Active site molecular modelling of xanthine oxidase inhibitors with antiinflammatory activity

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A molecular model for xanthine oxidase (XOD) ligand interactions is developed on the basis of the XOD inhibitory potency of a set of particularly substituted pyrrolo[2,3-d]pyrimidines having antiinflammatory activity. The interactions investigated are those common to purine analogues. The results explain the active site preference and the decreasing inhibitory potency of methyl–aldehyde–carbonic acid derivatives.

Keywords: molecular modelling, inhibitory potency, xanthine oxidase (XOD), SAR

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The xanthine oxidase (XOD) inhibitory potency of a set of particularly substituted pyrrolo[2,3-d]pyrimidines is used for the development of a molecular mechanism for XOD-ligand interactions common to purine analogues. By means of empirical energy and quantum mechanical calculations, the ability of *N*-1-tautomer formation at the pyrimidine site is recognized to be one of the decisive preconditions for receptor recognition and optimal activity. The influence of substituents at the heterobicycle on optimum binding to the enzyme is discussed.

The compounds used in this SAR study belong to the class of pyrrolo[2,3-d]pyrimidines or 7-deazapurines. Naturally occurring pyrrolopyrimidines carry a sugar moiety at position 7 and show antibiotic activity. A new class of synthetic pyrrolopyrimidines has been developed by the group of Roth and Eger[1–3].

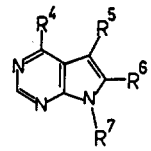
In animal tests these compounds showed remarkable antiinflammatory activity besides CNS-stimulating effects. All substitutable positions of the heterocycle, starting from compound III, have been investigated systematically. It was found that any substituent in position 2 leads to total loss of antiinflammatory activity[4–8].

Table 1 lists the compounds selected for this study. The biological data stems from an XOD test[9].

The use of XOD inhibition data is allowed for two reasons:

- The compounds to be tested are 7-deazapurines, structural analogues of the widely used XOD inhibitor Allopurinol.

Table 1. Compounds used in study



	R^4	R^5	R^6	R^7	I_{50} (μm l^{-1})
I	NH_2	COOH	COOH	Ph	4.7×10^{-2}
II	OH	COOH	COOH	Ph	7.4×10^{-3}
III	NH_2	CH_3	CH_3	Ph	3.8×10^{-1}
IV	OH	CH_3	CH_3	Ph	2.1×10^{-1}
V	OH	CH_3	CHO	Ph	3.2×10^{-1}
VI	OH	CHO	CH_3	Ph	6.9×10^{-2}
VII	OH	CH_3	CH_3	Isopr.	4.2×10^{-1}

- Because of the antiinflammatory activity of the compounds they should interact with the enzyme cyclooxigenase, which is a part of the prostaglandine–synthetase system. Cyclooxigenase and XOD belong to the same class of enzymes[10,11].

Basic work concerning SAR of pyrrolopyrimidines was done by Folkers and Burchard[12]. Following the work of Baker and coworkers on synthesis and pharmacological testing of enzyme inhibitors, and using the QSAR study on that topic made by Silipo and Hansch[13–15], no satisfying correlation was made. The authors believe that complex interactions, e.g. inhibition of enzymes, generally cannot be described satisfactorily by global parameters. For understanding of such complex interactions an exact step by step description of the interacting system is necessary.

METHODS

3D models of the interactions of pyrrolopyrimidine with receptor models are developed by simulating the receptor binding sites with amino acids, or parts of them. The coordinates of the constructed interaction complexes were used as input for supermolecular calculations using CNDO/2. Following equation (1):

$$E_{int} = E_{(AB)+} - E_{(A)} - E_{(B)+} \quad (1)$$

interaction energies are obtained by subtracting the single energies of the components from the energy of the complex. The molecular modelling software Sybyl* was used for construction of the interaction complexes. On a PS 300 picture system, Sybyl allows modelling and geometrical optimization of polymolecular structures. This procedure has the advantage of optimizing the molecular structures of the aggregates visually, while

