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Models of F·H Contacts Relevant to the Binding of Fluoroaromatic Inhibitors to Carbonic Anhydrase II

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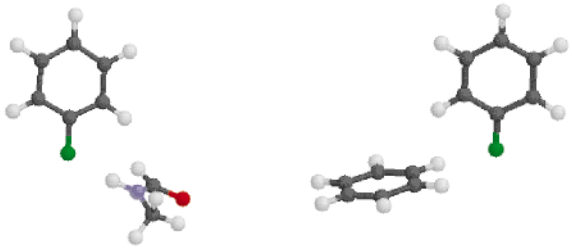
ABSTRACT



Complexes formed between fluorobenzene and *N*-methylformamide or benzene have been used as models of the interaction of fluoroaromatic drugs with carbonic anhydrase II. These structures have been investigated via ab initio and density functional methods, including HF, B3LYP, and MP2 procedures. The results of the calculations are consistent with the hypothesis, suggested originally by experimental X-ray crystal structures of the drug–receptor complexes, that favorable fluorine–hydrogen interactions affect binding affinity.

Weak, noncovalent interactions have become the focus of research in recent years in the field of structural biology and drug design. Aromatic contacts, one kind of weak interactions, have been used to help explain protein folding,[1−3] with aromatic interactions involving Phe and Tyr contributing significantly to tertiary structure.[1,3] Aromatic interactions have also been studied in the area of enzymatic catalysis, where researchers have identified a Tyr residue involved in the acceleration of the reaction catalyzed by the coenzyme thiamin.[4] Finally, interactions between aromatic groups have been the focus of research in host−guest chemistry, where the binding of a ligand to a receptor often involves such forces.[5] These favorable contacts may be useful in designing drugs that bind tightly to enzyme targets. The forces responsible for this "$\pi-\pi$" interaction have been studied extensively through the use of benzene and toluene dimers as models. In this paper, we have applied ab initio and density functional methods to elucidate the nature of the contacts between fluoroaromatic inhibitors of carbonic anhydrase II (CA) and the active site of this protein.[6]

Molecular mechanics calculations reveal that a benzene dimer in aqueous solution energetically prefers a T-shaped conformation relative to a stacked conformation.[7] This result

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can be explained primarily by dispersion forces and, to a lesser extent, electrostatics.[7−9] This conformation has also been identified via ab initio Hartree−Fock (HF) calculations, in a study of the binding of dihydrofolate reductase to its inhibitors.[10] The toluene dimer, which has been used as a model for Phe−Phe interactions in proteins, prefers a stacked conformation, with the added methyl group providing 40% more dispersion interaction than a benzene ring.[7] Another factor that contributes to the stability of the stacked conformation is the reduction of the hydrophobic surface area exposed to the solvent, which outweighs the repulsive interaction between the quadrupole moments of the toluene molecules. Although mechanics calculations indicate that the stacked conformation is preferred, exhaustive studies of protein data banks indicate that Phe−Phe interactions almost always occur in a T-shaped orientation.[1,2] Sterics,[7] electrostatics,[2] and dispersive forces[9] have been used to account for the apparent energetic preference for the T-shaped arrangement. Thus, even though $\pi-\pi$ interactions have been shown to prefer certain patterns, the relevant forces are not fully understood.

To shed light on the subject, Dougherty and co-workers investigated computationally the heterodimer formed by benzene and hexafluorobenzene.[11] These two molecules have quadrupole moments of similar magnitude but opposite sign,[12] which were proposed to result in a preference for a stacked conformation for the heterodimer. The interaction between fluoroaromatics and aromatics has also been studied experimentally in the solid state,[13,14] with regard to the quadrupolar interaction described above and to F−H interactions. Strongly favorable F−H interactions are uncommon but have been shown to exist.[15,16]

We have synthesized a small library of fluorinated inhibitors of CA and have measured their affinity for the protein.[6] We have found that binding is not solely a function of the number of fluorines attached to the aromatic ring of the inhibitor, but that instead the interaction between the host and the inhibitor depends, in part, on the relative placement of the fluorines. The calculations presented in this paper attempt to identify the lowest energy arrangement of models of (1) a fluoroaromatic inhibitor and a Phe side chain and (2) of the inhibitor and a backbone peptide bond. The aim of these calculations is to relate the binding energies from these models to the binding affinities in our biochemical system.

