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Benzodiazepine-based selective inhibitors of mitochondrial F_1F_0 ATP hydrolase

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Abstract—A series of benzodiazepine-based inhibitors of mitochondrial F_1F_0 ATP hydrolase were prepared and evaluated for their ability to selectively inhibit the enzyme in the forward direction. Compounds from this series showed excellent potency and selectivity for ATP hydrolase versus ATP synthase, suggesting a potentially beneficial profile useful for the treatment of ischemic heart disease.
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1. Introduction

Every year, large numbers of patients die from or experience complications from ischemic heart disease, which include acute myocardial infarction, congestive heart failure, cardiac arrhythmias, or other disorders. Myocardial ischemia exists when heart tissue demands for oxygen and substrates exceed the supply. These imbalances span a large range of various syndromes and biochemical pathways involved in the pathogenesis of low-grade to severe ischemic conditions. Major consequences of myocardial ischemia include mechanical and electrical dysfunction, muscle cell damage, and development of necrosis. Ultimately, if the ischemia is sufficiently severe there will be an immediate reduction (or cessation) of contractile function in the heart and a potential to generate arrhythmias.

The impairment of contractile function in ischemic muscle is associated with mitochondrial levels of adenosine triphosphate (ATP) and adenosine triphosphatases (ATPases).[1] ATPases typically catalyze the hydrolysis of ATP, to adenosine monophosphate (AMP) or adenosine diphosphate (ADP), plus phosphate ions and energy. The contractile function of the heart is regulated by the transport of calcium, sodium, and potassium ions, which in turn is modulated by ATP and ATPases. To maintain homeostasis the cells supply of ATP must be replenished as it is consumed (e.g., with muscle contraction). Thus, during steady state the rate of ATP synthesis is closely matched to its rate of consumption. However, unlike other ATPases which function typically to hydrolyze ATP and release energy, the mitochondrial F_1F_0-ATPase (mATPase)[2] has both hydrolytic and synthetic states. As 'ATP synthase', the mATPase catalyzes the production of ATP via oxidative phosphorylation of ADP and P_i.[3]

Under normoxic conditions, mATPase modulates ATP production via its two units, the F_1 (catalytic domain) and F_0 (inner membrane domain) complexes,[4] whereby electrons from ATPase substrates are transferred to oxygen and protons are transported out of the

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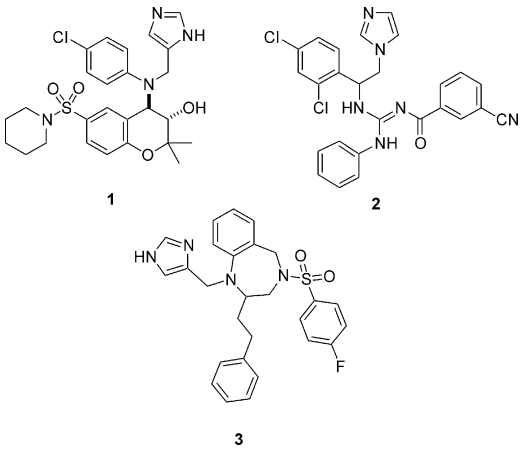
mitochondrial inner matrix, creating an electrochemical proton gradient. The energy resulting from proton flow back across the mitochondrial membrane is captured by the F_0 domain which drives the F_1 domain to synthesize ATP. Under ischemic conditions, however, this electrochemical gradient collapses, and mATPase switches to its hydrolytic state, with a concomitant down-regulation of F_1F_0-ATP synthase (up to 50–80% in ischemic muscle). This leads to a futile cycling and wasting of ATP which may increase cardiac contractility, vasoconstriction, sensitivity to vasoactive agents, and arterial blood pressure. The conversion of F_1F_0-ATP synthase to F_1F_0-ATP hydrolase is reversible, as addition of substrate and oxygen to the mitochondria of ischemic muscle can reactivate F_1F_0-ATPase and return ATP levels to control levels.[5] A native peptide, IF_1 inhibitor protein (IF_1)[6] may bind to the F_1 unit under ischemic conditions to inhibit the ATP hydrolase activity of the enzyme; however, IF_1 binding is highly pH dependent. Concentrations of its active form decrease at high pH, requiring saturating levels of active IF_1 to fully inhibit the enzyme.

Several inhibitors of mATPase have been described, including efrapeptin,[7] oligomycin,[8] aurovertin B,[9] and azide.[10] Oligomycin targets F_0 and reportedly postpones cell injury by preserving ATP during ischemia. However, until recent disclosures from these laboratories (**1**[11] and **2**[12]), the only inhibitors of mATPase known in the literature were large proteins or peptides which are not orally bioavailable.