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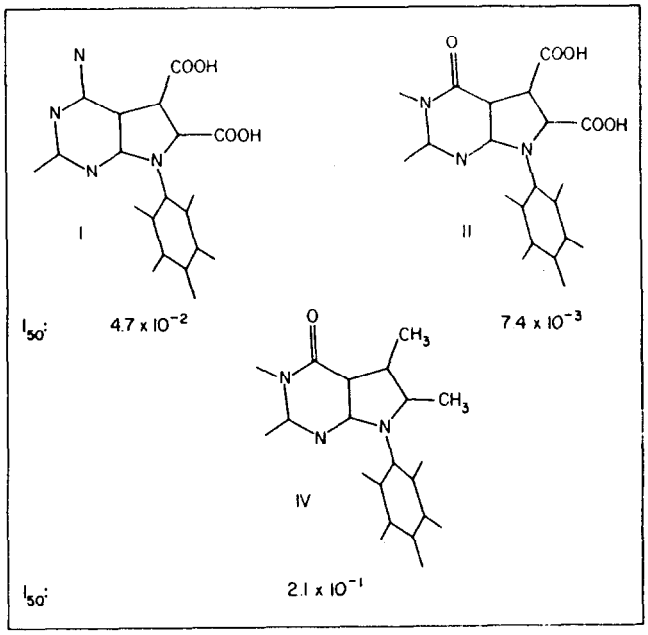

Figure 1. Structural comparison and inhibitory potencies of compounds I, II and IV

simultaneously checking the constructed interaction geometries by force field calculations. This way one succeeds in obtaining reliable coordinates as input for the semi-empirical calculations.

Molecular modelling and force field calculations were done on a VAX 11/730 computer, and quantum mechanical calculations were run on an IBM 3038 in the computer centre of the University of Berne.

RESULTS AND DISCUSSION

Compounds I and II (Figure 1) were used as starting materials for this molecular modelling study. The inhibitory potencies of the two compounds differ significantly, with compound II being six times more potent. Since the only difference in the structural features is found at the pyrimidine site, this may be causing the difference in biological response. Bergmann and Kwietny attempted to connect the importance of different heteroatom hybridization in purines with their chemical behaviour in enzymatic oxidation[16,17]. Tautomerization also seemed to be an important factor. Therefore, the present authors looked for the most likely protonation sites of I and II. In this concept, protonation sites should be important as a potential interaction site with a cationic model receptor.

Within the heterocyclic class considered, protonation should occur at N3 in the 4-aminopyrrolopyrimidine series and at N1 in the 4-oxo series. This assumption could be confirmed by the CNDO/2 calculations which showed that the N3 protonated 4-aminopyrrolopyrimidine and the N1 protonated 4-oxopyrrolopyrimidine were the energetically most stable forms of all possible protonated structures of these molecules. In the next step 3-dimensional interaction complexes have been constructed with a methylammonium ion serving as cationic model receptor[20] following the procedure mentioned above. Figures 2–5 show the different geometries developed for the 4-amino compound I. All interaction complexes were constructed in order to simulate an intermediate step of protonation. They consist of a hydrogen

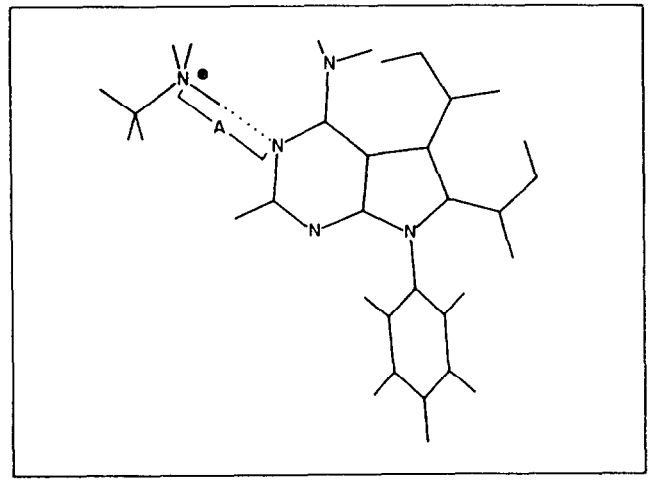

Figure 2. Interaction complex A

bond formed between a pyrimidine nitrogen and the methylammonium cation.

