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N-[1-Aryl-2-(1-imidazolo)ethyl]-guanidine derivatives as potent inhibitors of the bovine mitochondrial F_1F_0 ATP hydrolase

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Abstract—A series of substituted guanidine derivatives were prepared and evaluated as potent and selective inhibitors of mitochondrial F_1F_0 ATP hydrolase. The initial thiourethane derived lead molecules possessed intriguing in vitro pharmacological profiles, though contained moieties considered non-drug-like. Analogue synthesis efforts led to compounds with maintained potency and superior physical properties. Small molecules in this series which potently and selectivity inhibit ATP hydrolase and not ATP synthase may have utility as cardioprotective agents.

The mitochondrial F_1F_0 ATPase is responsible for the majority of ATP synthesis in mammalian cells. However, it can also hydrolyze ATP during cellular anoxia so the direction of this enzymes catalytic activity can have a profound effect on cellular metabolism.[1] During anoxia this electrochemical gradient collapses, and mitochondrial F_1F_0 ATPase switches to its hydrolytic state.[2] This hydrolysis of ATP serves no useful purpose and depletes the ischemic tissue of ATP leading to cell death.[3] The mitochondrial F_1F_0 ATP synthase activities in vesicles from ischemic muscle are substantially (~50–80%) less than those of control muscle. A naturally occurring inhibitor, IF_1 protein (IF_1), may be bound to the F_1 unit under ischemic conditions to inhibit the mitochondrial F_1F_0 ATP hydrolase activity of the enzyme. However, the activity of IF_1 is highly pH dependent and in severe conditions it can only provide modest control.[4] The conversion of mitochondrial F_1F_0 ATP synthase to hydrolase is reversible, since addition of oxygen to the mitochondria of ischemic muscle can reactivate the mitochondrial F_1F_0 ATPase and the ATP levels can return to control.

Several inhibitors of F_1F_0 ATPase have been described, including efrapeptin,[5] oligomycin,[6] aurovertin B,[7] and azide.[8] Oligomycin targets F_0 and reportedly postpones cell injury by preventing ATP loss during ischemia.[9] Prior to our recent disclosure of benzopyran derivatives related to compound **1**,[10] the known inhibitors of mitochondrial F_1F_0 ATPase have all been large molecules, and consequently not orally bioavailable. Through subsequent directed screening efforts, a second small molecule template was discovered, as represented by thiourethanes such as **2**. In this paper, we describe structural modifications within this series which led to cyano- and acyl-guanidine derivatives which posses potent and selective inhibitory activity against

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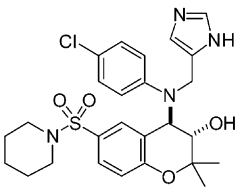
mitochondrial F_1F_0 ATP hydrolase without inhibition of the synthase. A select potent compound was found to be orally active in rats.



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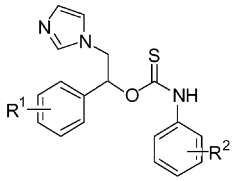
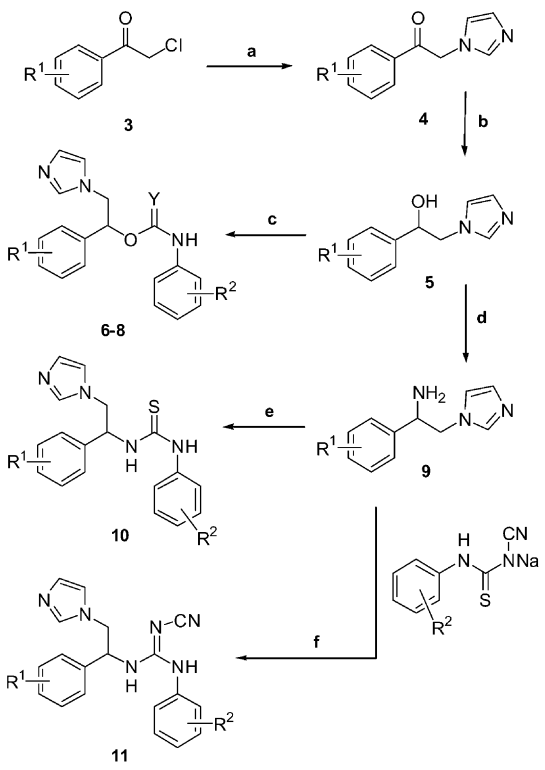