Gaussian98[17] was used to perform ab initio quantum mechanical calculations on the complexes formed by fluorobenzene with *N*-methylformamide or benzene. The fluorobenzenes are models of inhibitors of carbonic anhydrase (CA),[6] while benzene models the side chain of a phenylalanine residue in the active site of CA.[18] *N*-Methylformamide is used to model a peptide bond in the backbone of the protein.

Preliminary studies of complexes formed by benzene with 2-fluorotoluene and 2,3-difluorotoluene, respectively, at HF/6-31G* suggested the presence of an attractive H−F interaction.[19] Figure 1a shows the complex formed by fluorobenzene with *N*-methylformamide, which also includes an H−F contact (r = 2.045 Å; ∠NHF = 178°), at MP2/6-31G*. Figure 1b shows the *cis* form of 2- fluorobenzylamine in its optimized geometry, which is consistent with an intramolecular H−F interaction (r = 2.022 Å; ∠NHF = 127°). Figure 2a shows the MP2/6-31G* fluorobenzene−benzene complex. Figures 2b−2f show the same complex featuring geometries restricted as indicated in the caption.

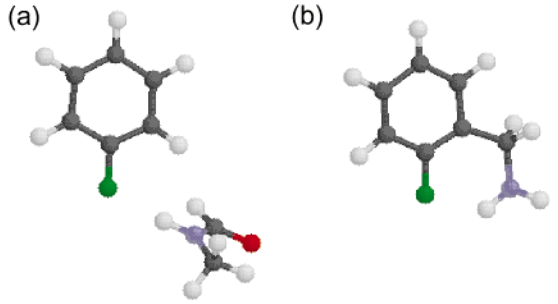

Figure 1. (a) Complex of *N*-methylformamide with fluorobenzene optimized at MP2/6-31G* and (b) optimized *cis* conformation of 2-fluorobenzylamine, at MP2/6-31G*.

Full optimization of the fluorobenzene−benzene complex, at the MP2/6-31G* level, gave the following geometrical parameters: r_{FH} = 2.98 Å; ∠CHF = 89.7°; an angle of 8.4° between the two planes. Table 1 shows the binding energies for structures **2a**−**f** at various levels of theory,[20] defined as the difference between the energy of the complex and the

Table 1. Binding Energies (kcal/mol) for Benzene−Fluorobenzene Complexes Shown in Figures **2a**−**f**

	B3LYP/ 6-31G** +BSSE[a]	B3LYP/ 6-31+G** +BSSE	MP2/ 6-31+G* +BSSE	MP2/ 6-31G**[b]	MP2/ 6-31G** a+BSSE[b]
2a	−0.39	−0.34	−2.43	−4.63	−0.76
2b	−0.07	−0.09	−2.37	−4.47	−0.79
2c	−0.38	−0.42	−1.59	−4.25	−1.49
2d	−0.39	−0.42	−1.88	−3.96	−1.06
2e	−0.31	−0.28	−2.32	−3.92	−1.76
2f	not bound			−0.94	not bound

[a] Single-point calculations, based on the final geometry obtained at B3LYP/6-31+G**. [b] Single-point calculations, based on the final geometry obtained at MP2/6-31G*.

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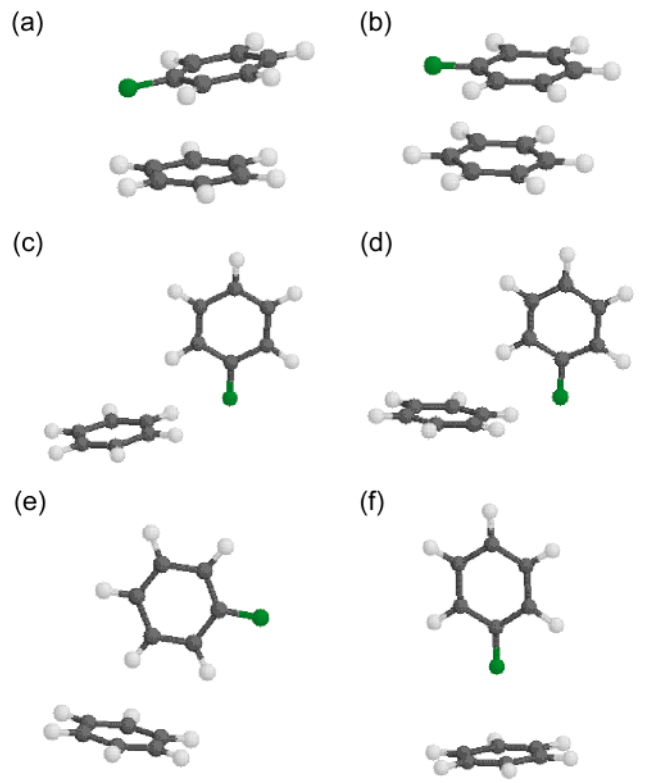

