Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 1487–1496

BIOORGANIC & MEDICINAL CHEMISTRY

A Novel Molecular Modelling Study of Inhibitors of the 17α-Hydroxylase Component of the Enzyme System 17α-Hydroxylase/17,20-Lyase (P-450$_{17\alpha}$)

Sabbir Ahmed*

School of Applied Chemistry, Kingston University, Penrhyn Road, Kingston upon Thames, KT1 2EE, UK

Received 1 September 1998; accepted 16 November 1998

Abstract—The enzyme 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$) has recently become the focus of research into the fight against hormone dependent prostate cancer. However, the specific nature of this enzyme, in particular, the dual role of its active site, remains unknown. In our drive to elucidate further information regarding P-450$_{17\alpha}$, and in light of our experience of other cytochrome P-450 enzymes, we chose to consider each part of this complex enzyme separately (i.e. the 17α-hydroxylase (17α-OHase) and the 17,20-lyase components). We therefore initiated a series of molecular modelling studies involving the construction of a 'substrate–heme complex' for each of the two components. Here, we consider the construction and use of the complex for the 17α-OHase component of this enzyme. Using this approach, we have successfully considered: the binding of steroidal and non-steroidal reversible inhibitors; the structural features necessary for potent inhibition; and, rationalised the mode of action of a number of compounds whose inhibitory activity has not been previously explained, for example, aminoglutethimide (an inhibitor of another related cytochrome P-450 enzyme, aromatase AR). The study concludes that the ability of the inhibitors of 17α-OHase to undergo polar–polar interaction with the active site and for the compounds to closely mimic the substrate plane is a major factor in determining potency. Factors such as log *P* (log of the partition coefficient value for the distribution of a compound between octanol and water) would then appear to determine the extent of overall inhibitory activity. Overall, the study suggests that the novel substrate–heme complex approach has provided a good approximation of the 17α-OHase active site and has proved to be a useful tool in drug design and discovery.

Introduction

Inhibitors of the enzyme complex 17α-hydroxylase/17,20-lyase (P-450$_{17\alpha}$) have been shown to have a beneficial role in the treatment of hormone dependent cancers, such as prostate cancer. P-450$_{17\alpha}$ is responsible for the conversion of C_{21} steroids (e.g. progesterone) to C_{19} steroids (e.g. androstenedione) (Figs. 1 and 2(a)), and numerous compounds (Fig. 2(b)) have been shown to possess inhibitory activity against this enzyme. For example, ketoconazole until recently was considered a possible candidate in the treatment of prostate cancer.[1] Other imidazole based inhibitors (Fig. 2(b)) have also been studied and a number of antimycotic compounds were found to be potent inhibitors,[2] although attempts to determine a simple structure–activity relationship for these compounds (within the same study) could not be found. From a molecular modelling study of these compounds, novel non-steroidal inhibitors have been proposed[3] and synthesised. Upon biochemical evaluation, these compounds were discovered to be good inhibitors. In his studies, Ahmed[3,4] considered the modelling of known antimycotic and novel imidazole based inhibitors and suggested a common theme of binding within these compounds, i.e. that they utilise the same hydrogen bonding site at the active site as the steroid substrate C(3) C=O [for progesterone] or C(3)-OH [for pregnenolone] groups. It is postulated that since the C(20) carbonyl is involved in the step following hydroxylation, i.e. the lyase of the C(20)−C(17) bond (Fig. 3), this carbonyl group is not involved in any hydrogen bonding interaction with the active site. However, the model obtained cannot be used to rationalise inhibitory activity of alternative inhibitors. Also, the differences in inhibitory activity observed between enantiomers could not be fully explained.

Derivatives of pyridyl acetic acid (Fig. 2(b)) have also been studied and modelled by superimposing the inhibitors onto the steroid substrate backbone.[5] Laughton et al.[6] recently undertook a homology based modelling study where they utilised another cytochrome P-450

Key words: 17α-Hydroxylase; inhibitors; P-450; substrate-heme complex; P-45017α.

* Tel.: +44-(0)-181-547-200, ext 2433; fax: +44-(0)181-547-7562; e-mail: ch_s534@kingston.ac.uk

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00077-2

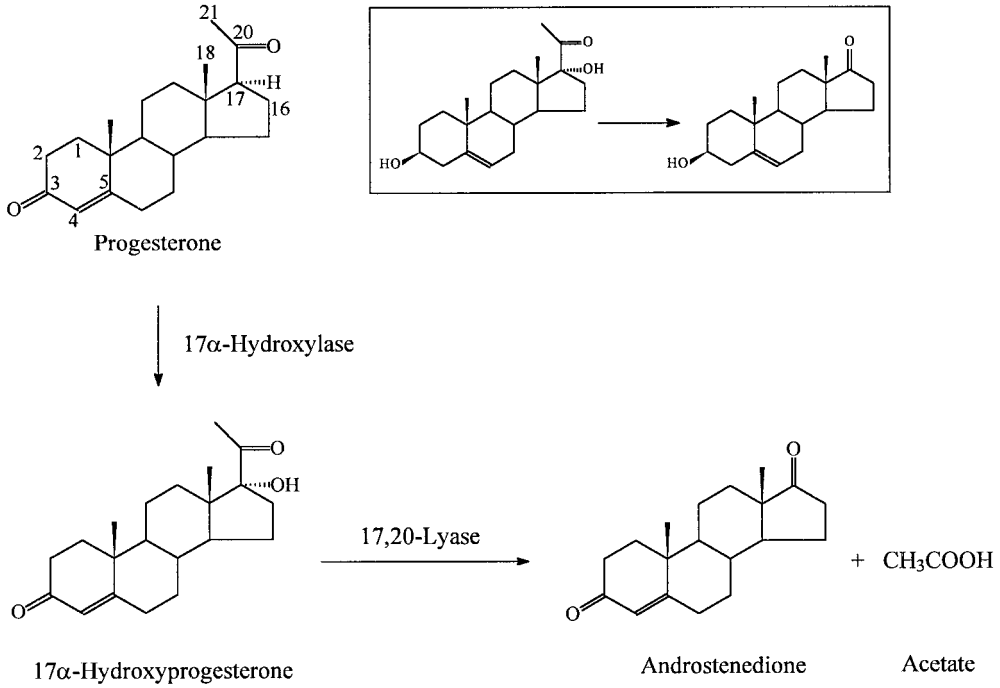

Figure 1. Conversion of progesterone (C_{21}) to androstenedione (C_{19}) (insert showing the action of P-$450_{17\alpha}$ on pregnenolone).

related enzyme and for which the crystal structure is known, namely P-450_{CAM} (the enzyme responsible for the hydroxylation of camphor), and although the authors supported the conclusions of the previous studies, they did not in their original report discuss in detail the modelling of inhibitors within their derived enzyme.