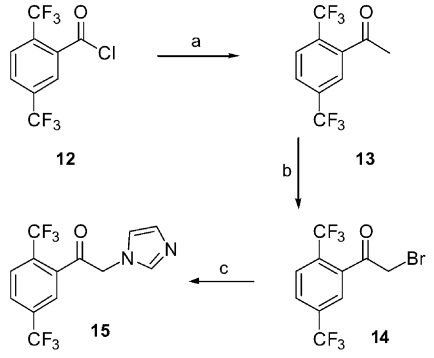
2

The guanidine derivatives were generally prepared according to Scheme 1. The commercially available substituted chloroacetophenones **3** were reacted with three molar equivalents of imidazole in acetonitrile to give 1-imidazoloacetophenones **4**. Reduction with sodium borohydride in ethanol provided alcohol **5**, which readily reacted with commercially available *N*-arylisothiocyanates to give thiocarbamates (**6a**–**e** and **7**). The carbamate analogues **8** were prepared from benzylic alcohols **5** and 2,4-dichlorophenylisocyanate. Conversion of alcohols **5** to amines **9** was carried out in two steps: reaction of **5** with diphenylphosphorylazide (DPPA) in presence of diazabicycloundecane (DBU) in THF,[11] and subsequent reduction of the resultant azide with triphenylphosphine in aqueous THF. Thioureas **10** were prepared by reacting amines **9** with appropriately substituted phenylthioisocyanates. Coupling of amines **9** with the sodium salts of *N*-cyano-*N*′-arylthioureas, prepared by addition of sodium cyanamide to arylthioisocyanates, in the presence of *N*,*N*-dimethyamino-ethyl-*N*′-propylcarbodiimide in DMF gave the desired cyanoguanidines (**11a**–**f**).[12]



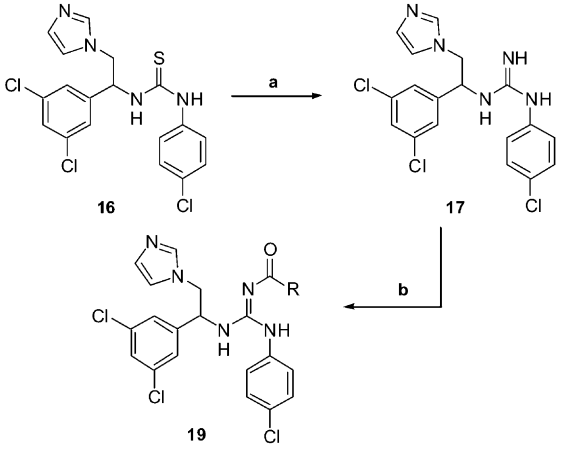
Scheme 1. General synthesis of thiocarbamates and cyanoguanidines: (a) imidazole, CH_3CN; (b) $NaBH_4$, EtOH; (c) NaH, DMF, 0 °C, R-NCS or RNCO; (d) DPPA, DBU, THF; then Ph_3P, THF, H_2O; (e) arylisothiocyanate; (f) *N*,*N*-dimethylaminoethyl-*N*′-propylcarbodiimide, DMF.

For the construction of 2,5-bis-trifluorophenyl substituted analogues, the required bromoacetophenone **14** was prepared by a two-step sequence (Scheme 2). The commercially available benzoyl chloride **12** was converted to acetophenone **13** by treatment with methylmagnesium bromide in the presence of tributylphosphine,[13] followed by the bromination of the resulting acetophenone **13** to give bromoacetophenone **14**. Treatment of **14** with imidazole gave the imidazolo-acetophenone **15**, which could then be carried on to the final product **11f** using the sequence described in Scheme 1.



Scheme 2. (a) MeMgBr, Bu_3P; (b) Br_2, HOAc; (c) imidazole, CH_3CN.

