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Unusually Weak Binding Interactions in Tetrazole−Amidine Complexes

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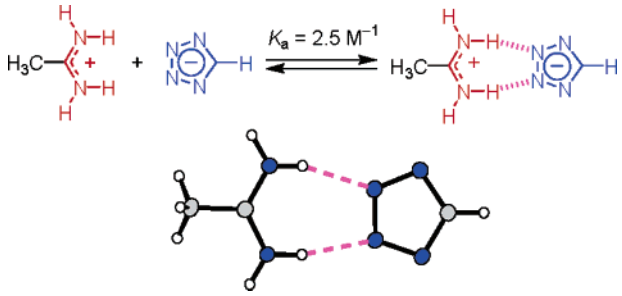
ABSTRACT



Tetrazoles frequently replace carboxylic acids in pharmaceutical drugs. However, while the binding modes of tetrazolate and carboxylate anions in amidinium complexes turns out to be similar, the association constant of the former is 2–3 orders of magnitude smaller in DMSO. Crystal structures revealed that the N···H–N hydrogen bonds in amidinium tetrazolates are bent (162° and 169°) and noticeably longer (N···N 2.96 Å) than corresponding hydrogen bonds in both amidinium carboxylates and ammonium tetrazolates.

Tetrazoles are acidic heterocycles that serve as important bioisosteric replacements for carboxylic acids in modern drug design.[1,2] The most prominent pharmaceutical application of tetrazoles is as angiotensin II receptor antagonists for the treatment of high blood pressure, and four out of six marketed drugs falling into this class contain a biphenyltetrazole pharmacophore.[3] However, no crystal structure of the membrane receptor binding site is available so far, while mutagenesis studies have led to conflicting evidence concerning the binding site of the drugs. Several reports suggest that the deprotonated tetrazole in these drugs interacts with a lysine and a histidine at the recognition site of the membrane receptor.[4,5] Others conclude that the tetrazole binds instead to a nearby arginine at the receptor binding site[6] or even that interactions involve both a lysine and an arginine.[7] What is clear, however, is that the replacement of a carboxylic acid by a tetrazole is not always beneficial in terms of pharmaceutical activity, in particular when the binding site contains an arginine.[8]

The arginine−carboxylate interaction possesses considerable importance in protein folding and a number of protein receptor−hormone (or protein receptor-drug) interactions. How does the analogous interaction between arginine and a tetrazole compare? Computational studies have indicated that a guanidinium carboxylate complex should be more stable

(1) The pK_a of the parent tetrazole is 4.6 (in 0.15 M aqueous KCl at 25 °C) and similar to that of acetic acid.

(2) Herr, R. J. *Bioorg. Med. Chem.* **2002**, *10*, 3379−3393.

(3) (a) Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D.; Timmermans, P. B. M. W. M. *J. Med. Chem.* **1996**, *39*, 625−656. (b) Naka, T.; Kubo, K. *Curr. Pharm. Design* **1999**, *5*, 453−472.

(4) (a) Noda, K.; Saad, Y.; Kinoshita, A.; Boyle, T. P.; Graham, R. M.; Husain, A.; Karnik, S. S. *J. Biol. Chem.* **1995**, *270*, 2284−2289. (b) Noda, K.; Saad, Y.; Karnik, S. S. *J. Biol. Chem.* **1995**, *270*, 28511−28514.

(5) In this respect, it is interesting to note that the crystal structure of a tetrazole-containing inhibitor of HIV-1 integrase shows the tetrazolate group of the inhibitor hydrogen-bonded to two protonated lysines of the protein receptor. Goldgur, Y.; Craigie, R.; Cohen, G. H.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040−13043.

(6) (a) Yamano, Y.; Ohyama, K.; Kikyo, M.; Sano, T.; Nakagomi, Y.; Inoue, Y.; Nakamura, N.; Morishima, I.; Guo, D.-F.; Hamakubo, T.; Inagami, T. *J. Biol. Chem.* **1995**, *270*, 14024−14030. (b) Vauquelin, G.; Fierens, F. L. P.; Gáborik, Z.; Minh, T. L.; De Backer, J.-P.; Hunyady, L.; Vanderheyden, P. M. L. *J. Renin Angiotensin Aldosteron Syst.* **2001**, *2*, S32−S36.