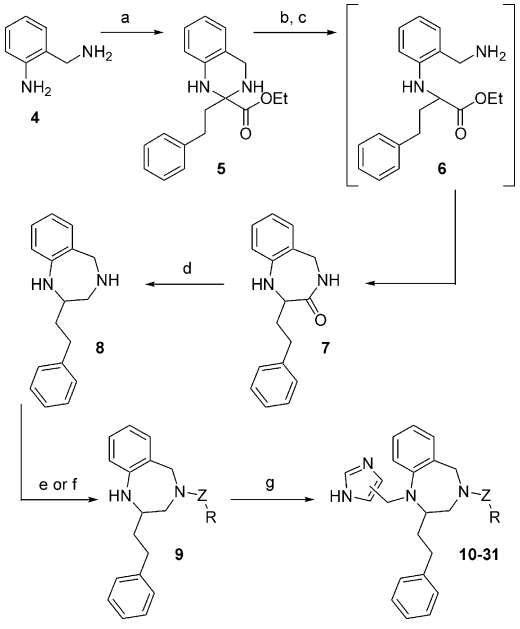
We report herein the discovery and preliminary structure–activity relationships (SAR) of a series of small-molecule, selective inhibitors of mitochondrial F_1F_0-ATP hydrolase. These small-molecule inhibitors are devoid of inhibitory activity against the corresponding ATP synthase and are of interest for the development of oral agents for the treatment of ischemic conditions mediated through this important pathway. An initial lead against this target (**3**) was discovered through directed screening of small molecule libraries using pharmacophore searching based on an earlier substituted guanidine lead in this program.[12] Substantial improvements in potency were rapidly achieved through analogue synthesis, while selectivity for hydrolase versus synthase inhibition was maintained throughout.

2. Chemistry

All compounds were prepared by appending a variety of peripheral aryl-containing moieties to an efficiently assembled 2-phenethylbenzodiazepine core (Scheme 1).[13] 2-Aminobenzylamine (**4**) was reacted with ethyl 2-oxo-4-phenylbutyrate in refluxing toluene using a Dean-Stark apparatus for removal of water to give tetrahydroquinazoline **5**. Reduction of **5** with triethylsilane in TFA and 1,2-dichloroethane gave tetrahydrobenzodiazepin-3-one **7** through the intermediacy of amino ester **6**. Reduction of **7** to give diamine **8** was effected with lithium aluminum hydride in THF. The diamine **8** was then either sulfonylated with a sulfonyl chloride or alkylated with an alkyl bromide under standard conditions to give *N*-sulfonyl or *N*-alkyl-substituted tetrahydrobenzodiazepines **9** respectively. Reductive amination with the appropriate imidazolyl aldehyde under standard conditions afforded inhibitors **10–31**.

3. Biological results and discussion

Compounds **10–31** were assayed for their ability to inhibit the activity of bovine mitochondrial F_1F_0-ATP hydrolase in submitochondrial particles in vitro (Table 1).[14] The initial lead for this series (**3**) selectively inhibited the hydrolase with an IC_{50} of 221 nM. A cursory evaluation of electronic variation of the peripheral aryl moiety ranging from electron-donating to neutral to electron-withdrawing (compounds **10–17**) revealed a modest impact on activity, with little evidence of a strong electronic component. Increasing the surface area of the



Scheme 1. (a) $Ph(CH_2)_2C(=O)CO_2Et$, toluene, reflux, Dean–Stark trap; (b) 1,2-DCE, TFA, Et_3SiH; (c) NaOH/MeOH; (d) LAH, THF; (e) $RS(=O)_2Cl$, NEt_3, CH_2Cl_2; (f) NaH, DMF, RCH_2Br; (g) imidazolylcarboxaldehyde, $Na(OAc)_3BH$, DCE, HOAc.

Table 1. Inhibition of bovine mitochondrial F_1F_0 ATP hydrolase assay results for compounds **3** and **10–31**

Compd[a]	R	Z	imidazole	F_1F_0 ATP hydrolase inhibition IC_{50}, μM[b]
3	4-F-Ph	SO_2	5-yl	0.221
10	Ph	SO_2	5-yl	0.282
11	4-OH-Ph	SO_2	5-yl	0.667
12	4-OMe-Ph	SO_2	5-yl	0.077
13	2,5-di-Cl-Ph	SO_2	5-yl	0.158
14	4-(AcNH)-Ph	SO_2	5-yl	2.981
15	4-CN-Ph	SO_2	5-yl	0.255
16	2-Cl-4-CN-Ph	SO_2	5-yl	0.939
17	3-NO_2-Ph	SO_2	5-yl	0.423
18	Naphth-1-yl	SO_2	5-yl	0.338
19	Thiophen-2-yl	SO_2	5-yl	0.636
20	Benzofurazan-7-yl	SO_2	5-yl	1.777
21	Quinolin-8-yl	SO_2	5-yl	2.935
22	Bn	SO_2	5-yl	2.405
23	CF_3	SO_2	5-yl	0.077
24	4-*t*-Bu-Ph	SO_2	5-yl	0.008
25	4-*t*-Bu-Ph	CH_2	5-yl	2.138
26	4-*t*-Bu-Ph	CH_2	4-Me-5-yl	2.352
27	4-*t*-Bu-Ph	SO_2	2-yl	>10.0
28	4-F-Ph	SO_2	2-Me-5-yl	9.623
29	4-F-Ph	SO_2	4-Me-5-yl	0.151
30	4-*t*-Bu-Ph	SO_2	4-Me-5-yl	0.077
31	3,4-di-Cl-Ph	SO_2	4-Me-5-yl	0.022

