CASE STUDY 6

Steroidal anti-inflammatory agents

CS6.1 Introduction to steroids

Steroids are important endogenous hormones found in many life forms. They all share a common tetracyclic structure, as shown in Figure CS6.1, but they vary in the substituents and functional groups that are present. The stereochemistry of the rings in fully saturated steroids is identical in mammalian steroids, where the three 6-membered rings have chair conformations. There are several asymmetric centres present, but only one stereoisomer occurs naturally for any particular steroid. For example, **cortisol** has seven asymmetric centres, but only the stereoisomer shown in Figure CS6.1 exists naturally.

Some of the terminology used in the nomenclature of steroids is worth explaining at this point. Substituents are often described as being alpha (α) or beta (β). α-Substituents are below the general 'plane' of the steroid, as represented in Figure CS6.1, and are represented by hatched wedges in two-dimensional diagrams, whereas β-substituents are above the plane and are represented by solid wedges. For example, in cortisol, the axial methyl groups (C18 and C19) are β-substituents, whereas the axial hydrogens at positions 9 and 14 are in the a position.

The position of double bonds in steroids is usually identified by the symbol delta (Δ). For example, Δ^4 signifies the double bond between C4 and C5 in cortisol. If there is any ambiguity, then the numbers of both carbons are indicated. For example, cholesterol has a double bond between C5 and C6, rather than C5 and C10, so this is indicated as $\Delta C^{5(6)}$.

Steroids are hydrophobic compounds owing to their extensive hydrocarbon skeleton. This is an important characteristic as the hormonal steroids have to cross cell membranes in order to interact with intracellular steroid receptors (see section 4.9 and Box 8.2). All of the important endogenous steroids have polar functional groups, such as alcohols, phenols, and ketones. These play a crucial role in the binding of steroids to their target receptors, but their presence does not alter the hydrophobic nature of the molecule as a whole. Because most steroids are hormones, they are present in very small quantities in the body (less than 1 mg). The exception is cholesterol, which is present in much larger quantities (250 g) and has a number of non-hormonal roles (Case study 1).

In this case study, we will be concentrating on those steroids released from the adrenal cortex of the adrenal gland—the **adrenocorticoids**. There are two types of adrenocorticoids—the **glucocorticoids** and the **mineralocorticoids**. The former act on carbohydrate, fat, and protein metabolism, mainly in the liver, muscles, and brain cells. They also have an important anti-inflammatory effect which is separate from their metabolic effects. The mineralocorticoids regulate electrolyte balance through sodium ion retention in kidney cells. The major endogenous glucocorticoids are **corticosterone**, **cortisone**, and **cortisol** (also known as **hydrocortisone**) (Figs CS6.1 and CS6.2). **Aldosterone** is the major endogenous mineralocorticoid. An imbalance of these steroids can lead to certain diseases. For example, an excess of glucocorticoids causes **Cushing's syndrome**, whereas a deficit results in

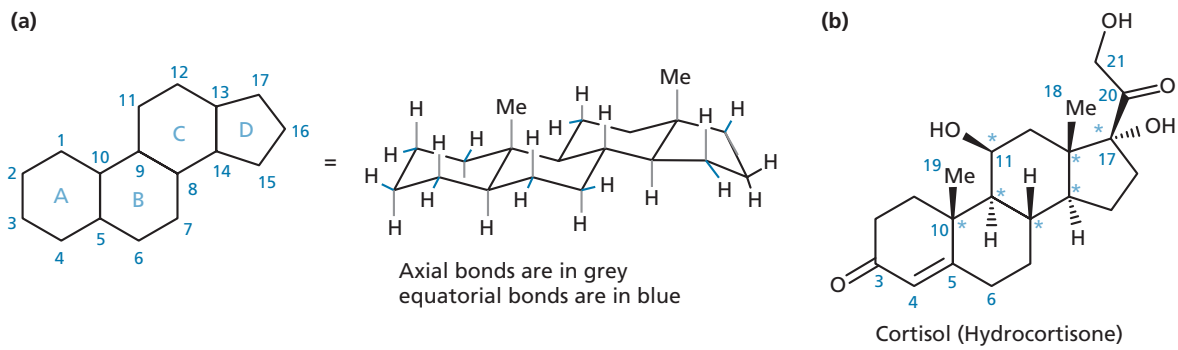


FIGURE CS6.1 (**a**) General tetracyclic structure of a steroid with numbering; (**b**) structure of cortisol (asymmetric centres indicated by stars). See also molecular modelling Exercises CS6.1 and CS6.2.

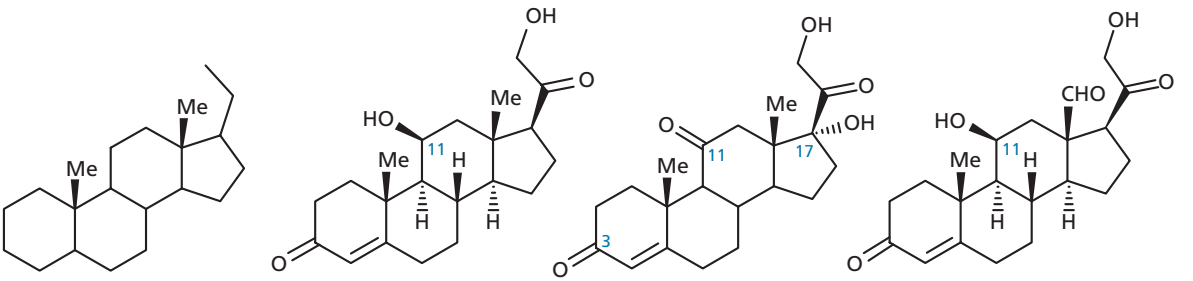


FIGURE CS6.2 Adrenocorticoids.

Addison's disease. An excess of mineralocorticoids leads to **Conn's syndrome**.

The glucocorticoids have an important clinical role in replacement therapy for Addison's disease, and have also been used as anti-inflammatories and immunosuppressants in the treatment of a number of conditions, such as asthma, hypersensitivity, rheumatoid arthritis, cancer, and diseases which have an autoimmune or inflammatory effect. The adrenocorticoids are examples of steroids having a **pregnane** skeleton—steroids having a two-carbon side chain at position 17 of the tetracyclic steroid skeleton (Fig. CS6.2).

One of the most important applications of glucocorticoids in medicine is as anti-inflammatory agents. Unfortunately, the endogenous glucocorticoids suffer from the fact that they have mineralocorticoid and immunosuppressant effects, which can cause oedema and increased susceptibility to infection. Moreover, the endogenous glucocorticoids affect a large number of enzymes in different cell types in order to control metabolism. This means that they have a large number of undesired side effects if they are taken as drugs to control inflammation. Consequently, glucocorticoids are best used as topical anti-inflammatory agents. A lot of research has gone into designing glucocorticoids that act locally at the site of administration and are metabolized rapidly in the blood supply such that they cannot act on other targets. Having said that, there are some glucocorticoids which *can* be administered orally and which have been designed to have fewer side effects.

CS6.2 Orally active analogues of cortisol

In 1947, it was found that cortisone could relieve the symptoms of rheumatoid arthritis. However, cortisone is readily converted in the liver to cortisol and it is now thought that the effects of cortisone are actually due to cortisol. A large number of analogues have been synthesized which have identified the features of cortisol that are important for corticosteroid activity. In essence, all the functional groups are important, and the removal of any of these groups either reduces or eliminates activity.

However, further studies have shown that the introduction of extra substituents can increase activity, which allows the removal of one of the original functional groups.

Introducing a 9α-fluoro substituent to give **fludrocortisone** increased activity 10-fold, but it also increased mineralocorticoid activity 300–600 times. In contrast, the introduction of an extra double bond at the Δ^1 position increased activity fourfold without increasing mineralocorticoid activity—see **prednisolone** and **prednisone** (Fig. CS6.3). Introducing substituents such as methyl or fluorine at the 6α-position has also been found to be beneficial because these groups serve to block metabolism at that position. For example, **methylprednisolone** has a 6α-methyl group.

A methyl group was introduced at C-16 to see whether it would block the metabolic reduction of the C-20 keto group of hydrocortisone analogues—a reaction that is known to lead to inactive metabolites. There is no evidence that such protection actually occurs and there is no obvious increase in glucocorticoid activity, but the presence of the methyl group does suppress the mineralocorticoid properties of sodium and water retention. It is thought that the 16-methyl substituent blocks the ability of these analogues to bind to the mineralocorticoid receptor. Further research revealed that the introduction of C-16 substituents, such as a methyl or hydroxyl group, counteracted the mineralocorticoid effect of a 9-fluoro substituent. This resulted in the development of **triamcinolone**, **dexamethasone**, **betamethasone**, and **flumetasone pivalate** (Fig. CS6.3), all of which have increased glucocorticoid activity and negligible mineralocorticoid side effects.

