ORGANIC LETTERS

1999
Vol. 1, No. 2
183−185

The Pattern of Fluorine Substitution Affects Binding Affinity in a Small Library of Fluoroaromatic Inhibitors for Carbonic Anhydrase

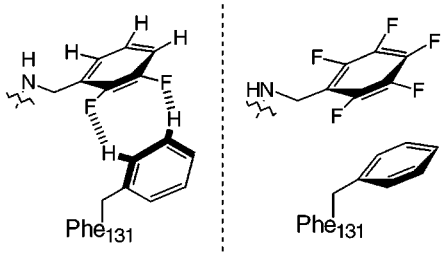
Jeffrey B. Doyon and Ahamindra Jain*

Department of Chemistry, Swarthmore College, Swarthmore, Pennsylvania 19081-1397

ajain1@swarthmore.edu

Received March 16, 1999

ABSTRACT



A library of fluoroaromatic inhibitors of carbonic anhydrase has been found to bind in a manner dependent on both hydrophobicity and the pattern of substitution of the fluoroaromatic ring. All of the compounds in the library bind to the protein with K_d < 3 nM. We have inferred two distinct binding modes from our data, which suggest two types of interactions that should be considered when designing fluorinated drugs.

The development of truly novel drugs begins with lead compounds, modified with groups that invoke novel interactions between receptor and ligand. We have used this approach previously to develop a small library of hydrophobic inhibitors of carbonic anhydrase II (CA), based on the known affinity of aromatic sulfonamides for this enzyme.[1] In this Letter, we have used one of these hydrophobic inhibitors, a benzyl amide, as a lead for the development of fluoroaromatic inhibitors of CA. These fluorinated compounds bind tightly to the protein due to their hydrophobicity and specific contacts between the fluoroaromatic ring and the protein. The tightest-binding inhibitor that we have identified in this study has K_d < 0.4 nM.

The 16 compounds comprising our library of inhibitors were prepared by coupling of fluoroaromatic benzyl amines to the *N*-hydroxysuccinimidyl ester of 4-carboxybenzenesulfonamide (Scheme 1). The benzyl amines were commercially available, in the case of seven members of the library. For the preparation of the other nine compounds, we either reduced a commercially available fluoroaromatic nitrile with $NaBH_4/CoCl_2$, in THF/H_2O,[2] or displaced a benzyl bromide with phthalimide, followed by hydrazinolysis. Each inhibitor was purified by SGC and was characterized by ^1H and ^{19}F NMR.[3] The actual concentration of inhibitor present in binding studies was determined by preparation of stock solutions of ≈20 mM in DMSO-d_6. These stock solutions were diluted 10-fold into DMSO-d_6 containing 1.94 mM DMF, which was used as an internal standard for the precise determination of inhibitor concentration by ^1H NMR integration of the methylene reso-

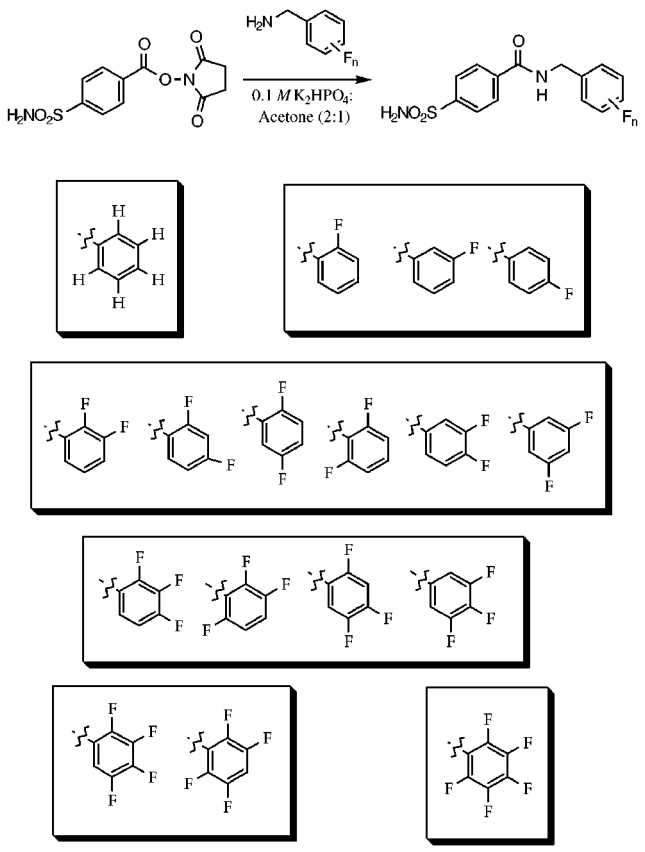
(1) Jain, A.; Alexander, R. W.; Christianson, D. C.; Whitesides, G. M. *J. Med. Chem.* **1994**, *37*, 2100−2105.

