Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 23, pp. 2795-2800, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00488-2

MOLECULAR MODELLING OF INHIBITORS OF 17α-HYDROXYLASE - A NOVEL APPROACH.

Sabbir Ahmed

Department of Pharmacy, University of Brighton, Cockcroft Building, Lewes Road, Brighton BN2 4GJ, UK.

ABSTRACT A novel molecular modelling study, involving inhibitors bound to a 'substrate-heme complex', is described for the binding of non-steroidal imidazole based inhibitors of the 17α-Hydroxylase (17α-OHase) component of the enzyme complex 17α-Hydroxylase/17,20-Lyase to gain insight into the active site of the overall enzyme.

In the treatment of hormone dependent cancers such as prostate cancer, inhibitors of the enzyme complex 17α-Hydroxylase/17,20-Lyase have been shown to have a beneficial rôle, for example Ketoconazole until recently was considered a hopeful candidate in the treatment of prostate cancer. In the absence of the crystal structure of the overall enzyme complex, we have sought to elucidate the probable positioning of the P-450 heme of the individual components of 17α-Hydroxylase/17,20-Lyase with respect to the substrate backbone (progesterone and/or pregnenolone). We have recently used a similar approach to consider the positioning of the heme within another P-450 enzyme, Aromatase (AR), and have produced a 'substrate-heme complex' as a representation of the AR active site[1], using which, we have successfully discussed the mode of action of several AR inhibitors (both steroidal and non-steroidal).

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. On the basis that the mechanism of 17α-OHase hydroxylation is similar to that of AR[2,3], 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 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 OH, resulting in the hydroxylation of the C(17) position of progesterone and the reformation of Fe^{III}. From a review of the literature, we discovered that 17α-OHase is also able to carry out the 16α-hydroxylation of progesterone[4] and we thus concluded that the Fe-O• radical must closely approach both C(17) and C(16) positions. We therefore constructed the appropriate structures (Figure 1) using Alchemy III[5] molecular modelling program and placed the Fe-O bond at an appropriate bond length and angle below the steroidal plane, in particular the C(17) and C(16) positions. This then resulted in the 17α-OHase 'substrate-heme complex' (Figure 2) which was subsequently utilised in the study of non-steroidal inhibitors.

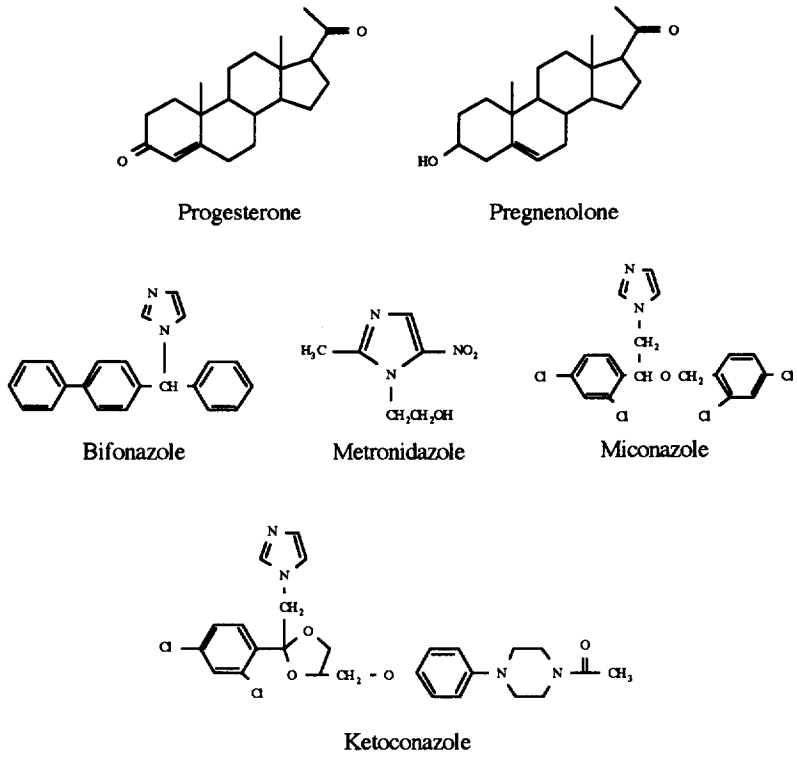

Figure 1. Substrates of 17α-OHase and some imidazole based inhibitors.