Alternative molecular modelling studies based on known, as well as novel, inhibitors have provided general information regarding the nature of the active site, however, more precise information has not been readily available on the different components of this enzyme. Furthermore, P-$450_{17\alpha}$ being a membrane bound enzyme has proved difficult to crystallise, as a result no crystal structure exists for this enzyme complex.

In our drive to elucidate a clearer picture of the active site, we concluded that a more effective approach may lie in the consideration of the individual hydroxylase and lyase components of the overall P-$450_{17\alpha}$ enzyme complex involving the use of the novel 'substrate–heme complex' approach. Also, we concluded from alternative studies undertaken within our laboratory[7] into similar enzymes, that the elucidation of the probable positioning of the P-450 heme of these two components (with respect to the substrate backbone, i.e. progesterone and/or pregnenolone) may provide more precise information regarding the active site and therefore the inhibition process. That is, we have previously reported a novel study where we considered the positioning of the heme with respect to the backbone of the steroid androstenedione, the natural substrate for another related cytochrome P-450 mono-oxygenase enzyme aromatase (AR), and have produced a substrate–heme complex as a representation of the AR active site.[7] Using this complex, we have discussed the mode of action of several type II AR inhibitors (both steroidal and non-steroidal)[7] and considered the probable mechanism(s) for the lyase of the C(10)–C(19) bond of androstenedione.[8]

In this report, we consider the 17α-OHase component of P-$450_{17\alpha}$, and attempt to elucidate the positioning of the P-450 heme with respect to the backbone of progesterone so as to construct the 'substrate–heme complex' (it is assumed that the conclusions/observations derived at for progesterone are applicable to pregnenolone). Using this complex as a simplified representation of the 17α-OHase, we consider the binding of some known reversible type II inhibitors (both steroidal and non-steroidal) (Figs. 2(a) and 2(b)) and suggest possible reasons for the difference in activity between inhibitors (and their enantiomers), as well as explaining the mode of action of aminoglutethimide, which to our knowledge, has not previously been reported for this enzyme.

Molecular Modelling

The structures of the porphyrin, progesterone, pregnenolone and the inhibitors (such as: bifonazole, ketoconazole, miconazole, econazole, metronidazole, aminoglutethimide (AG), derivatives of 4-pyridyl acetic acid, as well as the 2-pyridyl and 4-pyridyl analogues of 17-(3-pyridyl) androsta-5,16-dien-3β-ol[9] (Fig. 2(a) and 2(b)) were all constructed within Alchemy III[10] molecular modelling software, using suitable starting fragments from the structure library, on a P100 Intel Pentium microprocessor based IBM compatible microcomputer. For example, in the case of Bifonazole, a search of the Alchemy III fragment library resulted in the discovery of a benzene ring. Two benzene rings were bonded to each other resulting in the biphenyl moiety.

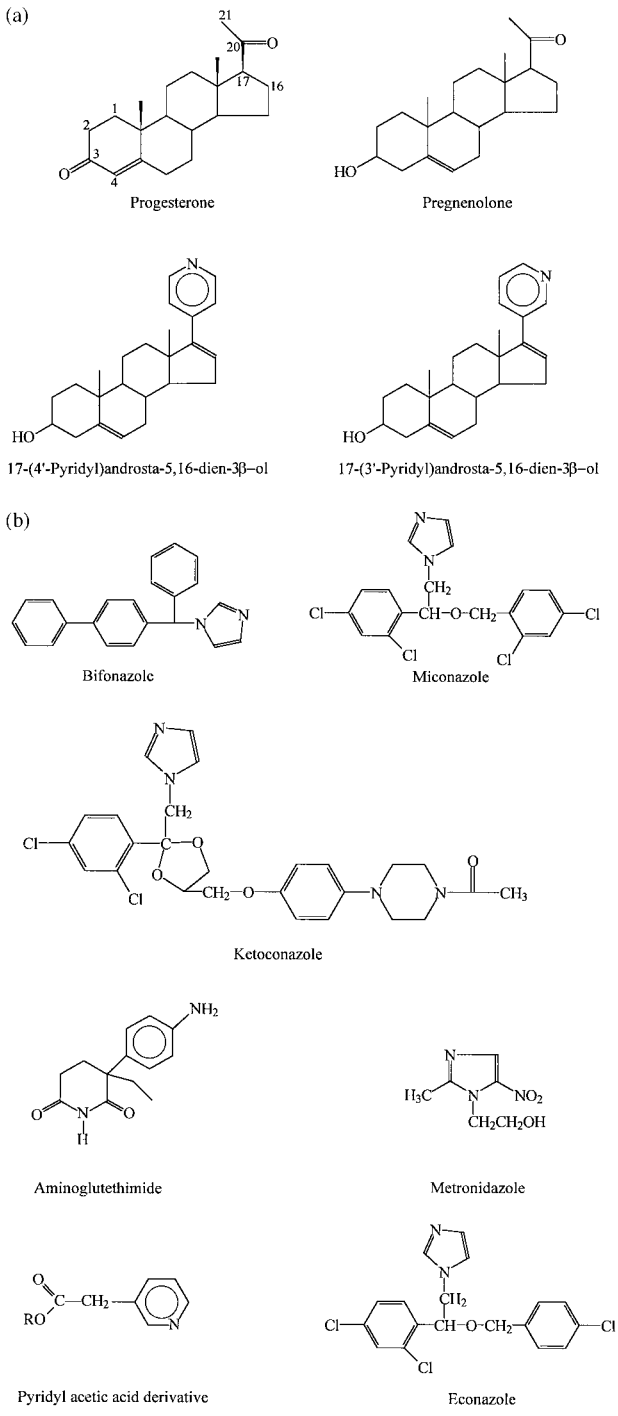

Figure 2. (a) Substrates and some steroidal reversible inhibitors of 17α-OHase. (b) Substrates and some non-steroidal reversible inhibitors of 17α-OHase.