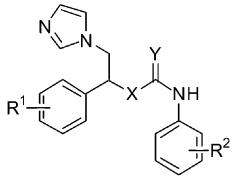
The acylguanidine derivatives **19** were prepared in a single step from unsubstituted guanidines **17** by acylation with carboxylic acids **18** in the presence of carbonyl diimidazole (Scheme 3). Guanidines **17** were generated from the thioureas **16** by treatment with methanolic ammonia (6 N) in the presence of HgO.



Scheme 3. (a) NH_3, MeOH, HgO; (b) RCO_2H (**18**), CDI.

Since the mitochondrial F_1F_0 ATPase is ubiquitous, several sources (rat, porcine, bovine, human) of the enzyme were evaluated. Because of its low cost and availability, the bovine enzyme was chosen for screening. Based on minimum structural requirements for inhibitors in our previously disclosed series based on analogues of compound **1**,[10] we undertook targeted screening of compounds with structural similarity. Upon doing so, we discovered that thiourethane

Table 1. Inhibition of bovine mitochondrial F_1F_0 ATP hydrolase assay results for **6**–**20**[a]



Compd	R^1	R^2	X	Y	F_1F_0 ATP hydrolase inhibition IC_{50} (μM)[b]
6a	2,4-Cl_2	4-Cl	O	S	0.43 ± 0.16
6b	2,4-Cl_2	H	O	S	3.60 ± 1.10
6c	2,4-Cl_2	4-Me	O	S	0.66 ± 0.12
6d	2,4-Cl_2	2,4-Cl_2	O	S	0.030 ± 0.013
6e	2,4-Cl_2	2,4-Me_2	O	S	0.23
7	2,4-Me_2	4-Cl	O	S	31.4
8	2,4-Cl_2	2,4-Cl_2	O	O	6.77
10	2,4-Cl_2	2,4-Cl_2	NH	S	2.41
11a	2,4-Cl_2	2,4-Cl_2	NH	NCN	0.60 ± 0.16
11b	2,4-Cl_2	4-Cl	NH	NCN	8.8
11c	2,4-Cl_2	2-Cl	NH	NCN	2.23
11d	2,4-Cl_2	2,3-Cl_2	NH	NCN	2.49 ± 0.72
11e	2,4-Cl_2	3-Cl	NH	NCN	9.17
11f	2,5-$(CF_3)_2$	2,4-Cl_2	NH	NCN	0.71 ± 0.34
19a	2,4-Cl_2	4-Cl	NH	CO(3-CN-Ph)	0.033 ± 0.02
19b	2,4-Cl_2	4-Cl	NH	CO(4-CN-Ph)	0.28
19c	2,4-Cl_2	4-Cl	NH	CO(4-Cl-Ph)	0.082 ± 0.03
19d	2,4-Cl_2	4-Cl	NH	COEt	2.27
20a[c]	2,4-Cl_2	4-Cl	NH	CO(3-CN-Ph)	0.018 ± 0.016
20b[c]	2,4-Cl_2	4-Cl	NH	CO(3-CN-Ph)	> 100

[a] The assays for the mitochondrial F_1F_0 ATP hydrolase and synthase are described.[14]
[b] For all compounds, mitochondrial F_1F_0 ATP synthase $IC_{50} > 100$ μM.
[c] Compounds **20a** and **20b** are the independent resolved enantiomers of compound **19a**, though the absolute stereochemistry is unknown.

compound **6a** is a potent inhibitor of mitochondrial F_1F_0 ATP hydrolase ($IC_{50} = 0.43$ μM), though does not inhibit the synthase ($IC_{50} > 300$ μM) (Table 1). In the aniline region of **6a**, it was discovered that chloro substitution is important, as the protio analogue **6b** is about 10-fold less active. This loss of activity was not fully restored in its 4-methyl analogue **6c**, possibly indicating the requirement of an electron withdrawing group. The dichloro compound **6d** was an order of magnitude more potent ($IC_{50} = 0.03$ μM), while maintaining selectivity for inhibition of the ATP hydrolase. The corresponding dimethyl analogue **6e** is approximately 10-fold less active ($IC_{50} = 0.23$ μM) than the corresponding dihalo compound **6d**, and dimethyl analogue **7** is nearly devoid of inhibitory activity, suggesting that halogen substitution is favorable in both the 1-aryl-2-imidazoloethyl and aniline regions of the molecule.

