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A switch in enantiomer preference between mitochondrial F_1F_0-ATPase chemotypes

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Abstract—The preferred absolute configuration of two series of F_1F_0-ATP synthase inhibitors was determined. Although the configuration of the active enantiomer in each series is different, each series presents the same 'triaryl' pharmacophore to the enzyme binding site.

Respiratory chain (or oxidative) phosphorylation is the process by which cells obtain energy under aerobic conditions, and ATP synthase (F_1F_0-ATPase) is the central enzyme in this process. Under normal conditions in mitochondria, this membrane-bound, multisubunit protein synthesizes ATP from ADP and P_i using a proton gradient established and maintained by the electron transport chain.[1] Under hypoxic conditions, the proton gradient disappears and F_1F_0-ATPase switches modes to act as an ATP hydrolase.[2] This hydrolase activity wastes ATP, making it unavailable to drive cellular processes, including those which maintain cellular function and viability.

Salvage of ATP through selective ATPase hydrolase inhibition may provide a novel mechanism for the treatment of unstable angina, MI, and other vascular diseases.[3] In pursuit of this objective, we and others have disclosed selective inhibitors of mitochondrial ATP hydrolase as potential antiischemic agents.[4] In this paper, we report the synthesis, absolute configuration, and ATPase inhibitory activity for several analogs in two closely related chemotypes (thiocarbamates and acylguanidines) and the surprising finding that the two series exhibit opposite stereospecificity for enzyme inhibition.

The synthesis of the enantiomeric thiocarbamate inhibitors **4** and **5** is outlined in Figure 1. The absolute configuration of **1a** was assigned as *R*-based on both the mode of synthesis[5] and by correlation to econazole, an antifungal agent of known absolute configuration.[6] This synthesis constitutes the first correlation of optical rotation data[7] to the absolute configuration of compounds **1**–**3**. The synthesis and separation of acylguanidines **6a** and **6b** were carried out as previously described.[4a]

Crystallization of **6b** from acetonitrile produced crystalline material suitable for the determination of a solid state structure and assignment of the absolute configuration by anomalous scattering.[8] On this basis, the absolute configuration of **6b** is confirmed as *S*- (Fig. 2).

Compounds **4**–**6** were evaluated as inhibitors of bovine F_1F_0-ATPase using the assay systems previously

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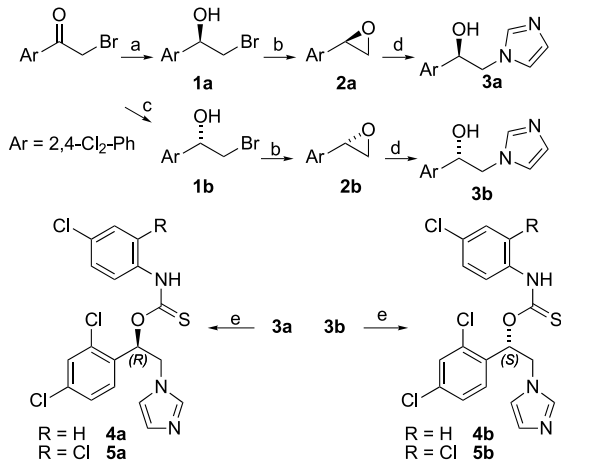
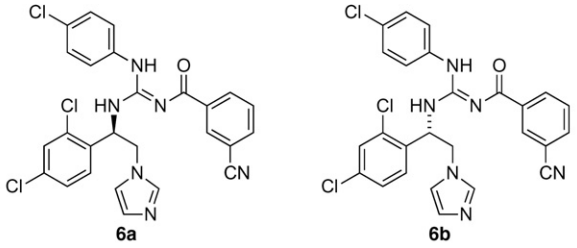


