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The derivation of a potential transition state for the reduction reaction catalysed by 17β-hydroxysteroid dehydrogenase—an approximate representation of its active site for use in drug design and discovery

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Abstract

In an effort to aid the drug discovery process, the approximate representation of the active site of 17β-hydroxysteroid dehydrogenase (17β-HSD) has been derived from the consideration of the proposed mechanism for the reduction reaction. Using the transition-state (TS), the mode of action of a number of inhibitors has been rationalised. The results of the study suggest that the area of the active site corresponding to the C(17) area of the steroidal backbone is relatively constrained, as such, groups which possess large groups that are flexible in nature may possess a decreased inhibitory activity. The model is therefore a good start point for the development of novel inhibitors of 17β-HSD and is a rapid technique for drug design and development.

Although the crystal structure is known for a number of enzymes, the use of such data often requires time and the use of complex programs. In an attempt to produce an in silico high throughput modelling system, we have considered a number of methods to approximate the active sites of a number of enzymes. We have considered previously enzymes such as 5α-reductase [1] (from the reduction reaction transition-state (TS) point of view) as well as the general P-450 family of enzymes [2–5]. Here, we consider the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD), which is responsible for the stereospecific NADPH-dependent conversion [6] of the C(17)═O group to the reduced β-hydroxy moiety in androgens and estrogens [in particular, the conversion of the weak estrogen, estrone, to the more potent estrogen, estradiol (Fig. 1)]. The reverse reaction, the oxidation of C(17)─OH to the carbonyl group, is undertaken by an isozyme of this enzyme, using $NADP^+$ as the co-factor. 17β-HSD has come under consideration as a potential target in the fight against hormone-dependent breast cancers since it has been shown that estradiol is an important mitogen in the initiation of this disease. Here, we report the initial results of the development of a model (involving the determination of the TS of the reduction reaction mechanism [6]) for the active site of 17β-HSD and the use of the model in the rationalisation of the inhibitory activity of a number of steroidal and flavanone based inhibitors [7].

Experimental

The structures of estrone, NADPH (partial structure was used due to a limitation of the software), and some of the inhibitors of 17β-HSD (Table 1) were all constructed within the CACHE [8] molecular modelling software suite on an Intel microprocessor-based IBM PC compatible microcomputer. Using atoms and available fragments from the structure library for amino acids, the tyrosine, serine, and threonine amino acids (presumed to be present at the active site) were also constructed. The completed structures were then refined performing a pre-optimisation calculation in mechanics using augmented MM2, followed by a geometry optimisation in Mopac [9] using PM3 parameters. In order to determine the TS, we argued that

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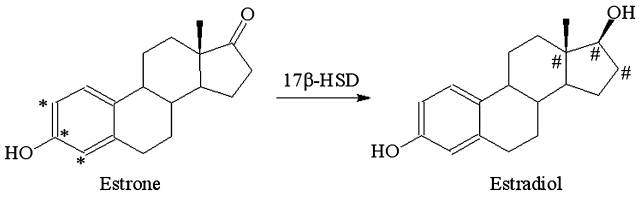
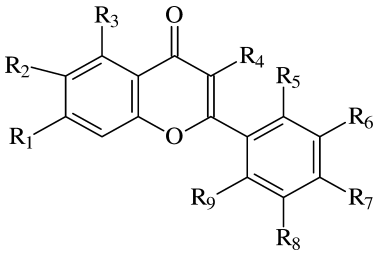


Fig. 1. Conversion of estrone and estradiol.

Table 1
Recently reported inhibitors of 17β-HSD [7]



	Substituents	IC_{50} (μM)
1	$R_2 = OH$	16.4
2	$R_1 = OH$	9.0
3	$R_1 = R_3 = R_7 = OH$	21.3

the NADPH moiety would be required to be close to the C(17)═O moiety and as such, the hydrogen of the pyridine ring of NADPH was attached to the C(17) carbon of estrone, via a weak bond. The remainder of the groups involved in the reduction reaction and the binding of the steroid within the active site were also attached, i.e., the hydrogen bonding carboxyl group to the C(3) hydroxy group, and appropriate amino acid hydrogen donors about the C(17) position of estrone. The initial structure was minimised using the MM2 and Mopac/PM3 routines (reactant file)—it should be noted that partial deletion of the NADPH molecule was required so as to allow calculations within Mopac atom limits. A 'product file' was also created (involving the conversion of the NADPH moiety to $NADP^+$) and the structures were minimised as previously described. Using routines within CACHE, the saddle point for the reaction was calculated. The resulting TS structure was then refined by performing a minimised gradient calculation using Mopac and PM3 parameters [9] following which the molecule's vibrational transitions were calculated (once again with Mopac using PM3 parameters) in order to 'verify' the TS. A negative vibration was observed upon viewing the molecule file (Fig. 3) ($-459.95\,cm^{-1}$).