The drug receptor interaction is characterized as an H-bonded type of intermolecular complex, which restricts the analysis of space around the substrate to several well defined geometries. The allowed geometries are dependent on the orbital geometries of the ring nitrogens and the ammonium nitrogen, as well as on the axis of symmetry along the hydrogen bond. The possible degrees of freedom of the interaction complexes are reduced further by observing the sterical requirements of the substrate.

In complex A (Figure 2) the heterobicycle and the methylammonium molecule are coplanar. Distance A between N3 and the ammonium nitrogen varies between 3.00 and 4.75 Å. Optimization of the complex geometry by empirical force field calculations yields a value of 60° for the N7–Cl torsion angle representing the angle between the aromatic and the heteroaromatic plane of the molecule. Quantum mechanical calculations gave equivalent results[18]. Complex B (Figure 3) represents the interaction between Nl and the model receptor. Similar to complex A, the methylammonium attacks coplanar at the *sp*2 hybrid orbital of N1. Investigation of the steric conditions by constructing van der Waals surfaces of the molecules shows that the attack in this case is difficult because of the steric requirements of the inclined phenyl ring. This view is confirmed by the results of

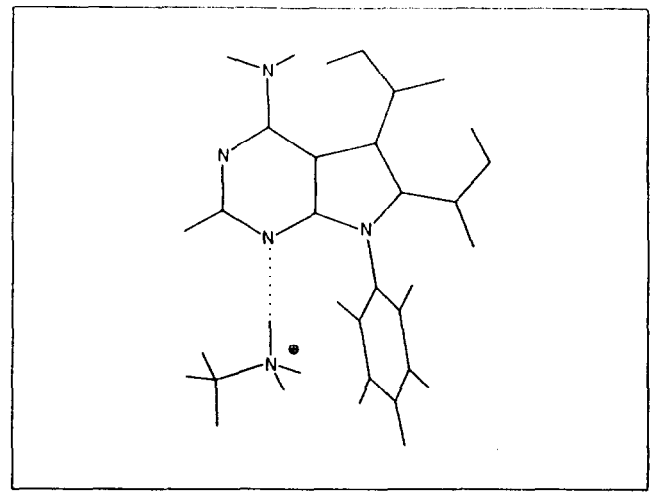

Figure 3. Interaction complex B

Table 2. Calculated interaction energies for the interaction complexes of I

Complex	Å	E_{int}(kJ mol^{-1})
A	3.0	−36.75
B	2.5–4.7	+ + +
C	3.0	−46.50
D	2.5–4.7	+ + +
E	2.5–4.7	+ + +

the calculated interaction energies presented in Table 2. To further support this hypothesis, complex C with a geometry identical to B was constructed showing the attack of the cation at N1 when N7 is unsubstituted (Figure 4). This results in a considerable increase of interaction energy because of the lack of steric interaction with the phenylring (Table 2). By arranging the potential binding site above the plane of the heterocycle it should be possible to evade steric hindrance by bulky substituents in position 7. This is shown in complexes

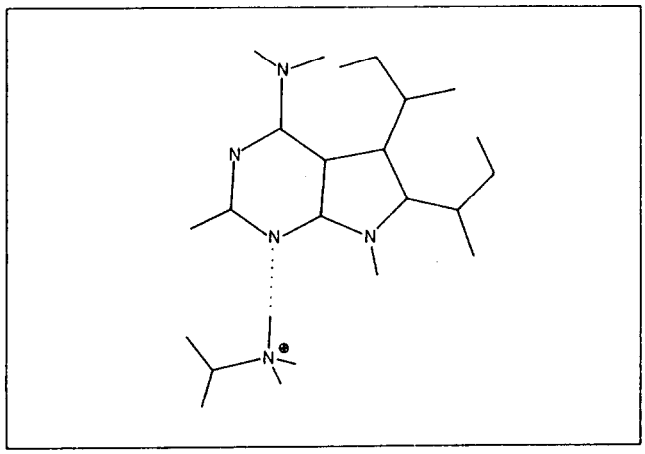

Figure 4. Interaction complex C

D and E (Figure 5). Here the ammonium ion is located above N1 at an angle of 60° or 90° with regard to the plane of the heterocycle. However, because of the *sp*2 hybridization of the N1, which implies participation of the *p* orbitals in the resonance system of the pyrimidine ring, extensive interaction energies cannot be expected. This is confirmed by the calculations (Table 2).

