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Small Molecule Mitochondrial F_1F_0 ATPase Hydrolase Inhibitors as Cardioprotective Agents. Identification of 4-(*N*-Arylimidazole)-Substituted Benzopyran Derivatives as Selective Hydrolase Inhibitors

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Abstract: In this paper we show that 4-aryl-CH_2-imidazole-substituted benzopyran compounds with 3*S*,4*R*-stereochemistry are cardioprotective by inhibiting the F_1F_0 mitochondrial ATP hydrolase. Compounds (e.g., **13**) with 3*R*,4*S*-stereochemistry act as mitochondrial K_{ATP} openers. This resulted from an inversion of stereochemistry for the F_1F_0 mitochondrial ATP hydrolase vs mitochondrial K_{ATP}. Structure−activity relationships for the inhibition of mitochondrial ATP hydrolase are also delineated. It is not clear how **13** (3*R*,4*S*) can selectively inhibit the hydrolytic activity of the F_1F_0 mitochondrial enzyme without interfering with the synthase activity.

Introduction. A consequence of ischemia is the depletion of ATP which compromises the cell's ability to function. Mitochondrial F_1F_0 ATPase, which is present in virtually all mammalian tissues, normally synthesizes ATP, but during ischemia a significant portion of ATP is hydrolyzed by this enzyme.[1] Because there is no apparent physiological benefit of ATP hydrolysis by F_1F_0 ATPase, it is termed "ATP wastage".[2] F_1F_0 ATPase is composed of approximately 15 different polypeptide chains that assemble into two functional domains on the inner mitochondrial membrane.[3] The F_1 domain protrudes from the membrane into the matrix and contains the nucleotide binding and catalytic sites. The F_0 domain is within the membrane and pumps protons into or out of the mitochondrial matrix as ATP is synthesized or hydrolyzed, respectively. The F_1 functional motif is highly conserved; the human and bovine subunits share 98% amino acid homology.

The synthetic or hydrolytic activity of the F_1F_0 ATPase depends on the presence or absence, respectively, of oxygen.[4] Electrons from metabolic cofactors are transferred down an energy gradient to oxygen in the inner membrane. The mediators of the electron transport are respiratory proteins that concomitantly pump protons out of the mitochondrial matrix, which establishes a proton motive force across the inner membrane. The F_1F_0 ATPase utilizes the proton motive force by pumping protons back into the matrix, which is translated into the synthesis of ATP. The protons are not involved in the chemical reaction, but rather in driving conformational changes in the F_1 domain that affect the binding and release of substrates and products. In the absence of oxygen (i.e., ischemia), the proton motive force collapses and the activity of the F_1F_0 ATPase switches from ATP synthesis to ATP hydrolysis with a reversal in proton flow through the F_0 domain (Figure 1).

The activity of an endogenous inhibitor protein of the F_1F_0 ATPase, IF_1,[5] indicates that the presence or absence of a proton motive force across the inner membrane could induce conformational changes in the F_1F_0 ATPase; however, conformational changes caused by a change in the proton motive force have not been directly observed. Under normoxic conditions of ATP synthesis (i.e., in the presence of a proton motive force), IF_1 does not bind to the mitochondrial F_1F_0 ATPase. Under ischemic conditions of ATP hydrolysis (i.e., the absence of a proton motive force), IF_1 binds to and inhibits the enzyme. The selectivity of IF_1 demonstrates that it is possible to inhibit the hydrolytic activity (ATP hydrolase) of the enzyme during ischemia without interfering with the synthesis of ATP (ATP synthase) during normoxic conditions. Although IF_1 provides a natural paradigm for selective inhibition of the hydrolase activity, its ability to prevent ATP wastage during ischemia is only modest.[2] The natural products, aurovertin and oligomycin, have been previously shown to inhibit the hydrolase and the synthase activity with very similar potencies.[2] The goal of our program was to identify potent and selective small molecule inhibitors of the ATP hydrolase to explore their potential for the treatment of ischemic disease.

