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A NEW PROPOSED MODEL OF ALDOSE REDUCTASE ENZYME INHIBITION ON THE BASIS OF AN ARTIFICIAL INTELLIGENCE APPROACH: A COMPUTER AUTOMATED STRUCTURE EVALUATION (CASE) STUDY

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Abstract

A large number of inhibitors of aldose reductase enzyme were submitted to the CASE (computer automated structure evaluation) program in order to ascertain the topological features relevant to activity. On the basis of the twenty-six biophores (activating fragments) and one biophobe (inactivating fragment), a new proposed interaction model was suggested for an aldose reductase enzyme with the chemical inhibitors. The critical relationship between enzyme inhibition and the structure of inhibitors is believed to depend on the relative positions of subordinate regions within the inhibitor structure.

1. Introduction

The artificial intelligence approach has been introduced to the chemical literature as a general tool which can be used by the researcher to reduce masses of experimental data to relevant information [1,2]. The general consideration of the artificial intelligence approach in drug design is to find the relevant factors which describe the biological activity in terms of molecular properties. The evaluation and prediction of the biological effect of chemical substances have been the center of preoccupation of medicinal chemists involved in drug development, as well as those concerned about the effect of chemicals which have been synthesized and tested previously. For the latter, a collection of compounds and the results of biological activity acquired on each compound may be studied in order to predict a property of the compounds to detect the discriminatory possessions or combination of properties between active and inactive molecules. In such a mechanistic study, we are able to draw a topological relationship between the structure of a molecule and its activity.

In recent years, several techniques have emerged that have the potential of helping to solve the problems consisting of the evaluation and prediction of biological activity via molecular properties of compounds. In this manner, the quantitative

structure–activity relationships (QSAR), the pattern recognition methods, discriminant analysis and molecular orbital calculations show great promise in solving this problem [3–6]. The QSAR methods are usually implemented as various regression analysis programs where linear, nonlinear or bilinear correlations have been seen between the observed biological activity of a series of congeneric substances. The parameters usually used in QSAR studies include hydrophobic, electronic and steric properties of the congeneric series in which the resulting observation, in some cases, may lead to correlations that are well enough accounted for to be used in developing new and optimized drugs. However, one of the greatest difficulties of QSAR studies is the selection of relevant properties to be used as descriptors of a molecular series under investigation [7]. In such cases, it is apparent that such a belief had been prompted mainly by the fact that no QSAR existed that was capable of performing qualitative correlations between molecular structure and biological response in a large database. On the other hand, human intelligence should be required to describe what the relation might be in terms of physicochemical properties in the relation of chemicals with biological systems. This is also limited to a qualitative consideration of meaningful descriptors which can be defined in order to reach significant results.

The above stated problem is general indeed, and expert systems can be used to solve problems in several diverse areas of developing new drugs. In the artificial intelligence approach, to a large degree, the success of an application depends on the outcome of intelligent and discriminative analysis of molecular properties that may be important in the mechanism of action of molecules. Therefore, the artificial intelligence required to constuct a computer program will be described in order to demonstrate the molecular capabilities of compounds.

The CASE (computer automated structure evaluation) program is particularly useful when comparing chemical substances showing the same kind of activity, especially if they belong to different chemical groups. However, no furnishings have been made within the program to account for chirality and cis/trans isomerism, although this remains a dynamic area of drug development.

The CASE program has been successfully applied to a number of biologically active compounds, in which attention was devoted to the identification of the most relevant structural features responsible for activity and to an illustration of the reliability of the artificial intelligence process for developing automatically the most prevalent molecular structure descriptors [8–11].

In this study, we wish to report a proposed biochemical interaction of the aldose reductase enzyme with organic molecules, previously obtained from the results of the application of the CASE program [12]. Known inhibitors of the aldose reductase enzyme vary widely in structure. However, few of them display a strong species specificity. These range from dicarboxylic compounds [13] to those containing the benzopyran and benzophenone [14], flavone [15], oxoquinoline [16], quinazoline [17], xanthose [18], naphthalene [18], hydantoin [19–21] and spiro-hydantoin [22] ring systems.