(2) Silverman, R. B.; Hawe, W. P. *J. Enzyme Inhib.* **1995**, *9*(3), 203−15.

(3) ^{13}C NMR was not used since α, β, and γ fluorines couple to carbon, affording spectra that are too complex to be used to assess purity.

10.1021/ol9905250 CCC: $18.00
Published on Web 06/04/1999

Scheme 1 . Fluorinated CA Inhibitors



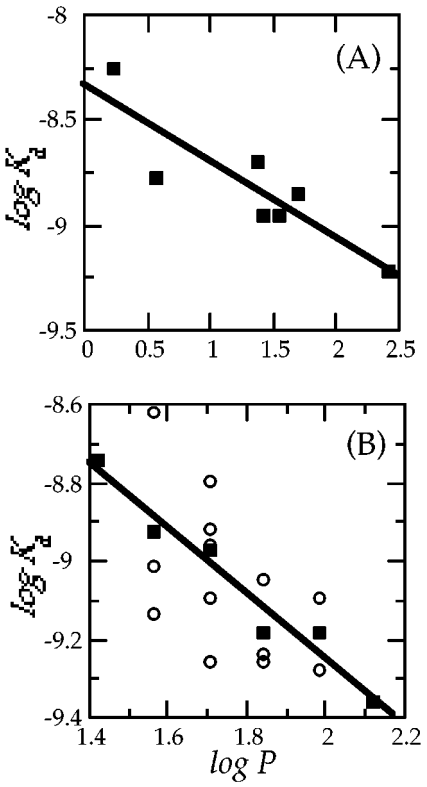


Figure 1. LFERs between log K_d and log P for hydrophobic (A) and fluorinated (B) inhibitors of CA. The slope of the dependence of binding affinity on hydrophobicity is −0.36 (r^2 = 0.775) for (A) and −0.83 (r^2 = 0.962) for (B). Note that the binding energies of several of the inhibitors are not resolved on this plot (see Table 1).

nance of each inhibitor relative to the methyl resonances from DMF.[4]

To elucidate the origin of tight binding of our library of inhibitors, we used CAChe[5] to calculate the octanol/water partition coefficient (log P) for each compound. A linear free energy relationship (LFER) between log K_d for each inhibitor[6] and its calculated value of log P is shown in Figure 1b.[7] The log K_ds for each extent of fluorination (non-, mono-, di-, tri-, tetra-, and penta-) have also been averaged, and these data, as a function of log P, have been fitted to a line with slope 0.83 (r^2 = 0.962). A similar plot of data from a comparable library of known nonfluorinated hydrophobic inhibitors[1] is shown in Figure 1a, and the slope of the best fit line to these data is 0.36 (r^2 = 0.775). These results are consistent with a model where hydrophobicity generally increases the affinity of inhibitors and where fluorine (Figure 1b) seems to be more hydrophobic than hydrocarbons (Figure 1a).[8] The large variability in the individual data points in Figure 1b, however, suggests that the *pattern* of fluorine substitution also affects the affinity of fluoroaromatic derivatives. Notably, in the case of mono- and difluorinated compounds, *para* substitution by fluorine seems to have little effect on binding affinity (Table 1).

On the basis of these results, on a crystal structure of the nonfluorinated benzyl amide derivative bound to CA,[1] and on preliminary data from ab initio calculations on a model system,[9] we propose the following two conformations for the interaction of fluoroaromatic inhibitors with the active site of carbonic anhydrase. In conformation I (Figure 2a), the *ortho* and *meta* hydrogens of Phe_{131} in the active site of CA interact with fluorines at the 2 and/or 3 position of our inhibitors via electrostatic contact(s) (F···H hydrogen bonds[10,11]). In conformation II (Figure 2b), the electron-rich aromatic ring of Phe_{131} interacts in a stacked manner with the electron-deficient ring of inhibitors bearing three or more fluorine atoms.[12,13] This conformation allows the molecular quadrupoles of the two aromatic rings to be aligned in their most favorable orientation.[14] These conformations are con-