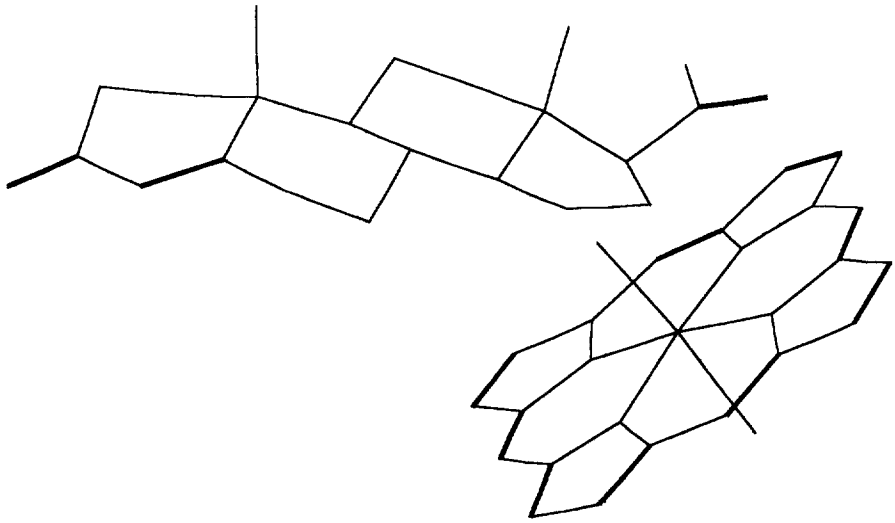

Figure 2. 17α-OHase substrate-heme complex.

Several workers have previously reported studies of inhibitors of 17α-OHase[6] and have shown that imidazole based inhibitors such as Bifonazole and Ketoconazole were potent, Miconazole and Econazole less potent and Metronidazole inactive against this enzyme.

In previous molecular modelling studies of 17α-Hydroxylase/17,20-Lyase, the inhibitors as shown in Figure 1, were fitted onto the substrate progesterone and the C(17) to inhibitor heme liganding hetero atom distance considered[7]. The basis of our novel approach utilises an idea which has now been widely accepted for AR. That is, in the inhibition process by hetero atom-(for example nitrogen or sulfur) containing compounds, the initial step is thought to involve the Fe to inhibitor hetero atom bond formation[8]. We postulate that this is also the case for 17α-OHase and that after this initial interaction, the inhibitors, containing polar groups capable of mimicking the steroidal C(3) carbonyl group, then search [due to the free rotation of the Fe to inhibitor hetero atom bond] for appropriate group(s) at the enzyme active site with which to interact. It is also our hypothesis that this search leads most inhibitors to the group responsible for H-bonding to the steroid C(3) carbonyl group of the steroidal backbone in the 17α-OHase substrate-heme complex. The use of hydrogen bonding groups which may be expected to bind the C(20) carbonyl of progesterone, or pregnenolone, within the active site is considered unlikely since the C(20)=O binding group at the active site would be required to be directly above, and close to, the porphyrin. Thus, it is our hypothesis that the binding of the substrate to the active site only involves the steroidal C(3) hydrogen bonding group.

We therefore carried out conformational analysis of the inhibitors above (Figure 1) using Powersearch[9] and attempted to mimic the inhibition process by binding the low energy conformer(s) of the inhibitors onto the 17α-OHase substrate-heme complex, i.e. the conformer(s) were bonded directly to the Fe of the heme and the Fe to inhibitor hetero atom bond rotated to find the minimum progesterone C(3) carbonyl to inhibitor carbonyl mimicking group distances - we postulate that this distance gives an indication of the strength of the interaction and therefore an approximate indication of the potency of the inhibitor. In compounds devoid of polar groups, we hypothesis that a logP (octanol/water partition coefficient) factor probably plays a part in determining the overall inhibitory activity of inhibitors of 17α-OHase. Therefore, we presume that there are only two main structural features at the active site which interact with the inhibitors and substrates: i) the porphyrin ring, and; ii) hydrogen bonding group which normally binds the steroid C(3) group. Thus, in modelling studies there is no requirement for the inhibitors to precisely mimic the substrate backbone and superimposition of inhibitors onto the substrate would appear to be misleading.

Binding Bifonazole to the complex and rotating the Fe-imidazole bond (Figure 3), we observe that the low energy conformer appears to be 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 group(s) corresponding to the progesterone C(3) carbonyl results in Bifonazole not undergoing hydrogen bonding with the active site. As a result, Bifonazole may be expected to possess a lower inhibitory activity compared to inhibitors containing such polar groups, but, as mentioned above, the logP of Bifonazole would appear to compensate for the lack of favourable polar-polar interaction.