(7) Flower, D. R. *Biochim. Biophys. Acta* **1999**, *1422*, 207−234.

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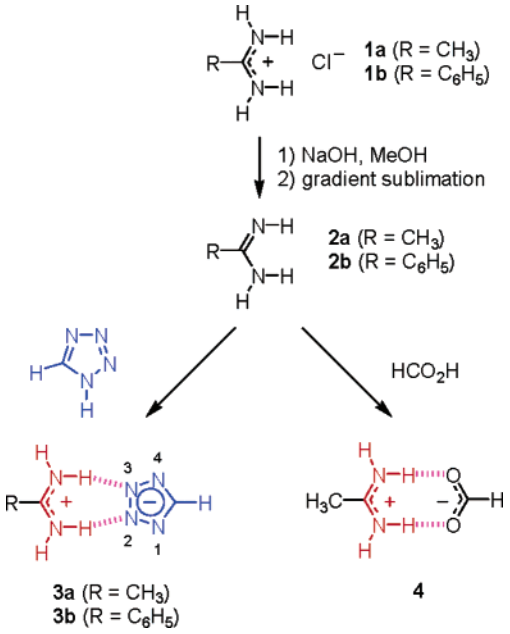
than a guanidinium tetrazolate.[9] To shed further light on tetrazole binding, we reinvestigated this problem using a simple amidine−tetrazole model system. Benzamidine is an obvious choice as a mimic for arginine because several para-substituted benzamidine derivatives are known to be potent, nonpeptide antagonists of the fibrinogen receptor, inhibiting platelet aggregation and preventing the coagulation of blood.[10] The benzamidine in these drugs occupies a recognition site for arginine in thrombin's Arg-Gly-Asp peptide sequence, the usual substrate for the protease.

Our previous work had already shown that tetrazoles form supramolecular complexes with imidazolines (a heterocyclic amidine) and *N*,*N*′-diethyl-substituted benzamidines, revealing distinct differences in binding modes between tetrazoles and carboxylic acids.[11,12] Tetrazolate ligands were found to display considerable flexibility in binding modes both in the crystal and in solution, although this was largely driven by the steric constraints imparted by the substituents at the nitrogens of the heterocyclic or *N*-substituted amidine. This paper describes the binding interactions between tetrazole and two *unsubstituted amidines*, benzamidine and acetamidine, which were selected as arginine mimics since they do not suffer from any such structural constraints.

Initial attempts to make the 1:1 amidine−tetrazole model complexes by ion exchange failed, and we decided to prepare the complexes by an acid−base reaction instead.[13] Treatment of the amidine hydrochlorides with a stoichiometric amount of sodium hydroxide in methanol gave the crude free amidine bases that were subsequently purified by gradient sublimation (Scheme 1). Acetamidine (**2a**) and benzamidine (**2b**) were stable in the absence of moisture. The free bases were then combined with an equimolar amount of tetrazole. A single recrystallization gave amidinium tetrazolates **3a**,**b** in high purity. Complex **4** was made analogously from acetamidine and formic acid.

Scheme 1. Synthesis of Amidine Complexes



Crystals suitable for X-ray crystallography were obtained by crystallization from methanol/acetonitrile. Figures 1 and 2 display the crystal structures of tetrazolate complexes **3a** and **3b**, as well as formate complex **4**. The most striking feature is the structural similarity in the binding modes of the tetrazolate and the formate. This was somewhat unexpected as theoretical calculations predict the highest charge density to be located on N^1 and N^4 of the tetrazolate ring (for numbering of the nitrogens, see Scheme 1).[9a,14] Consequently, these two nitrogen atoms should be preferred hydrogen-bond acceptor sites, thus favoring a side-on binding arrangement of the tetrazolate ligand (through N^1 and N^2) as seen in all previous crystal structures of complexes involving tetrazolates and N-substituted amidines.[11,12] However, binding of the tetrazolate to both NH_2 groups of an amidine molecule in **3a**,**b** clearly occurs end-on, through nitrogen atoms N^2 and N^3.