To this, a sp^3 carbon atom was added to one of the phenyl rings in the 4 position with respect to the remaining phenyl ring. Another benzene ring was then added to the sp^3 carbon atom, to which a previously prepared, minimised imidazole ring was added. Finally, hydrogens were added to the whole molecule, resulting in bifonazole. The completed structures were then subjected to an initial minimisation using the conjugate-gradient algorithm available within Alchemy III. The minimisations were carried out, in general, in excess of

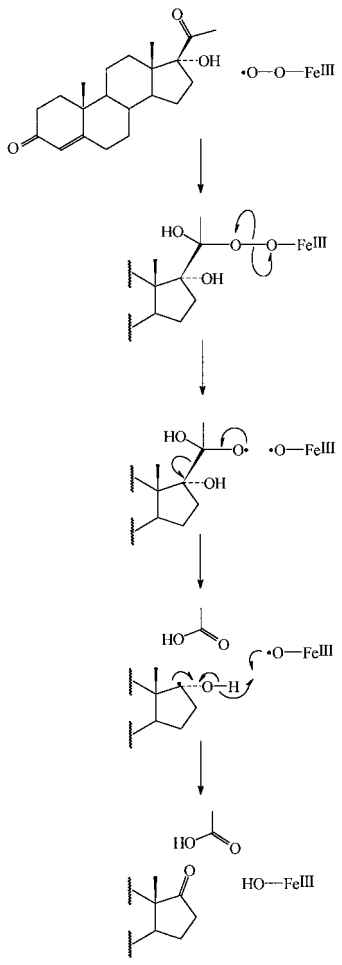

Figure 3. Proposed mechanism of action of P-$450_{17\alpha}$.

300 iterations and automatically terminated when the RMS gradient was less than 10^{-5}. Conformational analysis on the rotatable bonds within these inhibitors was carried out using the Powersearch[11] conformational analysis software using the systematic method with bond rotations of 20–40° and energy constraints of 10–40 kcal/mol (a range is given since each specific parameter was dependent upon the memory requirements of the calculation), resulting in the production of several conformers for each inhibitor. The lowest energy conformer ($\Delta E = 5$ kcal/mol) was retained for further studies.

Results and Discussion

Mechanism of hydroxylation and the position of the heme

To determine the orientation of the iron within the active site of 17α-OHase with respect to the steroid backbone, we considered the present hypotheses on the mechanism of hydroxylation of the steroid C(17) position (Fig. 3). On the basis that the mechanism of 17α-OHase is similar to that of AR,[12] (i.e. there is an initial involvement of a ferroxy radical) we hypothesised that the oxygen radical must be positioned within approximate bonding distance (and angle) to carry out hydrogen abstraction

from the C(17) such that when the Fe^{IV}-OH species is formed, the C(17) radical can be 'neutralised' by the formation of a bond with the Fe^{IV}-OH, resulting in the hydroxylation of the C(17) position of progesterone and the reformation of Fe^{III} heme. From a review of the literature, we discovered that 17α-OHase is also able to carry out the 16α-hydroxylation of progesterone[13] and we thus concluded that the Fe^{IV}-O• radical must therefore closely approach *both* the C(17) and C(16) positions. In the construction of the 17α-OHase substrate–heme complex, we therefore, attached the Fe-O moiety to the αC(17) position and minimised the initial structure. This then resulted in the 17α-OHase 'substrate–heme complex' which was subsequently utilised in the study of non-steroidal inhibitors (Fig. 4(a)). A substrate–heme complex was also constructed using pregnenolone as the substrate so as to compare the two complexes and consider differences between the two structures.

Further consideration of the 16- and 17-α hydroxylation properties of P-$450_{17\alpha}$ appears to suggest that the part of the active site involved with 17α-OHase activity may not be involved with the 17,20-lyase of the steroid backbone. That is, the proposed mechanism for the action of P-$450_{17\alpha}$ (Fig. 3) suggests that attack on the C(20) carbonyl group takes place prior to the attack on the C(17). If the heme is positioned between the C(16) and C(17) of the steroid backbone for attack on either position, then the intermediate would need to reposition itself so as to place the C(20) carbonyl group close to the heme for attack in the lyase step. The substrate would therefore, be required to move in an approximately horizontal and vertical plane, presumably requiring the existence of numerous hydrogen bonding sites in the horizontal plane for the intermediates to bind to the active site (Fig. 4(b) shows the different positions required for the attack on C(17) and C(20)). The existence of two hydrogen bonding sites has been reported in an homology based study of P-$450_{17\alpha}$, however, they were found to be situated in different planes, in an approximate 'L' arrangement, i.e. there appears to be two 'active sites' which are perpendicular to one another. Therefore, our approach to consider each active site separately in the elucidation of an overall model of the P-$450_{17\alpha}$ enzyme complex appears to be justified.

Reversible steroidal P-$450_{17\alpha}$ inhibitors

Reversible inhibitors of P-$450_{17\alpha}$, based on the substrates progesterone and pregnenolone, for example,