Test your understanding and practise your molecular modelling with Exercises CS6.2 and CS6.3.

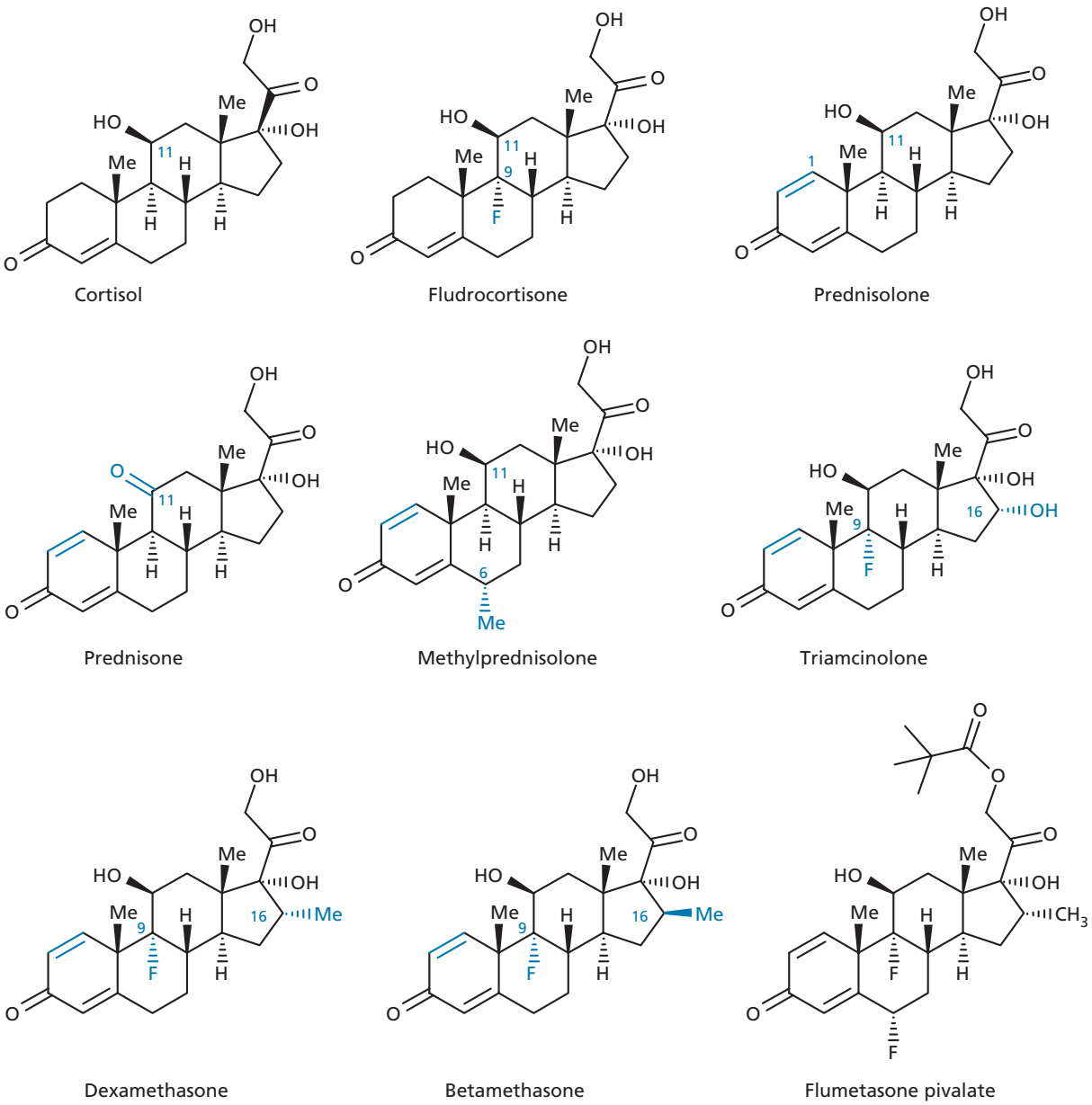


FIGURE CS6.3 Analogues of cortisol.

CS6.3 Topical glucocorticoids as anti-inflammatory agents

CS6.3.1 Cortisol analogues

Glucocorticoids are often applied topically to treat skin inflammations. **Triamcinolone acetonide** (Fig CS6.4) is one such agent. The acetonide group links the alcohol substituents at C16 and C17 of triamcinoline thus reducing the polarity of the molecule. This leads to better skin absorption and a 1000-fold increase in activity compared with triamcinolone itself. If the compounds are injected under the skin, they have equal activity. It is not yet clear whether the acetonide is acting as a prodrug and is rapidly metabolized once it reaches the tissues or whether the acetonide group increases binding to a hydrophobic region in the glucocorticoid receptor. **Fluocinolone acetonide**, **fluocinonide**, and **flunisolide** (Fig. CS6.4) are clinical agents that contain the same acetonide group (see also fludroxycortide; Box CS6.1).

Good skin absorption can also be achieved by esterifying one or more alcohol groups. The corresponding phosphate esters were less active, providing further evidence that lipophilicity is important to the activity of

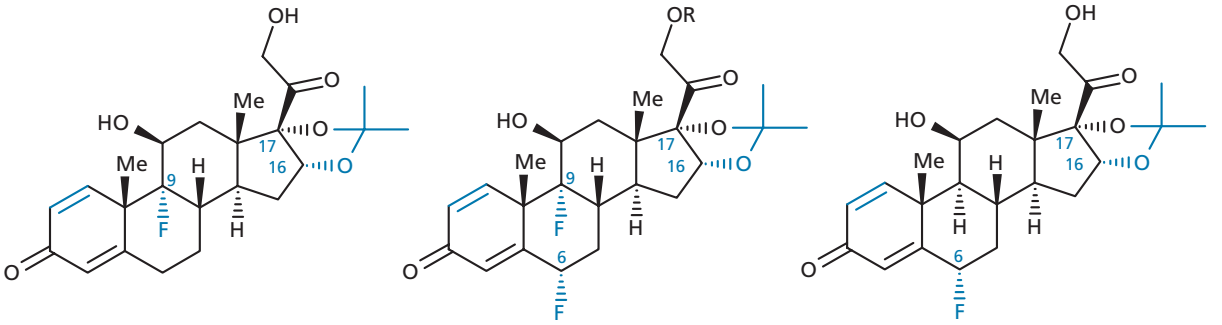


FIGURE CS6.4 Steroid acetonides used as topical agents.

topically applied anti inflammatories. Glaxo used this strategy to develop the clinically useful agents **betamethasone 17-valerate**, **betamethasone dipropionate**, and **beclometasone dipropionate** (formerly beclomethasone dipropionate) (Fig. CS6.5).

CS6.3.2 21-Deoxysteroids

Removal of the 21-OH group from cortisol eliminates activity, but activity can be restored by adding similar substituents to the ones described in the preceding section. Thus, the introduction of an extra double bond in the A ring, along with substituents at C6 and C9 results in **fluorometholone** (Fig. CS6.6).

Esterification of the 17-OH group results in better skin absorption and increased topical activity, for example **21-deoxybetamethasone 17-propionate** (Fig. CS6.6).

Introducing a halogen at position 21 was particularly beneficial for the 17-esters. The best activity was obtained using F or Cl, with short chain esters at C-17. The best

BOX CS6.1 Clinical aspects of glucocorticoids

The main clinical application for glucocorticoids is in the treatment of inflammation associated with conditions such as rheumatoid arthritis, asthma, and allergies. The agents used should have a low-to-negligible mineralocorticoid side effect. Ideally, they should be administered topically, whether that be as a cream or ointment for skin inflammations; drops for inflammations of the eye, ear, and nose; or aerosols for the prophylaxis and treatment of asthma. However, there are occasions when oral administration is acceptable and, in certain emergency situations, they can be injected, for example in severe asthma or anaphylactic shock. They can also be injected directly into joints or soft tissue for the treatment of joint inflammations. Long-term use of glucocorticoids is discouraged because it can lead to growth suppression in children, susceptibility to infection (especially chicken pox and measles), and suppression of the pituitary-adrenal glands. The last effect can result in serious medical problems if the treatment is stopped suddenly and so a steroid treatment card should be carried by any patients taking glucocorticoids on a long-term basis. Systemic administration can also result in a wide range of psychiatric conditions varying from nightmares to depression and suicidal tendencies, especially with patients who have a history of mental disorders. High doses can lead to Cushing's syndrome, but this is usually reversible when the treatment is withdrawn gradually.

Orally active glucocorticoids currently used in the clinic include **cortisol**, **cortisone acetate**, **deflazacort**, **dexamethasone**, **methylprednisolone**, **prednisolone**, **riamcinolone acetonide**, and the ester prodrugs of **betamethasone** and **dexamethasone**.