Although thiourethane **6d** is a potent inhibitor in vitro, this compound is not considered to be a drug like molecule. It was observed to exhibit chemical instability in aqueous buffer by reversion to isothiocyanate and aniline, and carries toxic liabilities associated with the thiourethane functionality.[15] We therefore turned our attention to the exploration of stable functionalities to replace the thiourethane moiety. Replacement of the thiourethane group in **6d** with a thiourea gave **10**, which is 50-fold less potent than **6d**. The carbamate derivative **8** and the cyanoguanidine derivative **11a** were also less potent inhibitors. Despite the reduced in vitro potency associated with this initial cyanoguanidine containing analogue **11a**, we focused on this moiety for further chemotype optimization, as this functionality is known to exist in more drug-like molecules and can be readily prepared.[16]

The structure–activity relationships of cyanoguanidine derivatives are shown in Table 1 (**11a**–**f**). Mono-chloro analogues **11b**, **11c**, and **11e** all retain a moderate level of activity, though all are inferior to 2,4-dichloro analogue **11a**. Unlike the thiourethane series of inhibitors, 2,4-dichloro substitution on the 1-aryl-2-imidazoloethyl moiety does not appear to be mandatory for activity, as evidenced by bis-trifluoromethyl analogue **11f** ($IC_{50} = 0.71$ μM). The favorable activity of this compound within this series may also serve to support the hypothesis that the electron-withdrawing character of the halogenated aryls contained in earlier analogues is an important contributing factor for inhibitory activity. As further modifications within the cyanoguanidine series failed to achieve additional gains in inhibitory potency beyond that of **11a**,**f** ($IC_{50} = 0.6$–0.7 μM), the strategy chosen involved making changes directly to the guanidine core.

The most expedient functionality to investigate was that of the acylguanidine, which can be readily prepared from the unsubstituted guanidines. The 3-cyanobenzoylguanidine containing analogue **19a** offered at least 100-fold improvement in potency relative to the corresponding cyanoguanidine compound **11b**, exhibiting an IC_{50} value of 0.033 μM. The 4-cyano analogue **19b** was about 10-fold less potent than its 3-cyano derivative **19a**. The 4-chloro analogue **19c** also retained

significant potency. While most of the benzoyl derivatives maintained excellent potency, the aliphatic compounds such as **19d** showed reduced activity. Because all of the inhibitors evaluated in this series were racemic mixtures, we then chose to separate the enantiomers of the most potent compound, 3-cyano derivative **19a**, using chiral HPLC (Chiracel AD® column; isopropanol/hexanes, 0.1% triethylamine). All of the potency was retained by single enantiomer **20a** ($IC_{50} = 0.018$ μM) of the racemic **19a** (the other enantiomer, **20b**, was devoid of any ATPase inhibitory activity), though the absolute stereochemistry of **20a** was not determined.

We next evaluated the oral bioavailability of the most potent compound (**20a**) of this series. The compound was dosed to rats ($n = 2$) in polyethyleneglycol:water: ethanol (1:1:1) and the plasma samples were analyzed by LC-MS/MS. The compound has an oral bioavailability of 47% and an iv half life of 2.1 h. The volume of distribution (V_{ss}) was 2.37 L/kg and the C_{max} was 21 μM. Thus we were able to identify an orally active compound with good pharmacokinetic properties which might be suitable for evaluation of pharmacological activity in animal models of ischemia.

Through modifications to peripheral aryl groups and the core functional group in a series of guanidine based analogues, we have identified a potent and selective inhibitor (**20a**) of mitochondrial F_1F_0 hydrolase. The compound does not inhibit the synthase component of the enzyme. It has good oral bioavailability and an excellent half life in rats. This is though to represent a suitable tool molecule for investigating the role of this enzyme in disease models. Interestingly, all of the compounds studied in this series showed complete absence of inhibition of the synthase component of this enzyme, although at present, the reasons for this selectivity are not clear. Since the enzymatic reaction of the mitochondrial F_1F_0 ATP hydrolase proceeds in discrete rotational steps, the forward and the backward reactions may each follow different steps, and therefore the determinants of selectivity may be manifested in the differences in these discreet steps.

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