Figure 2. Optimizations of benzene−fluorobenzene at MP2/6-31G* (a) unrestricted, (b) rings restricted to being in parallel planes, (c) rings restricted to being in perpendicular planes, (d) C−F bond restricted to being perpendicular to plane of benzene ring, (e) rings restricted to being perpendicular, but C−F bond forced to be parallel to plane of benzene ring, (f) C−F bond restricted to being perpendicular to plane of benzene ring and points at its center.

sum of the energies of each component, as well as the binding energies with the BSSE correction[21] included.

In general, the ab initio calculations carried out with a variety of partial geometric restrictions demonstrate that the potential energy surface is extremely flat. There is, for instance, very little preference one way or the other for a stacked versus a perpendicular arrangement. Interestingly, however, some of the most stable structures at a variety of levels of theory including HF, DFT, and MP2 allow the fluorine atom of fluorobenzene to approach closely one of the hydrogen atoms of the other aromatic ring. These structures feature an F−H distance of between 2.18 and 2.98 Å and are significantly different from the preferred structure of the benzene dimer.[7] The presence of low-energy structures featuring such a close approach between fluorine and hydrogen support the possibility of a favorable F−H interaction.

Furthermore, in the calculated structures of these complexes, there is a tendency for the C−F bond to tilt toward the C−H bond, in such a manner as to facilitate close contact between the fluorine and the hydrogen. This tendency is visible, for instance, in structure **2a**, where the F−H distance is 2.98 Å. This tilting further supports the hypothesis that fluorine and hydrogen experience an attraction.

To obtain further support for the significance of the F−H interaction in Figures 1a and 2c, we estimated the potential surface for rotation of the fluorobenzene ring about the axis perpendicular to the plane of the ring through its center (Figure 3). Single-point calculations at MP2/6-31G* for each of 12 conformations indicated that there is a >3 kcal/mol cost to rotation away from the geometries depicted in **1a** and **2c**.

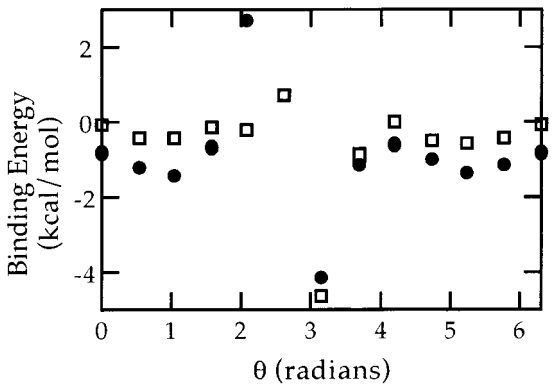

Figure 3. Potential surfaces for rotation of fluorobenzene moiety of structures **1a** (open squares) and **2c** (filled circles). The conformations shown in **1a** and **2c** correspond to π radians. The data point at $5/6\pi$ radians, for **2c**, have been omitted, since this conformation results in a steric interaction that costs ≈12 kcal/mol.

The presence of a favorable F−H interaction is in accord with data from X-ray crystallography.[22] These results show that the fluorobenzene moiety of the pentafluorinated inhibitor (Figure 4; $n = 5$) is perpendicular to the aromatic ring of the Phe residue, such that the F−H distance is only 2.4 Å. Structure **2c** from our calculations most closely resembles these experimental results. At the MP2/6-31G*//MP2/6-

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(19) 2-Fluorotoluene and 2,3-difluorotoluene exhibit binding energies with benzene of 1.18 and 0.90 kcal/mol at HF/6-31G* level. Both complexes implicate an F−H interaction. These binding energies are in the range of the HF/6-31G* binding energies of fluorobenzene−benzene, which support our use of the latter complex as a model for the interaction in our system.