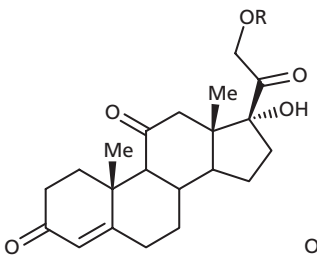
There are a large variety of topical agents used as creams, drops, or sprays, including **alclometasone dipropionate**, **beclometasone dipropionate**, **budesonide, cortisol**, **dexamethasone**, **diflucortolone valerate**, **fludroxycortide**, **flumetasone pivalate**, **fluorometholone**, **flunisolide**, **fluocinolone acetonide**, **fluocinonide**, **fluticasone propionate**, **halobetasol propionate**, **loteprednol etabonate**, **mometasone furoate**, **rimexolone**, and **triamcinolone acetonide**. Ester prodrugs of **betamethasone**, **clobetasol**, **cortisol**, **dexamathasone**, **fluocortolone**, and **prednisolone** are also available.

Preparations used for injections include **triamcinolone acetonide** and ester prodrugs of **betamethasone**, **cortisol**, **dexamethasone**, **methylprednisolone**, and **prednisolone**.

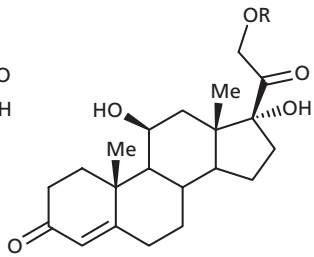
Agents used in the prophylaxis of asthma include **budesonide**, **ciclesonide**, **fluticasone propionate**, and **mometasone furoate**.

(Continued)

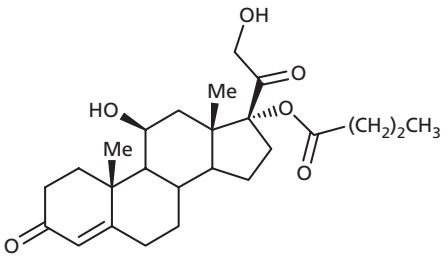
BOX CS6.1 Clinical aspects of glucocorticoids (*Continued*)



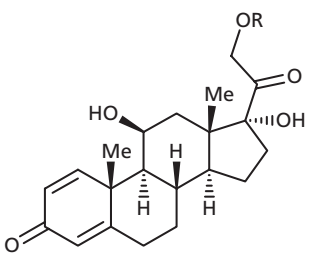
Cortisone; R = H
Cortisone acetate; R = Ac



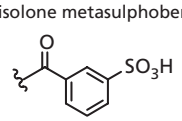
Hydrocortisone; R = H
Hydrocortisone acetate; R = Ac
Hydrocortisone phosphate; R = Phosphate
Hydrocortisone succinate; R = $CO(CH_2)_2CO_2H$

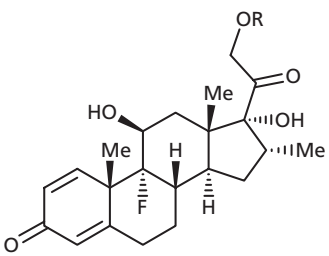


Hydrocortisone butyrate

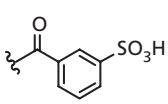


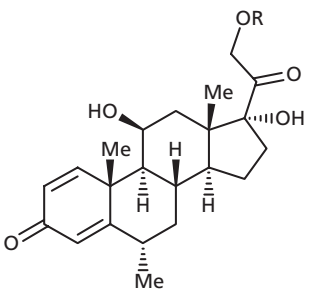
Prednisolone; R = H
Prednisolone acetate; R = Ac
Prednisolone sodium phosphate; R = PO_3Na_2
Prednisolone hexanoate; R = $CO(CH_2)_4CH_3$
Prednisolone metasulphobenzoate
R =



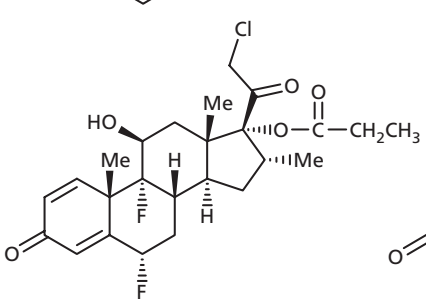


Dexamethasone; R = H
Dexamethasone acetate; R = Ac
Dexamethasone phosphate; R = PO_3^{2-}
Dexamethasone metasulphobenzoate; R =

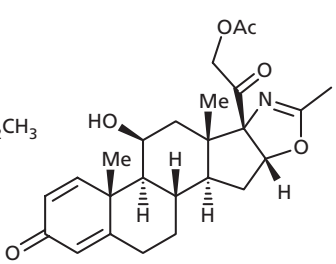




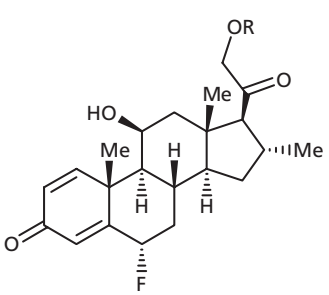
Methylprednisolone; R = H
Methylprednisolone acetate; R = Ac
Methylprednisolone succinate; R = $CO(CH_2)_2CO_2^-\ Na^+$



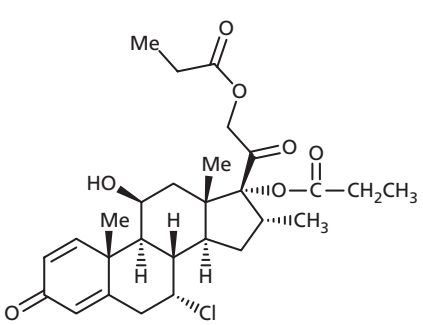
Halobetasol propionate



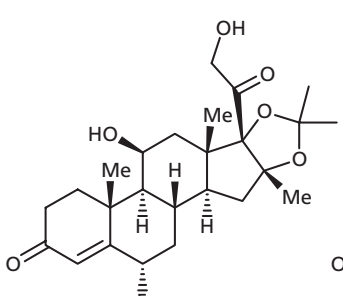
Deflazacort



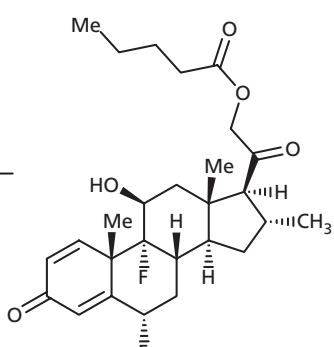
Fluocortolone; R = H
Fluocortolone pivalate; R = $COCMe_3$
Fluocortolone caproate; R = $COCH_2CMe_3$



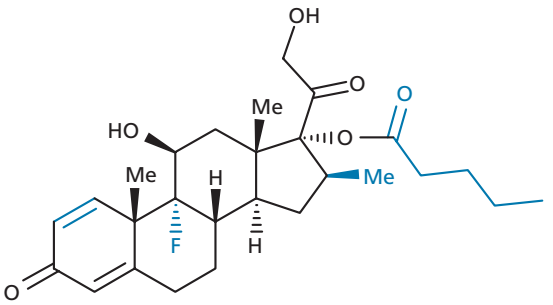
Alclometasone dipropionate



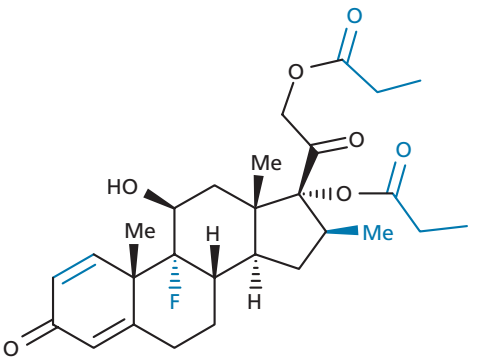
Fludroxycortide



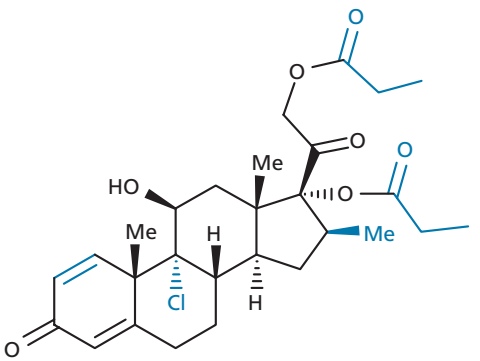
Diflucortolone valerate



Betamethasone 17-valerate



Betamethasone 17,21-dipropionate



Beclometasone 17,21-dipropionate

FIGURE CS6.5 Clinically useful esters and analogues of betamethasone.

compound arising from these studies was **clobetasol propionate** (Fig. CS6.6).