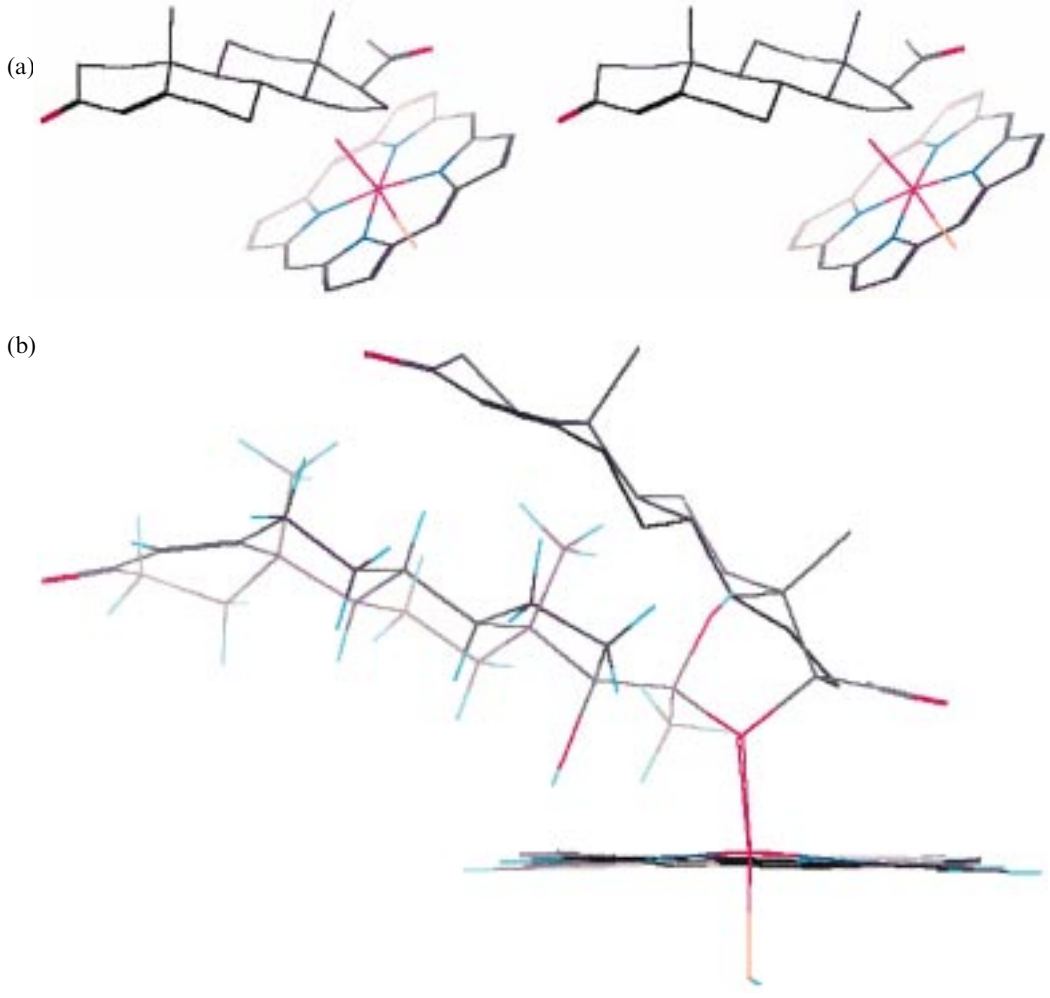

Figure 4 (a). Stereoplot of the substrate–heme-complex for the 17α-OHase component of P-$450_{17\alpha}$. (b) Diagram to show the differences between the substrate–heme-complex for the 17,20-lyase (with hydrogen atoms attached) and 17αOHase (hydrogens removed) components of P-$450_{17\alpha}$.

17-(3-pyridyl) androsta-5,16-dien-3β-ol, have recently been reported in the literature as being promising agents for the treatment of prostate cancer[9] This compound has been shown to be a potent inhibitor of P-450$_{17\alpha}$ ($IC_{50}=4$ μM against 17α-OHase; $IC_{50}=2.9$ μM against 17,20-lyase) (for comparison, ketoconazole under the same conditions possessed activities of $IC_{50}=65$ μM against 17α-OHase and $IC_{50}=26$ μM against 17,20-lyase).

The basis of our novel approach in the study of reversible steroidal inhibitors utilises an idea which has now been widely accepted as the proposed mode of action of non-steroidal reversible inhibitors of AR. That is, in the inhibition process by hetero atom (for example nitrogen or sulfur) containing compounds, the initial step is thought to involve formation of the bond between the heme iron and hetero atom of the inhibitor.[14] A similar sequence of events is thought to occur in the inhibition of 17α-OHase. Therefore, we attempted to mimic the inhibition process by directly binding the low energy conformer(s) of the reversible steroidal inhibitors to the iron atom of the heme of the 17α-OHase substrate–heme complex. We postulate that after this initial interaction, those inhibitors containing polar groups then 'search' (due to the free rotation of the iron to inhibitor hetero atom bond) for appropriate groups at the enzyme active site with which to interact. It is our postulate that this search leads most inhibitors to the group responsible for hydrogen bonding to the steroid C(3) carbonyl of the steroidal backbone in the 17α-OHase substrate–heme complex, i.e. polar–polar interaction occurs between the active site and the inhibitor, stabilising the inhibitor–enzyme complex. We therefore postulate that the distance between the inhibitor polar group and the steroid C(3) carbonyl group (within the substrate–heme complex) is inversely related to the strength of the polar–polar interaction and therefore gives an approximate indication of the potency of the inhibitor (Table 1). We also suggest that whilst this distance may determine the inhibitory activity of these inhibitors, other factors such as log *P* (the log of the partition coefficient, *P*, between octanol and water) also play an important part in determining the overall activity, the exact number and nature of these factors is still however unclear.

Thus, the iron to inhibitor hetero atom bond was rotated to find the minimum progesterone C(3) carbonyl to inhibitor carbonyl mimicking group distances. It is unlikely that hydrogen bonding groups exist which bind the C(20) carbonyl of progesterone, or pregnenolone, within the active site since such binding groups would be required to be situated directly above, and close to, the porphyrin. Thus, we hypothesise that the binding of the substrate of 17α-OHase to its active site involves only the steroidal C(3) hydrogen bonding group.

Table 1. Inhibitory activity of three steroidal inhibitors against 17α-OHase and C(3)=O to inhibitor polar group distance

Inhibitor	IC_{50}/μM	Steroid C(3)=O to inhibitor polar group distance (Å)
17-(3-Pyridyl) androsta-5,16-dien-3-βol	4	3.6
17-(2-Pyridyl) androsta-5,16-dien-3-βol	270	4.8
17-(4-Pyridyl) androsta-5,16-dien-3-βol	4000	5.5

Binding 17-(3-pyridyl) androsta-5,16-dien-3β-ol to the substrate–heme complex and rotating the iron to pyridine nitrogen bond, we observe that the compound is able to match the orientation of the substrate (Fig. 5), but possesses a minimum substrate C(3)=O to inhibitor C(3)−OH distance of 3.6 Å, larger than would be expected. Also, the inhibitor appears to be bound in such a way that steric interaction occurs between the active site wall (i.e. it appears to be larger than the substrate), results which appeared initially to disfavour our novel methodology. However, in a similar study involving the binding of this inhibitor to the substrate–heme complex representing the 17,20-lyase moiety, a much better overlap of the inhibitor onto the substrate was observed and as a result, a smaller substrate C(3)=O to inhibitor C(3)−OH distance was seen (< 1 Å).[15] This is consistent with the greater inhibitory activity observed against 17,20-lyase than 17α-OHase.