The three crystal structures display an extended array of hydrogen bonds, which effectively involves every amidine NH proton and tetrazolate nitrogen (as well as formate oxygen atom). Both the formate and tetrazolate are almost coplanar to the amidinium group of the acetamidine, with torsion angles between the two binding groups being 1.11(2)° and 5.5(4)°, respectively. Only the amidine group of

(8) Examples include prostaglandin $F_{2\alpha}$ analogues: (a) Schuster, V. L.; Itoh, S.; Andrews, S. W.; Burk, R. M.; Chen, J.; Kedzie, K. M.; Gil, D. W.; Woodward, D. F. *Mol. Pharmacol.* **2000**, *58*, 1511−1516. Tyrosine phosphatase inhibitors: (b) Liljebris, C.; Larsen, S. D.; Ogg, D.; Palazuk, B. J.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 1785−1798. And glutamate receptor antagonists: Frølund, B.; Greenwood, J. R.; Holm, M. M.; Egebjerg, J.; Madsen, U.; Nielsen, B.; Bräuner-Osborne, H.; Stensbøl, T. B.; Krogsgaard-Larsen, P. *Bioorg. Med. Chem.* **2005**, *13*, 5391−5398.

(9) (a) Zablocki, J. A.; Miyano, M.; Rao, S. N.; Panzer-Knodle, S.; Nicholson, N.; Feigen, L. *J. Med. Chem.* **1992**, *35*, 4914−4917. (b) Suvire, F.; Floridia, R.; Giannini, F.; Rodriguez, A.; Enriz, R.; Jauregui, E. *Molecules* **2000**, *5*, 583−584.

(10) (a) Alig, L.; Edenhofer, A.; Hadváry, P.; Hürzeler, M.; Knopp, D.; Müller, M.; Steiner, B.; Trzeciak, A.; Weller, T. *J. Med. Chem.* **1992**, *35*, 4393−4407. (b) Ewing, W. R.; Becker, M. R.; Manetta, V. E.; Davis, R. S.; Pauls, H. W.; Mason, H.; Choi-Sledeski, Y. M.; Green, D.; Cha, D.; Spada, A. P.; Cheney, D. L.; Mason, J. S.; Maignan, S.; Guilloteau, J.-P.; Brown, K.; Colussi, D.; Bentley, R.; Bostwick, J.; Kasiewski, C. J.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. *J. Med. Chem.* **1999**, *42*, 3557−3571. (c) Adler, M.; Davey, D. D.; Phillips, G. B.; Kim, S.-H.; Jancarik, J.; Rumennik, G.; Light, D. R.; Whitlow, M. *Biochemistry* **2000**, *39*, 12534−12542.

(11) (a) Peters, L.; Fröhlich, R.; Boyd, A. S. F.; Kraft, A. *J. Org. Chem.* **2001**, *66*, 3291−3298. (b) Kraft, A.; Peters, L.; Fröhlich, R. *Acta Crystallogr. Sect. C* **2002**, *58*, o272−o274.

(12) Kraft, A.; Osterod, F.; Fröhlich, R. *J. Org. Chem.* **1999**, *64*, 6425−6433.