The aldose reductase enzyme, involved in the sorbitol pathway (fig. 1) which is an important mechanism in the regulation of mammalian glucose metabolism, has been found to play a physiologically significant role in the initiation of diabetic complications such as cataract formation, neuropathy and retinopathy [23]. Under

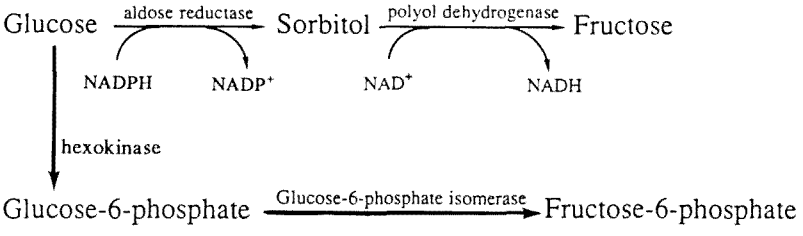

Fig. 1. Sorbitol pathway.

normal glycemic conditions, aldose reductase has very little affinity to glucose [24]. Therefore, glucose is primarily converted by hexokinase into metabolic products. This is not so under diabetic conditions, in which increased concentrations of sorbitol become predominant, leading to tissue damage. Diabetic cataracts are believed to be caused by the reduction product of glucose called sorbitol via the aldose reductase enzyme, which is major under pathophysiologic conditions [24]. Although the exact mechanism of the sorbital pathway is still largely unknown, it is apparent that selectively active aldose reductase inhibitors are able to reduce and prevent the pathological perspective of diabetic complications.

Structure–activity relationships of aldose reductase enzyme inhibitors have been extensively studied [25], and considerable effort has been spent in attempts to develop clinically useful aldose reductase inhibitors. The need for better aldose reductase inhibitor activity in this class makes it desirable to extend our actual knowledge about the structural factors responsible for activity. Unfortunately, not only is the mechanism of aldose reductase inhibition unknown, but also a satisfactory correlation between aldose reductase inhibition and molecular structure descriptors has not yet been obtained.

2. Method

The computer automated structure evaluation (CASE) methodology [7] differs from traditional quantitative structure–activity relationships in which the descriptors automatically generated by the program are not physicochemical parameters, but represent molecular fragments inherent in the compound's structural composition.

The CASE study based on the artificial intelligence technique may be outlined as follows:

(1) Construction of a database including an approximately equal number of active and inactive inhibitors of the aldose reductase enzyme. The program requires as input the molecule structures of compounds to be evaluated, together with the experimentally measured biological activity to be studied. The molecules are coded by the use of the Klopman Line Notation [8].

(2) Clustering the activity values suitable to a CASE format.

(3) Tabulation and analysis of the fragments that are generated from the structure of the molecules of the constructed database.

(4) Elimination of irrelevant data and determination of causalities.

(5) Selection of statistically significant fragments and proposal of the mechanistic approach to the interaction.

CASE proceeded by making a fragmentation of each molecule structure into units of 3 to 10 heavy atoms together with their associated hydrogens. Each fragment is designated positive or negative if it belongs to an active or inactive molecule, respectively. The collected and statistically analyzed fragments, due to the discrepancy of a random distribution of structural subunits between active and inactive pools, are taken as indicator fragments in which the subunits are relevant to the property being examined. One of the major advantages of the CASE methodology is that the selection of descriptors from the fragment pool, usually intensively performed by the investigators, is accomplished automatically. In spite of the fact that the data pool of potential descriptors obtained from activating fragments is huge (thousands for a 50–100 compound database), it is apparent that human evaluation appears to be out of reach. Once the database has been analyzed, new structures can be submitted and inspected for activating/deactivating fragments and the expected activity can then be estimated. Indeed, the program acts as a "learning machine" and the database can be updated when more data become available, thus providing a better prediction.