When we considered analogues of 17-(3-pyridyl) androsta-5,16-dien-3β-ol, i.e. 17-(2-pyridyl) androsta-5,16-dien-3β-ol) ($IC_{50}=270$ μM against 17α-OHase) and 17-(4-pyridyl) androsta-5,16-dien-3β-ol) ($IC_{50}=4000$ μM against 17α-OHase), we observe that the minimum substrate C(3)=O to inhibitor C(3)−OH distance increased for these compounds (e.g. 4.8 Å for 17-(4′-pyridyl) androsta-5,16-dien-3β-ol (Fig. 6) and 5.5 Å for 17-(2′-pyridyl) androsta-5,16-dien-3β-ol] (Table 1)). We hypothesise that the inability of these compounds to effectively utilise the hydrogen bonding site results in the lowering of their activity compared to 17-(3′-pyridyl) androsta-5,16-dien-3β-ol (the log *P* for these compounds being similar). It is believed that substitution of the pyridyl ring at the 2- or 4-position causes these compounds to adopt an orientation such that the C(3) polar group is positioned away from the substrate's C(3)=O (as can be observed in Fig. 6), thereby decreasing the hydrogen bond strength, resulting in reduced binding and therefore lowered inhibitory activity. The reduced hydrogen bonding hypothesis is further supported by the consideration of other derivatives of the 3-pyridyl compounds which do not possess C(3)=O or C(3)−OH groups and/or hydrogen bonding groups which do not closely approach the C(3)=O of the substrate–heme complex—some of these compounds are found to possess IC_{50}'s > 10,000 μM. That these compounds possess any inhibition is probably due to their high log *P*, as we shall observe later, log *P* appears to play a major role in *P*-450$_{17\alpha}$ inhibition by non-steroidal inhibitors.

Reversible non-steroidal P-450$_{17\alpha}$ inhibitors

Several workers have previously reported studies of inhibitors of 17α-OHase[2] and have shown that imidazole based inhibitors such as bifonazole and ketoconazole

Figure 5. 17-(3'-Pyridyl) androsta-5,16-dien-3β-ol bound to 17α-OHase complex.

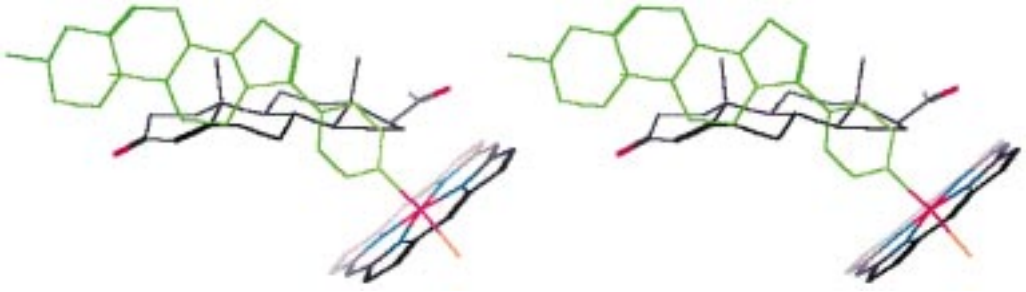

Figure 6. 17-(4'pyridyl) androsta-5,16-dien-3β-ol bound to 17α-OHase substrate–heme complex.

possess potent activity. Miconazole and econazole have been shown to be less potent and metronidazole inactive against this enzyme. In more recent work, compounds based on pyridyl acetic acid[16] and phenyl alkyl imidazole[17] have also been proposed as inhibitors of 17α-OHase. In previous molecular modelling studies of these inhibitors, the compounds were superimposed onto the substrate, progesterone, and the C(17) to inhibitor heme liganding hetero atom distance considered.[4] It is our hypothesis that the sequence of events which occurs with the hetero atom-containing reversible inhibitors of AR, and with the hetero atom-containing reversible steroidal inhibitors of 17α-OHase above, is also observed with the reversible non-steroidal inhibitors of 17α-OHase. Thus, the low energy conformer(s) were bonded directly to the iron atom of the heme and the iron to inhibitor hetero atom bond rotated to find the minimum progesterone C(3) carbonyl group to inhibitor carbonyl mimicking group distances.

In compounds devoid of polar groups such as bifonazole, we hypothesise that a log *P* factor plays a major part in determining the overall inhibitory activity of inhibitors of 17α-OHase. Therefore, we postulate that there are only two main structural features at the active site which are able to interact with the inhibitors (and substrates): (i) the porphyrin ring, and (ii) a hydrogen bonding group which normally binds the steroid C(3)=O (progesterone) or C(3)-OH (pregnenolone) groups. Thus, from the results of the present study, there appears to be no requirement for the inhibitors to precisely mimic the substrate backbone and superimposition of inhibitors onto the substrate, as carried out previously,[4] would therefore appear to be misleading.

By binding bifonazole to the complex and rotating the iron–imidazole bond (Fig. 7), we observe that the binding of the low energy conformer appears to be such that it is able to occupy the same area as the steroid backbone, thereby avoiding any unfavourable interactions with the remainder of the active site. The lack of polar groups corresponding to the progesterone C(3) carbonyl bond results in bifonazole being unable to undergo polar–polar interaction with the active site, and it would thus be expected that bifonazole would possess a lower inhibitory activity compared to inhibitors containing such polar groups. However, as previously mentioned, the log *P* of bifonazole (4.77, compared to the log *P* of progesterone of 3.87) would appear to compensate for the lack of favourable polar–polar interaction. As can be observed in Figure 7, with bifonazole, whilst the biphenyl moiety appears to occupy the same space as the steroid backbone, the remaining phenyl ring is directed away from the porphyrin, resulting in an overall approximate 'L' shape. It can be concluded from the potent inhibitory activity of bifonazole that this area of the active site (occupied by the single phenyl ring) is devoid of any structural features—the presence of any part of the active site about this position would be expected to lead to a lowering of the inhibitory potency due to steric factors. In an earlier report[6] it was suggested that the active site of P-450$_{17\alpha}$ is an approximate 'L' shape. Our initial study of the 17α-OHase component, using a different technique, would appear to support this observation.