[a] All compounds tested were of greater than 98% purity as determined by LC/MS and ^{1}H NMR analysis.
[b] For all compounds, mitochondrial F_1F_0 ATP synthase IC_{50} > 30 μM.

aryl ring at this same position, or changing to heterocyclic aryls (compounds **18–21**) likewise failed to show any improvement in activity. From these initial efforts, however, it was noted that H-bond donors (**11**,**14**) were particularly ill-tolerated. In contrast, more lipophilic susbtituents were somewhat favorable (**12**,**13**), and additional lipophilically-substituted analogues were explored. Direct replacement of the aryl moiety with a benzyl homologue (**22**) eroded activity ten-fold, whereas substitution with a trifluoromethyl group, as in compound **23**, improved activity to 77 nM. Potency could be further improved by an additional order of magnitude to 8 nM with a 4-*tert*-butyl-substituted phenyl group (**24**).

Along with the aforementioned improvements in potency, however, potent inhibition of cytochrome P450 (CYP) isoform CYP2C9 was observed (IC_{50} = 38 nM for compound **24**), and efforts were made to identify key portions of the molecule that might be altered to diminish or remove this unwanted property while maintaining mATPase activity. Several changes that were investigated greatly diminished target potency, and so were not pursued further, including replacement of the sulfonyl moiety with CH_2 (**25**), and changing the point of attachment of the imidazole ring (**27**). Methylation of the imidazole ring gave mixed results: methylation at the 2-position as in compound **28** abolished mATPase activity, however, methylation at the 4-position could be tolerated with modest impact on target potency while significantly reducing CYP2C9 inhibition (IC_{50} = 3 μM and 2 μM for compounds **30** and **31**, respectively).

The present series of mATPase inhibitors includes a number of potent and selective inhibitors of the hydrolase activity of this enzyme. Lead optimization efforts successfully achieved a 40-fold improvement in mATPase potency while maintaining selectivity for inhibition of the hydrolase versus the synthase activity of this enzyme.[15] Modifications to the imidazole portion of the initial lead compound helped reduce a potential CYP liability, with only modest impact on target potency. Additional structure–activity studies focusing on unexplored regions of this scaffold have the potential of leading to the identification of suitable drug candidates acting through this novel pathway. Such agents are expected to restore energy balance to ischemic cells, and may thereby find utility in the clinical treatment of ischemic heart disease and its associated complications.

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13. Representative experimental procedures for Scheme 1 are illustrated for the preparation of compound **29**. (a) **2-Phenethyl-1,2,3,4-tetrahydroquinazoline-2-carboxylic acid ethyl ester (5)**. A solution of 2-aminobenzylamine (2.4 g, 20 mmol), and ethyl 2-oxo-4-phenylbutyrate (3.8 mL, 20

