Cardioselective Antiischemic ATP-Sensitive Potassium Channel (K_{ATP}) Openers. 6. Effect of Modifications at C6 of Benzopyranyl Cyanoguanidines

Charles Z. Ding, George C. Rovnyak, Raj N. Misra, Gary J. Grover, Arthur V. Miller, Syed Z. Ahmed, Yolanda Kelly, Diane E. Normandin, Paul G. Sleph, and Karnail S. Atwal*

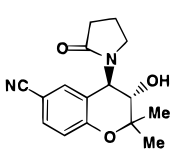
The Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000

Received April 21, 1999

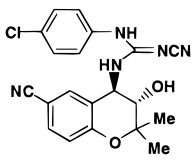
The effect on potency and selectivity of modifications at the C6 position of the cardioprotective K_{ATP} opener BMS-180448 (**2**) is described. Structure−activity studies show that a variety of electron-withdrawing groups (ketone, sulfone, sulfonamide, etc.) are tolerated for cardioprotective activity as measured by EC_{25} values for an increase in time to the onset of contracture in globally ischemic rat hearts. Changes made to the sulfonamido substituent indicate that compounds derived from secondary lipophilic amines are preferred for good cardioprotective potency and selectivity. The diisobutyl analogue **27** (EC_{25} = 0.04 μM) is the most potent compound of this series. The cardiac selectivity of **27** results from a combination of reduced vasorelaxant potency and enhanced cardioprotective potency relative to the potent vasodilating K_{ATP} openers (e.g., cromakalim). The diisobutylsulfonamide analogue **27** is over 4 orders of magnitude more cardiac selective than cromakalim (**1**). These results support the hypothesis that the cardioprotective and vasorelaxant properties of K_{ATP} openers follow distinct structure−activity relationships. The mechanism of action of **27** appears to involve opening of the cardiac K_{ATP} as its cardioprotective effects are abolished by the K_{ATP} blocker glyburide.

Introduction

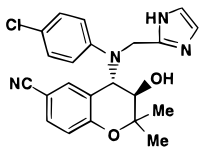
In experimental studies, K_{ATP} openers have direct cardioprotective properties which are independent of vasodilation.[1] The first generation K_{ATP} openers (e.g., cromakalim, **1**) possess a narrow window of efficacy for their use as cardioprotective agents. Their potent peripheral and coronary vasodilating properties can compromise the tissue already at risk. Thus, the identification of K_{ATP} openers that are more selective for the ischemic myocardium is desired for this class of agents to be safe and effective as cardioprotective agents. In recent publications, we have reported that the cardioprotective and vasorelaxant potencies of K_{ATP} openers follow distinct structure−activity relationships.[2] On the basis of those studies, we identified cardioprotective K_{ATP} openers (BMS-180448, **2a**; BMS-191095, **2b**) which, despite being equipotent to the reference agents (e.g., cromakalim) as cardioprotectants, are significantly less potent as vasorelaxants.[3] Thus, compounds such as **2a** and **2b** are valuable tools to explore the utility of this class of compounds for the treatment of myocardial ischemia.



Cromakalim (1)



BMS-180448 (2a)



BMS-191095 (2b)

Structure−activity studies on the cyanoguanidine portion of **2a** failed to improve cardioprotective potency beyond the micromolar level.[4−6] Thus, we turned our attention to the benzopyran portion of this compound and explored the effect of modifications at C6. Those studies provided the C6-sulfonamido class of compounds with improved cardioprotective potency and selectivity relative to **2a,b**. The structure−activity relationships leading to this class of compounds are detailed in this publication.

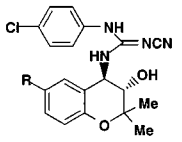
Chemistry

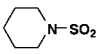
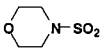
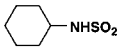
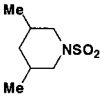
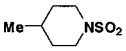
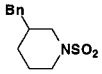
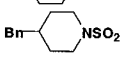
Compounds (**3**−**28**) prepared for SAR studies are summarized in Table 1. The benzopyranyl cyanoguanidines described in this paper were prepared according to Scheme 1. For most of the analogues, the amine (**32**) can be prepared from the commercially available 4-substituted phenol (**29**) via the epoxide (**31**) by standard methods.[4−6] While the racemic epoxide **31** can be obtained from the olefin **30** by epoxidation with 3-chloroperoxybenzoic acid, the chiral epoxide (**31**) is prepared from **30** by the Jacobsen epoxidation.[7] The amine (**32**) is coupled to the thiourea **33** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride to give the desired product in 50−60% yield. Details of this method have been described.[8]