(20) At MP2/6-31G*, with and without BSSE, we observed the same trends in the binding energies as are shown in Table 1.

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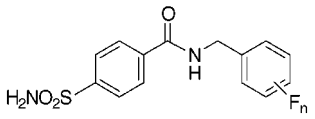

Figure 4. General structure of fluorinated inhibitors of carbonic anhydrase II.

31G** level of theory, **2c** is within 0.3 kcal/mol of the structure calculated to be of lowest energy.

The fact that **2c** is not calculated to be the very lowest energy structure need not be taken as evidence against a favorable F−H interaction. Many factors could alter geometric preferences in our simple model system compared to the real inhibitor−receptor complex. Our model system not only omits all of the protein structure aside from the Phe ring but also neglects the solvent, which could play an important role. Furthermore, as is apparent from the data in Table 1, the BSSEs are substantial. This is particularly true for the MP2 calculations, where the counterpoise corrections are larger than the (corrected) binding energies themselves! The BSSEs thus cast considerable doubt on the energy ordering of the different geometries, as it is well-known that counterpoise corrections are a crude estimate of the BSSE at best.[23] However, the only reliable solution to this dilemma, which is to use a *very* large basis set, was not feasible for systems as large as those we have studied here. Consequently, the most we can say with confidence is that the calculations on the model system are at least consistent with the possibility of a favorable F−H interaction.

Examination of the experimental data pertaining to the binding of fluoroaromatic inhibitors to CA[6] shows trends that are consistent with the presence of an F−H contact. Based on the mode of binding of arylsulfonamides to CA,[18] it is clear that the structure of these inhibitors (Figure 4) prevents any F−H interaction between a fluorine at the 4-position of the benzyl amide group and the protein. This isomer of the monofluorinated inhibitors does not, therefore, bind as tightly to CA as the 2- or 3-fluoro derivatives (K_d = 2.4, 0.73, and 0.97 nM, respectively). Molecules that have a 3-fluoro group afford the tightest binding inhibitors, suggesting that fluorine at this position may invoke a strong F−H interaction with an NH group in the peptide backbone. This F−H attraction is stronger than fluorobenzene−benzene F−H interactions, as shown by the fact that the binding energy of *N*-methylformamide to fluorobenzene, in Figure 1, is 4.51 kcal/mol at the MP2/6-31G* level, compared to 1.25 kcal/mol for the aromatic/aromatic interaction in Figure 2c.

The fact that the 2,5-difluoro derivative binds more tightly than the 2,6-compound (0.55 vs 1.2 nM) can be explained in two ways: the fluorine at the 5-position may participate in an F−H attraction with the backbone or the fluorines at the 2- and 6-positions may form intramolecular F−H hydrogen bonds with the NH group of our inhibitor, thus weakening their interaction with the protein. To explore the second possibility, 2-fluorobenzylamine was studied at the MP2/6-31G* level, and it was found that the fluorine prefers to be *trans* to the NH group, by 1.4 kcal/mol. If there are two *ortho* fluorines, however (as is the case with the 2,6-derivative), one of them will have to form an intramolecular F−H bond. The fact that the 2,3-derivative binds only slightly more tightly than the 2,6 compound (1.1 vs 1.2 nM), while 2,5 binds much more tightly (0.55 nM), supports our conclusion that fluorine at the 5-position must make an F−H bond with the peptide backbone. Assuming that all other interactions between the inhibitors and the protein are comparable, this type of specific F−H contact may determine the relative binding energies of fluorinated inhibitors.

To follow up on these ab initio calculations of models of fluorinated inhibitors bound to CA, we are considering the minima accessible to complexes of these groups when they are restricted by the active site of the protein. Specifically, by fixing the methyl carbon of the inhibitor model, and the methyl carbon−ring carbon bond of the Phe model, we hope to be able to confirm that the structures obtained by crystallography are energetic minima, at some level of theory. The specific computational approach required to replicate the conformation found by crystallography should provide valuable information about the nature of the interactions that are responsible for tight binding in this series of inhibitors and may provide more general predictive power regarding F−H contacts in drug design.

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