(4) Jain, A.; Huang, S.-G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1994**, *116*, 5057−5062.
(5) Version 4.02, Oxford Molecular Group, 1998.
(6) K_ds were determined by the procedure described in ref 5.
(7) Hansch, C. W.; McClarin, J.; Klein, T.; Langridge, R. *Mol. Pharmacol.* **1985**, *27*, 493−498.
(8) An alternate interpretation would be that addition of fluorines to the benzyl amide ring lowers the pK_a of the sulfonamide, which should also increase the affinity of inhibitors, resulting in a more steep LFER.
(9) Manuscript in preparation. Preliminary data from calculations at MP2/6-31G* suggest that an F···H bond provides about 4 kJ/mol.
(10) Dunitz, J. D.; Taylor, R. *Chem. Eur. J.* **1997**, *3*, 89−98.
(11) Thalladi, V. R.; Weiss, H.-C.; Blaser, D.; Boese, R.; Nangia, A.; Desiraju, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 8702−8710.
(12) West, A. P.; Mecozzi, S.; Dougherty, D. A. *J. Phys. Org. Chem.* **1997**, *10*, 347−350.

Table 1. Dissociation Constants for Fluorinated Inhibitors of Carbonic Anhydrase

fluorine substitution pattern	K_d[a] (nM)
2	0.73
3	0.97
4	2.4
2, 3	1.1
2, 4	1.6
2, 5	0.55
2, 6	1.2
3, 4	1.6
3, 5	0.81
2, 3, 4	0.90
2, 4, 5	0.55
3, 4, 5	0.58
2, 3, 4, 5	0.80
2, 3, 5, 6	0.53
2, 3, 4, 5, 6	0.44
perhydro	1.8

[a] K_d values have uncertainties of ±10%, estimated from the errors in fits of data from multiple titrations with each inhibitor and from the variation in the K_ds obtained from separate experiments.

sistent with the known crystal structure of the nonfluorinated inhibitor bound to CA, since in that structure the distances between the closest carbons of the inhibitor and Phe_{131} are 4−5 Å. Rotation of carbon−carbon single bonds would therefore allow fluorines of the inhibitor and hydrogens of Phe_{131} to lie within 3 Å. It is important to note, however, that these two conformations alone do not allow us to fully explain the pattern of binding affinities reported in Table 1.

We are now in the process of obtaining crystallographic data for complexes of several of our fluoroaromatic inhibitors bound to CA. We are also measuring $^{19}F \rightarrow {}^{1}H$ NOEs of

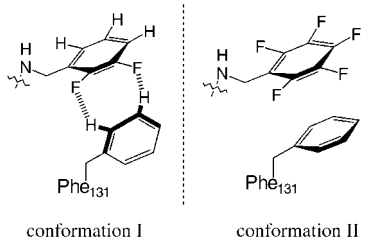


Figure 2. Proposed conformations of fluorinated inhibitors bound to CA. In conformation I, an electrostatic interaction is involved between fluorines of the inhibitor and hydrogens of Phe_{131}. In conformation II, the molecular quadrupoles of the fluorinated inhibitor and Phe_{131} are expected to interact favorably.

inhibitors bound to CA, to infer the interactions between these small molecules and residues in the active site.[15] Finally, we are expressing a mutant CA bearing pentafluorophenylalanine specifically at position 131 in the protein,[16] to test our hypothesis regarding the favorable interaction between stacked, opposite molecular quadrupoles. We hope that these data, when considered together, will illustrate the importance of the novel modes of interaction depicted in Figure 2 in the design of fluorinated drugs.

Acknowledgment. Edgar Lee and Ron Gefen contributed to the early stages of this project. Swarthmore College and the Howard Hughes Medical Institute provided financial support.

Supporting Information Available: ^{1}H and ^{19}F NMR spectra of each inhibitor and detailed descriptions of synthetic procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

OL9905250

(13) An ^{19}F NMR spectrum of the complex of the pentafluorobenzyl derivative bound to CA shows that the *ortho* and *meta* fluorines are shielded in the bound state, relative to their chemical shift when not bound. This shielding is consistent with conformation II.

(14) Wilcox and co-workers have recently presented data that refute this interpretation. Kim, E.; Paliwal, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 11192−11193.

(15) Dugad, L. B.; Cooley, C. R.; Gerig, J. T. *Biochemistry* **1989**, *28*, 3955−3960.

(16) Furter, R. *Protein Sci.* **1988**, *7*, 419−426.