Material and Methods. Details of all enzyme preparations are reported in the Supporting Information. Rat heart submitochondrial particles (SMP) were prepared from mitochondria purified through a Percoll gradient.[6]

ATP Hydrolysis. The hydrolysis of ATP in submitochondrial particles in microtiter plates was measured using a pyruvate kinase−lactate dehydrogenase-coupled enzyme system in microtiter plates by a decrease in absorbance at 340 nm. The assay was done in 50 mM Tris acetate pH 7.5, 2 mM $MgCl_2$, 0.5 mM ATP, 2 mM phosphoenolpyruvate ATP. The hydrolysis of ATP in submitochondrial particles was measured using a pyruvate kinase−lactate dehydrogenase-coupled enzyme system in microtiter plates by following a decrease in absorbance at 340 nm. The SMP were added and briefly preincubeated at room temperature before measuring NADH oxidation on a Molecular Devices UVMax plate reader equipped with a 340 nm filter and interfaced to a MacIntosh computer equipped with Soft Max software. The change in absorbance at 340 nm was converted into μmol of ADP min^{-1} using the extinction coefficient of NADH (6220 mol^{-1}). Protein concentrations were determined using the Bradford assay and used to calculate specific activity (μmol of ADP min^{-1} mg^{-1}).

ATP Synthesis. The ATP synthase activity was measured by monitoring the increase in the absorbance at 340 nm using a hexokinase−glucose-6-phosphate dehydrogenase-coupled assay. The buffer for this assay

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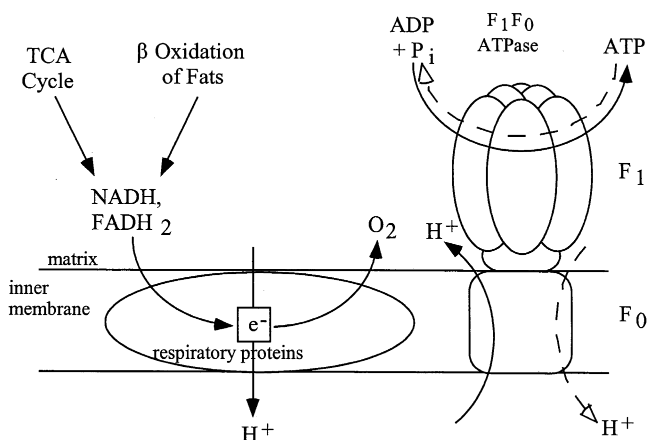


Figure 1. Schematics of the mitochondrial inner membrane, oxidative phosphorylation, and the structure and function of the F_1F_0 ATPase. Under normal physiologic conditions, metabolic products (NADH, FADH) provide electrons to respiratory proteins that conduct the electrons down an energy gradient to the ultimate acceptor oxygen. Concomitantly protons are pumped across the inner membrane, creating a proton motive force composed of a membrane potential and a pH gradient. The F_1F_0 ATPase (right) utilizes the proton motive force to synthesize ATP by pumping protons into the matrix through the F0 domain (solid arrows). In the absence of oxygen, the protomotive force collapses, oxidative phosphorylation is arrested, and the F_1F_0 ATPase hydrolyzes ATP with a concomitant pumping of protons out of matrix (dashed arrow).

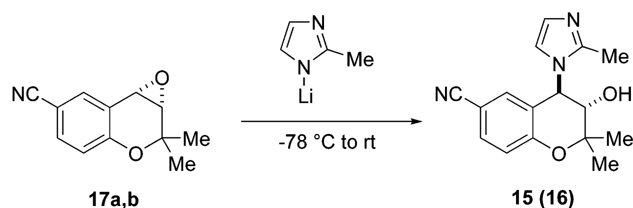
was 10 mM HEPES at pH 8. The diluted enzyme (25 μL) was mixed with glucose, $MgCl_2$, KPO_4, potassium succinate, and AMP (to inhibit adenylate kinase). The mixture was allowed to incubate for 30 min with gentle shaking. The inhibitor (in 50% DMSO; final DMSO concentration 5%) was added, and the incubation was continued for 15 min. The reaction was initiated by adding 25 μL of the coupling enzymes.

Separation of F_1 from F_1F_0 ATPase. The F_1F_0 ATPase complex was purified from beef heart mitochondria following established protocols.[7] Mitochondria were suspended in TrisHCl, 10% glycerol, and EDTA. Membrane protein was solubilized with 1% (v/v) laurylmaltose. Fractions with ATPase activity were pooled and purified by anion exchange chromatography.