CS6.3.3 11-Ketosteroids

In general, replacing the 11β-OH group of cortisol with a keto group results in a drop in activity and it is believed that the ketone group has to be reduced *in vivo* for the compound to be active. However, activity can be restored by introducing suitable substituents elsewhere. Halogens at positions C-9 and C-21 are particularly important in this respect, for example **clobetasone butyrate** (Fig. CS6.7).

CS6.3.4 Analogues with modified C-17 side chains

The two-carbon chain at C-17 is generally important for activity, but it was found that activity could be retained if the side chain was replaced with a carboxylic acid as long as both it and the 17-OH group were esterified. If only one or other of the functional groups was esterified, then there was no activity. This was an important discovery as it meant that the di-esters would be active at the site of administration but would be hydrolysed to inactive compounds as soon as they reached the blood circulation, thus reducing the chances of unwanted side effects elsewhere in the body. A variety of esters were synthesized which demonstrated that the 17α-propionate and 17β-fluoromethyl esters were ideal (structure I, Fig. CS6.8). Further variations led to the discovery that the 17β-fluoromethyl thioester was also beneficial, leading to the clinically important **fluticasone propionate** (Fig CS6.8). This agent has a high affinity for target receptors, high potency, and low oral bioavailability (1%) because of low solubility and rapid metabolism in the liver.

CS6.3.5 Glucocorticoids used in asthma treatment

Glucocorticoids are used as anti-inflammatory agents in the treatment of asthma and are administered by inhalation in order to reduce the risks of side effects caused by their presence in the blood supply. However, it is not

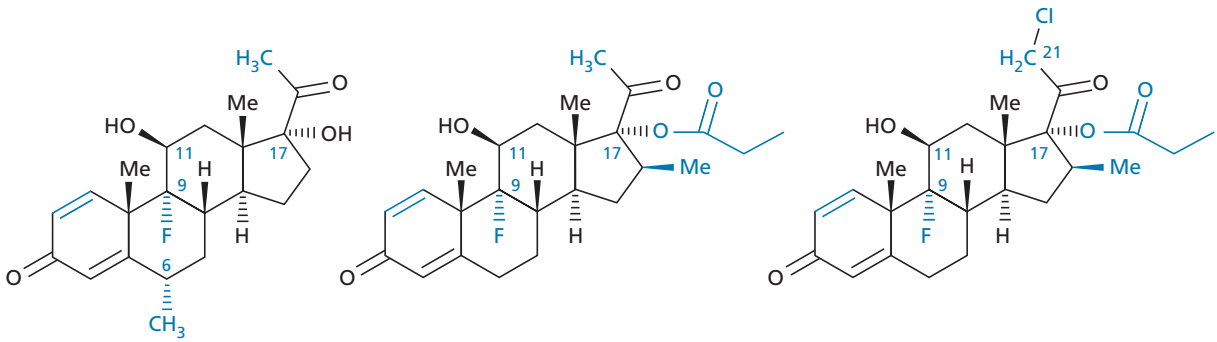


FIGURE CS6.6 21-Deoxysteroids with glucocorticoid activity.

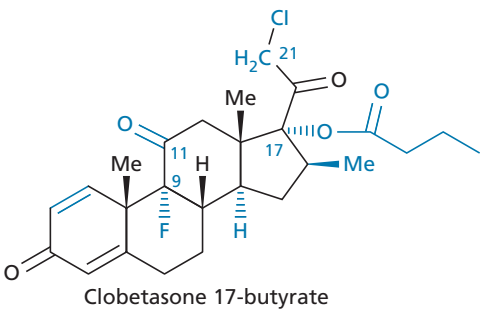


FIGURE CS6.7 Clobetasone 17-butyrate.

possible to completely prevent these agents reaching the blood supply; a certain percentage of inhaled glucocorticoid is swallowed and absorbed orally. However, most of the glucocorticoids used in asthma treatment are rapidly metabolized in the liver. Of more significance is the proportion of inhaled dose that gets absorbed into the blood supply through the lungs. Therefore, it is important that glucocorticoids used in asthma treatment are susceptible to metabolic deactivation in the blood; for example by esterases.

Beclometasone dipropionate (Fig. CS6.5) represented a breakthrough in asthma treatment and is currently used as an inhaler, as are **budesonide**, **ciclesonide**, **mometasone furoate**, and **fluticasone propionate** (Figs CS6.8 and CS6.9). Budesonide is an example of a new generation of non-halogenated glucocorticoids. One would actually expect a drop in activity as a result of the lack of halogen substituents, but the nature of the acetal is key in providing high topical anti-inflammatory activity. The acetal group increases the hydrophobic nature of the compound leading to prolonged residence in lung tissue. Budesonide has been found to have high receptor affinity and a higher anti-inflammatory potency than fluticasone propionate. In contrast, its systemic glucocorticoid activity is 4–7 times lower owing to extensive first-pass metabolism in the liver by the cytochrome P450 enzyme (CYP3A4) to much less potent metabolites. **Ciclesonide** is the latest in this series and is an example of a **soft steroid**. The structure acts as a prodrug and is activated by esterases in lung tissue which hydrolyse the C-21 ester to reveal a free alcohol group. This is the active compound and has a prolonged duration of action in lung tissue. However, it has negligible activity elsewhere in the body,

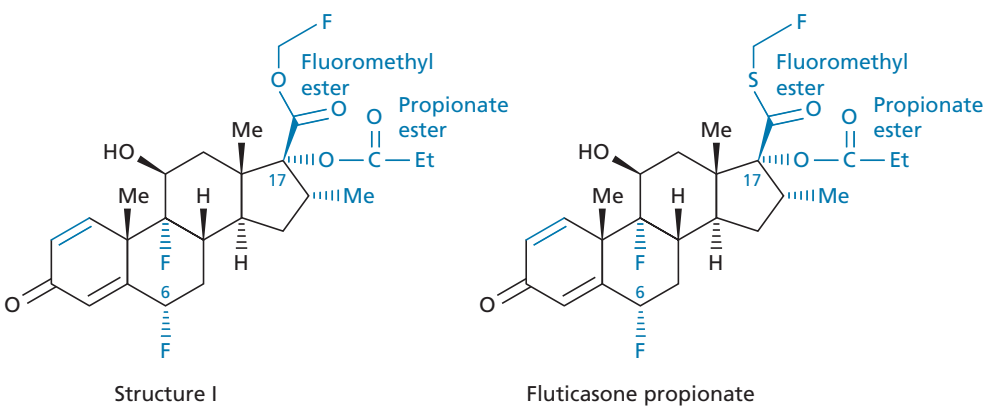


FIGURE CS6.8 Development of fluticasone propionate.

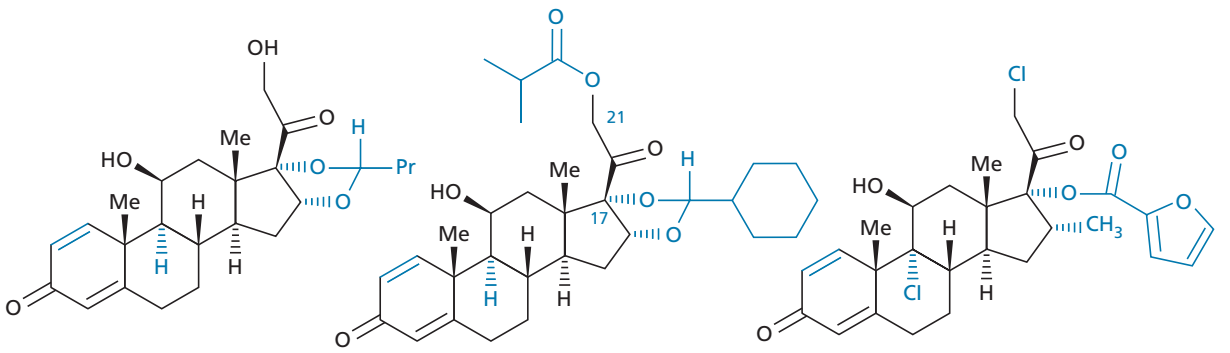


FIGURE CS6.9 Glucocorticoids used in the treatment of asthma.

despite it being able to reach the circulatory system. This is because it is rapidly metabolized by cytochrome P450 enzymes to inactive metabolites.

The use of heterocyclic esters at C-17 also results in high topical anti-inflammatory activity, as in mometasone furoate.

CS6.3.6 Glucocorticoids used in ophthalmology

A number of steroids have been used as topical anti-inflammatory agents in ophthalmology, such as **dexamethasone** (Fig. CS6.3), **fluorometholone** (Fig. CS6.6), **betamethasone sodium phosphate**, **hydrocortisone acetate**, **prednisolone acetate**, **prednisolone sodium phosphate**, and **rimexolone** (Fig. CS6.10). Rimexolone is surprisingly short of many of the features that are present in other anti-inflammatory glucocorticoids. For example, it lacks the 17α-OH group, as well as halogen substituents.