The olefin amine **34** used in the synthesis of compound **6** was prepared from the corresponding ketone **33** by methyllithium addition followed by dehydration under acidic conditions (Scheme 2). The sulfonamido analogues **9** and **12**−**28** were prepared from the known sulfonyl chloride **35**[9] by a sequence of steps which involved treatment with an appropriate amine to give the sulfonamide (**36**) followed by conversion to the final product under standard conditions outlined in Scheme 1 (Scheme 3). The phosphinate precursor **38** for the synthesis of compound **10** was prepared from the corresponding iodide **37** under palladium catalyzed conditions (Scheme 4).[10] The amide precursor **42** for the

10.1021/jm990196h CCC: $18.00
Published on Web 08/17/1999

Table 1. Cardioprotective and Vasorelaxant Potencies of C6 Substituted Benzopyran Analogues



Compound	R	Antiischemic Potency[a] EC_{25} (μM) or % inc. @ 1 μM	Vasorelaxant Potency[b] IC_{50} (μM)	Ratio[c] EC_{25}/IC_{50}
Cromakalim (1)		8.9	0.032 ±0.012	278
2a		2.5	1.8±0.8	1.4
2b		1.4	>30	<0.05
3	Ph(O=)C	0.9	7.1±2.2	0.13
4	$PhCH_2(O=)C$	0.4	6.8±0.6	0.06
5	$PHCH_2$	11.1	10.0±4	1.1
6	$Ph(=CH_2)C$	3%	10.7±0.06	-
7	$Me_3C(O=)C$	1.8	5.4±0.2	0.33
8	$PhSO_2$	0.8	0.5±0.07	1.6
9	$PhNHSO_2$	1.9	3.3±1.0	0.6
10	Ph (OMe)(O)P	2.4	44.1±28.9	0.05
11	PhNHCO	>10	>100	-
12		0.27	1.9±0.9	0.14
13	Et_2NSO_2	0.3	1.1±0.3	0.27
14		1.0	3.1±0.9	0.32
15		0.33	-[d]	-
16		0.6	-[d]	-
17		2.2	11.7±3.4	0.19
18	NH_2SO_2	>10	41.3±19.9	-
19		0.34	0.5±0.03	0.68
20		0.23	32.1±11.9	0.007
21		0.65	-[d]	-
22		1.1	-[d]	-
23		0.94	>30	-
24		0.43	8.8±7.08	0.05
25	$(Me_2CH)_2NSO_2$	0.24	-[d]	-
26	$CF_3CH_2(Et)NSO_2$	0.26	-[d]	-
27	$(Me_2CHCH_2)_2NSO_2$	0.040	9.4±7.43	0.004
28	$Me_2CHCH_2(Me)NSO_2$	0.25	-[d]	-

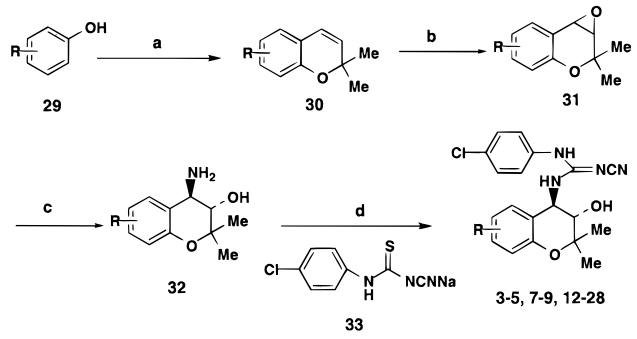
[a] EC_{25} for antiischemic (cardioprotective) potency is determined by measurement of increase in time to contracture in globally ischemic isolated perfused rat hearts. Time to contracture is defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg. Each point on the concentration−response curve is an average of three to four rat hearts, and the EC_{25} value is within ±20%. The formula weight of the compound was used to prepare the testing solution of the compound to account for the solvent present. [b] IC_{50} for vasorelaxant potency is determined by relaxation of rat aorta precontracted with methoxamine. IC_{50} value is presented as a mean with $n = 4$ or higher. [c] Selectivity is defined as the ratio of the EC_{25} value for cardioprotection to the IC_{50} value for vasorelaxant potency. [d] Not determined.