(13) Acetamidinium hydrochloride **1a** (2.00 g, 21.2 mmol) was dissolved in methanol (20 mL). To this was added a solution of NaOH (853 mg, 21.3 mmol) in MeOH (60 mL). The mixture was then concentrated in a vacuum, and the residue was sublimed at 60 °C/0.4 mbar to yield **2a** in form of a colorless oil (150 mg, 2.59 mmol, 23%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.75 (s, 3 H), 5.36 (br s, 3 H). A solution of tetrazole in acetonitrile (0.45 M, 5.45 mL, 2.45 mmol) was added to a solution of acetamidine **2a** (142 mg, 2.45 mmol) in MeOH (2 mL). The mixture was concentrated in a vacuum, and the residue was recrystallized from hot MeOH/MeCN to yield **3a** as a colorless solid (165 mg, 53%). DSC: 149 °C (145 J g^{-1}). ^{1}H NMR (400 MHz, DMSO-d_6, 60 mg/0.7 mL): δ 2.19 (s, 3 H), 8.23 (s, 1 H), 9.28 (br s, 4 H). ^{13}C NMR (50 MHz, DMSO-d_6, 60 mg/0.7 mL): δ 18.69 (CH_3), 149.16 (tetrazole CH), 168.42 (amidine C=N). IR (KBr, cm^{-1}): ν 3277, 1703, 1530, 1442, 1377, 1281. Anal. Calcd. for $C_3H_8N_6$ (128.14): C, 28.24; H, 6.36; N, 64.61. Found: C, 28.12; H, 6.29; N, 65.59.

(14) Biot, C.; Bauer, H.; Schirmer, R. H.; Davioud-Charvet, E. *J. Med. Chem.* **2004**, *47*, 5972−5983.

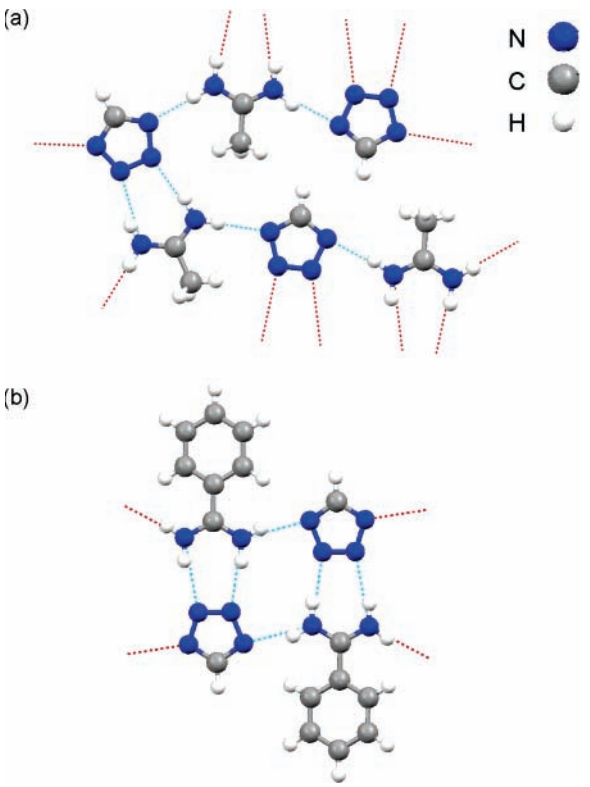


Figure 1. (a) Crystal structure and hydrogen-bonded arrangement of acetamidinium tetrazolate complex **3a**. (b) Crystal structure and hydrogen-bonded arrangement of benzamidinium tetrazolate complex **3b**.

the benzamidinium complex is tilted slightly further, by 22.8(1)°, to the plane of the tetrazolate.

Complex **4** possesses almost linear hydrogen bonds between the formate oxygen and amidinium nitrogen, with O···H−N angles of 178.6(2) and 176.6(2)° and O···N distances of 2.841(2) and 2.870(2) Å. In contrast, the hydrogen bonds in the corresponding acetamidinium tetrazolate **3a** are noticeably bent, with N···H−N angles in the range 161(2)−169(2)°. The H-bonds in **3a** are also significantly longer,

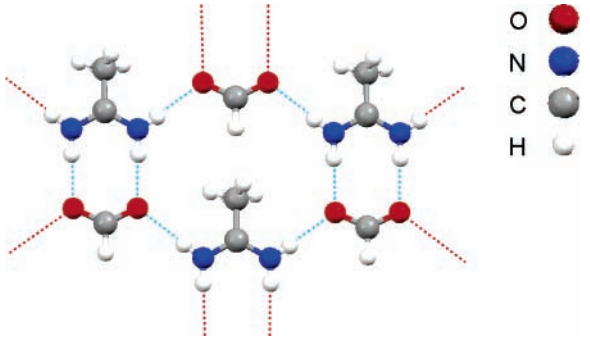


Figure 2. Crystal structure and hydrogen-bonded arrangement of complex **4** (CH_3 group disordered).