Unfortunately, glucocorticoids can cause side effects, such as glaucoma and cataract formation. The latter is thought to be associated with the C-20 keto group forming Schiff bases with lysine residues on proteins, followed by a rearrangement reaction involving the C-21 hydroxyl group to give amine-linked adducts. Indeed, efficacy appears to go hand in hand with toxicity.

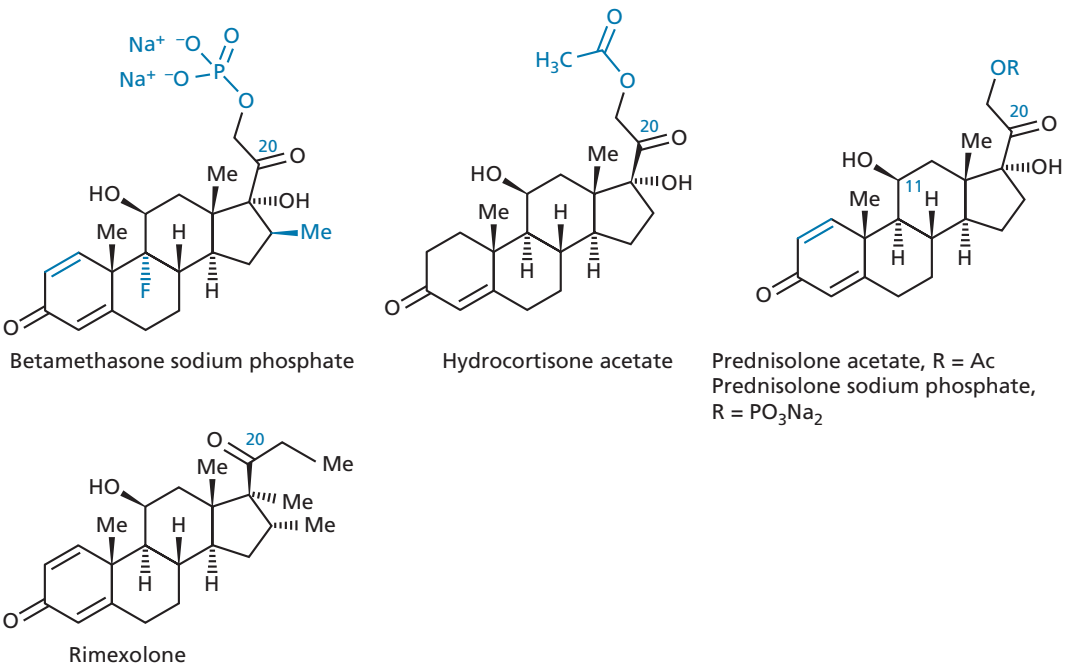


FIGURE CS6.10 Glucocorticoids used in ophthalmology.

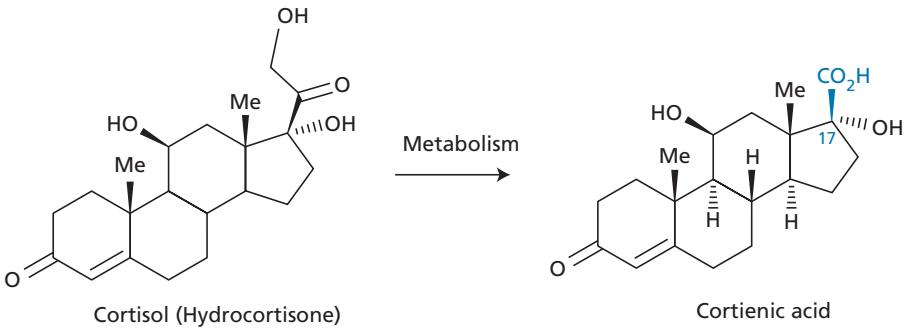


FIGURE CS6.11 Metabolism of cortisol to cortienic acid.

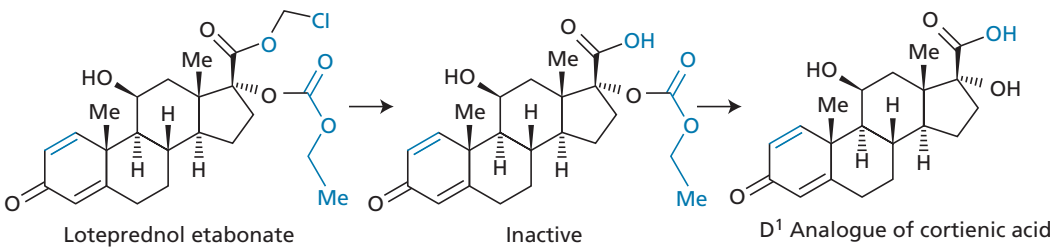


FIGURE CS6.12 Metabolism of loteprednol etabonate.

To tackle this problem, it was decided to design soft drugs which would metabolize quickly in the circulation to inactive compounds. The ideal drug would be one which was metabolized at a reasonable rate in the blood supply, but survived long enough to act as an anti-inflammatory agent at its intended target. This required the correct balance of activity, solubility, lipophilicity, tissue distribution, protein binding, and rate of metabolic deactivation. The lead compound for the design of these compounds was **cortienic acid**, which was known to be an inactive metabolite of hydrocortisone resulting from oxidation of the dihydroxyacetone side chain (Fig. CS6.11).

The aim was to now restore activity by adding suitable esters to the functional groups at C-17. As the esters would be susceptible to hydrolysis by esterases in the blood, any activity introduced in this manner would be lost completely after hydrolysis had taken place. Other features that were known to be beneficial to anti-inflammatory activity were also included in various analogues, such as an extra double bond in the A ring or fluorination at C-6 or C-9. A first generation of compounds was synthesized that illustrated the following important features for activity:

- a fluoromethyl or chloromethyl ester at C-17β
- a carbonate or ether group at C-17α.

This led to the discovery of **loteprednol etabonate** (Fig. CS6.12), which has a much better therapeutic ratio than the traditional corticosteroids. The compound contains the extra double bond in the A ring which is good for activity, as well as two hydrolysable esters. As predicted, it is metabolized in two stages to the Δ^1 analogue

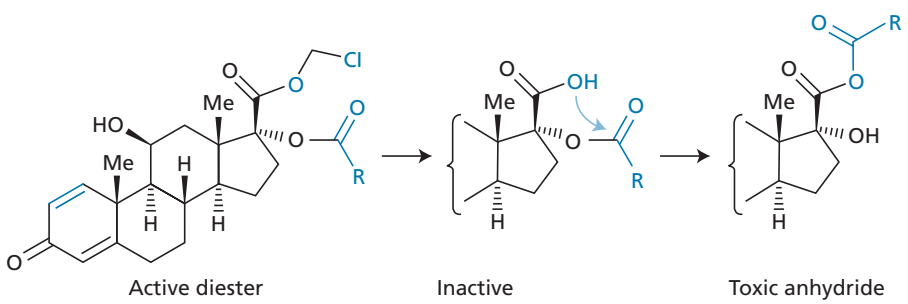


FIGURE CS6.13 Intramolecular reaction leading to toxic anhydrides.

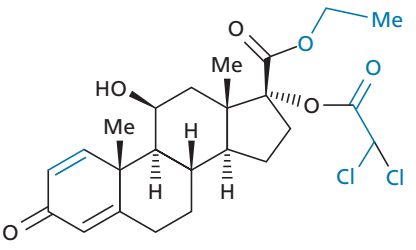


FIGURE CS6.14 Etiprednol dicloacetate.

of cortienic acid. Ester hydrolysis occurs first to give an inactive metabolite, followed by hydrolysis of the less reactive carbonate ester.

The use of a carbonate ester over a normal ester at C-17α was a deliberate strategy to prevent the possibility of the intramolecular reaction shown in Figure CS6.13, which would result in toxic anhydrides being formed.