Binding of a cation can reasonably be assumed only at N3 of the 4-amino derivative I. In the case of N7 unsubstituted compounds N1 can be seen as an additional or competing binding site. Interaction geometries of the 4-oxo compound II were constructed following the same procedure.

In complex F (Figure 6) the methylammonium ion

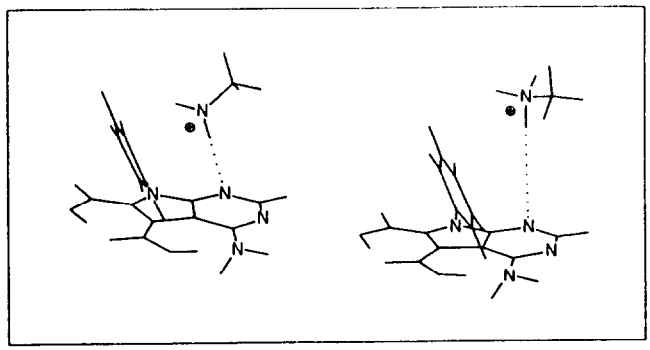

Figure 5. Interaction complexes D and E

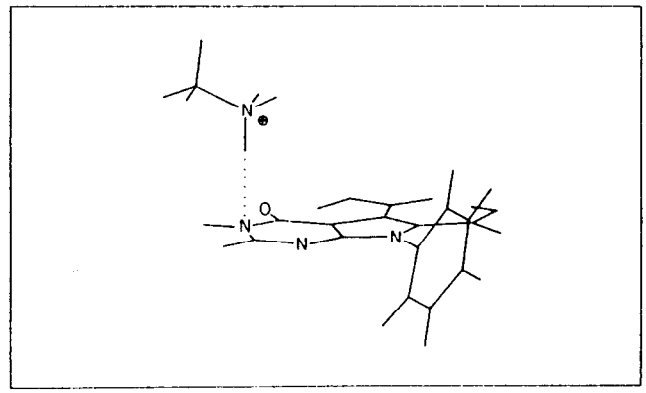

Figure 6. Interaction complex F

is located above the heterocyclic plane at an angle of 90° taking into consideration that N3 is a *sp*2 trigonal planar lactame type nitrogen and therefore has the lone pair in the *p* orbital, in contrast to the 4-amino compound. Also, face to face interaction with the carbonamide hydrogen makes no sense by chemical reasoning.

Complex G (Figure 7) shows a similar configuration, with the cation attacking N1, while the 4-oxo compound is existing as N1 tautomer. This configuration also favours an attack at the *p* orbital of the trigonal planar N1 simultaneously evading steric interaction with the phenyl substituent if the contact takes place from the 'open' side with regard to the angle of inclination of the phenyl ring with the heterocyclic plane. The results of the quantum mechanical calculations are presented in Table 3. As expected from the calculations of potential

Table 3. Calculated interaction energies for the interaction complexes of II

Complex	Å	E_{int} (kJ mol^{-1})
F	3.0	−34.29
G	3.0	−74.60

protonation sites mentioned above, it can be seen that complex G yields a much better interaction energy than does complex F. The N1 tautomer (Complex G) shows the best interaction energy of all the complexes calculated.

