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MOLECULAR MODELLING OF INHIBITORS OF AROMATASE - A NOVEL APPROACH

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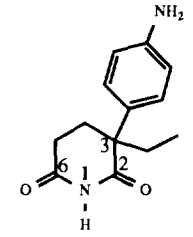
Abstract : A novel molecular modelling study, involving inhibitor(s) bound to the iron of a substrate-heme complex, is described for non-steroidal inhibitors of Aromatase (AR). Study of aminoglutethimide (AG) suggests that it may mimic the steroid C(17) and not the C(3) carbonyl group. Possible reasons for difference in activity of enantiomers of AG, 3-(4'-pyridyl)-3-ethyl piperidine-2,6-dione and 10-thiiranylestr-4-ene-3,17-dione are also suggested.

In the absence of the crystal structure of Aromatase (AR), several workers have previously described models for the action of inhibitors of AR. Banting et al[1] were the first to produce a model of the AR active site from the consideration of several non-steroidal inhibitors modelled onto the AR substrates androstene-3,17-dione (AD) and testosterone. Although useful at the time, this model soon became redundant as it could not explain or predict some potent inhibitory activity obtained by some compounds, such as those studied by Ahmed[2].

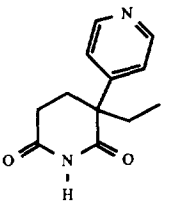
Furet et al have attempted to determine the relative binding of azole-type inhibitors, such as CGS16949A[3] (Figure 1), with respect to the reversible steroidal inhibitor (19*R*)-10-thiiranylestr-4-ene-3,17-dione, utilising previously reported iron-ligand crystallographic data. In our approach, we have sought the approximate position of the Fe with respect to the natural substrate, by determining the highest concentration of inhibiting moieties of both reversible and non-reversible steroidal inhibitors, when fitted onto the substrate, since all these compounds would appear to bind to the active site in a similar manner to AD involving hydrogen bond formation with the active site via the steroidal C(3) and C(17) carbonyl groups.

Using the molecular modelling package Alchemy III[4] and the conformational analysis program Powersearch[4], we constructed potent steroidal inhibitors [for example (19*R*,*S*)-10-thiiranylestr-4-ene-3,17-dione, 10β-propargylestr-4-ene-3,17-dione, 10β-vinylestr-4-ene-3,17-dione, (19*R*,*S*)-10-oxiranylestr-4-ene-3,17-dione and 10β-aziridinylestr-4-ene-3,17-dione] and carried out conformational analysis on the flexible parts of the inhibitors. The low energy conformer(s) of the steroidal inhibitors were then fitted onto AD, and each other, by specification of four points, utilising both the C(3) and C(17) C=O groups. From these fittings, we approximated the position of the heme to be about the C(1) of the steroidal backbone. To determine the position of the heme above the steroidal plane, we considered the present hypotheses on the mechanism of hydroxylation of the steroid C(19) methyl. On the basis that the mechanism of AR involves a ferroxy radical[5], we hypothesised that the oxygen atom must be positioned within approximate bonding distance [and angle] to the C(19). We

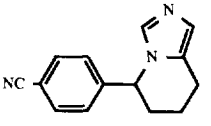
therefore placed the Fe-O bond at the appropriate bond length and angle above the steroid A ring, resulting in the 'substrate-heme complex' (Figure 2), which was utilised in further studies of the non-steroidal inhibitors. To check our approximate position of the iron, we refitted the low energy conformers of the reversible steroidal inhibitors onto the substrate-heme complex and found that our positioning corresponded well, in that the inhibiting moieties of all these inhibitors were found to closely approach the Fe.



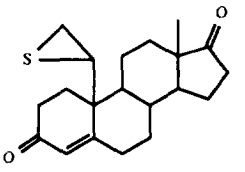
Aminoglutethimide



3-(4'-Pyridyl)-3-ethyl piperidine-2,6-dione



CGS-16949A



(19-*R*,*S*)-10-Thiiranylestr-4-ene-3,17-dione

Figure 1. Inhibitors of Aromatase.