In general, with the antimycotic-type compounds, containing groups capable of mimicking the steroid C(3) polar group, interaction appears to be possible between the polar residue of the active site (which would hydrogen bond to the C(3) carbonyl of the steroid substrate) and polar groups of the inhibitors, i.e. two favourable interactions are postulated: (i) inhibitor hetero atom to heme bond formation, and (ii) inhibitor polar group to active site polar group interaction. Both of these interactions help to stabilise the enzyme–inhibitor complex,

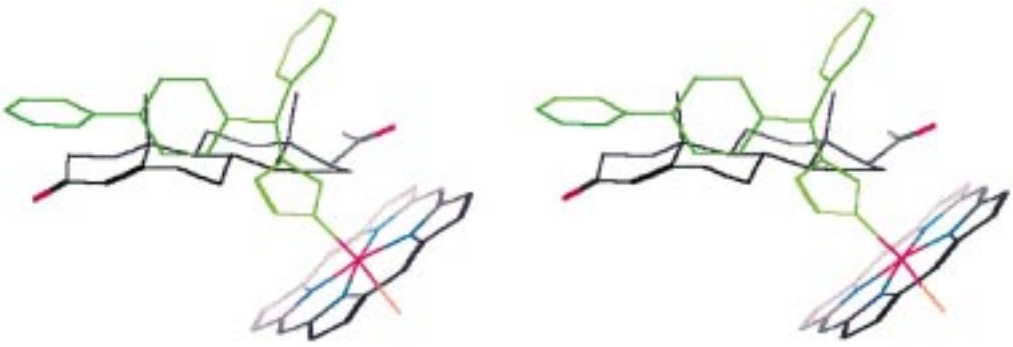

Figure 7. Bifonazole bound to the 17α-OHase substrate–heme complex.

a loss (or ineffective interaction) of either may therefore be expected to lower the inhibitory activity.

Using the substrate–heme complex we are able to suggest reasons for the observed difference in inhibitory activity between the four diastereoisomers of ketoconazole[17]—results that have been left unanswered. It has been shown that of the four diastereoisomers, the 2*S*,4*R* is the most active, the 2*R*,4*S* the least active, whilst the remaining two diastereoisomers are found to possess intermediate inhibitory activity. From the present study we hypothesise that the binding profile of the 2*S*,4*R* enantiomer allows the low energy conformer to fit within the active site such that it can utilise the two important interactions outlined above without undergoing unfavourable conformational change or steric interactions, similar to that seen for bifonazole, i.e. the approximate 'L' shape is adopted (Fig. 8), resulting in a substrate–heme complex C(3)=O to 4-chlorophenyl distance of 0.5 Å (Table 2). The low energy conformer of the 2*R*,4*S* enantiomer, however, is thought to have to undergo conformational change in order to utilise both favourable interactions, resulting in reduced inhibitory activity. That is, on binding the low energy conformer of 2*R*,4*S* ketoconazole to the substrate–heme complex, we find that the assumed non-hydrogen bonding 'tail' of this compound is positioned such that there are several steric interactions between the inhibitor and the porphyrin. The interactions can be removed through an unfavourable conformational change (Fig. 9) ($\Delta E > 5$ kcal/mol), resulting in a substrate–heme complex C(3)=O to 4-chlorophenyl distance of 1.8 Å. This increase in distance compared to the 2*S*,4*R* enantiomer would result in decreased interaction (i.e. weaker polar–polar interactions) to the active site and therefore lead to a further decrease in inhibitory activity.

Considering the binding of the remaining two diastereoisomers, we see that the 2*S*,4*S* (which is the second most active enantiomer) is able to take up an orientation

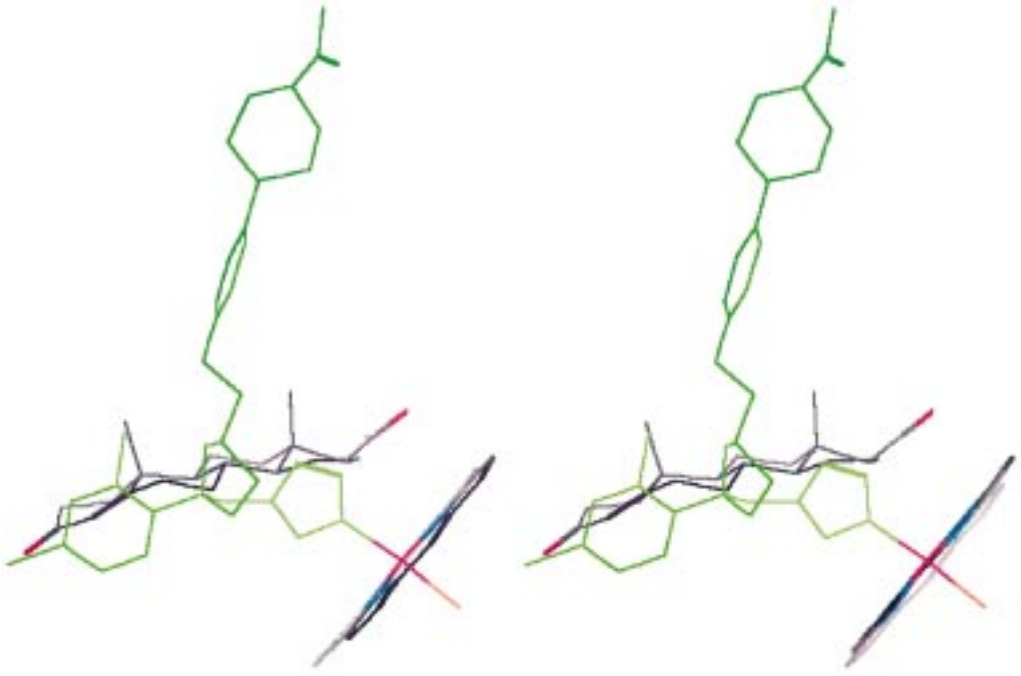

Figure 8. (2*S*,4*R*) Enantiomer of ketoconazole bound to the 17α-OHase substrate–heme complex.

Table 2. Inhibitory activity of the four diastereoisomers of ketoconazole against P-450$_{17\alpha}$ activity and C(3)=O to 4-Cl atom distance (without undergoing any conformational change)

Inhibitor	$\frac{IC_{50}}{\mu M}$	Steroid C(3)=O to inhibitor 4-Cl distance (Å) for 17α-OHase
2*S*, 4*R*	0.05	0.5
2*S*, 4*S*	0.59	0.7
2*R*, 4*R*	2.04	1.8
2*R*, 4*S*	2.38	2.1

similar to the 2*S*,4*R* form, i.e. the 'L' shape, but that a conformational change is required with a $\Delta E \sim 2$ kcal/mol. It is also discovered that a greater substrate–heme complex C(3)=O to 4-chlorophenyl distance is observed for this enantiomer (compared to the 2*S*,4*R* form), both factors therefore explaining its 10-fold decrease in inhibitory activity. The 2*R*,4*R* enantiomer (similar in activity to the 2*R*,4*S*) however, is found to orientate itself such that the 'tail' is directed along the horizontal plane to the steroid backbone and away from the porphyrin, resulting in a similar substrate–heme complex C(3)=O to 4-chlorophenyl distance as the 2*R*,4*S* enantiomer, the conformational change required ($\Delta E > 5$ kcal/mol) and the greater distance thus explaining the greatly lowered activity of this enantiomer. From the consideration of the conformers of the two pairs of diastereoisomers, it would appear that deviation from the 'L' shape results in lowered inhibitory activity.