The following conclusion can be drawn from the results: the ability of tautomer formation implying binding at N1 seems to be essential for optimal interaction. Molecules which are not able to form the N1 tautomer would be only poorly recognized by the receptor and

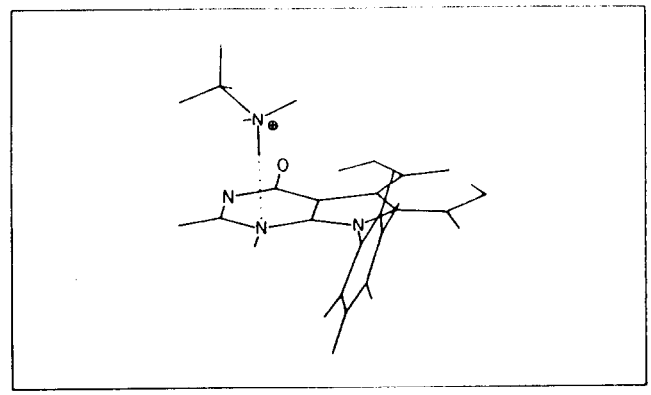

Figure 7. Interaction complex G

therefore could not react at all, or show very low reaction rates. This hypothesis is consistent with the biochemical findings of Bergmann and coworkers[16,17], Springer *et al.*[21], Moder and Leonard[22] and Rosemeyer and Seela[23].

On the basis of the literature it would seem that the interactions described above represent the first step in the complex formation of purine type inhibitors with XOD.

The conclusions drawn are supported by recent n.m.r. investigations on the London–Schmidt Model[24]. By means of Transferred Nuclear Overhauser Effects (TRNOE), nucleoside triphosphates could be shown to undergo complex formation with aspartate transcarbamylase: pyrimidine ring nitrogens react with electrophilic groups at the receptor site. Adenosin, which is the aza-analogue of the described 4-amino-pyrrolopyrimidine I, is assumed to bind via N1 (N3 in the pyrrolopyrimidine series) as described above.

The next point of interest is the importance of the substituents, both on the heterocyclic and the aromatic moiety for binding at XOD. Previous calculations of electron density distributions together with n.m.r. results indicate their negligible influence on the electrical state of the pyrimidine ring. These were interpreted as if the substituents mainly have local significance for the interaction process[18,19]. In the authors' view, they do not interfere with the recognition step, but decide upon the quality of interaction with the active centre, thereby determining the degree of enzymatic attack on the substrate. This can be demonstrated on the basis of the pyrrolopyrimidine derivatives listed in Figure 8. The strength of inhibition increases with increase of polarity of the substituents at position 5. This is easily understandable since the natural substrates in that position bear an *sp*2 nitrogen.

Compound VII shows clearly that the phenyl ring at position 7 intensifies the interaction with the enzyme (IV is twofold more active than VII) (see Figure 9). However, compound II unequivocally indicates the importance of substituents at the pyrrol moiety for activity of the compounds: due to the 4-amino substituent II forms only a weak recognition complex, but having all the other substituents necessary for high activity, it finally is more potent than the 5,6-dimethyl-4-oxo compound IV.

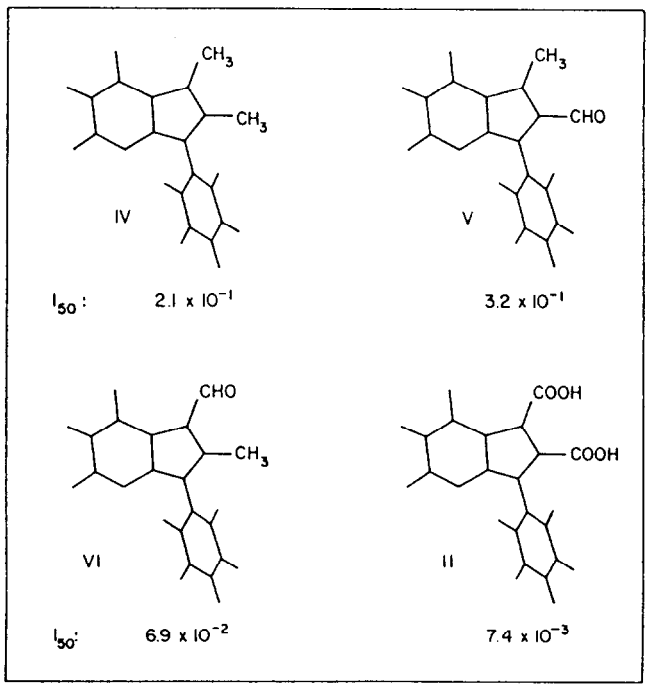

Figure 8. Structural comparison and inhibitory potencies of compounds II, IV, V and VI