Etiprednol dicloacetate (Fig. CS6.14) is a second-generation soft drug where two normal esters have been employed. The two chloro groups increase the rate of

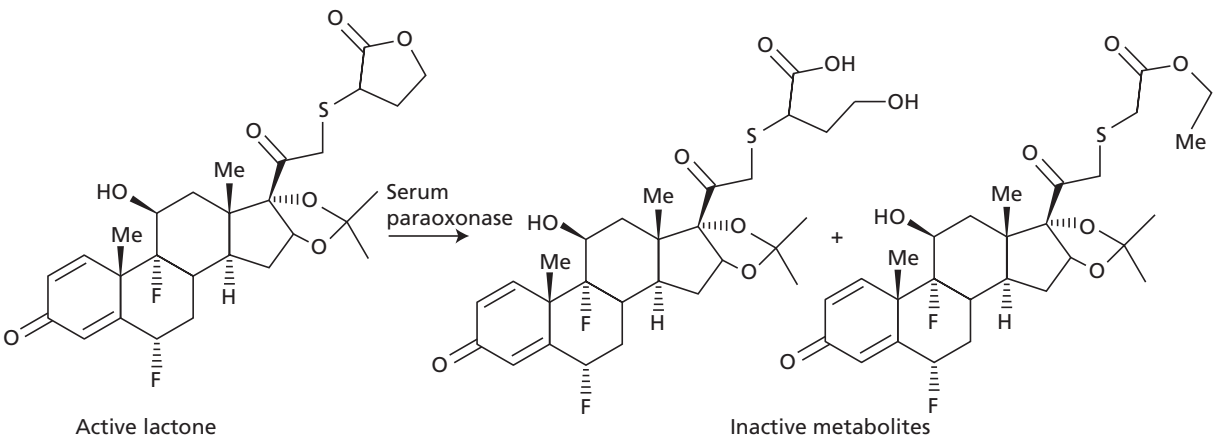


FIGURE CS6.15 Inactivation of an active lactone by the enzyme serum paraoxonase.

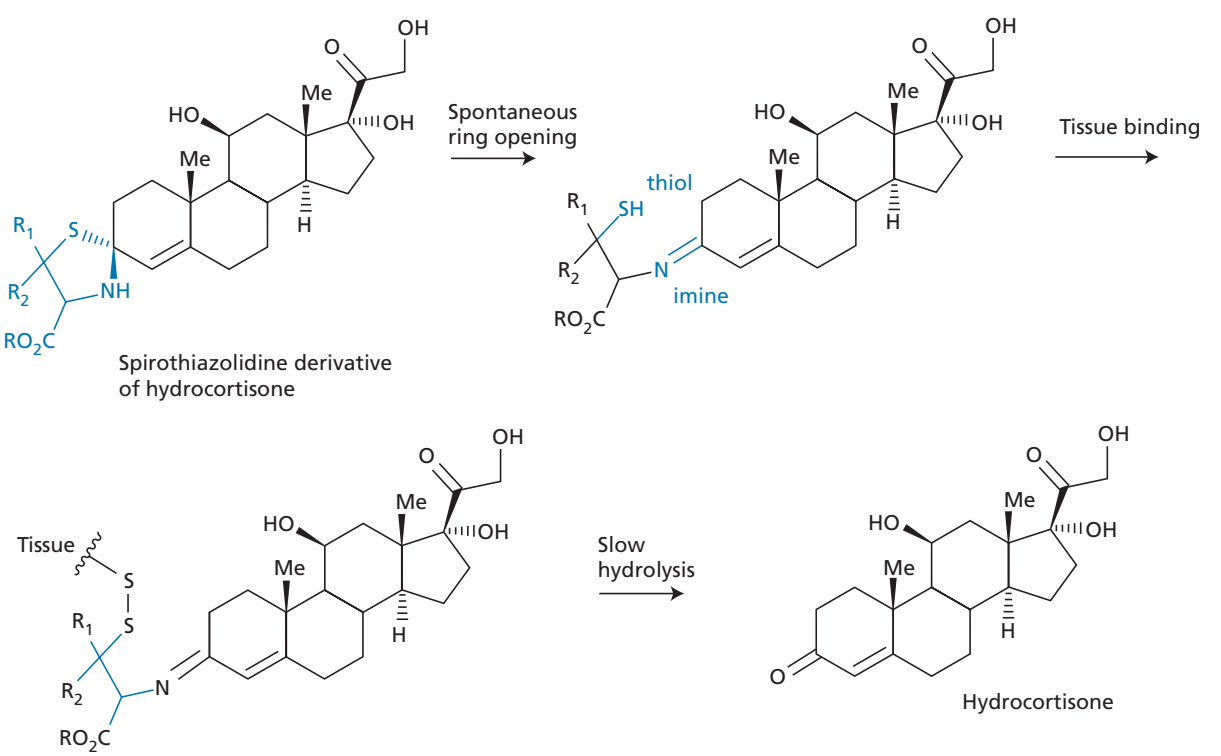


FIGURE CS6.16 Sustained release of hydrocortisone.

hydrolysis of the 17α-ester, which means that this ester is hydrolysed first instead of the 17β-ester, thus avoiding the risk of anhydride formation.

The absence of the chlorine substituent from the 17β-ester is potentially a problem as this is part of the pharmacophore for activity. However, molecular modelling studies demonstrated that one of the two chlorine substituents on the 17α-ester could occupy the same position in space as the original chlorine substituent.

Soft drugs containing a lactone group are of potential interest as anti-asthmatic agents (Fig. CS6.15). The lactone in the figure displays sufficient activity and stability in lung tissue to be effective. However, when it reaches the plasma, it undergoes rapid hydrolysis to form inactive metabolites. This is owing to the enzyme **serum paraoxonase**, which is present in plasma and the liver, but not in lung tissue.

CS6.3.7 Sustained release of topical anti-inflammatory agents

An interesting example of a pro-soft drug approach in drug design involves the design of a sustained chemical release system for hydrocortisone (Fig. CS6.16). When the spirothiazolidine derivative of hydrocortisone is applied topically it undergoes a spontaneous ring opening to form an imine and a thiol. The latter group reacts with the thiol group of cysteine residues in proteins, and becomes tethered to local tissue via a disulphide bond. Eventually, the imine is hydrolysed to release the drug. The compound has been found to be more active than hydrocortisone itself, and less of it crosses the dermis into the blood supply.

Test your understanding and practise your molecular modelling with Exercises CS6.1–CS6. 8.

CASE STUDY 7

Current research into antidepressant agents

CS7.1 Introduction

> I am worn out with grief;
> every night my bed is damp from my weeping;
> my pillow is soaked with tears.
> I can hardly see;
> my eyes are so swollen
> from the weeping caused by my enemies.
>
> Psalm 6, verses 6 and 7

Major depression is a common ailment that affects up to 10% of the population. It is estimated that 18 million people suffer from it in the USA and 340 million worldwide. The World Health Organization believes that by the year 2020, depression could be the second leading ailment in the world after heart disease. Depression is common in the elderly, and it is estimated that 21% of women and 13% of men will suffer major depression at some point in their lives. Symptoms include misery, apathy, pessimism, low self-esteem, feelings of guilt, inability to concentrate or work, loss of libido, poor sleep patterns, loss of motivation, and loss of appetite. Sufferers of long-term depression are more prone to other diseases and their lifespan can be shortened.

The causes of depression are many and varied. Some people are genetically predisposed to depression, but, in many cases, a stressful life-changing event precipitates the condition. Such events include loss of employment, divorce, bereavement, rejection, victimization, false accusation, and slander. Often, the sufferer has no control or redress over what has taken place, and the sense of helplessness and hopelessness that results exacerbates the situation.

Those suffering severe depression describe each day as a living nightmare. The same distressing thoughts whirl round in their minds pulling them deeper and deeper into a bottomless psychological whirlpool from which there seems to be no escape. Each day is an ordeal to be endured and, for some, it can be too much. Some turn to alcohol or illicit drugs for temporary oblivion; a few turn to suicide for permanent oblivion. Those who have never suffered depression have no concept of the disease, and telling the sufferer to 'snap out of it' or 'pull yourself together' is worse than useless.

CS7.2 The monoamine hypothesis

The pharmacological processes that cause depression are still an area of a research, but the accepted theory proposes that a deficit of monoamine neurotransmitters in certain parts of the brain causes the condition. This is known as the **monoamine** or **monoaminergic hypothesis**. The principal neurotransmitters believed to be involved are **dopamine**, **noradrenaline**, and **serotonin** (also known as **5-hydroxytryptamine**, 5-HT). There are various lines of evidence which support this. For example, the antihypertensive agent **reserpine** lowers monoamine levels in the brain and is known to cause depression as a side effect. Moreover, the clinically important antidepressant agents are known to increase monoamine levels by a variety of mechanisms. However, there are anomalies which indicate that there is more to the story than an increase in monamine levels. For example, **amphetamine** and **cocaine** are agents that increase noradrenaline and serotonin transmission, but are ineffective as antidepressants. There is also evidence that a wide range of endogenous hormones and neurotransmitters play a role in depression: substance P, corticotrophin-releasing factor, arginine, vasopressin, neuropeptide Y, melanin-concentrating hormone, acetylcholine, glutamic acid, gamma-aminobutyric acid, glucocorticoids, cytokines, enkephalins, and anandamide. Nevertheless, most clinically useful agents in use today are responsible for raising monoamine levels.

CS7.3 Current antidepressant agents

First-generation antidepressants were introduced about 50 years ago, and include the **monoamine oxidase inhibitors** (MAOIs), which are discussed in section 23.12.5, and the **tricyclic antidepressants** (TCAs), which are described in section 23.12.4. Unfortunately, these drugs have low target selectivity and many side effects.