Table 2. Physical Properties of Various Analogues

compound	mol. formula	melting point (°C)[a]	microanalysis[b]	optical rotation α_D (deg)
2a,b	see refs 3a,b			
3	$C_{26}H_{23}ClN_4O_3$	174−175 (A)	C, H, N, Cl	+13.6 (c = 0.5, MeOH)
4	$C_{27}H_{25}ClN_4O_3 \cdot 0.2H_2O$	155−160	C, H, N, Cl	−3.9 (c = 0.1, MeOH)
5	$C_{26}H_{25}ClN_4O_2 \cdot 0.6H_2O$	120−122	C, H, N, Cl	
6	$C_{27}H_{25}ClN_4O_2 \cdot 0.21H_2O$	softens at 105	C, H, N, Cl	
7	$C_{24}H_{27}ClN_4O_3 \cdot 0.69H_2O$	230−232	C, H, N, Cl	
8	$C_{25}H_{23}ClN_4SO_4 \cdot 0.58H_2O$	158−160	C, H, N, Cl, S	+31.3 (c = 0.1, MeOH)
9	$C_{25}H_{24}ClN_5SO_4 \cdot 1.5H_2O$	158	C, H, N, Cl, S	
10	$C_{26}H_{26}ClN_4O_4P \cdot 0.37H_2O$	softens at 150	C, H, N, Cl, P	
11	$C_{26}H_{24}ClN_5O_3 \cdot 0.3H_2O \cdot 0.1EtOAc$	204−205	C, H, N, Cl	
12	$C_{24}H_{28}ClN_5SO_4 \cdot 0.20H_2O$	174	C, H, N	+18.0 (c = 0.25, MeOH)
13	$C_{23}H_{28}ClN_5SO_4 \cdot 0.26H_2O \cdot 0.15$toluene	164	C, H, N, Cl, S	+7.8 (c = 0.6, MeOH)
14	$C_{23}H_{26}ClN_5SO_5 \cdot 0.36H_2O$	160	C, H, N, Cl, S	+27.2 (c = 0.6, MeOH)
15	$C_{23}H_{26}ClN_5SO_4 \cdot 1.8H_2O \cdot 0.11CHCl_3$	127−130	C, H, N, Cl, S	+7.4 (c = 0.7, MeOH)
16	$C_{25}H_{30}ClN_5SO_4 \cdot 1.3H_2O$	157−161	C, H, N, S	+1.1 (c = 0.4, MeOH)
17	$C_{25}H_{30}ClN_5SO_4 \cdot 0.64H_2O$	165	C, H, N, Cl, S	+3.5 (c = 1.3, MeOH)
18	$C_{19}H_{20}ClN_5SO_4 \cdot 0.34H_2O$	184	C, H, N, Cl, S	
19	$C_{26}H_{32}ClN_5SO_4 \cdot 0.3H_2O \cdot 0.25CHCl_3$	176−178	C, H, N	−15.0 (c = 0.4, MeOH)
20	$C_{26}H_{32}ClN_5SO_4 \cdot 1.7H_2O \cdot 0.1CHCl_3$	138−140	C, H, N, Cl, S	−4.0 (c = 0.3, MeOH)
21	$C_{25}H_{30}ClN_5SO_4 \cdot 0.35H_2O$	157−160	C, H, N, Cl, S	+12.7 (c = 0.3, MeOH)
22	$C_{31}H_{34}ClN_5SO_4$	150	C, H, N, Cl, S	
23	$C_{31}H_{34}ClN_5SO_4$	140	C, H, N, Cl, S	
24	$C_{26}H_{34}ClN_5SO_4 \cdot 0.20H_2O$	188−190	C, H, Cl, N, S	
25	$C_{25}H_{32}ClN_5SO_4$	150	C, H, N, Cl, S	
26	$C_{23}H_{25}F_3ClN_5O_4S \cdot 0.03H_2O$	130−140	C, H, N, Cl, S	
27	$C_{27}H_{36}ClN_5SO_4 \cdot 0.90H_2O$	145−148	C, H, N	+0.5 (c = 0.37, MeOH)
28	$C_{24}H_{30}ClN_5SO_4 \cdot 0.20H_2O$	142−144	C, H, N, Cl, S	−1.7 (c = 0.5, MeOH)

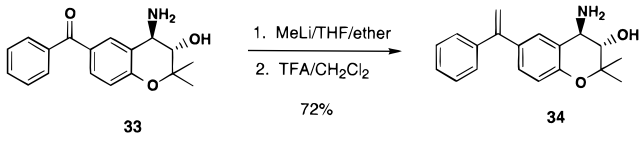
[a] Melting point is reported for all unrecrystallized compounds. [b] Microanalysis is within ±0.4% except for compounds **9** and **16** for which deviation higher than 0.4% was noted; ^{1}H and ^{13}C NMR and mass spectra are consistent with the structures assigned.

Scheme 1[a]

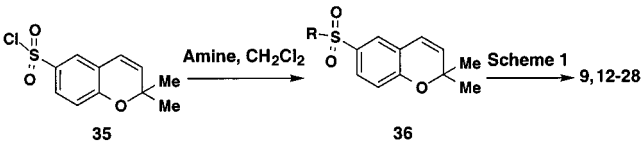


[a] Reaction conditions: (a) see references 4−6, 15; (b) dichloromethane, bleach, the Jacobsen's catalyst; (c) THF, 2-propanol, ammonium hydroxide; (d) 1-(3-(dimethylamino)propyl)-2-ethylcarbodiimide hydrochloride.