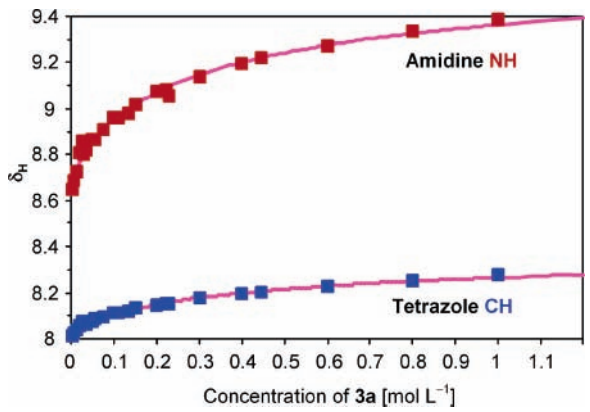


Figure 3. Concentration dependence of the tetrazolate CH and amidine NH ^{1}H NMR chemical shifts for complex **3a** in DMSO-d_6 at 25 °C. The curves represent the calculated isotherms for 1:1 binding, whereas the filled squares are experimental values. Data were obtained from two independent experiments.

with N···N distances of 2.919(3)−2.985(3) Å. Similar angles and distances are observed in complex **3b**. In both amidinium−tetrazolate complexes, the tetrazole binds in the same way as a carboxylic acid. Both the nonlinear hydrogen bond angles and the longer H-bonds serve to weaken the attraction between the tetrazolate and amidinium ions.

We note that the tetrazolate ions show shorter H-bonds when they hydrogen bond side-on through N^1 and N^4 to neighboring amidinium ions [with N···N distances of 2.919(3)−2.963(3) Å and N···H−N angles of 167(2)−177(2)° for **3a**]. This is in keeping with the theoretical calculations referred to above. Such a difference in hydrogen bond lengths was not observed for complex **4**. Its intermolecular H-bonds between neighboring complexes (O···H−N angle 176.6°, O···N distance 2.87 Å) were almost identical in length to the intracomplex hydrogen bonds between the formate and amidinium ion, forming an eight-membered ring.

Saltlike complexes **3a**,**b** and **4** were soluble in polar solvents, including water, methanol, and DMSO. Supramolecular binding was only observed in DMSO, and even in this solvent, weak binding was evident from the high concentrations that were needed before chemically induced shifts could be observed. A Job plot supported a 1:1 stoichiometry in DMSO.[15] Figure 3 depicts a typical NMR dilution curve of complex **3a** showing the concentration dependence of the tetrazolate CH and amidine NH singlets. Nonlinear regression analysis revealed a rather weak association constant, K_a, of 2.5 ± 0.5 M^{-1} for acetamidine complex **3a**, and even the analogous benzamidine complex **3b** gave rise to only a marginally higher K_a of 18 ± 3 M^{-1}.[16] In contrast, the K_a of complex **4** was found to be 1600 ± 100 M^{-1}, about 2−3 orders of magnitude larger than the binding of tetrazolates in complexes **3a**,**b**.[17]

Electrostatic arguments, as previously suggested,[9a] cannot be the sole reason for the small association constants in

(15) (a) Job, P. *Ann. Chim.* **1928**, *9*, 113−203. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626−4636.