Considering the binding of miconazole (Fig. 10), or the related antimycotic econazole (which is found to bind in a similar manner to miconazole), it would appear that both of these compounds are able to utilise the polar–polar interaction with the active site as well as iron-imidazole bond formation, similar to that seen for ketoconazole. The lower inhibitory activity possessed by these compounds would therefore appear to be due to the protrudance of the chloro group beyond the C(3)=O of the substrate backbone of the substrate–heme complex, compared to the 2*S*,4*R* enantiomer of ketoconazole. That is, the chloro atom (of the 4-chlorophenyl group) is probably involved in steric interaction with the group at the active site responsible for hydrogen bonding to the C(3) carbonyl group of the substrate. From consideration of the binding of the 2*S*,4*R* ketoconazole, miconazole and econazole, we observe that all three are able to adopt the 'L' shape seen earlier for bifonazole, providing further evidence for the absence of steric factors above the porphyrin system and therefore adds further support to the 'L' shaped active site hypothesis.

Although no enantiomeric data exists for bifonazole, miconazole or econazole with regards to 17α-OHase inhibition, we attempted to consider the effect of each enantiomer. That is, in the above cases, the *R* enantiomer of both miconazole and econazole was utilised, resulting in the remaining (non-hydrogen bonding) phenyl ring being directed away from the porphyrin structure in a vertical plane. The use of the *S* enantiomer resulted in the phenyl ring being directed along the horizontal plane away from the porphyrin ring, similar to the 2*R*,4*R* enantiomer of ketoconazole. We therefore hypothesise that the *R* enantiomer of both miconazole and econazole is the most likely favoured form.

Binding metronidazole to the substrate–heme complex (Fig. 11), we observe that a steric interaction is possible between the hydrogens of the methyl side chain on the C(2) of the imidazole ring and the porphyrin of the enzyme active site. Another, and possibly more important factor, may be the strong electron-withdrawing NO_2 group on the imidazole ring, which is postulated to reduce the availability of the nitrogen lone pair of electrons. We therefore hypothesise that a combination of these two factors, and the low log *P* of metronidazole, are responsible for the lack of inhibitory activity observed with this compound. This is further supported by other compounds containing similar groups on the imidazole ring, such as nimorazole, which is also observed to lack inhibitory activity.[3]

Thus far, we have discussed reversible non-steroidal inhibitors which have been considered in previous studies and for which explanations for their mode of action have been postulated.[3–5] Inhibitors such as aminoglutethimide[18] (AG), however, have been largely ignored since modelling this compound using previous rationale resulted in distances which were considered unlikely. For example, modelling *R*-AG and *S*-AG onto the progesterone A-ring (using the C(3)=O for superimpositioning in a manner similar to that undertaken for similar P-450 enzymes previously.[14] and measuring the steroid C(17) to phenylamine nitrogen distance (the heme liganding group) resulted in distances of 10.81 and 10.05 Å, respectively—such long range interactions between Fe and N lone pair of electrons would clearly

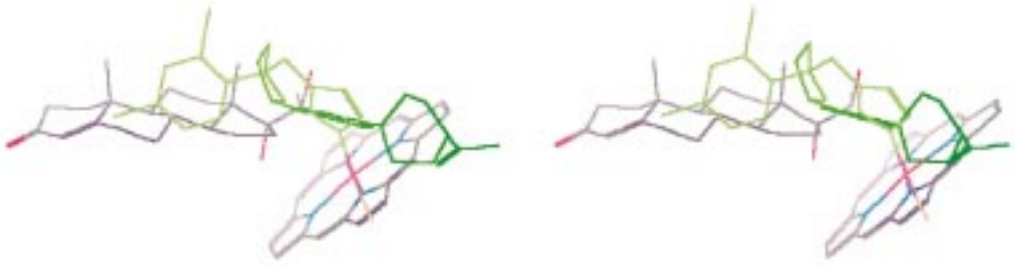

Figure 9. (2*R*,4*S*) Enantiomer of ketoconazole bound to the 17α-OHase substrate–heme complex.

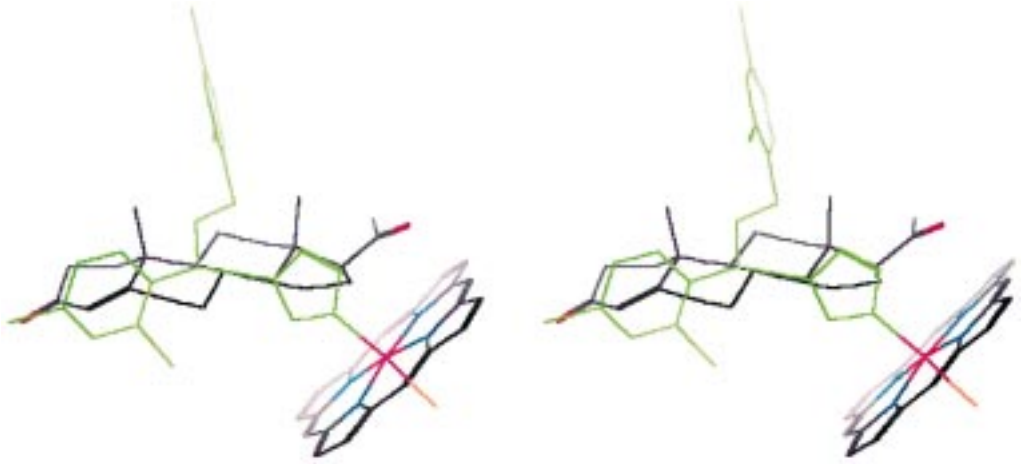

Figure 10. Miconazole bound to the 17α-OHase substrate–heme complex.