Scheme 2

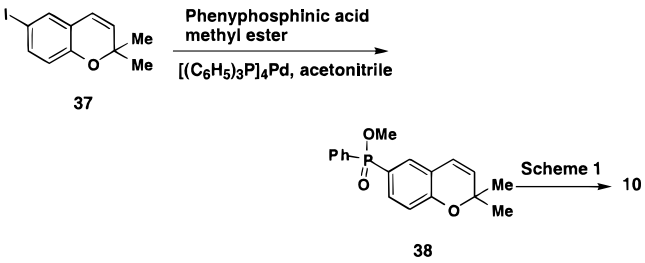


Scheme 3

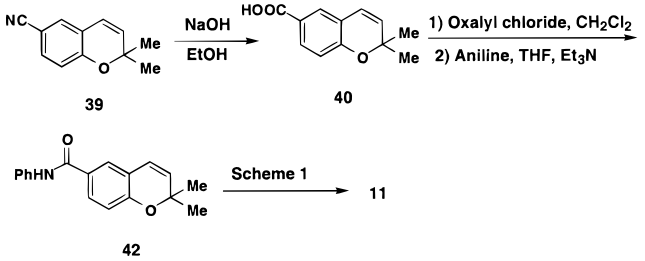


synthesis of compound **11** was generated from the corresponding nitrile **39** by base hydrolysis (10 N NaOH, EtOH), conversion of the acid **40** to the acid chloride **41**, followed by treatment of **41** with aniline (THF, Et_3N, 86% overall) (Scheme 5). Physical properties of the various analogues prepared are summarized in Table 2.

Scheme 4



Scheme 5



Results and Discussion

Since the details of the molecular mechanism of action of K_{ATP} openers are still elusive, we employed functional tests to determine cardioprotective and vasorelaxant potencies. Cardioprotective potencies were determined by measurement of EC_{25} values for an increase in time to the onset of contracture in globally ischemic isolated perfused rat hearts.[11] Time to contracture is defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg compared to the baseline value. Contracture develops due to rigor bond formation which is presumably caused by depletion of high energy phosphates. Vasorelaxant potencies were determined by measurement of IC_{50} values for the relaxation of the methoxamine contracted rat aorta, as

described previously.[4−6] The ratio of the EC_{25} value for the time to contracture and the IC_{50} value for the vasorelaxant potency indicates selectivity in vitro for the ischemic myocardium. Due to the nature of the tests employed, potency values for cardioprotection and vasorelaxation are empirical in nature.

With the exception of compound **18** which was prepared as a racemate, all compounds had 3*S*,4*R*-stereochemistry at the benzopyran centers. Previous studies have shown that for the benzopyranyl-cyanoguanidine K_{ATP} openers (e.g., **2a**) most of the cardioprotective potency resides in the 3*S*,4*R*-enantiomer.[2] The starting point for these studies was the benzophenone analogue **3** which showed a slightly improved cardioprotective potency (EC_{25} = 0.9 μM) compared to **2a** (EC_{25} = 2.5 μM) (Table 1). This improvement in cardioprotective potency was not accompanied by a similar improvement in vasorelaxant potency, which supports the hypothesis that the structure−activity relationships for cardioprotective and vasorelaxant activities of K_{ATP} openers are distinct. The higher cardioprotective potency of **3** relative to **2a** suggested that further improvement in cardioprotective potency might be achieved by modification of the C6 substituent. Thus we studied the effect of substitution at C6 by keeping the rest of the molecule constant.

A comparison of cardioprotective and vasorelaxant potencies of various analogues differing in the C6 substituent is summarized in Table 1. The homologue **4** of **3** has an EC_{25} value of 400 nM in the isolated perfused rat hearts, making it 6-fold more potent than **2a**. The benzyl (**5**) and the olefin (**6**) analogues show reduced cardioprotective potencies relative to the benzophenone lead **3**, indicating that an electron-withdrawing group at C6 is required for optimum cardioprotective potency. The cardioprotective potency of the *tert*-butyl analogue **7** of **3** suggests that the aromatic ring can be replaced with a bulky alkyl group. We explored alternate electron-withdrawing groups at C6. Replacement of the ketone of **3** with a sulfone (**8**) maintains cardioprotective potency but results in reduced cardiac selectivity by virtue of enhanced vasorelaxant potency. While the sulfonamide and the phosphinic ester analogues **9** and **10**, respectively, have cardioprotective potencies similar to that of **3**, the amide analogue **11** is less potent. These results suggest that the ketone of **3** can be replaced with other electron-withdrawing groups. The C6 substituent most likely plays the role of a hydrogen-bonding acceptor.

Further work was concentrated on the sulfonamide series of compounds due to the flexibility that they offer for rapid exploration of structure−activity relationships. Most of these compounds were evaluated for cardioprotective potency. Only selected compounds were evaluated for vasorelaxant potency to determine selectivity. The piperidine derivative **12** is 7-fold more potent than the benzenesulfonamide **9**. Compound **12** is an order of magnitude more potent than the previous lead compound **2a**. While the diethyl analogue **13** retains the cardioprotective potency of **12**, the morpholine analogue **14** is less potent. Comparison between **12** and **14** suggests that the polar groups are less tolerated in this region of the molecule. As shown by the comparison among **12**, **15**, and **16**, the size of the ring has minimal impact on cardioprotective potency. The disubstituted sulfonamides (e.g., **12**) appear to be more potent than the monosubstituted (**9**, **17**) and protio (**18**) analogues. Taken together, these data indicate that a disubstituted sulfonamide carrying a lipophilic substituent is preferred for cardioprotective potency.