3. Results and discussion

The CASE analysis was performed on 482 aldose reductase inhibitors [12] in order to identify molecular features of the inhibition mechanism. Fourty-eight compounds were randomly excluded from the database for later submission to the program as a test set. Chemical structures used for the aldose reductase database can be seen in fig. 2. Compounds were classified as inactive (–), marginally active (+), active (++), very active (+++) and extremely active (++++). The aldose reductase inhibition activity was measured in terms of pIC_{50} values. Submission of the training database (434 compounds) to the CASE program resulted, via a multiple stepwise regression analysis, in twenty-six biophores (activating fragments) and one biophobe (inactivating fragments) (fig. 3) out of a total of 120 relevant fragments generated automatically, in which 103 were found to be activating and 17 inactivating [12].

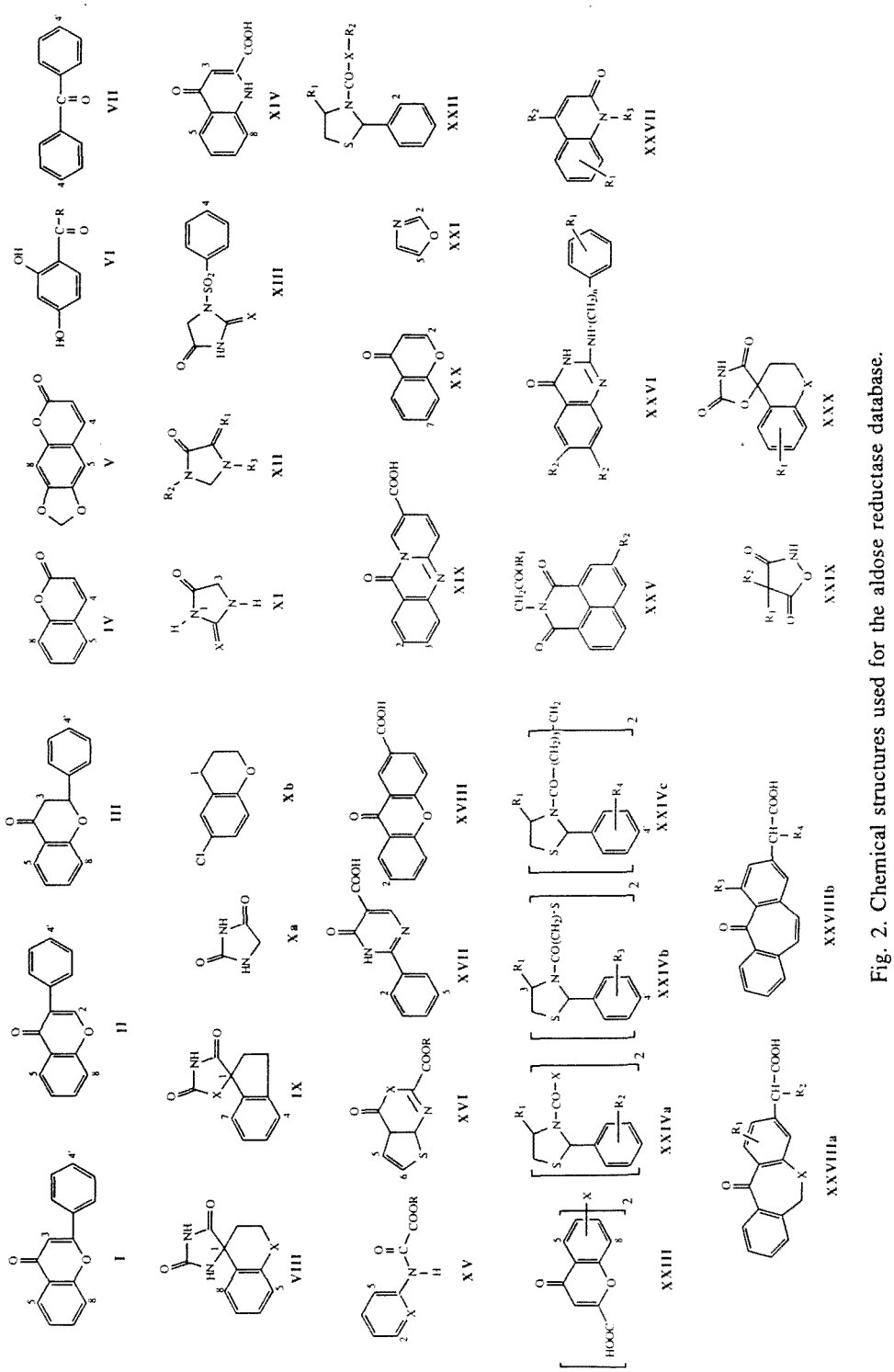

Fig. 2. Chemical structures used for the aldose reductase database.