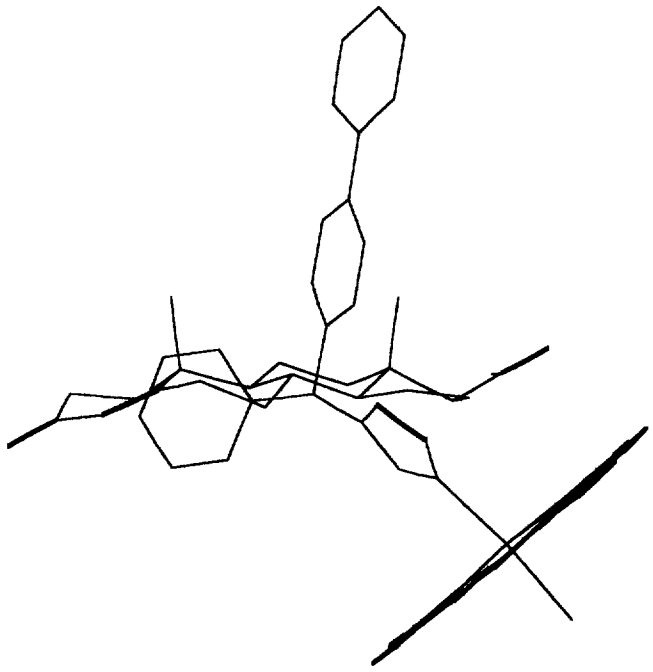

Figure 3. Bifonazole bound to the 17α-OHase substrate-heme complex.

With Ketoconazole and Miconazole (Figures 4 and 5), polar-polar 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 the 4-chlorophenyl part of the inhibitors, i.e. with these types of inhibitors two favourable interactions exist : 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, a loss of either may be expected to lower the inhibitory activity, but we postulate that a large logP may help to overcome the reduction in the polar-polar interaction, similar to that seen earlier for Bifonazole. Using the substrate-heme complex we are able to suggest reasons for the observed difference in inhibitory activity between the four enantiomers of Ketoconazole[10]. It has been shown that of the four, the 2*S*,4*R* is the most active enantiomer whilst the 2*R*,4*S* is the least active. From the present study we hypothesise that the binding profile of the 2*S*,4*R* enantiomer probably allows the low energy conformer of this enantiomer to fit within the active site such that it can utilise the two important interactions outlined above without major conformational change (ΔE=1.2kcal/mol between lowest energy and binding conformer). The 2*R*,4*S* enantiomer on the other hand is thought to undergo conformational change (ΔE=5.7kcal/mol) so as to utilise the favourable interactions resulting in reduced inhibitory activity. The lower inhibitory activity possessed by Miconazole (whilst possessing a larger logP than Ketoconazole) would appear to be due to the protrusion of the chlorine 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, i.e the chlorine 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. Inhibition data for the enantiomers of Bifonazole or Miconazole are not available.

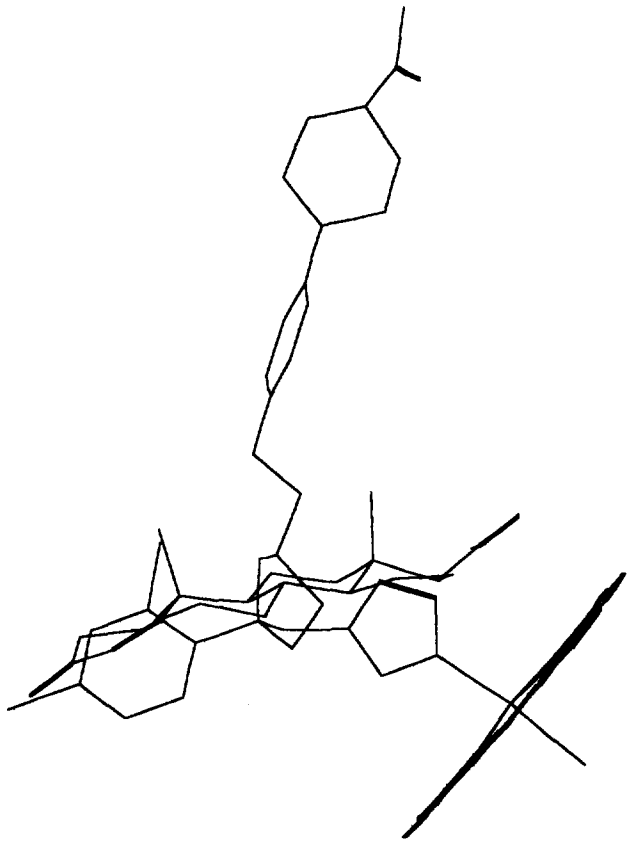

Figure 4. (2*S*,4*R*) Ketoconazole bound to the 17α-OHase substrate-heme complex.

