three-dimensional quantitative determination of the distribution of radioactivity within the human body. It is becoming increasingly important for the measurement of physiological, biochemical and pharmacological function [55]. Compared to other imaging techniques available, it offers the best combination of selectivity, specificity, and sensitivity when used with appropriate radiopharmaceuticals [56]. The radiopharmaceuticals are labelled with positron emitting radionuclides such as ^{11}C, ^{13}N, ^{15}O and ^{18}F. Positrons (β^+) are formed during the decay of nuclides that have an excess of protons in the nucleus compared to the number of neutrons. PET is based on the simultaneous detection of two γ-rays of 511 keV each, emitted during positron annihilation. When these proton-rich radionuclides decay, they emit a positron and a neutrino. The positron travels a short distance in the tissue, losing most of its energy, before interacting with a rest electron which results in annihilation of both particles. The mass of the positron and electron is converted into energy with the production of two γ-rays travelling at 180° to each other, with an energy of 511 keV. These γ-rays can be detected by pairs of co-linearly aligned detectors that are arranged in multiple rings around the patient (Figure 13). These rays that interact with the detectors within a predefined time-window are registered as decay events. The radioactivity detected along lines at a series of angles is then

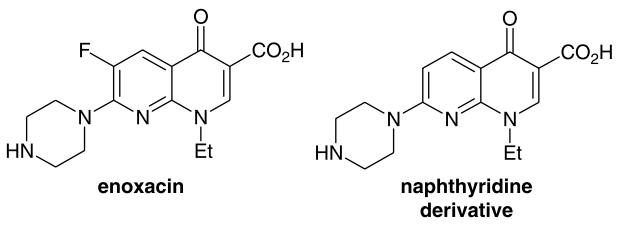


Figure 12. Structures of enoxacin and its non-fluorinated naphthyridine derivative, naphthyridine. Enoxacin has a 15-fold greater gyrase activity [48].

Figure 13. A patient undergoing a PET scan, showing the circular detector around the patient (http://www.samc.com/UMAP.asp?ID = 888).

used to reconstruct tomographic images of regional radioactivity distribution [57].

Fluorine-18 (^{18}F) is one of the most important radioisotopes used in PET. From a physical point of view, ^{18}F has the most favourable nuclear properties for imaging with PET:

- It has a relatively long half-life (110 minutes) compared to the other PET radionuclides mentioned. This allows fluorine to be produced in a regional centre and be distributed to local hospitals, whereas the other radionuclides need to be produced on site. Its production is commonly carried out in a cyclotron via the ^{18}O(p,n) process using ^{18}O enriched water for fluoride production or ^{18}O-oxygen gas for elemental fluorine production. The half-life of ^{18}F also gives it an appropriate decay time for imaging that can facilitate kinetic studies.
- It has a high percentage of β^+ emission (97%).
- It has relatively low positron energy (635 keV) so imaging can potentially be done at the highest resolution.

The other favourable properties of ^{18}F are that the fluorine atom is small, can form hydrogen bonds as a hydrogen bond acceptor and the C–F bond is very strong. Therefore fluorine substitution for a hydrogen atom or hydroxyl group does not cause significant steric changes, although the physicochemical properties may change the biological behaviour of the labelled compound. One of the few limitations of ^{18}F is that because there are no naturally occurring fluorine containing compounds in the body, it cannot act as a true isotopic tracer. Despite this there are a wide range of ^{18}F radiopharmaceuticals used in PET [56,58,59].

The main radiopharmaceutical in use is [^{18}F]-fluorodeoxyglucose (FDG) which can show glucose uptake and energy consumption in various cells, and its main uses are in the fields of oncology and neuroscience. FDG is taken up into cells by glucose transporters and phosphorylated by hexokinase in position 1, in the same manner as glucose. However following rapid phosphorylation, FDG-6-phosphate cannot be metabolised further as this is blocked by the fluorine in position 2, which results in it being trapped within the cell. Therefore the intracellular FDG concentration is proportional to the glucose utilisation of the tissues [57,60]. This is of use in oncology as tumour cells have a higher glycolytic rate and therefore take up FDG more actively than normal cells. Clinical situations where FDG-PET can be used includes initial diagnosis, evaluation of response to therapy and identifying reoccurrence. The tumour types most commonly explored using FDG-PET are lung nodules, non-small-cell lung carcinomas, lymphomas, melanomas, colorectal cancers, and head and neck tumours [61]. FDG-PET has also been applied to research and clinical practise for quantitative analysis of cerebral glucose metabolism [62]. PET imaging in clinical neurology can be used for differential diagnosis, describing pathophysiological changes in the course of a disease and the evaluation and follow-up of disease. FDG-PET can be used to indicate reduced glucose metabolism in a range of conditions such as Alzheimer's disease, Creutzfeldt-Jakob disease and epilepsy [63]. There are also a number of studies involving the dopamine transporter and D_2 receptor in the human brain which have been studied using ^{18}F-DOPA which is especially useful in Parkinson's disease and schizophrenia [64].

In terms of evaluating cancers, there are two types of radiopharmaceuticals for cancer imaging. One type is used to evaluate tumour growth activity, which is universal for most types of cancers. This evaluation can be performed using tracers for energy metabolism, DNA or RNA synthesis and protein synthesis. The other type is used to evaluate specific biological characteristics of cancers, which can be performed using substrates for enzymes or ligands of specific receptors involved [65]. Therefore this presents an opportunity to label such radiopharmaceuticals with ^{18}F for use in PET, allowing further advances in the nuclear imaging field of oncology. There are already a wide range of radiopharmaceuticals labelled with ^{18}F being studied for use in PET for a number of different conditions, a number that would be expected to grow in the near future.

Conclusion and future perspectives

This review addresses the broad applications of strategically placed fluorine groups in medicinal chemistry and drug development. The main rationale for introducing fluorine into compounds is either to improve the metabolic stability, alter the physicochemical properties or improve the binding affinity of these compounds. These strategies are already widely used and established by medicinal chemists in the processes of drug discovery and development. At present, fluorine is often introduced into molecules *ad hoc* but it can be speculated that it could be involved in a more rational approach to drug design where various techniques could be used to determine where to substitute fluorine atoms to maximise activity.

The area of PET is continually advancing and its use for functional imaging will continue to grow and with this, so will the use of labelled ^{18}F molecules to study a wide range of systems within the body. A current area of development is the combination of PET with computed tomography (CT), which has been studied in oncology. Different information can be given about the tumour from the anatomical detail from CT and functional imaging from PET. This has advantages over PET alone in terms of speed

of throughput, increased confidence and fewer equivocal interpretations as well as benefits in diagnostic accuracy [66]. Further trials are needed to assess the use of such a technique in other clinical situations as well as the possible development of other radiopharmaceuticals, where ^{18}F could potentially have a role. PET can also have uses outside of medicine, in drug development, where the pharmaceutical industry can label drugs to establish their distribution *in vivo* and determine efficacy of drugs before large scale trials are conducted, which can potentially avoid large cost and time investments or justify them if positive results are obtained. Another area of interest is the investigation of the incorporation of ^{18}F into drug molecules so that non-invasive imaging and treatment could be conducted at the same time. Therefore one of the most important applications of fluorine in medicinal chemistry in the future is likely to be its use in PET and the development of new radiopharmaceuticals and labelled drugs for the purposes of drug development, diagnosis, treatment and follow-up.

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