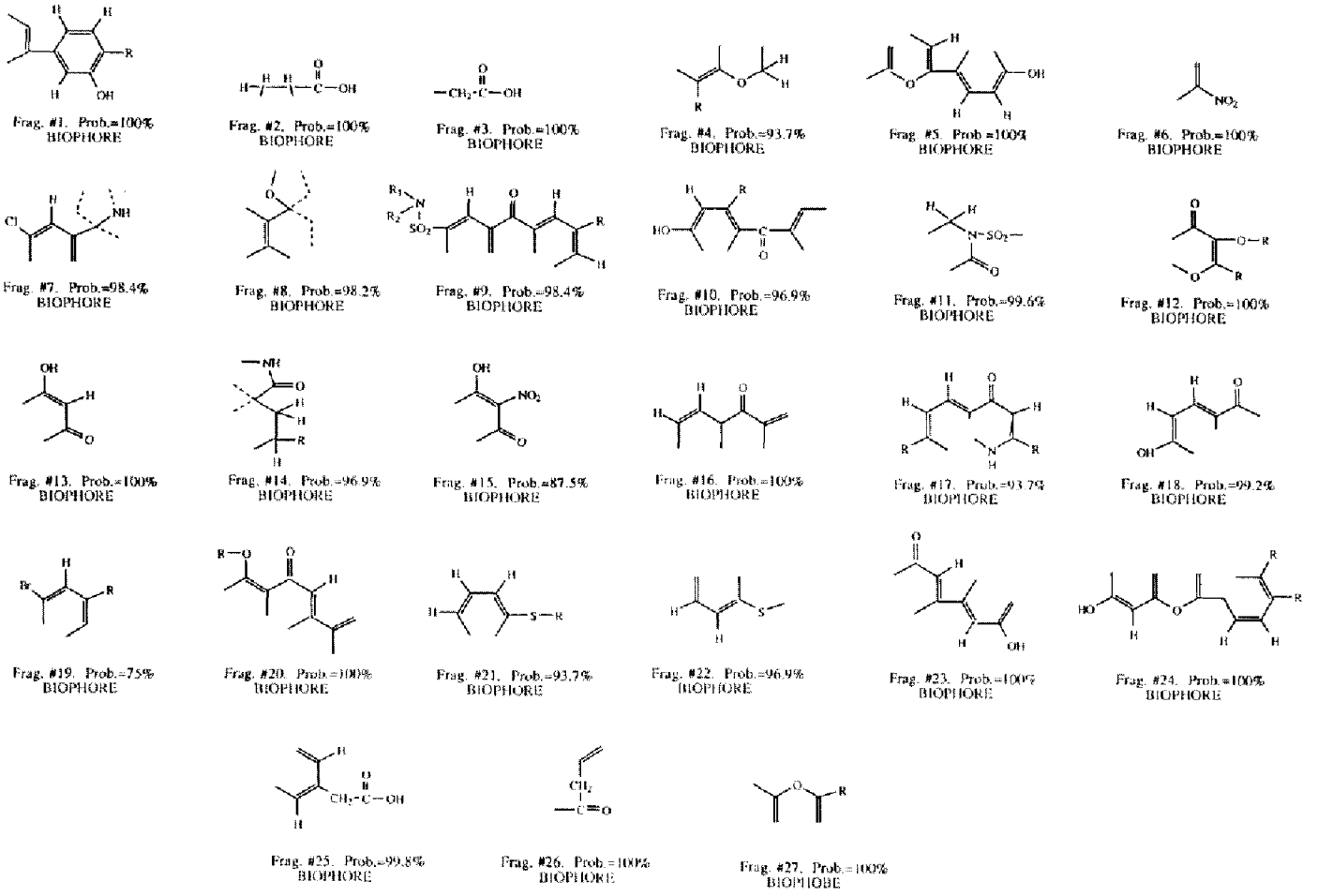

Fig. 3. Activating and inactivating fragments.

On the basis of the biophores and the biophobe, we have previously described a mechanistic feature of aldose reductase inhibitors.

It is suggested that a wide range of aldose reductase inhibitors bind at a common inhibitor site on the aldose reductase enzyme [26]. This suggestion is based on certain common molecular orbital studies (the observed correlation between LUMOs and inhibitory potencies of each inhibitor) obtained from simple Hückel and CNDO/2 calculations, which suggest that aldose reductase inhibition mostly depends on the molecular structure of an inhibitor and should have at least a specific group which is adequate to undergo a reversible nucleophilic attack, such as carbonyl or thiocarbonyl. Although molecular orbital theory has been employed with increasing success to assist in the definition of the electronic moiety of compounds, a limited estimation was obtained with some electronic charge on atomic centers and the highest occupied and lowest unoccupied molecular energies. However, this fact is also confirmed by the study of several protein-modification reagents and irreversible aldose reductase inhibitors, which both indicated that the carbonyl region of the inhibitor reversibly undergoes a reaction with a nucleophilic amino acid residue at the enzyme active site [25]. Therefore, the proposal leading to tetrahedral intermediate formation between the carbonyl function and the hydroxyl of tyrosine residue is generally accepted in spite of the fact that several alternative roles for the carbonyl may be inferred, in addition to a new interaction mechanism which is postulated between the aldose reductase active site and inhibitory compounds in the light of the CASE study published previously [12].