Since the piperidine (**12**) and the diethylsulfonamide (**13**) analogues were among the most potent compounds, additional work was devoted to making modifications to these substituents. Substitution of the piperidine ring with methyl groups (**19**−**21**) is tolerated for cardioprotective potency. However, a large substituent such as a benzyl group (**22**, **23**) offers no further improvement in cardioprotective potency. Thus, there appears to be certain restrictions as to the size of the substituent on the piperidine ring. No further enhancement in cardioprotective potency is seen with the fused analogues (e.g., **24**). The diethyl (**13**) to diisopropyl (**25**) change was without an effect on cardioprotective potency as was the diethyl (**13**) to trifluoroethyl-ethyl change (**26**). Further increase in the size of the alkyl groups attached to the sulfonamido nitrogen provided the diisobutylsulfonamide analogue **27** (EC_{25} = 0.04 μM) which turned out to be the most potent compound of this series. Compound **27** is over 20-fold more potent than our starting compound **3** (EC_{25} = 0.9 μM). Both isobutyl groups of **27** appear to be necessary for optimum potency as the isobutyl-methyl analogue **28** (IC_{50} = 0.25 μM) is less potent.

For previous compounds (e.g., **2a,b**) in this series, the cardiac selectivity was achieved by reduction of vasorelaxant potency relative to the reference K_{ATP} openers such as cromakalim (**1**).[4−6] The improvement in cardiac selectivity for **27** results from enhancement in cardioprotective potency relative to **1** and **2a,b**. Taking into account the ratio of cardioprotective to vasorelaxant potency as a measure of cardiac selectivity, the sulfonamide analogue **27** is over 100-fold more cardiac selective than the structurally related compound **2a**. These results further support that the structure−activity relationships for cardioprotective and vasorelaxant activities of K_{ATP} openers are distinct. We hypothesize that these differences in structure−activity relationships are due to the existence of different receptor subtypes in smooth muscle and the cardiac tissue.[12] Alternatively, the receptor environment (e.g., plasmalemal vs mitochondrial) could also account for these differences in structure−activity relationships.

Further studies were undertaken to confirm the K_{ATP} opening mechanism for the cardioprotective activity of **27**, the most potent analogue of this series. Since the biochemical tools to investigate the mechanism of K_{ATP} openers are limited at the present time, we relied on pharmacological studies using the K_{ATP} blocker glyburide. We studied the effect of the K_{ATP} blocker glyburide on the cardioprotective activity of **27** in globally ischemic isolated rat hearts (Figure 1). Time to the onset of ischemic contracture was used as a measure of cardioprotective activity. Three groups of rat hearts (n = 4) were given vehicle, compound **27** (0.3 μM), and compound **27** (0.3 μM) plus glyburide (0.3 μM). Previous studies have shown that this concentration of glyburide has no effect on the time to the onset of contracture.[13] When given alone, compound **27** signifi-

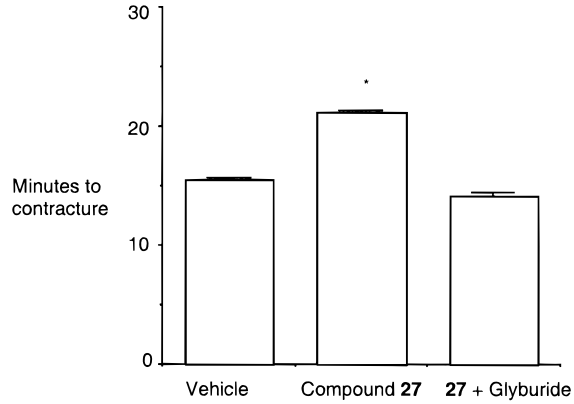


Figure 1. Effect of the K_{ATP} blocker glyburide (300 nM) on the increase in time to the onset of contracture by compound **27** (300 nM). While having no effect when given alone, glyburide completely abolished the increase in time to the onset of contracture induced by **27**.

cantly increased (21 min) time to the onset of contracture compared to the vehicle treated hearts (15.6 min). The increase in time to contracture by **27** was abolished by co-treatment with glyburide. In fact, the combination of glyburide and compound **27** was slightly proischemic, as shown by a small decrease in time to contracture compared to the vehicle treated hearts. The proischemic activity seen for a combination of **27** and glyburide is similar to the results reported for other K_{ATP} openers such as cromakalim.[13] Thus the profile of cardioprotective activity of **27** in isolated perfused rat hearts is similar to that seen for other agents of this class. Since glyburide is known to be a specific blocker of K_{ATP},[14] these results suggest that the cardioprotective effects of **27** are most likely mediated via opening of K_{ATP} in the myocardium.

