Notes

Twisted Amides Inferred from QSAR Analysis of Hydrophobicity and Electronic Effects on the Affinity of Fluoroaromatic Inhibitors of Carbonic Anhydrase

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Abstract: QSAR has been used to elucidate the origin of the hydrophobicity and binding affinity of a small library of fluoroaromatic inhibitors of F131V carbonic anhydrase II. Our analysis predicted the presence of a twisted amide conformation for several bound inhibitors, which we confirmed crystallographically. We also determined that the hydrophobicity of the inhibitors as a whole results from the fragment hydrophobicities of their fluorobenzyl rings, corrected for field effects and the presence of an intramolecular F·H contact in solution. The loss of this interaction on binding to the enzyme makes the affinity sensitive to the same terms, but with the opposite dependence on the F·H contact. In the case of the four inhibitors bound as twisted amides, this F·H contact must be retained to some extent in the bound state in order for their affinities to be consistent with our QSAR analysis of the entire set of 17 molecules.

Rational drug design requires the consideration of a range of factors that impact the affinity as well as the accessibility of drugs.[1] One approach involves QSAR, in which the molecular structure of a drug is systematically varied to modulate its physicochemical properties.[2] Ideally, by quantifying how these properties affect specific drug behavior, drug action can be optimized and predicted.[3]

In the case of inhibitors of human carbonic anhydrase II (CA), which are used clinically to treat the symptoms of glaucoma,[4] hydrophobicity has been found to be a key determinant of the affinity of inhibitors.[5,6] More recently, we have shown that the binding of fluoroaromatic CA inhibitors, to both wild-type and F131V CA, is not exclusively determined by their hydrophobicity.[7,8] Using cocrystal structures of inhibitors bound to wild-type and F131V CA, we have found that simple calculations of dipolar and quadrupolar interactions between the fluorobenzyl ring of these inhibitors and residues in the active site of the protein predict the binding affinities of five of our molecules surprisingly well.[9] We have further concluded that inhibition of F131V CA involves a hydrophobic contact with P202, as well as a hydrogen bond network involving T200, P201, an ordered water molecule, and the amide of our inhibitors.[8,9] We now wish to report an alternative analysis of the binding of our inhibitors to F131V CA that incorporates experimental measurements of their hydrophobicity as well as considerations of electronic effects and an intramolecular F·H contact in the free and bound state.

To elucidate the factors that determine hydrophobicity, we considered plots of log *P*-values of our fluoroaromatic inhibitors determined by microemulsion electrokinetic chromatography (MEEKC)[8,10] versus the sum of fragment hydrophobicities.[11] While this plot showed no correlation, the inclusion of a correction factor accounting for the presence of an *o*-fluorobenzyl amide[12] gave an R^2-value of 0.84. Further correction for the inductive effect of fluorine substituents[13] on the dipole of the fluorobenzyl ring gave a correlation having $R^2 = 0.92$ (Figure 1), from which we concluded that the hydrophobicity of these inhibitors is determined by their extent and pattern of fluorination. The distribution of fluorine around the aromatic ring presumably affects the polarization of the amide functional group by two mechanisms (Figure 2). Ab initio calculations suggest that ortho fluorines interact with the amide hydrogen,[14] increasing the polarity of the molecule. Field effects due to fluorination create a local

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(11) The fragments that we considered are the benzyl group and each of its attached fluorines (ref 8). The rest of the molecule was present in all cases, so its contribution was not included in this summation.

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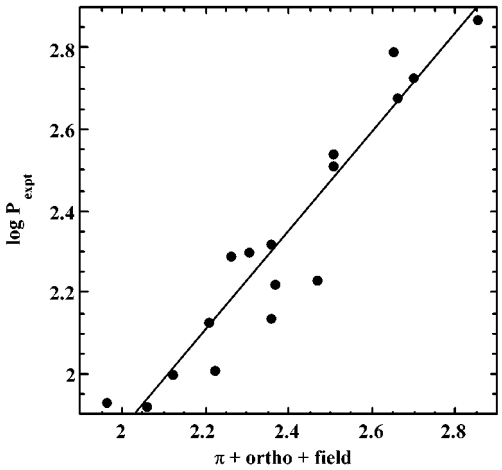

Figure 1. Correlation between log *P*-values determined by MEEKC and calculated hydrophobicities, including corrections for field effects and intramolecular hydrogen bonds. Please see Supporting Information for attempted correlations involving fewer parameters.

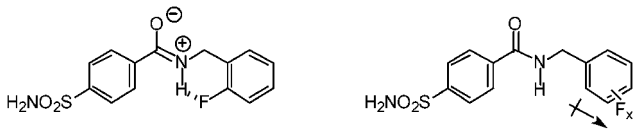

Figure 2. Effects on amide polarization of an intramolecular F···H interaction (increased polarization) and of a field effect arising from the net dipole of a fluorobenzyl group (decreased polarization).

dipole, which may either favor or disfavor polarization of the amide. This variation in polarization affects the overall hydrophobicity of the molecule.