Citrate Synthase and Cytochrome *c* Oxidase. Citrate synthase (Sigma) and cytochrome *c* oxidase were assayed by the protocols described in the literature.[8] Either commercially prepared enzyme (Sigma, ICN) or beef heart mitochondria was added to a cuvette containing 3% reduced cytochrome *c* (ICN) and the inhibitor compound in KPO_4 pH 7.0 at 37 °C. Initial velocities were determined by following the cytochrome *c* oxidation at 550 nm. For the Na^+/K^+ ATPase, the enzyme was preincubated at 37 °C for 10 min. The reaction was initiated by adding NaCl in the presence or absence of ouabain (0.1 mM) and the oxidation of NADH monitored at 340 nm. Ca^{2+} ATPase activity was measured in MOPS, KCl, $MgCl_2$, and $CaCl_2$. The initial rates were calculated using the extinction coefficient of NADH.

Cardioprotective Activity. The cardioprotective activity, as measured by increasing time to ischemic contracture, was determined in isolated perfused globally ischemic rat hearts.[9] When the coronary perfusate is shut off, the heart goes in contracture due to the lack of ATP. Compounds were initially screened at 10 μM in DMSO (0.04%) solution. Those demonstrating greater than 25% increase in time to the onset of contracture were subjected to concentration–response studies to determine EC_{25} values.

Scheme 1[a]



[a] **15/16** not recrystallized.

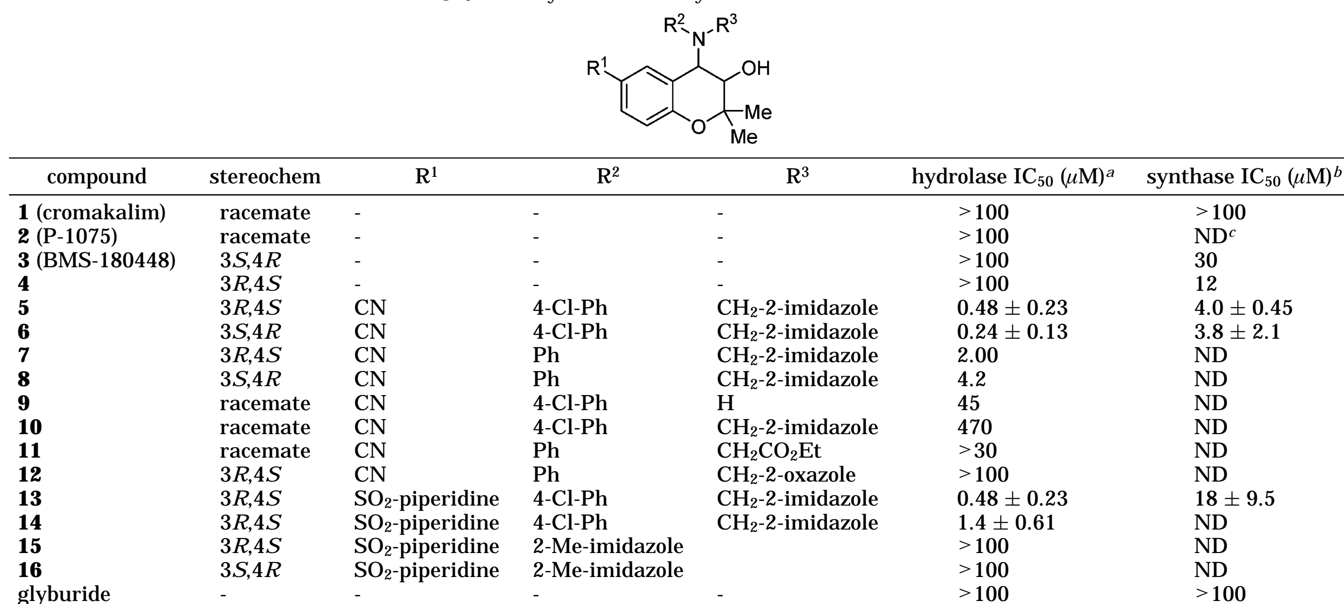
Chemistry. Most of the compounds disclosed in this paper have been reported previously.[10] The only compounds that were not described previously are the enantiomeric imidazole analogue **15** and **16**, which were prepared by opening the benzopyranyl epoxide **17a**,**b**[10,11] with the anion of imidazole (Scheme 1). The epoxides **17a**,**b** with both 3*S*,4*R* and 3*R*,4*S* absolute stereochemistry are known.[10,11] The spectral data and the experimental procedure are detailed in the Supporting Information.