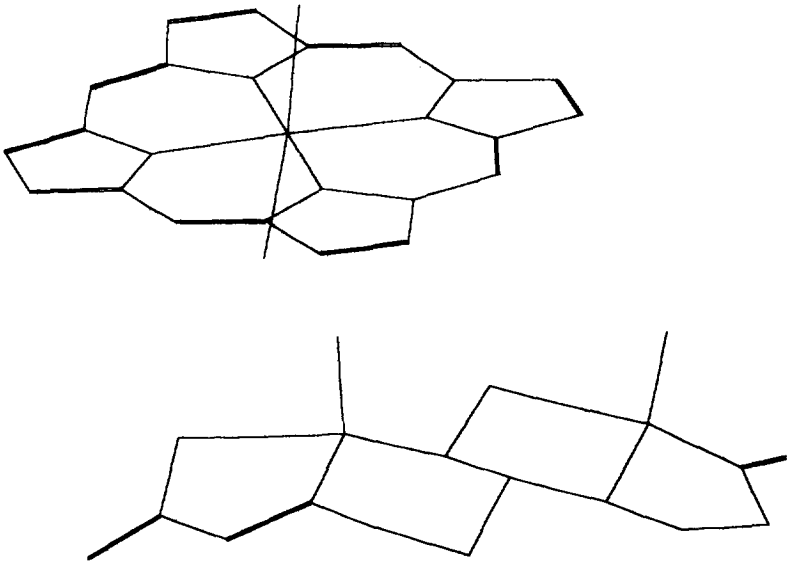


Figure 2. Substrate-heme complex.

In considering the development of a novel approach to the modelling of non-steroidal inhibitors, we reviewed the previous modelling studies and discovered that, although the studies involved modelling compounds onto the steroid backbone, there was an assumption that the initial step in the inhibiting process involved bond formation between the Fe of the heme and the hetero atom lone pair of electrons on the ligand[1]. The basis of our study therefore involves the mimicking of the inhibition process by the hetero atom- (for example N or S) containing non-steroidal inhibitors. Also, we postulate that after this initial interaction, inhibitors, containing groups capable of mimicking the steroidal C(3) or C(17) C=O groups, then 'search' [due to the rotation of the Fe-hetero atom bond] for the appropriate group(s) at the enzyme active site with which to interact. We therefore modelled this process by bonding the low energy conformers of non-steroidal inhibitors directly to the Fe of the heme, resulting in an inhibitor-substrate-heme complex, an approach which differs from that of Furet et al who fitted inhibitors to a model representing an imidazole and a thiirane ring complexed to a porphyrin nucleus. The Fe-hetero atom bond was then rotated (to mimic the free rotation of the Fe-inhibitor bond within the AR active site) to find the minimum C(3) and C(17) to inhibitor polar binding group distances. It is our hypothesis that this distance and the overall orientation of the inhibitor within the active site are two factors involved in determining the overall inhibitory activity.

The results obtained with derivatives of CGS16949A agreed with the conclusions of Furet et al. Results obtained with the aminoglutethimide (AG) type inhibitors were, however, quite different to those previously reported[1]. That is, in all previous studies, it had been assumed that the carbonyl groups on AG would mimic the C(3) carbonyl group of the steroidal substrate, whilst the phenylamine ring was directed towards the C(19). In our study, however, we discovered that AG appears to utilise the same hydrogen bonding group at the active site as the steroid C(17) carbonyl (Figure 3). It was found that the C=O groups of the piperidine-2,6-dione ring closely approach the steroidal C(17) carbonyl [closest distance was 1.8Å], whereas the closest distance to the C(3) was 3.6Å. This observation has not, to our knowledge, been previously reported and would appear to contradict previous studies[1,6]. Also, using data from the AG-substrate-heme complex we suggest that the difference in activity between *R*- and *S*-AG is related to their orientation about the steroid backbone. That is, on rotation of the Fe-N bond [so as to obtain the minimum inhibitor C(17)=O mimicking group to steroid C(17)=O distance] the *S* enantiomer is positioned such that the bulk of the molecule is placed beyond the C(11), C(12) and C(17) area - an area where space is presumed to be restricted, an attempt to optimise the steric interaction results in increased steroid C(17) C=O to AG C=O group distance and therefore lower potency. The *R* enantiomer, however, is found to be positioned such that the bulk of the inhibitor overlaps the steroidal backbone, and is therefore able to mimic the steroidal substrate more closely than the *S*. As seen in Figure 3, however, this ability of *R*-AG to mimic the substrate is not ideal and may account for the lesser potency of AG compared to some of the more recently developed inhibitors.