With this understanding of the factors relevant to the hydrophobicity of fluorobenzyl-linked CA inhibitors, we attempted to relate their binding affinity for F131V CA to their polarity. Unfortunately, experimental log *P*-values failed to correlate with binding energies. We then extracted and analyzed the relationships between the individual components of hydrophobicity (illustrated in Figure 1) and binding energy. Correlations with the sum of fragment hydrophobicities, uncorrected and corrected for field effects, were unsuccessful. Field effects should alter the acidity of the amide hydrogen, which could affect affinity by modulating the strength of the hydrogen bond network between bound inhibitors and F131V CA.[8] When the ortho correction was applied, but with the opposite sign relative to Figure 1 (vide infra), the 18 inhibitors were distributed into two groups (Figure 3). Inhibitors lacking fluorine at the 4- or 6-position are more sensitive to hydrophobicity ($m = -0.57$) than those containing fluorine at these positions ($m = -0.29$).[15]

The fact that ortho fluorination enhances binding while decreasing hydrophobicity was unexpected. Examination of crystal structures of several *o*-fluorobenzyl-derived inhibitors, however, indicated the origin of this effect. In solution, *o*-fluorobenzylamides should be more polar due to a likely intramolecular F·H contact (Figure 2).[14] On binding to CA, the fluorobenzyl ring is twisted away from the amide and this interaction is lost. This conformation of the inhibitors is more hydrophobic than indicated by

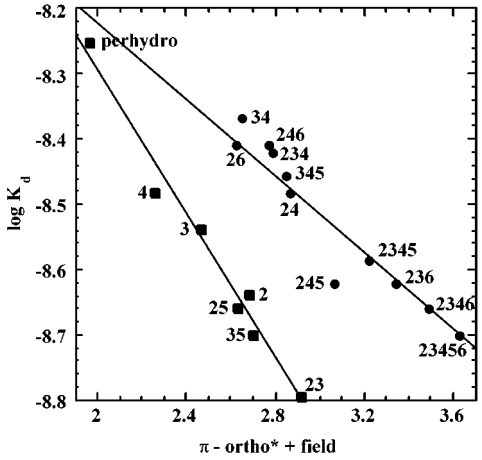

Figure 3. Correlation between log K_d-values for binding of fluoroaromatic inhibitors to F131V CA and fragment hydrophobicities, including corrections for field effects and intramolecular F···H contacts as described in the text. No ortho correction has been included for four inhibitors, as described in the text, indicated here by the ortho* on the *x*-axis. Please see Supporting Information for attempted correlations involving alternate parameters.

Table 1. F131V CAII-Inhibitor Dissociation Constants and Inhibitor Amide Distortions

fluorination pattern	K_d (nM)[a]	amide distortion[b] (degrees)
perhydro[c]	5.6	1
2-	2.3	1
4-	3. 3	9
2,3-	1.6	1
2,4-	3.3	11
2,5-	2.2	22
2,6-[d]	3.9	22
2,3,4-	3.8	24
2,4,6-	3.9	18
3,4,5-	3.9	2
2,3,4,5,6-[d]	2.0	26

[a] K_d-values reflect inhibitor dissociation from the primary binding site, measured by a fluorescence-based assay (ref 8). Uncertainties in these values are ±10%, on the basis of data from 2 to 3 separate measurements. [b] Dihedral angle deviation from perfect planarity, in the bound state. [c] Inhibitor with no fluorines on benzyl ring. [d] Inhibitor with fluorines at all positions on benzyl ring.

the log *P*-values measured in solution. Therefore, to obtain a measure of hydrophobicity relevant to binding, the ortho correction must be subtracted.

At this point, one anomaly remained, which suggested another structural feature relevant to binding affinity. The 2,5-, 2,6-, 2,3,4-, and 2,4,6-fluorinated inhibitors only fit the correlation if no ortho correction is applied at all (plotted as ortho* on the *x*-axis in Figure 3). Examination of the crystal structure of the 2,6-difluorobenzylamide-linked compound[9] suggested the origin of this effect. This molecule binds to CA with its amide twisted out of plane by 22°, allowing a weak polar interaction between the amide proton and fluorine at the ortho-position ($r_{N\cdots F}$ = 3.4 Å). Twisting of the amide prevents the orbital overlap required for the delocalized structure in Figure 2. The dipole moment of the amide is thereby attenuated, increasing the hydrophobicity of the molecule as a whole. Crystallographic studies of complexes of the 2,5-, 2,3,4-, and 2,4,6-substituted inhibitors[16] were subsequently

(15) Fluorines at the 4- and 6-position tend to be exposed to solvent and not in contact with ordered water molecules.

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found to support this hypothesis, since the amide is indeed twisted out of plane in each of these four crystal structures of F131V CA complexes with fluorobenzylamide-linked inhibitors (Table 1).

QSAR has enabled us to further interpret the affinity of fluoroaromatic inhibitors for carbonic anhydrase. We have found that both hydrophobicity and binding affinity are determined by the fragment hydrophobicities of our fluoroaromatic inhibitors, as well as the field and electronic effects of their substituents. Due to a change in the conformation of some ortho-fluorinated inhibitors on binding to CA, an intramolecular F···H contact has opposite effects on hydrophobicity and binding affinity. Ortho fluorines decrease the hydrophobicity of inhibitors but increase their affinity for F131V CA. This type of detailed understanding of the roles of hydrophobicity and electronic effects on the affinity of small molecule ligands for their receptors should enhance our ability to design similarly fluorinated inhibitors in other systems.

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Supporting Information Available: Correlations referred to in the text involving analysis of hydrophobicity and binding affinity; a table of fragment hydrophobicities, corrected for field effects and ortho fluorination, experimentally determined log *P*-values, and log K_ds of inhibitors; and a table of amide dihedral angle distortions for *o*-, *m*-, and *p*-substituted *N*-phenylmethyl acetamides, from the Cambridge Structural Database. This material is available free of charge via the Internet at http://pubs.acs.org.

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