The full spectrum of biological activity of aldose reductase inhibitors is, most likely, not yet known. The inhibitors have been found to display complex interaction with the enzyme itself. Extensive recent review articles [23,27] have discussed the pharmacology and stereospecific behavior of inhibitors in general. On the basis of the literature, it would seem that the possible interactions outlined below represent the first step in the complex formation of inhibitors with aldose reductase enzymes. The conclusions outlined are supported by recent investigations [26] as follows:

(1) The effect of the carbonyl function could be shown to undergo tetrahedral intermediate formation with the tyrosine hydroxyl group at the enzyme active site.

(2) A generally planar structure consisting of two lipophilic regions (aromatic) is required to accommodate the enzymes' hydrophobic site for hydrophobic interaction.

(3) Hydrogen bonding sites located at the planar structure increase the binding of an inhibitor to the enzyme.

In this three-point binding view, we do not interfere with the carboxyl group, which was thought initially to be involved in the charge-transfer reaction in spite of the fact that a decreased capability of this reaction via esterification leads to the fact that the carboxyl group does not change in inhibition potency. However, as we

have stated previously, the ionization potential of the carboxyl moiety might be vigorously effective on the inhibitory site of the enzyme in deciding upon the quality of the interaction with the cationic center bearing amino acids residues [24] such as arginine, lysine and/or histidine. This can be easily demonstrated on the basis of the fact that the strength of inhibition increases with increasing polarity of the inhibitors.