Results and Discussion. The F_1F_0 ATPase from rat and bovine cardiac mitochondria are virtually identical. We could not distinguish the F_1F_0 ATPase activity of one species from that of another with respect to inhibitor sensitivity or hydrolase/synthase selectivity. This is not surprising given the conservation of this enzyme's structure and function. For example, the amino acid sequence of the β subunit from human F_1 catalytic domain shares 98% homology with the bovine sequence.[12] We used bovine and rat heart submitochondrial particles to screen compounds. Since the IC_{50} values in the two systems were nearly identical, only the rat heart IC_{50}'s are reported in the paper.

ATP dependent potassium channel (K_{ATP}) openers, which are known to work by opening the mitochondrial K_{ATP}, were initially selected for screening against F_1F_0 ATP hydrolase and synthase.[14] A number of studies have shown that K_{ATP} openers whose mechanism of action involves resetting the mitochondrial membrane potential, prevent ATP hydrolysis.[15] We have also shown that F_1F_0 mitochondrial ATP hydrolysis inhibitors (e.g., oligomycin) protect the isolated perfused rat heart by preventing ATP hydrolysis.[15] Thus, a mitochondrial K_{ATP} activation[13] and F_1F_0 mitochondrial hydrolase inhibition[15] appear to operate via a common mechanism involving conservation of ATP.[16]

We screened several different classes of K_{ATP} openers, and their potencies for the inhibition of mitochondrial ATP hydrolase are given in Table 1. The classical K_{ATP} openers cromakalim (racemic) and P-1075 were devoid of activity against the ATP hydrolase, as were the two enantiomers (**3**, **4**) of the cyanoguanidine analogue BMS-180448. However BMS-191095 (**5**) showed a submicromolar potency against the rat enzyme ($IC_{50} = 0.48$ μM). The enantiomer **6** (3*S*,4*R*) of **5**, which has little activity as a K_{ATP} opener, showed a slightly better potency ($IC_{50} = 0.24$ μM) as a ATP hydrolase inhibitor. We then screened these compounds for inhibition of rat F_1F_0 ATP synthase activity and found that this class of compounds are also modest inhibitors of F_1F_0 ATP

Table 1. Inhibition of Rat Mitochondrial F_1F_0 ATP Hydrolase and Synthase in Submitochondrial Particles



compound	stereochem	R^1	R^2	R^3	hydrolase IC_{50} (μM)[a]	synthase IC_{50} (μM)[b]
1 (cromakalim)	racemate	-	-	-	>100	>100
2 (P-1075)	racemate	-	-	-	>100	ND[c]
3 (BMS-180448)	3*S*,4*R*	-	-	-	>100	30
4	3*R*,4*S*	-	-	-	>100	12
5	3*R*,4*S*	CN	4-Cl-Ph	CH_2-2-imidazole	0.48 ± 0.23	4.0 ± 0.45
6	3*S*,4*R*	CN	4-Cl-Ph	CH_2-2-imidazole	0.24 ± 0.13	3.8 ± 2.1
7	3*R*,4*S*	CN	Ph	CH_2-2-imidazole	2.00	ND
8	3*S*,4*R*	CN	Ph	CH_2-2-imidazole	4.2	ND
9	racemate	CN	4-Cl-Ph	H	45	ND
10	racemate	CN	4-Cl-Ph	CH_2-2-imidazole	470	ND
11	racemate	CN	Ph	CH_2CO_2Et	>30	ND
12	3*R*,4*S*	CN	Ph	CH_2-2-oxazole	>100	ND
13	3*R*,4*S*	SO_2-piperidine	4-Cl-Ph	CH_2-2-imidazole	0.48 ± 0.23	18 ± 9.5
14	3*R*,4*S*	SO_2-piperidine	4-Cl-Ph	CH_2-2-imidazole	1.4 ± 0.61	ND
15	3*R*,4*S*	SO_2-piperidine	2-Me-imidazole		>100	ND
16	3*S*,4*R*	SO_2-piperidine	2-Me-imidazole		>100	ND
glyburide	-	-	-	-	>100	>100

[a] The IC_{50} for the ATP hydrolase activity was measured by decrease in absorbance at 340 nm using a pyruvate kinase/lactate dehydrogenase system. [b] ATP synthase activity was measured by monitoring the increase in absorbance at 340 nm using a hexokinase/glucose-6-phosphate dehydrogenase coupled assay. [c] ND, not determined.

synthase. The imidazole-based K_{ATP} openers were modestly (approximately 40-fold) potent and selective for the inhibition of ATP hydrolase. Previous studies have shown that the 3*R*,4*S* stereochemistry is preferred by the K_{ATP} openers. The stereochemical requirement for ATP hydrolase inhibition were opposite to that of the K_{ATP} openers. The less potent K_{ATP} enantiomer (3*S*,4*R*) was preferred by the F_1F_0 mitochondrial hydrolase.