The details of the mechanism of action of K_{ATP} openers are beginning to evolve. We have previously shown that cardioprotective potencies of K_{ATP} openers correlate with their binding affinities to the ^{3}H-P1075 binding site in cardiac and skeletal muscle membranes.[15] The key to the identification of the binding site was the presence of nucleotides in the binding medium. The cardiac K_{ATP} is hypothesized to be composed of two subunits: SUR_{2A} and $K_{ir}6.2$.[16] The binding site for K_{ATP} openers appears to reside on the SUR_{2A}. As for the binding site in membranes,[15] the binding to SUR_{2A} requires the presence of ATP. Taken together, these data suggest that K_{ATP} openers most likely express their cardioprotective effects by binding to the SUR_{2A}. A correlation between binding affinities of K_{ATP} openers to SUR_{2A} and their cardioprotective potencies needs to be established to link cardioprotective activity to the molecular site of action.

In conclusion, we have shown that modifications at C6 of **2a** lead to compounds (e.g., **27**) with improved cardioprotective potency and selectivity. The structure−activity studies indicate that the sulfonamido analogues derived from secondary lipophilic amines are more potent than those derived from primary amines. The sulfonamides in general appear to be more potent than the corresponding amides. The diisobutylsulfonamide analogue **27** (EC_{25} = 0.04 μM) is the most potent compound of this series. Unlike previous compounds (e.g., **2a**) in this series for which the cardiac selectivity was obtained by reduction of vasorelaxant potency, the cardiac selectivity of **27** results from a combination of reduction in vasorelaxant potency and enhancement of cardioprotective potency relative to the potent vasodilating K_{ATP} openers (e.g., cromakalim). The mechanism of action of the cardioprotective activity of **27** still involves opening of the cardiac K_{ATP} as its cardioprotective effects are abolished by co-treatment with the K_{ATP} blocker glyburide.

Experimental Section

Chemistry. All melting points were taken on a capillary melting point apparatus and are uncorrected. The infrared spectra were recorded with a Perkin-Elmer 983 spectrophotometer in KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were measured on JEOL GX-400 and FX-270 spectrometers with tetramethylsilane as an internal standard. Mass spectra were obtained with a Finnigan TSQ-4600 spectrometer. Flash chromatography was run with Whatman LPS-1 silica gel and Merck kieselgel 60 (230−400 mesh ASTM). All compounds were characterized by ^{1}H and ^{13}C NMR and mass spectra. Microanalysis of all crystalline compounds is consistent with the structures assigned. The amount of solvent present in the molecular formula was determined by ^{1}H NMR spectra and microanalysis. Karl Fischer analysis was performed in selected cases to confirm the amount of water present.

The benzopyranylamines (**32**) can be prepared from the corresponding phenols (**29**) by methods described in the literature.[4−6,17]

A typical procedure is illustrated for the preparation of (3*S*-*trans*)-*N*-(4-chlorophenyl)-*N*′-cyano-*N*″-[6-[(diethylamino)sulfonyl]-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl]guanidine (13). A. *N*,*N*-Diethyl-2,2-dimethyl-2*H*-1-benzopyran-6-sulfonamide (36, R = NEt_2). To a stirred solution of diethylamine (1.5 g, 20.5 mmol) in a (1:1) mixture of water and CH_2Cl_2 (20 mL) at 0 °C was added 2,2-dimethyl-2*H*-1-benzopyran-6-sulfonyl chloride[9] (1.0 g, 3.9 mmol) in portions. The resultant mixture was stirred at 0 °C for 20 min and room temperature for 2 h. The organic layer was separated, and the aqueous layer was reextracted with dichloromethane. The combined organic extracts were dried over $MgSO_4$ concentrated in vacuo to give the title compound as an oil (1.12 g, 100%). ^{1}H NMR (CDCl3) δ 1.22 (t, J = 7.0 Hz, 6 H), 1.54 (s, 6 H), 3.30 (q, J = 7.0 Hz, 4 H), 5.78 (d, J = 10 Hz, 1 H), 6.40 (d, J = 10 Hz, 1 H), 6.90 (d, J = 8.8 Hz, 1 H), 7.50 (d, J = 2.3 Hz, 1 H), 7.60 (dd, J = 2.3; 8.8 Hz, 1 H). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 14.17, 28.25, 41.96, 77.46, 116.57, 121.14, 125.38, 128.23, 131.86, 156.25.