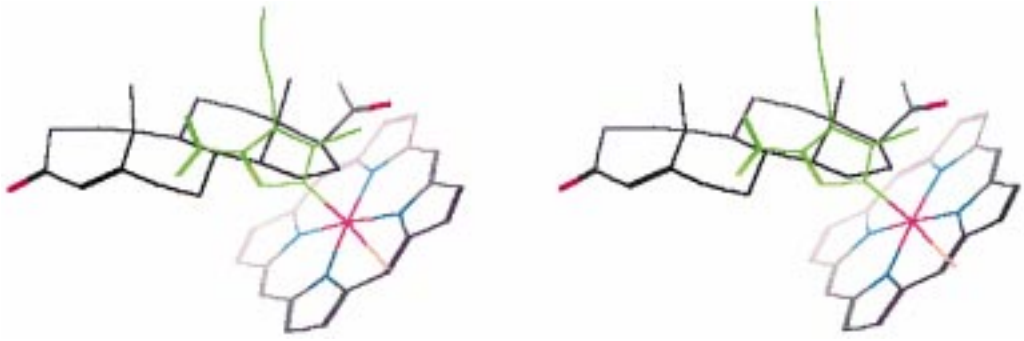

Figure 11. Metronidazole bound to the 17α-OHase substrate–heme complex.

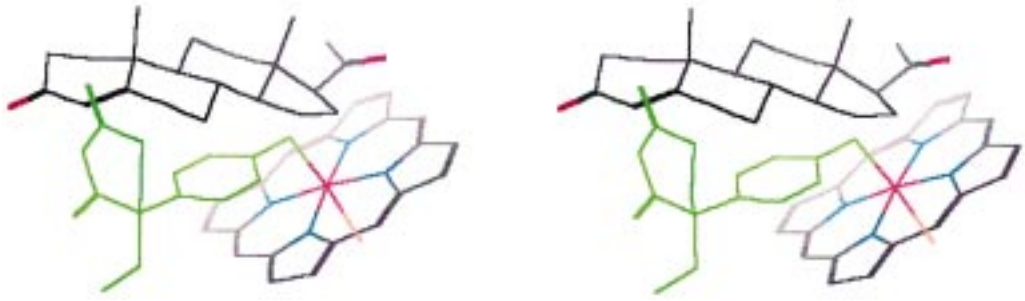

Figure 12. *R*-AG bound to the 17α-OHase substrate–heme complex.

not be expected. Using the substrate–heme complex, we believe we are now in a position to suggest a possible mode of action for the inhibition of 17α-OHase by AG. On binding *R*-AG to the complex (Fig. 12), we discovered that the carbonyl groups of the piperidine-2,6-dione ring are able to approach the C(3)=O of the complex, resulting in a minimum substrate–heme complex C(3)=O to piperidine-2,6-dione carbonyl distance of 2.4 Å (2.2 Å with *S*-AG). This relatively large distance, as well as the use of a weak ligand (phenylamine) to bind to the heme and a low log *P* (calculated log $P = 0.67$), would therefore account for the very poor inhibitory activity observed with AG. To our knowledge, this is the first report of the mode of action of AG within the P-$450_{17\alpha}$ enzyme system and would suggest that, as we have previously shown with modelling of AG on AR, the exact mimicking of the substrate backbone is not an essential requirement for the modelling of inhibitors.

Conclusion

To conclude, the present structure–activity relationship determination study using the novel substrate–heme complex approach has allowed us to successfully consider

and mimic the inhibition process taking place within the 17α-OHase moiety of the active site of P-450$_{17\alpha}$ and also to study the probable mode of action of some reversible steroidal and non-steroidal inhibitors (and some of their enantiomers). From the results of the study, we have been able to rationalise the differences in inhibitory activity between: a series of reversible steroidal inhibitors; a number of imidazole based inhibitors; and, the diastereoisomers of ketoconazole. Furthermore, using this new approach we have suggested reasons for the inhibitory activity of compounds such as AG. To our knowledge, the rationalisation of the inhibitory activity of such different types of inhibitors (and in particular their enantiomers) for 17α-OHase have not been reported elsewhere. The results of this study, and that of the related P-450 enzyme aromatase, adds further support to the novel substrate–heme complex approach developed within our laboratories and to the hypothesis that the cytochrome P-450 family of enzymes possess a single mechanism of action. However, the 'true' picture of the active site still awaits the crystal structure of P-450$_{17\alpha}$.

References and Notes

1. Labrie, F.; Belanger, A.; Simard, J.; Labrie, C.; Dupont, A. *Cancer suppl.* **1993**, *71*, 1059.
2. Ayub, M.; Levell, M. J. *J. Steroid Biochem.* **1987**, *28*, 521.
3. Ahmed, S. Ph.D. thesis, University of Wales College Cardiff, Dec. 1990.
4. Ahmed, S. *Drug Des. Dis.* **1994**, *12*, 77.
5. Laughton, C. A.; Neidle, S. J. *Med. Chem.* **1990**, *33*, 3055.
6. Laughton, C. A.; Neidle, S.; Zvelebil, M. J. J. M.; Sternberg, M. J. E. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 1160.
7. Ahmed, S.; Davis, P. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1673.
8. Ahmed, S.; Davis, P. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2789.
9. Potter, G. A.; Barrie, S. E.; Jarman, M.; Rowlands, M. G. *J. Med. Chem.* **1995**, *38*, 2463.
10. Alchemy III™ molecular modelling package, Tripos Associates Inc., 1699 South Hanley Road, Suite 303, St. Louis, Missouri 63144, USA.
11. Powersearch™ conformational analysis program, Tripos Associates Inc., 1699 South Hanley Road, Suite 303, St. Louis, Missouri 63144, USA.
12. Akhtar, M.; Corina, D. L.; Miller, S. L.; Shyadehi, A. Z.; Wright, J. N. *J. Chem. Soc. Perkin Trans. 1* **1994**, *3*, 263.
13. Swart, P.; Swart, A. C.; Waterman, M. R.; Estabrook, R. W.; Mason, J. I. *J. Clin. Endocrin. Metab.* **1993**, *77*, 98.
14. Banting, L.; Smith, H. J.; James, M.; Jones, G.; Nazareth, W.; Nicholls, P. J.; Hewlins, M. J. E.; Rowlands, M. G. *J. Enz. Inhib.* **1988**, *2*, 215.
15. Ahmed, S. *Biophys. Biochem. Res. Comm.*, in press.
16. McCague, R.; Rowlands, M. G.; Barrie, S. E.; Houghton, J. *J. Med. Chem.* **1990**, *33*, 3050.
17. Rotstein, D. M.; Kertesz, D. J.; Walker, K. A. M.; Swinney, D. C. *J. Med. Chem.* **1992**, *35*, 2818.
18. Pearce, S.; Cariuk, P.; Smith, J. H.; Nicholls, P. J. Presented at the British Pharmaceutical Conference, Cardiff, September 1990.