A limited effort was undertaken to explore structure–activity relationships, and the data are summarized in Table 1. Removal of chlorine from the aromatic ring (**7**, **8**) maintains significant inhibition of F_1F_0 mitochondrial hydrolase, indicating halogen substitution is not required for hydrolase inhibition. Removal of the imidazole-containing fragment (**9**) caused nearly a 100-fold loss of potency against the hydrolase. The imidazole-containing fragment (**10**) without the benzopyran attachment had little potency as an ATP hydrolase inhibitor. These results strongly suggest that the imidazole and perhaps benzopyran moieties are required for potency. We then attempted to investigate if the imidazole heterocycle could be replaced. The ester replacement **11** for imidazole is devoid of hydrolase inhibitory potency, further indicating that imidazole is required for activity. The same conclusion can be drawn by comparing the imidazole analogue **6** with the corresponding oxazole **12** (IC_{50} > 100 μM). Substitution on the phenyl ring of the benzopyran resulted in the identification of the sulfonamido analogue **13** with good hydrolase inhibitory potency (IC_{50} = 0.48 μM) and selectivity (~40-fold).

We then investigated the imidazole analogues **15** and **16**. As shown in the table, both compounds were devoid of hydrolase inhibitory activity. These data suggest that the aryl-N-CH_2-imidazole of **13** plays a crucial role in enzyme inhibition. Thus with limited studies, we identified compound **13** with good potency and selectivity.

The enzyme can carry out the hydrolysis of ATP with only the catalytic F_1 subunit, and the membrane subunit F_0 is not needed for this function. We separated the F_1 and F_0 subunits of the ATPase by methods described in the literature.[14] The classical inhibitors aurovertin and oligomycin inhibit the hydrolytic activity of the enzyme with only the catalytic subunit F_1. When **13** was studied with F_1 subunit only, there was no inhibition of the enzyme up to a concentration of 100 μM. Thus, this compound is effective when both the F_0 and F_1 subunits are present. Although the mechanism of inhibition is not understood at the molecular level, these studies do suggest that the membrane subunit F_0 is involved in the hydrolysis reaction.

We determined the specificity of **13** on two other mitochondrial enzymes, citrate synthase and cytochrome *c* oxidase. There was no effect of **13** on either enzyme up to a concentration of 100 μM. The activity of cytochrome *c* oxidase is especially of interest as this enzyme utilizes oxygen in the respiratory chain. Therefore, **13** is without an affect on the two other metabolically important mitochondrial enzymes.

Since **13** appears to be interacting with the domain of F_1F_0 ATPase responsible for pumping electrons across the inner mitochondrial membrane, it was also tested against two other ion motive ATPase found in the myocardium, the Na^+/K^+ and Ca^{2+} ATPase. The activity of these enzymes was also not effected by this compound between 0.1 and 100 μM. These studies imply that the compound appears to be selective for the F_1F_0 ATPase among ion motive ATPase enzymes.

Having established the potency and selectivity of **13**, we concentrated on finding the cardioprotective potency of these molecules in isolated rat hearts using a previously described protocol.[9] Previous studies from our laboratories have shown that the cardioprotective potency of **14** are reversed by the K_{ATP} blocker glyburide. Those studies suggested that the opening of K_{ATP} is involved in their mechanism of action. Since the most selective compounds for inhibition of F_1F_0 ATP hydrolase vs K_{ATP} opening are based on 3*S*,4*R* stereochem-