Conformational analysis of the inhibitors was performed on flexible parts of the inhibitors in order to determine probable low energy conformers (with a sequence of conformers generated by sequentially searching a number of geometry labels, calculations being performed in mechanics using augmented MM2 parameters). In order to obtain a measure of the fit, the inhibitors and the TS were read into Alchemy III [10] molecular modelling software (using Sybyl2 file format) for the superimpositioning study involving the specification of three or more points on both the assumed binding ring of the inhibitor and ring A or ring D of estrone, using the polar groups in the fitting process (each pair of three or more points is highlighted using * or # on the estrone and estradiol backbones, respectively, Fig. 1).

Results and discussion

Transition-state of 17β-HSD

Consideration of the TS shows that the steroid C(17) area of the steroid backbone is seen to be crowded with the hydrogen bond donors as well as the proton donators which play a part in the overall mechanism of the reduction reaction. Furthermore, the NADPH reducing moiety is positioned close to the C(17) position of the steroidal backbone such that the nearest NADPH atom to the steroid is found to be 2.4 Å (Fig. 2). This observation is consistent with experimental data which show that inhibitors containing bulky groups about the C(17) or C(16) positions can result in poorer inhibitory activity [11]. We therefore propose that these larger groups are involved in steric interaction with the NADPH molecule leading to destabilisation of the enzyme–inhibitor complex—Figs. 3A and B show the low energy conformers of the 6- and 7-hydroxyflavone, respectively (the latter was found to possess twice the potency of the former). Consideration of the superimpositioning of the derivatives of the flavones onto the TS shows that in the case of the 6-hydroxyflavone (Fig. 3A), the crowded nature of the active site results in strong steric interactions between the inhibitor and groups which are presumed to exist at the active site of 17β-HSD, thereby reducing the inhibitory activity compared to the 7-hydroxy flavone, which does not appear to be involved in any unfavourable interactions (Fig. 3B).

Using the TS, we also considered a range of steroidal inhibitors of 17β-HSD, in particular, those based on the estradiol backbone [11] (Table 2). The results of the superimpositioning of these compounds onto the TS show a similar trend to those observed with the flavone-based inhibitors. Consideration of inhibitors **5** and **6** (Figs. 4A and B) shows that the R_2 group in these two

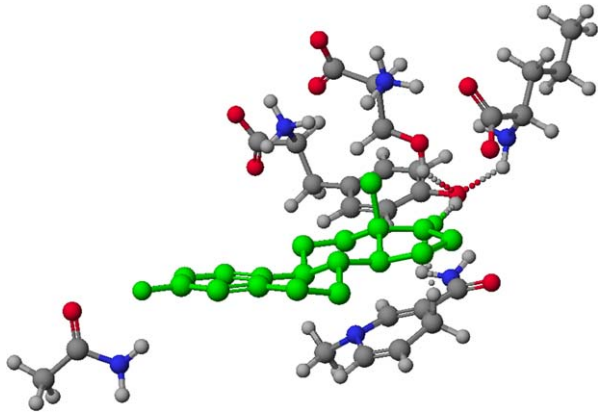


Fig. 2. Showing the derived TS as a representation of the active site of 17β-HSD (with estrone in green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

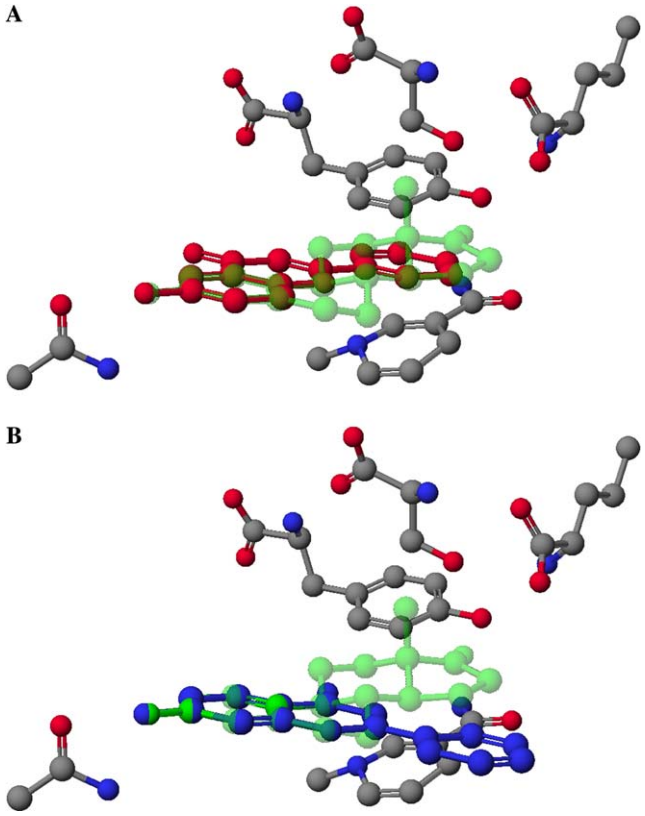


Fig. 3. (A) Partial diagram to show the superimpositioning of the 6-hydroxyflavone (red) onto estrone (shaded) of the derived TS. (B) Partial diagram to show the superimpositioning of the 7-hydroxyflavone (blue) onto estrone (shaded) of the derived TS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