B. (1a*S*-*cis*)-*N*,*N*-Diethyl-1a,7b-dihydro-2,2-dimethyl-2*H*-oxireno[c][1]benzopyran-6-sulfonamide (31, R = SO_2NEt_2). To a mixture of commercial sodium hypochlorite solution (15.0 mL, 0.705 M, 10.5 mmol) and Na_2HPO_4 buffer (6 mL, 50 μM) was added 1 N sodium hydroxide at 0 °C until pH ∼ 11.3. To the resulting reaction mixture were added title A compound (1.1 g, 3.7 mmol), (*S*,*S*)(+)-*N*,*N*′-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediamino-manganese(III) chloride (the Jacobsen's catalyst) (30 mg, 1 mol %) and 4-phenylpyridine-*N*-oxide (20 mg). The resultant biphasic mixture was stirred at 0 °C for 18 h and poured into methylene chloride (50 mL), and the organic layer was separated. The aqueous layer was extracted with methylene chloride (2 × 50 mL), and the combined organic extracts were washed with saturated NH_4Cl solution and brine. After drying over $MgSO_4$, the solvent was removed in vacuo to give the title compound as an oil (1.05 g, 91%). ^{1}H NMR ($CDCl_3$): δ 1.23 (t, J = 7.0 Hz, 6 H), 1.38 (s, 3 H), 1.70 (s, 3 H), 3.30 (q, J = 7.0 Hz, 4 H), 3.64 (d, J = 4.1 Hz, 1 H), 4.04 (d, J = 4.1 Hz, 1 H), 6.98 (d, J = 8.8 Hz, 1 H), 7.78 (dd, J = 1.8 Hz, 8.8 Hz, 1 H), 7.90 (d, J = 1.8 Hz, 1 H).

C. (3*S*-*trans*)-4-Amino-*N*,*N*-diethyl-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-sulfonamide (32, R = SO_2NEt_2). To a stirred solution of title B compound (910 mg, 2.5 mmol) in a mixture of THF and 2-propanol (15 mL, 2:1) was added ammonium hydroxide (3 mL), and the reaction

mixture was heated in a sealed tube at 75 °C (oil bath temperature) for 24 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was diluted with ethyl acetate (100 mL) and extracted with saturated $NaHCO_3$. The organic layer was dried and concentrated in vacuo, and the residue was crystallized from ethyl acetate-cyclohexane to give a white solid (850 mg, 70%). $[\alpha]_D = +50.9°$ ($c = 0.90$, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.24 (s, 3 H), 1.43 (m, 2 H), 1.53 (s, 3 H), 1.65 (m, 4 H), 2.97 (m, 4 H), 3.35 (d, $J = 9.0$ Hz, 1 H), 3.68 (d, $J = 9.0$ Hz, 1 H), 6.90 (d, $J = 8.8$ Hz, 1 H), 7.53 (dd, $J = 1.8$, 8.8 Hz, 1 H), 7.80 (d, $J = 1.8$ Hz, 1 H).

D. (3*S-trans*)-*N*-(4-Chlorophenyl)-*N′*-cyano-*N″*-[6-[(diethylamino)sulfonyl]-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl]guanidine (13). To a stirred solution of the title C compound (400 mg, 1.2 mmol) and *N*-chlorophenyl-*N′*-cyanothiourea, monosodium salt[8] (283 mg, 1.34 mmol) in DMF (7 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (257 mg, 1.34 mmol) at room temperature under argon. The reaction mixture was stirred at room temperature for 18 h. The resultant mixture was poured into a mixture of ethyl acetate (100 mL) and saturated NH_4Cl solution (50 mL). The ethyl acetate layer was separated, and the aqueous layer was reextracted with ethyl acetate (2 × 50 mL). The combined organic layer was washed with brine (50 mL). After the organic layer was dried over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography on silica gel (ethyl acetate:hexane/1:1) to give a colorless solid after trituration with ether (400 mg, 65%). $[\alpha]_D^{25} = +7.8°$ ($c = 0.60$, CH_3OH). ^{1}H NMR ($CDCl_3/CD_3OD$): δ 1.22 (t, $J = 7.0$ Hz, 6 H), 1.37 (s, 3 H), 1.60 (s, 3 H), 3.30 (q, $J = 7.0$ Hz, 4 H), 3.82 (d, $J = 10$ Hz, 1 H), 5.15 (d, $J = 10$ Hz, 1 H), 7.00 (d, $J = 8.8$ Hz, 1 H), 7.45 (m, 4 H), 7.65 (m, 1 H), 7.80 (br s, 1 H). ^{13}C NMR ($CDCl_3/CD_3OD$): δ 157.59, 133.14, 130.59, 129.23, 128.62, 127.15, 118.77, 81.31, 53.92, 43.53, 27.09, 18.68, 14.76.