Examination of the CASE projection in fig. 3 and the distribution of certain functions provide insight into the similarities and differences of the conformations displayed by the fragments. In thirteen observations out of the twenty-six activating fragments, the carbonyl group has been found to be present, since its interactive value is limited by not being part of a free rotational system except in fragment 10. However, the similarity of the carbonyl function in fragment 10 demonstrates that the carbonyl function is being maintained in part of a conjugated system. The compounds derived from structure-I, -II and -III can be seen to be distinguished in terms of activity, which could be attributed to the position of the second phenyl ring in the environment of the carbonyl group. In structure-III, the saturated carbonyl bearing ring decreased the potency of binding. Fragments 1, 5, 20, 23 and 24 (the probability of these fragments being in the activating pool is extremely high) are generated most likely from structure-I; in contrast, only one fragment (nr. 4) represented the biophoric interaction from structure-III, in which it is thought that the position of the R group would become forceful in this fragment. The conclusion reached is that the above statement might be important in the arrangement of the environmental variation of the carbonyl group. The second phenyl ring at position 3 suppresses the interaction with the enzyme. This is quite contrary to the previously hypothesized three-point model. In considering possible binding modes of the phenyl ring to aldose reductase on the basis of CASE fragments, the following assumptions can be made. In terms of the number of phenyl ring skeletons, the fragments are equally complex. This process involves both similarities and differences. In fragments 1, 5, 10, 18, 23 and 24, they are all hydroxyl bearing phenyl rings; fragments 4, 17 and 24 have several types of substitution when comparing fragments 6, 7 and 19, which have nitro, chloro and bromo substitution, respectively. Only two phenyl moieties (fragments 22 and 23) showed a very similar thioether bridge in the activating fragment pool. The use of this fact is based on the assumption that similar molecules have similar properties, but we are not able to announce that the phenyl rings are incorporated in the inhibition of the enzyme in the same way in the activity type. However, the inconsistency in the phenyl environment could be related to the spatial conformation of a molecule approaching the enzyme's inhibitor site. This might involve several types of stabilization of the phenyl ring via hydrogen bonding and/or enhanced hydrophobic interaction. Halogens display hydrogen bonding acceptor capability as well as hydroxyl groups on the phenyl ring. Indeed, the introduction of a nitro group into the phenyl ring noticeably increased the aldose reductase inhibitory activity and favored the interaction due to, most likely, the benefit of the increased electron density around oxygen atoms leading to an enhanced electron-

withdrawing property. Based on the above assumptions, at least two phenyl rings may be required for optimum inhibitory activity in which the first phenyl moiety should denote certain functions to manifest hydrogen bonding (primary hydrophobic area at the enzyme inhibitory site), and the second one involved substituents with increased electronic density around the aromatic ring (secondary hydrophobic area). The carbonyl or thiocarbonyl group is then placed in the middle of the two phenyl rings having functions that should stabilize its tetrahedral intermediate formation.

The next point of interest is the importance of carboxyl (fragments 2, 3, 25 and 26) and $-SO_2-$ (fragments 9 and 11) functions located both on the heterocyclic and the aromatic moiety for binding to the enzyme. The carboxyl moiety showed evidence that it is preferably found within a side chain and may interact with the cationic site of the aldose reductase enzyme. This site includes arginine, lysine and/or histidine residues [24]. A naturally occurring binding site in the enzyme therefore offers itself as being cationic due to the fact that many of the inhibitors comprising carboxylic acids include 4-oxo-4H-1-quinoline-2-carboxylic acids [28], oxanilic acids [29], 3, 4-dihydro-4-oxothieno[2, 3-d]pyrimidine carboxylic acids [30],