Second-generation antidepressants were introduced in the 1980s and are represented by agents known as **selective serotonin reuptake inhibitors** (SSRIs) (Box 10.1).

These represented a major step forward in treatment because they are more selective and have fewer side effects. However, like the TCAs and MAOIs, they have a slow onset of action and it can take 2–6 weeks before patients feel any benefit. Another problem with their use is their negative effect on libido.

Third-generation antidepressant agents include **selective noradrenaline reuptake inhibitors** (section 23.12.4), and dual action **serotonin and noradrenaline reuptake inhibitors** (SNRIs) (section 23.12.4).

CS7.4 Current areas of research

Currently, there is research into novel agents designed to interact with the following targets:

- transport proteins for dopamine, serotonin, and noradrenaline
- adrenergic receptors, such as the α_2-adrenoceptor
- serotonin receptors, such as the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, and 5-HT_7 receptors.

Dual action agents that act on two of the above targets are of particular interest. Examples include agents that:

- block the reuptake of both noradrenaline and serotonin
- block α_2-adrenoceptors (section 23.11.2) and activate 5-HT receptors
- block serotonin reuptake and are antagonists for the 5-HT_{1A} receptor. The 5-HT_{1A} receptor is an autoreceptor present on the presynaptic neurons that release serotonin. When activated, this receptor inhibits the release of serotonin from the neuron and so an antagonist should counteract this effect
- block serotonin reuptake, and act as antagonists for the 5-HT_{2A} receptor. This receptor is responsible for the sexual dysfunction side effect associated with SSRIs.

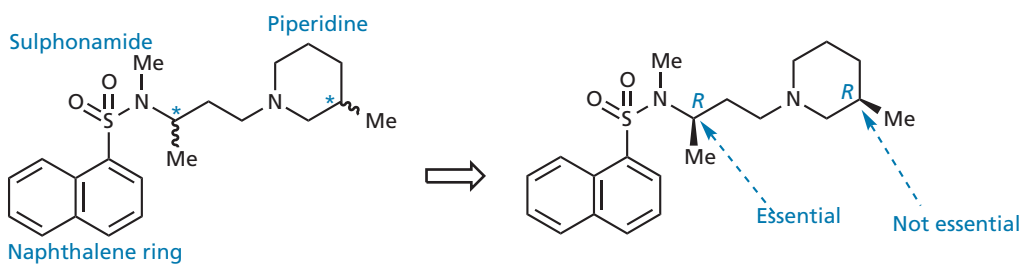
In this case study, we shall look at a research project aimed at discovering antagonists for the 5-HT_7 receptor.

CS7.5 Antagonists for the 5-HT_7 receptor

There are seven main types of serotonin receptors (5-HT_1–5-HT_7) and several subtypes of these. The 5-HT_7 receptor is the most recent serotonin receptor to be discovered and appears to play an important role in psychiatric disorders, such as depression. It has been shown that antagonists of this receptor have an antidepressant activity in animal studies, although the mechanism by which this takes place is unclear. At first sight, it may seem odd that a serotonin antagonist should have an antidepressant activity, as antidepressant activity is normally associated with increased serotonin levels and increased activation of serotonin receptors. However, it should be borne in mind that different receptors for the same neurotransmitter serve different purposes and some act as autoreceptors to provide a negative feedback control for neurotransmitter release. For example, the α_2-adrenergic receptor is a presynaptic autoreceptor which has the effect of inhibiting noradrenaline release (sections 23.6.3 and 23.11.2). It is conceivable that activation of 5-HT_7 receptors might lead to a drop in serotonin levels by a similar manner. Therefore, an antagonist that is selective for this receptor over other serotonin receptors could be advantageous.

Workers at SmithKline Beecham carried out high-throughput screening of their compound bank for structures having affinity for the 5-HT_7 receptor and identified the sulphonamide (I; Fig. CS7.1) as a lead compound with slight selectivity. The structure has two asymmetric centres and was tested as a mixture of the two possible diastereomers. As there are two enantiomers for each diastereomer, this means that there are four possible stereoisomers (*R,R*; *S,S*; *R,S*; and *S,R*) All four stereoisomers were tested separately and the *R,R* isomer (II) was found to have the best affinity.

The affinity for the *R,S*-diastereomer was still 6.2, which indicated that the stereochemistry of the asymmetric centre in the piperidine ring was not essential.



I; pK_i 7.2 II; pK_i 6.9

FIGURE CS7.1 Identification of a lead compound.

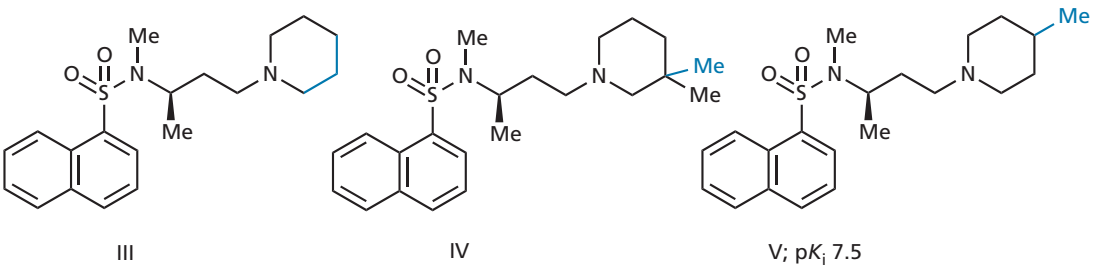


FIGURE CS7.2 Methods of removing the asymmetric centre in the piperidine ring.

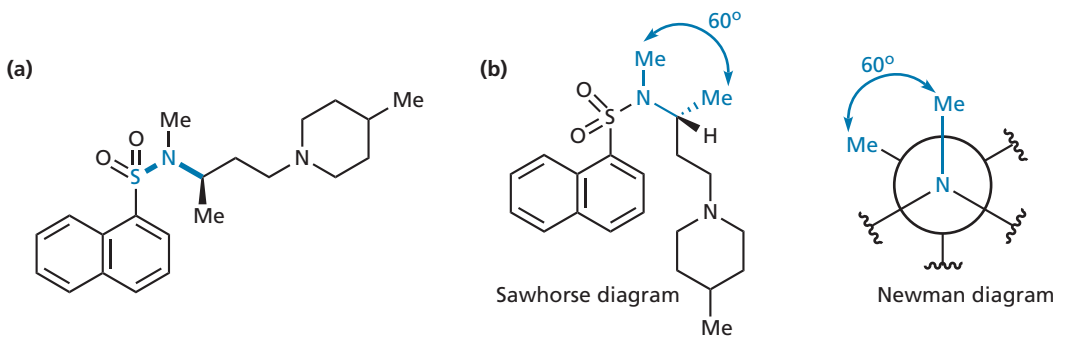


FIGURE CS7.3 Conformational analysis shows that (**a**) the bonds shown in blue have restricted rotation and (**b**) there is a stable conformation having a torsion angle of 60°.

Therefore, it was decided to remove this asymmetric centre as this would simplify the synthesis of analogues (*simplification; section 13.3.8*) and avoid the need to separate and purify diastereomers for each analogue produced. The obvious way of removing the asymmetric centre was to remove the methyl substituent, but the resulting structure III (Fig. CS7.2) had no affinity. This indicated the importance of the methyl group, which suggests that it might be interacting with a hydrophobic pocket in the binding site. Another method of removing the asymmetric centre was to add a second methyl substituent at the same position. However, the resulting structure IV had no affinity either, implying that the second methyl group might be bad for steric reasons. The problem was solved eventually by shifting the methyl group to position 4 of the piperidine ring, which not only removed the asymmetric centre but improved affinity (*simplification and group shift; sections 13.3.8 and 14.2.6*).

A conformational analysis of the flexible chain linking the two ring systems was now carried out (*conformational analysis; section 17.8*). This revealed that all the bonds are relatively free to rotate apart from the bonds shown in bold (Fig. CS7.3). Concentrating on conformations involving these bonds an energy minimum was found when the two methyl substituents are gauche with respect to each other, corresponding to a dihedral angle of 60°.

As the gauche conformation is an energy minimum, it represents a stable conformation and the molecule will spend a greater amount of time in this conformation than in others. Therefore, there is a possibility that this might correspond to the active conformation (*active conformation; section 13.2*). If this is the case, locking the molecule into this conformation should increase binding affinity (*rigidification; section 13.3.9*).

Rigidification can be carried out by introducing a ring that incorporates both methyl groups and the connecting bonds, for example structures VI and VII where the ring is six-membered and five-membered respectively (Figs CS7.4 and CS7.5). Before synthesizing these structures, docking experiments were carried out using a 5-HT_7 receptor homology model (*docking, section 17.12; homology models, section 17.14.1*). These predicted that the *R*-enantiomer of structure VI would have greater binding affinity than the *S*-enantiomer. Both enantiomers were duly synthesized and the *R*-enantiomer had 25-fold better affinity as predicted. It also had slightly better affinity than structure V. Structure VII containing the five-membered ring was then synthesized (*ring contraction; section 13.3.4*), which resulted in an increase in affinity.