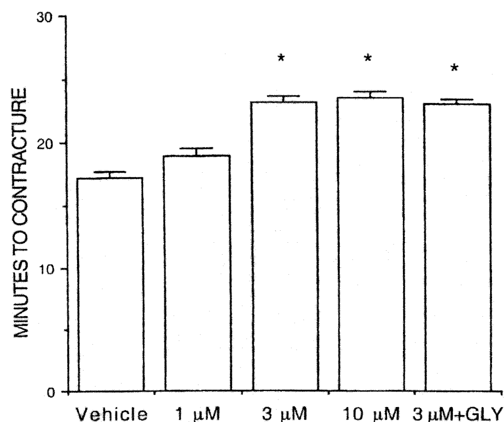


Figure 2. Effect of the K_{ATP} blocker glyburide (GLY) on the cardioprotective effects of **13** in isolated perfused rat hearts. Glyburide (0.3 μmol) while having no effect of its own, has no effect on the cardioprotective effects (as measured by the time to the onset of contracture) of compound **13** (3 μmol). *Significantly different from control.

istry, we evaluated these compounds for cardioprotective potency. The cardioprotective activity was measured in ischemic rat hearts subjected to global ischemia. Time to the onset of contracture during ischemia was used as an index of the severity of ischemia. The heart goes into contracture (also called stone heart) for lack of oxygen which results in depletion of ATP and calcium overload. The potential cardioprotective agents increase time to the onset of contracture.

Compound **13** increased time to the onset of contracture at the 3 μM dose. The time to the onset of contracture increased from 18 min to over 22 min at 3 μM, indicating good cardioprotective potency. The effect of a 10 μM dose is somewhat questionable due to the poor aqueous solubility of this compound at this dose. The effect of the K_{ATP} blocker glyburide on the cardioprotective affects of **13** was also studied to distinguish this mechanism from the K_{ATP}-mediated effects.[9,10] The affect of 0.3 μM of the K_{ATP} blocker glyburide (no effect on the enzyme, Table 1) on the cardioprotective affect of **13** was evaluated in the same model. Glyburide (0.3 μM) had no affect on increase in the time to the onset of contracture on its own or in the presence of **13**. This profile is typical of compounds whose mechanism of cardioprotection does not involve K_{ATP}. These studies suggest that compound **13** shows a cardioprotective activity by a non-K_{ATP} mechanism. (Figure 2).