Figure 1. (a) (−)-DIP-Cl, THF −25 °C, 16 h, 79%; (b) 5 N aq. NaOH, THF; (c) (+)-DIP-Cl, THF −25 °C, 16 h, 98%; (d) imidazole, NaH, DMF; −5 °C to rt, 16 h, 47%; (e) 4-Cl-C_6H_4-NCS or 2,4-Cl_2-C_6H_3-NCS, *tert*-butylimino-tri(pyrrolidino)-phosphorane, CH_3CN, rt, 6 h, 74%.

described[3] and the data are collected in Table 1. The pairwise comparisons of enantiomers in both chemotypes (**4a/b**, **5a/b**, and **6a/b**) indicate enantiomer preferences. The preference for (*R*)- is moderate (28-fold) for **4a** and **4b**, but is stronger (116-fold) for **5a/b**. The strongest preference (5600-fold) is for **6b** versus **6a**, however, the (*S*)-enantiomer is the more potent inhibitor.

Although initially surprising in light of the structural similarity of the two chemotypes, the SAR is consistent with the distinct conformational preferences of the two chemotypes. The H-bonded conformation in the solid-state structure of **6b** represents the low energy conformation about the acylguanidine.[9] To overlay the three aromatic rings of enantio-matching **5b** onto the corresponding groups in **6b**, **5b** would have to adopt a conformation that is 1.7 kcal/mol higher than the preferred thiocarbamate conformer, a situation that is energetically unfavorable. However, one of the low energy conformers of enantio-opposite **5a** can be superimposed onto **6b** if the overlay matches, respectively, the imidazole, the C-linked dichlorophenyl and the N-linked dichlorophenyl of **5a** with the imidazole, the C-linked dichlorophenyl ring and the *cyanobenzoyl* groups of **6b**. Qualitatively, this alignment of the aromatic rings and their substituents appears better than all of the possible alignments of **5b** and **6b** (Fig. 3).

Additionally, the role of the cyanobenzoyl moiety as a pharmacophore is consistent with our previous SAR.[10] So, although the absolute configurations of the two chemotypes are different, the potent enantiomers (**5a** and **6b**) each present the hydrolase with an appropriate 'triaryl' pharmacophore array.



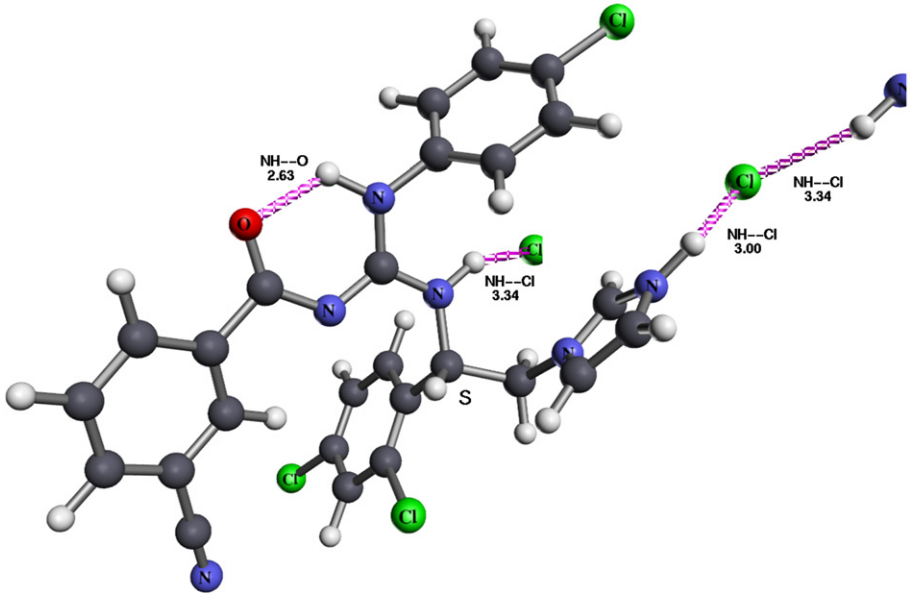


Figure 2. Solid state structure of **6b**.