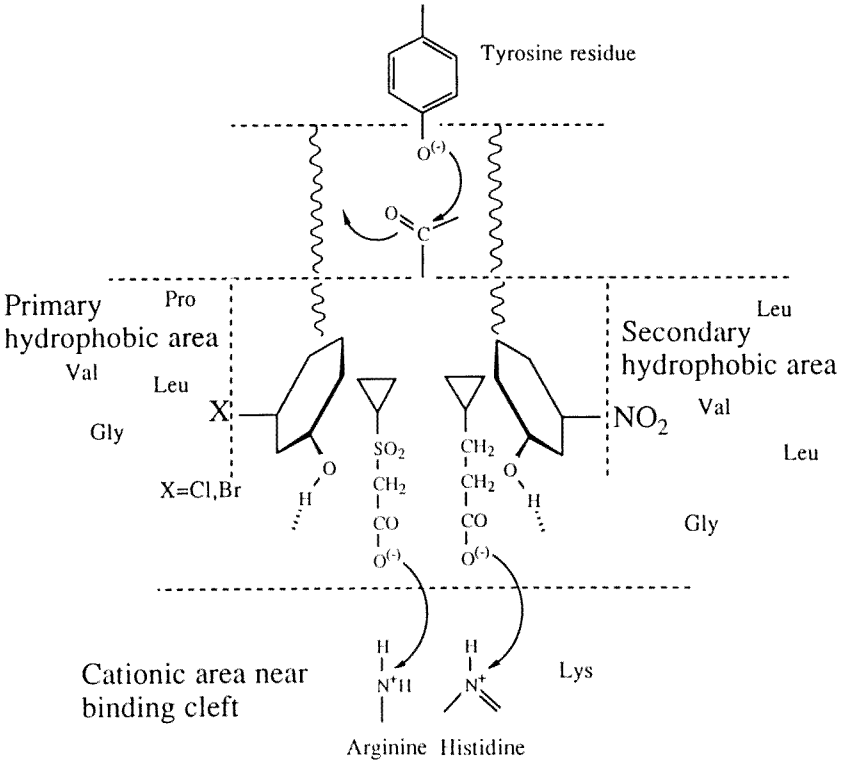

Fig. 4. A schematic representation of hypothetical relationships of AR enzyme inhibitors with their activity site.

xanthone-2-carboxylic acids [31], 11-oxo-11H-pyrido[2, 1-b]quinazoline-2-carboxylic acids [23] and 1, 6-dihydro-6-oxo-2-phenylpyrimidine-5-carboxylic acids [32]. The compounds consisting of sulfonyl functionality have been found to represent desirable activity including 7-sulfamoylxanthone-2carboxylic acids [18], 1-(aryl and -alkyl sulfonyl)hydantoins [20] and 5-[5′-substituted-2′-[(N-alkyl-amino)sulfonyl]phenyl]hydantoin derivatives [21]. It is suggested that the cationic area would presumably be located coplanar with the primary and the secondary hydrophobic plane due to the fact that fragments consisting of carboxyl moiety should be preferably located at the side chain to display some flexibility for interaction. Assuming this geometry, optimized by CASE mechanistic selectivity of the compounds studied, no similar coplanar geometry can be constructed in the case of the side-chain-carboxyl function interacting with the enzyme. Although the position of the carboxyl group may be restricted by the steric hindrance of the phenyl substituents, the location of the cationic area, above or below the phenyl rings, in certain cases may distribute vigorous interactions despite the missing steric effect. A schematic representation of hypothetical relationships is shown in fig. 4. Therein, the carboxyl moiety (polar tail) is positioned in such a manner that it can interact with amino acid residues which are located at the cationic site near the hydrophobic binding cleft. The two hydrophobic heads of the molecule are such that they fit into the lipophilic pockets which are created by several non-polar acting amino acid residues [33]. Furthermore, the proton-donor region of the phenolic hydroxyl group would lie close to, or in, the lipophilic pocket.