We have shown that the non-K_{ATP} openers of the imidazole class of agents (**13**) are cardioprotective agents. The mechanism of cardioprotection appears to involve inhibition of the hydrolytic activity of the mitochondrial F_1F_0 ATPase. The catalytic F_1 subunit alone cannot carry out the reaction. The mitochondrial membrane subunit F_0 appears to be an integral part of the mechanism. It is also not clear at this stage how **13** can selectively inhibit the hydrolytic activity of the enzyme. Since the enzyme operates in discrete rotational steps, it is possible that the forward reaction by the enzyme is not the same as the backward reaction. This would create different steps for the forward reaction compared to the backward reaction. Regardless of how the selectivity is achieved, we have identified small molecule inhibitors of the hydrolytic activity of F_1F_0 mitochondrial ATPase that are selective for hydrolase over synthase activity. These compounds are cardioprotective in the isolated perfused rat hearts and would serve as important tools to further investigate the role of this enzyme in disease.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jennings, R. B.; Murry, C. E.; Steenbergen, C. J.; Reimer, K. A.; Development of Cell Injury in Sustained Acute Ischemia. *Circulation* **1990**, *82* [Suppl. 2], II 2 − II 12.
(2) (a) Rouslin, W.; Erickson, J. L. E.; Solaro, R. J. Effects of Oligomycin and Acidosis on Rates of ATP Depletion in Ischemic Heart Muscle. *Am. J. Physiol.* **1986**, *250*, H503−H508. (b) Jennings, R. B.; Reimer, K. A.; Steenbergen, C. J. Effect of Inhibition on the Mitochondrial ATPase on net ATP in Total Ischemia. *J. Mol. Cell Cardiol.* **1991**, *23*, 1449−1458.
(3) Abrahams, J. P.; Leslie, A. G. W.; Lutter, R.; Walker, J. Structure at 2.8 A^0 Resolution of F1-ATPase from Bovine Heart Mitochondria. *Nature* **1994**, *370*, 621−628.
(4) Harris, D. A.; Das, A. M. Control of Mitochondrial ATP Synthesis in the Heart. *Biochem. J.* **1991**, *280*, 561−573.
(5) (a) Lippe, G.; Sorgato, M. C.; Harris, D. A. The Binding and Release of the Inhibitor Protein are Governed Independently by ATP and Membrane Potential in Ox Heart Submitochondrial Vesicles. *Biochim. Biophys. Acta*, **1988**, *933*, 12−21. (b) Power, J.; Cross, R. L.; Harris, D. A.. Interaction of Ox Heart Mitochondria with its Naturally Occurring Inhibitor Protein. *Biochim. Biophys. Acta* **1983**, *724*, 128−141.
(6) Smith, A. L. Preparation, Properties and Conditions for Assay of Mitochondria: Slaughterhouse Material, Small Scale. *Methods Enzymol.* **1967**, *10*, 81−86.
(7) Buchanan, S. K.; Walker, J. E.. Chromatographic Purification of F(1)F(0)-ATPase and Complex-1 from Bovine Heart Mitochondria − FPLC-Anion Exchange and Dye Ligand Chromatography. In *Practical Guide to Membrane Protein Purification, Separation, Detection, and Characterization of Biological Macromolecules*; Vonjagow, G., Schwagger, H., Eds.; Academic Press Inc.: San Diego, 1994, pp 125−133..
(8) Wharton, D. C.; Tzagoloff, A. Cytochrome Oxidase from beef Heart Mitochondria. *Methods Enzymol.* **1967**, *10*, 245−250.
(9) Grover, G. J.; D'Alonzo, A. J.; Darbenzio, R. B.; Parham, C. S.; Hess, T. A.; Bathala, M. S.. In Vivo Chracaterization of the Mitochondrial Selective K_{ATP} Opener (3R)-*trans*-4-((Chlorophenyl)-N-(1H-imidazol-2-ylmethyl)dimethyl-2H-1-benzopyran-6-carbonitrile Monohydrochloride: Cardioprotective, Hemodynamic, and Electrophysiological Effects. *J. Pharmacol. Exp. Therapeut.* **2002**, *303*, 132−140.
(10) Rovnyak, G. C.; Ahmed, S. Z.; Ding, C. Z.; Dzwonczyk, S.; Ferrara, F. N.; Humphery, W. G.; Grover, G. J.; Santafianos, D.; Atwal, K. S.; Baird, A. J.; McLaughlin, L. G.; Normandin, D. E.; Sleph, P. G.; Traeger; S. C. Cardioselective Antiischemic ATP−Selective Potassium Channel (K_{ATP}) Openers. 6. Effect of Modifications at the C6 of Benzopyran Cyanoguanidines. *J. Med. Chem.* **1997**, *40*, 24−34.
(11) Ding, C. Z.; Rovnyak, G. C.; Misra; R. N.; Grover, G. J.; Miller, A. V.; Ahmed, S. Z.; Kelly, Y.; Normandin, D. E.; Sleph, P. G.; Atwal, K. S.. Cardioselective Antiischemic ATP−Selective Potassium Channel (K_{ATP}) Openers. 5. Identification of 4-(N-Aryl)-S−Substituted Benzopyan Derivatives with High Selectivity. *J. Med. Chem.* **1999**, *42*, 3711−3717.
(12) Ohta, S.; Kagawa, Y. Human F1-ATPase Molecular Cloning of cDNA for the Beta Subunit. *J. Biochem.* **1986**, *99*, 135−141.
(13) Grover, G. J.; Newburger, J.; Sleph, P. G.; Dzwonczyk, S.; Taylor, S. C.; Ahmad, S. Z.; Atwal, K. S. Cardioprotective effects of the Potassium Channel Opener Cromakalim: Stereoselectivity and Effects on Myocardial Adenine Nucleotides. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 156−162.
(14) Garlid, K. D.; Paucek, P.; Yarov-Yarvoy, V.; Sun X.; Schindler, P. A. The Mitochondrial K_{ATP} Channel as a Receptor for Potassium Channel Openers. *J. Biol. Chem.* **1996**, *271*, 8796−8799.
(15) Green, D. W.; Murray, H. G.; Sleph, P.; Wang, P.; Baird, A. J.; Rogers, W. J.; and Grover, G. J. Preconditioning in isolated Rat Hearts is not Dependent on Mitochondrial F_1F_0 ATPase Inhibition or ATP Conservation. *Am. J. Physiol.* **1998**, *274*, H90−H97.
(16) Walker, J. E.; Fearnley, I. M.; Gay, N. J.; Gibson, B. W.; Northrop, F. D.; Powell, S. J.; Runswick, M. J.; Saraste, M.; Tybulewicz, V. L. J.. Primary Structure and the Subunit Stoichiometry of F_1-ATPase from Bovine Mitochondria. *J. Mol. Biol.* **1985**, *184*, 677−701.

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