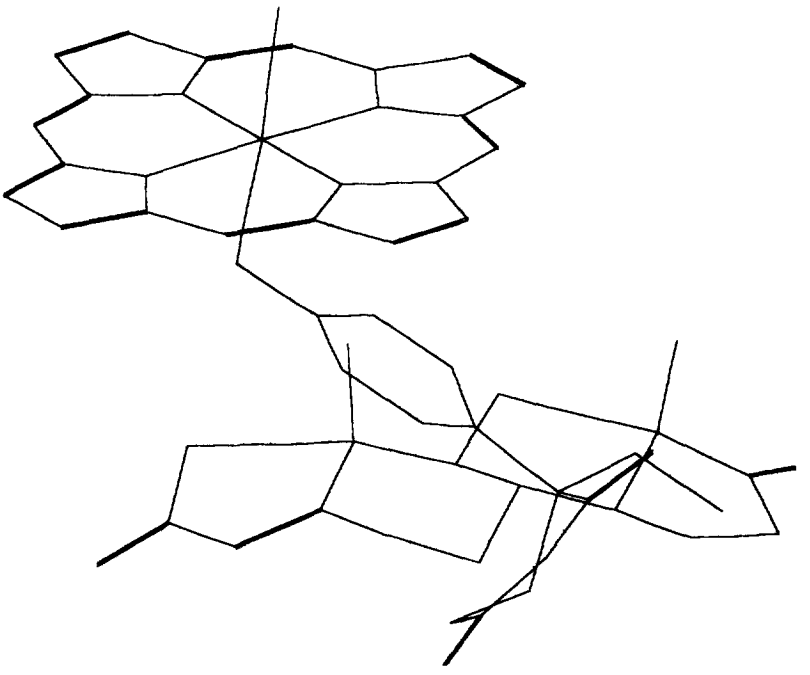


Figure 3. *R*-AG bound to Substrate-heme complex.

When the binding of 3-(4'-pyridyl)-3-ethyl piperidine-2,6-dione (PYG) to the substrate-heme complex was studied, we discovered that the carbonyl groups of PYG approached the C(3) carbonyl group of the steroidal backbone, similar to results obtained by other workers[1,6]. Indeed, the closest distance observed to the C(3) carbonyl was 1.8Å, whereas the closest steroid C(17) carbonyl to PYG carbonyl distance was approximately 4.5Å. By considering the binding of the different enantiomers of PYG, we are also able to put forward a possible explanation for the greater activity of the *R*-enantiomer compared to the *S*-. Binding the enantiomers to the substrate-heme complex, we discovered that the initial orientations of the enantiomers of PYG used in the previous studies[6] appeared to be opposite to ours (Figure 4). From our results, we hypothesise that a steric factor is involved in lowering the activity of the *S*-enantiomer, in that it appears to be positioned such that the bulk of the inhibitor is placed in what is considered to be a restricted area about the C(1) and C(2) region of the steroid backbone.

Using the substrate-heme complex, we also considered the *N*-octyl derivative of PYG and suggest a possible reason for the apparent lack of inhibition and binding of this compound to the AR heme. In their study of the derivatives of PYG, Laughton et al[6] showed that of the *R*- and *S*-enantiomers of the *N*-octyl derivative of PYG, only the *S*-enantiomer possessed

inhibitory activity (IC_{50}=9μM) and suggested that an electronic factor may be involved. We postulate that the octyl substituent on the piperidine-2,6-dione ring results in the *S*-enantiomer taking up an orientation within the active site such that it utilises the C(6)=O [as opposed to the C(2)=O] group of the piperidine-2,6-dione ring, resulting in the octyl chain pointing away from the steroid C(3)=O group. With the *R*-enantiomer however, the octyl chain is found to be directed towards the steroid C(3)=O group, where it is probably involved in an unfavourable steric interaction with the active site 'wall'. Also, it appears that unlike the *S*-enantiomer, the *R*-enantiomer is not able to utilise the alternative C(6)=O of the piperidine-2,6-dione ring since a reorientation about the Fe-N bond would result in the octyl chain being positioned about the C(1) and C(2) of the steroid backbone. As a result, the *R*-enantiomer has been found to be a very poor inhibitor of AR[6].