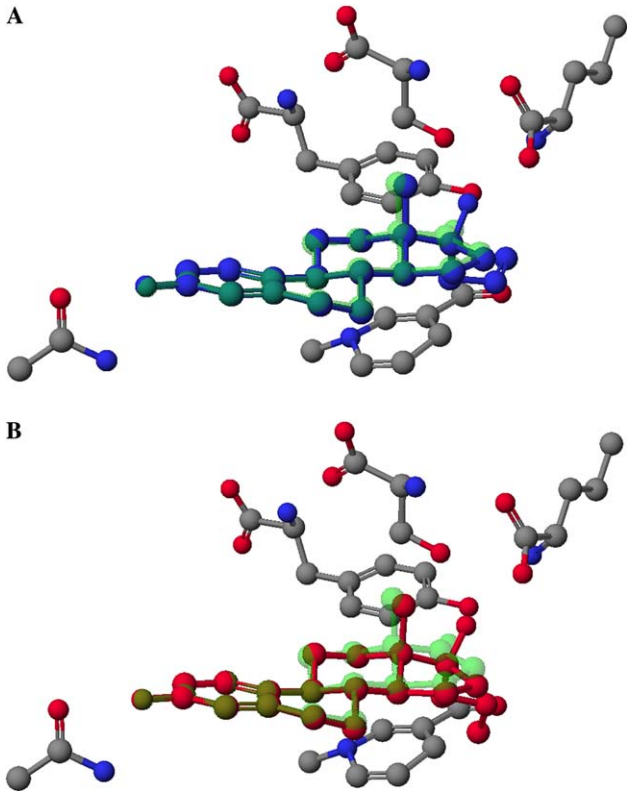
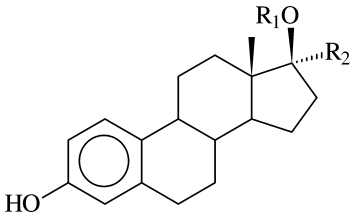


Fig. 4. (A) Partial diagram to show the superimpositioning of the steroidal inhibitors (**5** in blue) onto the derived TS. (B) Partial diagram to show the superimpositioning of the steroidal inhibitors (**6** in red) onto the derived TS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

Table 2
Steroid based inhibitors of 17β-HSD [11] ([I] = 10 μM)



	R_1	R_2	% Inhibitory activity
4	TBDMS	$CH_2CH_2CONBuMe$	0
5	H	$CH_2CH{=}CH_2$	29
6	H	$CH_2CH_2CH_3$	51

compounds is positioned such that it does not result in close interaction with the NADPH moiety. Superimpositioning of inhibitor **4**, however, shows that a strong interaction between the inhibitor and the reducing agent (NADPH) is indeed possible (resulting in close distances between the two molecules being less than 1 Å), therefore explaining the lack of inhibitory activity shown by this compound. This observation therefore adds further support to the conclusions from the superimpositioning of the flavone-based compounds.

The steroidal inhibitors, however, are found to be, in general, weaker inhibitors than the flavones—we believe that this is a result of the highly restricted nature of the C(17) area of the substrate backbone, as a result, the R_1 and R_2 groups within the estrogen-based compounds are involved in steric hindrance with the groups present in this area of the 17β-HSD active site, explaining their reduced inhibitory activity. In particular, compound **4** is found to undergo strong steric interactions with the groups at the active site preventing this compound from entering the active site, and resulting in a complete lack of inhibitory activity. However, as can be observed from Figs. 4A and B, the lack of the ether group and the flexibility of the alkyl groups (propenyl group in **5** and propyl chain in **6**) allow these compounds to possess greatly reduced steric interaction with the active site in comparison to **4** and therefore greater inhibitory activity.

Conclusion

From the results of our study, we conclude that the ability of inhibitors to fit within the crowded active site (or indeed occupy similar area/volume of space to the natural substrate) is the major factor in their inhibitory activity. Furthermore, any interaction with the NADPH, or indeed any other moiety within the active site, results in an unfavourable interaction that further decreases the inhibitory activity. A good inhibitor would therefore be expected to possess reduced steric interactions with the area of the active site corresponding to the steroid C(17) backbone whilst undergoing strong hydrogen bonding interaction with the C(3) area of the active site. Using the derived representation, we have designed novel and potent inhibitors of 17β-HSD (to be reported elsewhere) and believe that it is possible to automate the above so as to produce a system for the de novo design of further novel compounds.

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