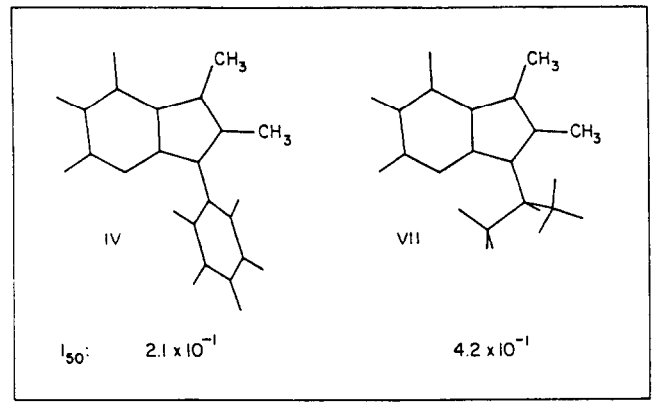

Figure 9. Structural comparison and inhibitory potencies of compounds IV and VII

These qualitative observations agree well with results of Baker and coworkers, obtained when investigating substituted guanines. Therefore, in the next stage the aim is to construct potential binding site models for the phenyl ring and for the substituents in positions 5 and 6.

In considering possible binding modes of the pyrrol moiety to XOD, the following assumptions can be made. Physiological substrates of XOD are the purines bearing an *sp*2 nitrogen at the position which is equivalent to carbon 5 in the pyrrolopyrimidine series. A naturally occurring binding site in the enzyme active centre therefore offers itself as being cationic and if so, presumably would be located coplanar with the purine plane. Assuming this geometry, optimized by natural selectivity, no similar coplanar geometry can be constructed in the case of the pyrrolopyrimidines interacting with the enzyme, because of the steric hindrance of the 5-substituents. However, location of the cationic binding site, above or below the purine plane, in certain cases allows strong interactions despite missing steric fit of the substrate. Because of the powerful long range potentials of ions, interactions of cations and anions, e.g. the interaction of a carboxylate with a cationic binding site, do not need an especially optimized interaction geometry to produce substantial binding energies. On the contrary, methyl and aldehyde groups lacking the advantage of ionic interactions exhibit only weak binding energies.

Based on this concept, interaction complexes were constructed, with pyrrol-2,3-dicarbonic acid serving as a model for the substrate and propylguanidinium cation representing arginine as one possible amino acid at the receptor binding site. In view of the fact that quantum mechanical calculations of ionic interactions result in unrealistically high interaction energies, a special supermolecular geometry was used (Figure 10). It represents an intermediate state of the protonation process with a distance of 2Å between the carbonic acid hydrogen and the *sp*2 configurated nitrogen of guanidine. The geometry chosen is equivalent to a totally planar interaction of a purine substrate with an argininium cation. The quantum mechanical calculation of this complex yields an interaction energy of -55 kJ mol^{-1}.

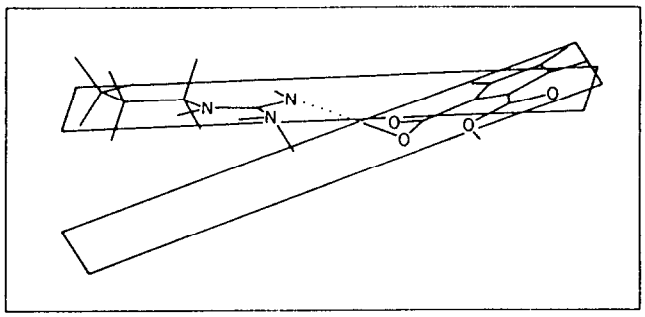

Figure 10. Interaction complex geometry of pyrrole-2,3-dicarbonic acid with n-propylguanidine

The results represented are able to explain both the regioselectivity observed and the ranging of inhibitory potency of methyl–aldehyde–carbonic acid derivatives.

Further investigations are in progress to elucidate the role of the phenyl at position 7, especially with regard to the question of coplanar or noncoplanar conformation with respect to the plane of the heterocycle, and to develop an interaction complex with the molybdenium located at the active centre of XOD.

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