References

[1] K. Lindsday, B.G. Buchanan, E.A. Feigenbaum and J. Lederberg, *Application of Artificial Intelligence for Organic Chemistry: The DENDRAL Project* (McGraw-Hill, New York, 1980).
[2] R.O. Duda and E.H. Shortlife, Science 220(1983)261.
[3] R. Osman, H. Weinstein and J.P. Green, ACS Symp. Ser. 112(1979)21.
[4] K. Yuta and P.C. Jurs, J. Med. Chem. 24(1981)241.
[5] S. Dowe, K. Fronke, O.L. Monshjan, W.A. Schkuljev and L.W. Chashanjan, J. Med. Chem. 22(1982)521.
[6] L.B. Kier, in: *Molecular Orbital Theory*, ed. L.B. Kier (Academic Press, New York, 1971).
[7] G. Klopman, J. Amer. Chem. Soc., 106(1984)7315.
[8] G. Klopman and M. MacGonigal, J. Chem. Inf. Comput. Sci. 21(1981)48.
[9] G. Klopman and R. Contreras, Mol. Pharmacol. 27(1985)86.
[10] G. Klopman and A.N. Kalos, J. Theor. Biol. 118(1986)199.
[11] G. Klopman, O.T. Macina, E.J. Simon and J.M. Miller, J. Mol. Struct. 134(1986)299.
[12] G. Klopman and E. Buyukbingol, Mol. Pharmacol. 34(1988)852.
[13] J.H. Kinoshita, D. Dvornik, M. Kraml and K.H. Gabbay, Biochim. Biophys. Acta 158(1968)472.
[14] P.F. Kador and N.E. Sharpless, Biophys. Chem. 8(1978)81.
[15] S.D. Varma, I. Mikuni and J.H. Kinoshita, Science 188(1975)1215.
[16] J. DeRuiter, A.N. Brubaker, W.L. Whitmer and J.L. Stein, Jr., J. Med. Chem. 29(1986)2024.
[17] P.F. Kador, N.E. Sharpless and J.D. Goosey, Prog. Clin. Biol. Res. 114(1982)243.
[18] J.R. Pfister and L.D. Waterbury, J. Med. Chem. 23(1980)1264.
[19] K. Inagaki, I. Miwa, T. Yashiro and J. Okuda, Chem. Pharm. Bull. 30(1982)3244.

[20] I. Miwa, M. Hirano, K. Inagaki, C. Belbeoch and J. Okuda, Biochem. Pharmacol. 36(1987)2789.
[21] J.P. Rizzi, R.C. Schnur, N.J. Hutson, K.G. Kraus and P.R. Kelbaugh, J. Med. Chem. 32(1989)1208.
[22] M.J. Peterson, R. Sarges, C.E. Aldinger and D.P. MacDonald, Metabolism 28, Suppl. 1 (1979)456.
[23] P.F. Kador, W.G. Robinson, Jr. and J.H. Kinoshita, Ann. Rev. Pharmacol. Toxicol. 25(1985)691.
[24] J.H. Kinoshita, S. Fukushi, P.F. Kador and L.O. Merola, Metabolism 28(1979)462.
[25] P.F. Kador and N.E. Sharpless, Mol. Pharmacol. 24(1983)521.
[26] P.F. Kador, J.H. Kinoshita and N.E. Sharpless, Metabolism 35(1986)109.
[27] P.F. Kador, J.D. Goosey, N.E. Sharpless, J. Kolish and D.D. Miller, Eur. J. Med. Chem. 16(1981)293.
[28] J.H. Kinoshita, P.F. Kador and M. Catiles, J. Amer. Med. Assoc. 246(1981)257.
[29] K.H. Gabbay and J.B. O'Sullivan, Diabetes 17(1968)239.
[30] J.H. Kinoshita, L.O. Merola, K. Satoh and E. Dikmak, Nature (London) 194(1962)1085.
[31] P.F. Kador, J.H. Kinoshita and N.E. Sharpless, J. Med. Chem. 28(1985)841.
[32] D. Dvornik, Ann. Rep. Med. Chem. 13(1978)159.
[33] P.R. Andrews, D.L. Craik and J.L. Martin, J. Med. Chem. 27(1984)1648.