Table 1. Data for inhibition of mitochondrial F_1F_0-ATP hydrolase and synthase by compounds **4-6**

Compound	Absolute configuration	Inhibition of mATP hydrolase (nM)	Inhibition of mATP synthase (nM)
4a	*R*	140 ± 41	>100,000 (racemate)
4b	*S*	3600 ± 1100	>100,000 (racemate)
5a	*R*	19 ± 2.7	>100,000
5b	*S*	2200	>100,000 (racemate)
6a	*R*	>100,000	>100,000
6b	*S*	18 ± 16	>100,000

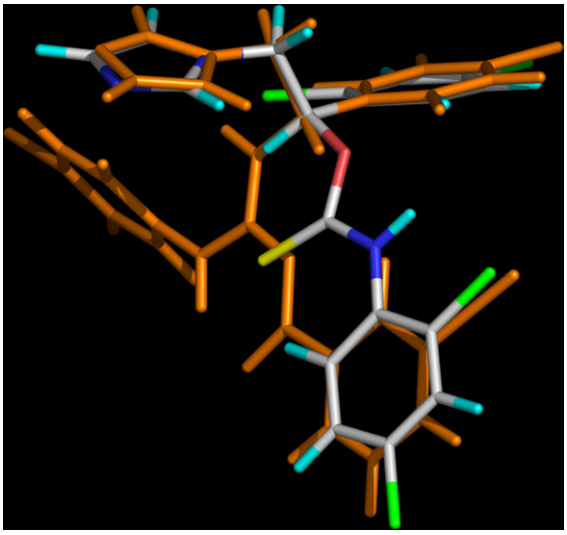


Figure 3. Overlay of thiocarbamate **5a** (colored by atom type) and acylguanidine **6b** (salmon).

In summary, we have determined the absolute configurations for two related series of potent mATPase inhibitors. We found that the stereospecificity for hydrolase inhibition in the two series is opposite to one another and postulate that the SAR is consistent with presentation of a 'triaryl pharmacophore' by both chemotypes.

References and notes

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7. **1a** $[\alpha]_D$ −43 (*c* = 7.6 mg/mL); **1b** $[\alpha]_D$ +40 (*c* = 7.6 mg/mL); **2a** ($[\alpha]_D^{rt}$ −55 (*c* = 8.6 mg/mL); **2b** ($[\alpha]_D$ +56 (*c* = 6.3 mg/mL); **3a** ($[\alpha]_D$ −79 (5.1 mg/mL); **3b** ($[\alpha]_D$ +83 (5.0 mg/mL); **4a** ($[\alpha]_D$ +85 (*c* = 5.6 mg/mL), 95% ee); **4b** ($[\alpha]_D$ −90 (*c* = 5.1 mg/mL), 97% ee); **5a** ($[\alpha]_D$ +68 (*c* = 5.2 mg/mL), 98% ee); **5b** ($[\alpha]_D$ −73 (*c* = 5.1 mg/mL), >99% ee). Rotations were measured at rt in $CHCl_3$. Enantiomeric excess (% ee) was determined by chiral chromatography.
8. Crystallographic data (excluding structure factors) for **6b** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 251489 and 251490. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
9. Semi-empirical gas-phase conformations and heats of formation were calculated using AMPAC's SAM-1 parameter set. For SAM-1, see Dewar, M. J. S.; Jie, C.; Yu, J. *Tetrahedron* **1993**, *49*, 5003–5038, AMPAC 6.55, Semichem Inc., Box 1649, Shawnee KS 66222.
10. The conformationally similar cyanoguanidine-based inhibitors lack this group and are generally inferior inhibitors (Ref. 4a). In addition, the overlay aligns the *m*-cyano substituent in **6b** and the *o*-chloro group in **5a**. The presence and location of both of these substituents are important for inhibitor potency (Ref. 4a and Table 1).