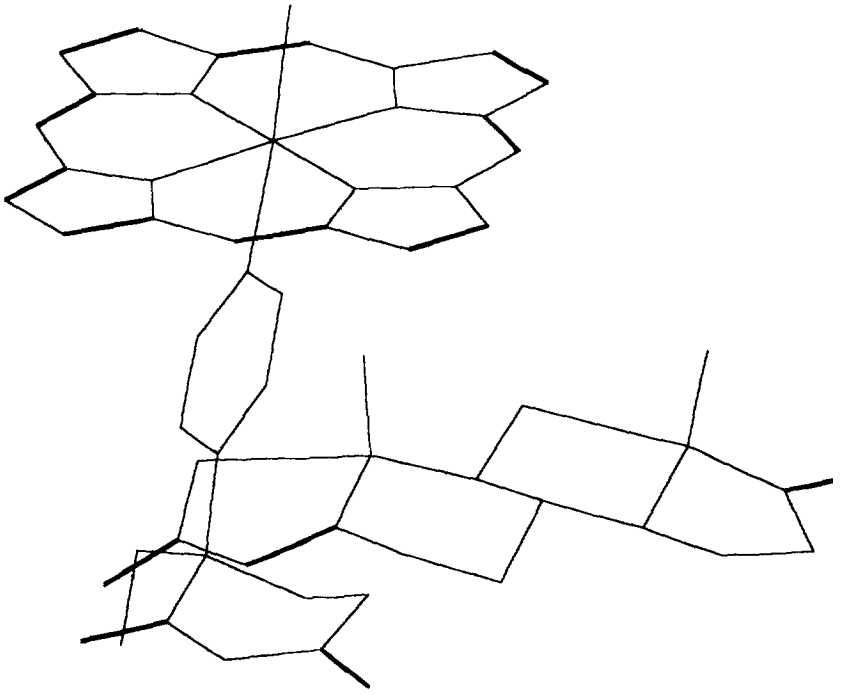


Figure 4. *R*-PYG bound to Substrate-heme complex.

As mentioned earlier, Furet et al utilised (19*R*)-10-thiiranylestr-4-ene-3,17-dione as a representation of the natural substrate in their study of azole type compounds but did not consider the *S* form. The *R*-enantiomer has been shown to be 75 times more potent than the *S*, and by fitting the two forms of this highly potent inhibitor to the substrate-heme complex, we are able to suggest a possible reason for this difference. That is, the *R* enantiomer was found to mimic the substrate androstenedione in an exact manner, resulting in the sulfur atom closely

approaching the Fe, close to the oxygen atom of the ferroxy. When the *S* enantiomer was fitted onto AD of the substrate-heme complex, it was discovered that rotation of its C(10)-C(19) bond was required to position the sulfur correctly below the Fe of the heme, which resulted in an increase in energy of approximately +8Kcal/mol. This relatively large energy difference is believed to be the reason for its lowered inhibitory activity.

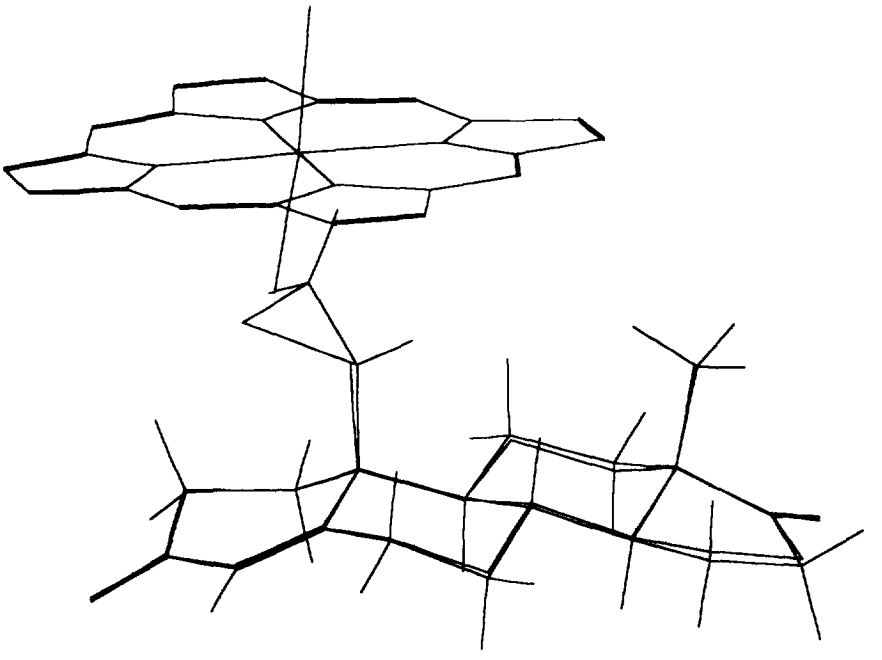


Figure 5. [(19*S*)-10-Thiiranylestr-4-ene-3,17-dione] fitted onto the Substrate-heme complex.

In conclusion, the present approach using the substrate-heme complex and the direct binding of the inhibitors to the complex, allows us to mimic the 'inhibition process' in AR by its inhibitors, and to explain differences in activity of compounds and their enantiomers.

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