The naphthalene ring system is not essential for activity and it was possible to replace it with a single aromatic ring to give structure VIII (*simplification or*

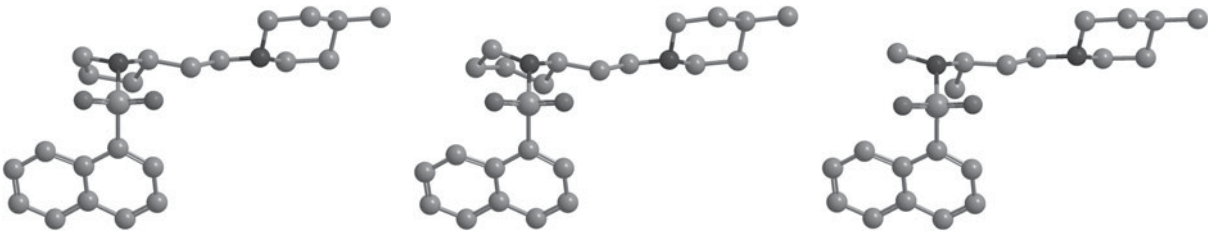


FIGURE CS7.4 Three-dimensional representations of the lead compound and rigidified analogues.

ring variation; sections 13.3.8 and 13.3.5). A number of different aromatic substituents were tested at different positions (*variation of aromatic substituents; section 13.3.1.2*) and it was found that a phenolic group was best for activity giving SB 269970. It is possible that this group is participating in a hydrogen bonding interaction with the binding site, as a methoxy substituent has less affinity. This was confirmed by docking the structure into the model binding site and identifying a possible hydrogen bonding interaction.

The selectivity of SB 269970 was tested against various receptors, and it was found to have greater than 250-fold selectivity over 13 other receptors, as well as a 50-fold selectivity over 5-HT_{5A}. Further testing with a commercial screening package (Cerep) showed that it had a 100-fold selectivity over a total of 50 other receptors, enzymes, or ion channels. The compound has been shown to be an inverse agonist (section 8.5).

As SB 269970 contains a phenolic group, it is prone to phase II conjugation reactions (section 11.5.5), which leads to rapid excretion. The phenolic group is involved in an important binding interaction and so, rather than removing it entirely, it was replaced with a metabolically stable bioisostere (*bioisosteres; sections 13.3.7 and 14.1.5*) that would still be capable of forming the important hydrogen bond. This was achieved by fusing a five-membered heterocycle onto the aromatic ring such that an NH group would be placed at the same position as the original phenol. Various heterocycles were tried with an indole ring system being the best (structure IX, Fig. CS7.5).

Unfortunately, the compound was rapidly cleared from the blood and had zero bioavailability when tested in rats, and so attention now turned to the methyl substituent on the piperidine ring, as this was also likely to be susceptible to metabolism (section 11.5.2). Molecular modelling showed that it might be possible to replace the

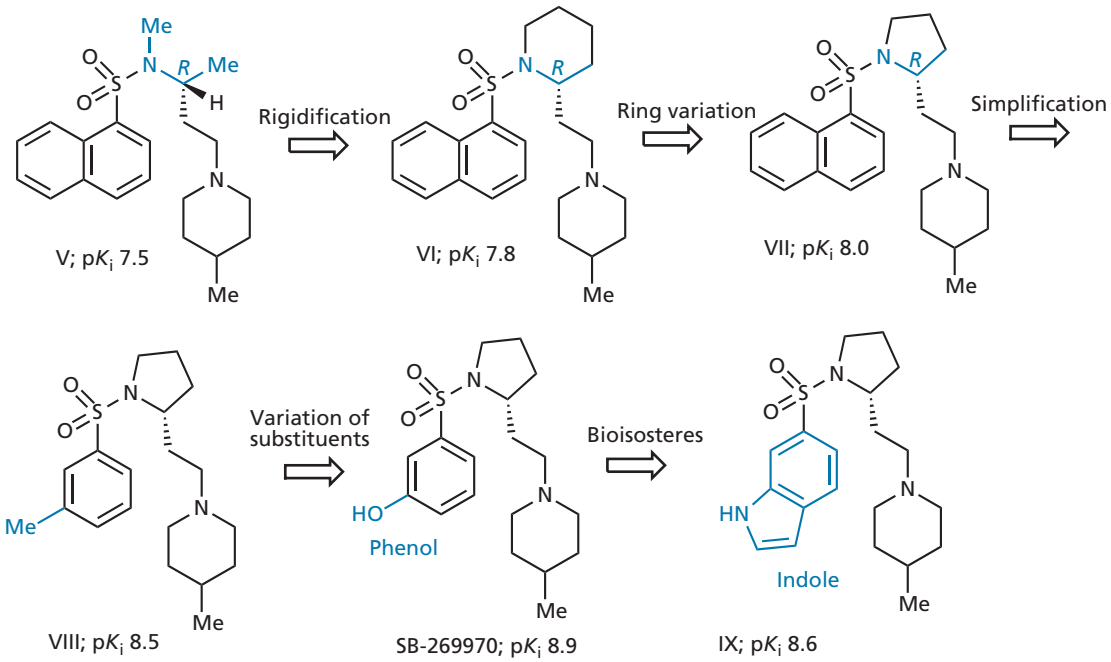


FIGURE CS7.5 The development process from lead compound to structure IX.

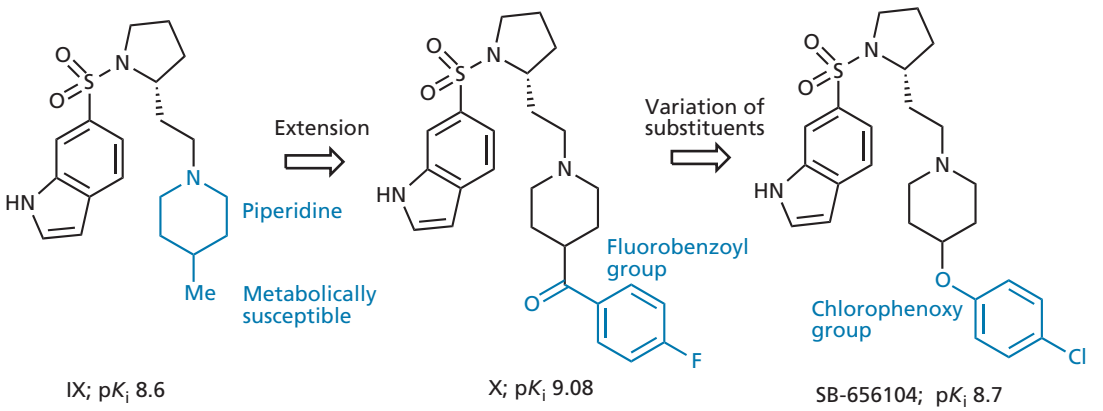


FIGURE CS7.6 Development of SB 656104.

methyl group with a substituent that would extend into a large hydrophobic pocket close by in the binding site. It was decided to try a substituent containing an aromatic ring. This would not only remove the susceptible methyl group, but offer the possibility of increased binding with the hydrophobic pocket (*extension; section 13.3.2*). Various substituents were tried and a fluorobenzoyl substituent was one of the best (structure X; Fig. CS7.6). Unfortunately, structure X had increased affinity for the α_{1B} adrenoceptor, as well as the $5HT_7$ receptor. Variation of the substituents (section 13.3.1.2) at either end of the aromatic ring showed that the chlorophenoxy group had much better selectivity (SB 656104; Fig. CS7.6). Although binding affinity for the $5HT_7$ receptor had dropped, this structure had the best balance of properties. Crucially, it lasted far longer than SB 269970 in the blood supply and had an oral bioavailability of 16%. This compound was taken forward as the basis for further studies.

FURTHER READING

Forbes, I. T., Douglas, S., Gribble, A. D., Ife, R. J., Lightfoot, A. P., Garner, A. E., et al. (2002) SB-656104-A: A novel 5-HT_7 receptor antagonist with improved in vivo properties. *Bioorganic and Medicinal Chemistry Letters* **12**, 3341–3344.

Leopoldo, M. (2004) Serotonin7 receptors (5-HT_7Rs) and their ligands. *Current Medicinal Chemistry* **11**, 629–661.

Pacher, P. and Kecskemeti, V. (2004) Trends in the development of new antidepressants. Is there a light at the end of the tunnel? *Current Medicinal Chemistry* **11**, 925–943.

Stromgaard, K. (2009) Recognising antidepressants. *Chemistry World* July, 33.