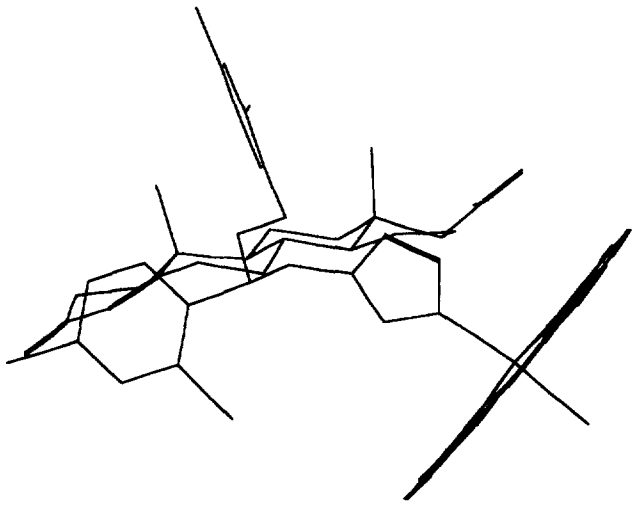

Figure 5. Miconazole bound to the 17α-OHase substrate-heme complex.

Binding Metronidazole to the substrate-heme complex (Figure 6), we observe that a steric interaction may occur between the hydrogens of the methyl side chain on C(2) of the imidazole ring and the porphyrin of the enzyme active site. Another more important factor may be the strong electron withdrawing group (NO_2) on the imidazole ring, which is postulated to reduce the availability of the lone pair of electrons on the nitrogen undergoing co-ordination with the heme. We therefore hypothesise that these two factors are responsible for the lack of inhibitory activity observed with this compound, this is further supported by the observation that other compounds containing similar groups on the imidazole ring (such as Nimorazole) are also observed to lack inhibitory activity[6].

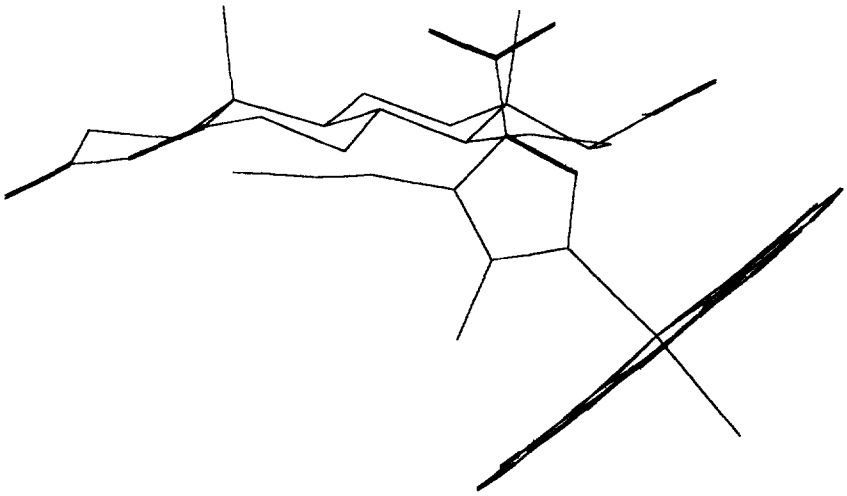

Figure 6. Metronidazole bound to the 17α-OHase substrate-heme complex.

In conclusion, the present study has allowed us to consider and mimic the inhibition process taking place within the 17α-OHase active site and study the probable mode of action of some imidazole based non-steroidal inhibitors and their enantiomers.

REFERENCES

1. Ahmed, S. and Davis, P. J., *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1673-1678.
2. Wright, J. N. and Akhtar, M., *Steroids*, **1990**, *55*, 142-151.
3. Akhtar, M., Wright, J. N., Shyadehi, A. Z., and Robichaud, P, *P. App. Chem.*, **1994**, 66, 2387-2390.
4. Swart, P., Swart, A. C., Waterman, M. R., Estabrook, R. W. and Mason, J. I., *J. Clin. Endocrin. Metab.*, **1993**, *77*, 1, 98-102.
5. Alchemy III™ molecular modelling package, Tripos Associates Inc., 1699 South Hanley Road, Suite 303, St. Louis, Missouri 63144, USA.
6. Ayub, M. and Levell, M. J., *J. Steroid Biochem.*, **1987**, *28*, 5, 521-531.
7. Ahmed, S., *Drug Des. Dis.*, **1994**, *12*, 77-88.
8. 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*, 215-229.
9. Powersearch™ conformational analysis program, Tripos Associates Inc., St. Louis, USA.
10. Rotstein, D. M., Kertesz, D. J., Walker, K. A. M., Swinney, D. C., *J. Med. Chem.*, **1992**, *35*, 2818-2825.

(*Received in Belgium* 17 *July* 1995; *accepted* 15 *October* 1995)