amidinium tetrazolates. While charge distribution in the tetrazolate may lead to some reduction in the anion's electrostatic attraction by an amidinium cation, the crystal structures of complexes **3a**,**b** reported in our paper provide evidence of hydrogen bonds between the amidinium ion and the two nitrogens N^2/N^3 of the tetrazolate, even though these nitrogens possess the lowest charge density. The weaker binding is more likely due to the lengthening of N···H−N hydrogen bonds in complexes **3a**,**b** by 0.1 Å to, on average, 2.96 Å compared with H-bonds between a tetrazolate and a protonated amine (average 2.85 Å), since the N···H−N angles of the hydrogen bonds in amidinium tetrazolates and ammonium tetrazolates are essentially the same.[18,19]

These findings suggest why tetrazoles are not always suitable substitutes for carboxylic acids, particularly when the crucial binding interaction requires the tetrazolate to form two strong hydrogen bonds to the guanidinium group of an arginine. Notable examples include inhibitors of the fibrinogen receptor,[9a] as well as inhibitors of the prostaglandin transporter,[8a] where replacement of a crucial carboxylic acid group by a tetrazole lowered the inhibitor efficiency by over 2 orders of magnitude and, in one case, even led to an inactive compound. However, when binding involves other cationic sites such as lysine or histidine, tetrazoles should still remain highly effective acidic pharmacophores and valuable bioisosteric replacements for carboxylic acids. In all likelyhood, tetrazole-containing angiotensin II receptor antagonists fall into this category.

Acknowledgment. We gratefully thank the School of Engineering & Physical Sciences at Heriot-Watt University for support.

Supporting Information Available: Experimental procedures, characterization details, potentiometric titration of tetrazole, NMR dilution studies, Job plot, and X-ray crystal structural information. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The hyperbolic shape of the dilution curve was fitted to 1:1 host−guest complexation: (a) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442−6448. (b) Wilcox, C. S. In *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1991; pp 123−143. (c) Macomber, R. S. *J. Chem. Educ.* **1992**, *69*, 375−378.

(17) Carboxylates bind to amidinium ions with K_a values that are typically of the order of 1000−3500 M^{-1} in DMSO at room temperature, although K_a tends to be lower if the carboxylate is substituted with a strongly electron-withdrawing residue: (a) Deng, Y.; Roberts, J. A.; Peng, S.-M.; Chang, C. K.; Nocera, D. G. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2124−2127. (b) Papoutsakis, D.; Kirby, J. P.; Jackson, J. E.; Nocera, D. G. *Chem. Eur. J.* **1999**, *5*, 1474−1480. (c) Kirby, J. P.; van Dantzig, N. A.; Chang, C. K.; Nocera, D. G. *Tetrahedron Lett.* **1995**, *36*, 3477−3480. (d) Roberts, J. A.; Kirby, J. P.; Nocera, D. G. *J. Am. Chem. Soc.* **1995**, *117*, 8051−8052. (e) Krechl, J.; Smrčková, S.; Kuthan, J. *Collect. Czech. Chem. Commun.* **1990**, *50*, 460−468.

(18) For examples, see: (a) Lyakhov, A. S.; Voitekhovich, S. V.; Gaponik, P. N.; Ivashkevich, L. S. *Acta Crystallogr. Sect. C* **2003**, *59*, o22−o23. (b) Alkorta, I.; Rozas, I.; Elguero, J. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2671−2675. (c) Chertanova, L. F.; Struchkov, Y. T.; Sopin, V. F.; Kovalenko, V. I.; Timokhov, V. N.; Fronchek, E. V. *Chem. Heterocycl. Compd.* **1989**, 655−657.

(19) The low dissociation constant of ammonium phenyltetrazolate of 2.87×10^{-5} M in DMSO at 25 °C (determined by electrical conductivity measurements) correlates well with the crystallographically observed short hydrogen bond in ammonium tetrazolates. Tsentovskii, V. M.; Bashkirtseva, V. E.; Evgen'ev, M. I.; Ivanova, Z. P.; Poplavskii, V. S.; Ostrovskii, V. A.; Koldobskii, G. I. *Chem. Heterocycl. Compd.* **1984**, 1238−1240.