mmol, 1.0 equiv) in 100 mL of toluene was heated at reflux for 18 h using a Dean–Stark apparatus for removal of water. The solvent then was removed under reduced pressure to give ester **5** as a brown oil (6 g, 95%). ^{1}H NMR ($CDCl_3$) δ 1.20 (m, 3H), 2.10 (m, 2H), 2.33 (m, 1H), 2.40 (m, 1H), 4.00 (dd, 2H), 4.70 (m, 2H), 6.60 (d, 1H), 6.75 (t, 1H), 6.90 (m, 1H), 7.05 (t, 1H), 7.15–7.35 (m, 5H). (b) **2-Phenylethyl-2,3,4,5-tetrahydrobenzodiazepin-3-one** (**7**). To a stirred solution of tetrahydroquinazoline **5** (4.5 g, 14.5 mmol) in 1,2-DCE (40 mL) was added TFA (10 mL) at 0 °C, followed by triethylsilane (4.0 mL, 25 mmol, 1.7 equiv), and the mixture was allowed to warm to rt over 2 h. The solvent was then removed under reduced pressure, and the residue was dissolved in MeOH (50 mL). To this stirred solution at 0 °C was added 1 N NaOH to adjust the pH to 13. After 18 h, the precipitate was collected to give benzodiazepinone **7** as a tan solid (2.5 g, 65%). ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 1H), 2.30 (m, 1H), 2.79 (m, 2H), 3.55 (d, $J=5$, 1H), 3.86 (dd, $J=7$, 16, 1H), 4.32 (dd, $J=12$, 6.5, 1H), 4.86 (dd, $J=16$, 7, 1H), 6.50 (d, $J=8$, 1H), 6.65 (m, 2H), 6.90 (d, $J=7.4$, 1H), 7.07 (m, 1H), 7.20 (5H); ^{13}C NMR ($CDCl_3$) 31.34, 31.71, 44.49, 52.94, 116.76, 117.51, 120.45, 125.28, 127.66, 127.68, 127.93, 128.35, 140.32, 144.54. (c) **2-Phenylethyl-2,3,4,5-tetrahydrobenzodiazepine** (**8**). To a stirred suspension of lithium aluminum hydride (0.33 g, 8.6 mmol, 1.0 equiv) in THF (20 mL) at rt under argon was added a solution of benzodiazepinone **7** (2.3 g, 8.6 mmol) in 20 mL THF through an addition funnel, and the mixture was allowed to stir at rt for 18 h before quenching with the addition of water (1 mL). The resultant suspension was filtered, and the filtrate was concentrated under reduced pressure to give benzodiazepine **8** as a yellow oil (2.1 g, 98%), which was used directly in the next step. MS m/z 253 $[M+H]^+$. (d) **4-(4-Fluorophenylsulfonyl)-2-phenylethyl-2,3,4,5-tetrahydrobenzodiazepine** (**9**). To a stirred solution of benzodiazepine **8** (280 mg, 1.1 mmol) in CH_2Cl_2 was added NEt_3 (0.23 mL, 1.7 mmol, 1.5 equiv) and 4-fluorobenzenesulfonyl chloride (220 mg, 1.1 mmol, 1.0 equiv) sequentially. The mixture was allowed to stir at rt for 3 h, and then saturated K_2CO_3 solution was added, followed by solid K_2CO_3. The suspension was filtered, and the filtrate was concentrated under reduced pressure to give the sulfonamide **9** (ZR = 4-F-$PhSO_2$) as a yellow oil (300 mg, 68%). ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 2H), 2.72 (m, 2H), 3.00 (m, 1H), 3.30 (dd, 1H), 3.58 (dd, 1H), 4.28 (d, $J=15$, 1H), 4.45 (d, $J=15$, 1H), 6.58 (d, $J=8.0$, 1H), 6.80–7.40 (m, 10H), 7.60 (m, 2H); ^{13}C NMR ($CDCl_3$) 32.32, 34.68, 52.39, 55.61, 115.73, 116.06, 119.70, 121.20, 126.24, 127.15, 128.27, 128.61, 129.69, 129.81, 135.79, 140.65, 147.11. (e) **4-(4-Fluorophenylsulfonyl)-1-(4-methylimidazol-5-ylmethyl)-2-phenylethyl-2,3,4,5-tetrahydrobenzodiazepine** (**29**). To a stirred solution of sulfonamide **9** (ZR = 4-F-$PhSO_2$) (80 mg, 0.20 mmol) in 1,2-DCE (1 mL) and acetic acid (0.5 mL) was added 4-methyl-5-imidazolecarboxaldehyde (26 mg, 0.24 mmol, 1.2 equiv), and the mixture was allowed to stir at rt for 30 min. Sodium triacetoxyborohydride (130 mg, 0.60 mmol, 3.0 equiv) was then added, and additional quantities of the reagents were added to ensure completion of the reaction. The reaction mixture was then poured into an EtOAc and NH_4OH solution, and the organic layer was separated, dried, and concentrated under reduced pressure to give the title compound **29** as a solid (95 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 1.43 (m, 2H), 2.03 (m, 3H), 2.44 (m, 1H), 2.63 (m, 1H), 3.13 (m, 1H), 3.71 (m, 2H), 4.12 (m, 3H), 4.60 (d, $J=10.8$, 1H), 6.90–7.40 (m, 12H), 7.81 (m, 2H); MS m/z 505 $[M+H]^+$.

14. Assays using the bovine enzyme in submitochondrial particles were performed as described in ref 11.
15. None of the compounds in the present series exhibited any measurable inhibition of F_1F_0 ATP synthase at concentrations up to 30 μM. While the molecular basis for the ability of these agents to inhibit this enzyme in the forward direction only is presently unknown, current hypotheses center on the potential for the discreet rotational steps unique to this enzyme to be different in forward and reverse directions.