(3*S-trans*)-4-Amino-3,4-dihydro-2,2-dimethyl-6-(1-phenylethenyl)-2*H*-1-benzopyran-3-ol (34). To a solution of ketone **33** (667 mg, 2.25 mmol), prepared in a standard fashion,[4−6,17] in dry THF (15 mL) cooled to −78° was added dropwise methyllithium solution (7.0 mL, 1.4 M in ether, 9.8 mmol). After 5 min the resulting reaction mixture was quenched by addition of methanol (1 mL) and warmed to 0 °C. To the resulting reaction mixture was added brine (50 mL), and it was extracted with ether. The combined ether extract was dried (Na_2SO_4) and concentrated to give the crude alcohol as a pink oil which was taken up in dichloromethane (15 mL), cooled in an ice bath, and treated with trifluoroacetic acid (1 mL). The reaction mixture turned yellow. After storage at 5 °C for 18 h, the reaction mixture was added to 25 mL of 1 N sodium hydroxide and extracted with methylene chloride. The combined organic extract was dried (Na_2SO_4) and concentrated to give the title compound (475 mg, 72%) as a white solid, mp 108−109 °C.

The amine **34** can be converted to the final product **10** by the procedure described for the synthesis of compound **13** (step D).

6-(Methoxyphenylphosphinyl)-2,2-dimethyl-2*H*-1-benzopyran (38). To a reaction mixture containing **37** (929 mg, 9.2 mmol), prepared in a standard fashion,[4−6,17] phenylphosphinic acid, methyl ester[18] (2.39 g, 8.36 mmol), and 4-methylmorpholine (929 mg, 9.2 mmol) in acetonitrile (25 mL) at room temperature under argon was added tetrakis-triphenylphosphine palladium (0) (485 mg, 0.42 mmol).[10] The resulting heterogeneous reaction mixture was heated at 80 °C for 45 min to give a yellow solution. It was cooled to room temperature and concentrated in vacuo. The residue was partitioned between ethyl acetate and 1 N hydrochloric acid. The aqueous layer was separated and extracted with ethyl acetate. The organic extracts were combined, washed with 5% sodium thiosulfate solution, dried ($MgSO_4$) and concentrated in vacuo to give a dark oil. The crude oil was purified by flash chromatography on silica gel (ethyl acetate:hexane/4:1) to afford the title compound (2.18 g, 83%) as a pale yellow oil. The olefin can be converted to the final product **10** by the same procedure as described for the synthesis of **13** (Scheme 1).

2,2-Dimethyl-*N*-phenyl-2*H*-1-benzopyran-6-carboxamide (41). A. 2,2-Dimethyl-2*H*-1-benzopyran-6-carboxylic acid (40). A light yellow solution of **39**[4−6,17] (25.0 g, 135 mmol) in ethanol (125 mL) was treated with 10 N sodium hydroxide (125 mL). The reaction mixture was heated at reflux temperature for 7 h, then cooled to 0 °C, and slowly treated with concentrated hydrochloric acid (100 mL). The solid was collected by filtration and washed several times with water. The light yellow solid was dried in vacuo at 90 °C for 20 h to afford the desired product (27.6 g, 100%) as a light yellow solid, mp 155−157 °C.

B. 2,2-Dimethyl-*N*-phenyl-2*H*-1-benzopyran-6-carboxamide (41). To a light yellow solution of title A compound (1.0 g. 4.9 mmol) in dichloromethane (8 mL) and DMF (3 drops) at 0 °C under argon was added oxalyl chloride (521 mL, 5.97 mmol). The resulting yellow solution was stirred at 0 °C for 30 min and room temperature for 30 min. The crude reaction mixture was concentrated in vacuo to give 2,2-dimethyl-2*H*-1-benzopyran-6-carbonyl chloride (**40**) which in anhydrous THF (8 mL) was added to a solution of aniline (670 mL, 7.35 mmol) and triethylamine (3 mL) in THF (8 mL) at 0 °C under argon. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The crude reaction mixture was concentrated, diluted with ethyl acetate (100 mL), and filtered. The filtrate was washed with brine, dried ($MgSO_4$), and concentrated. The resulting solid was triturated with ethyl acetate/hexane to afford the desired compound (1.17 g, 86%) as a white solid, mp 152−153 °C.

The olefin can be converted to the final product by the same procedure as described for the synthesis of compound **13**.

Biological Assays. EC_{25} values for increasing time to contracture were determined in isolated perfused globally ischemic rat hearts. Compounds were initially evaluated at 10 μM concentration. Those demonstrating greater than 25% increase in time to the onset of contracture were subjected to concentration−response studies to determine EC_{25} values. To compare the antiischemic and peripheral vasodilator potencies, we determined IC_{50} values for the relaxation of the methoxamine contracted rat aorta. Experimental details of both methods are described.[4−6]

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

References

(1) (a) Coetzee, W. A. ATP-Sensitive Potassium Channels and Myocardial Ischemia: Why Do They Open? *Cardiovasc. Drugs Ther.* **1992**, *6*, 201−208. (b) Hearse, D. J. Activation of ATP-sensitive Potassium Channels: a Novel Pharmacological Approach to Myocardial Protection. *Cardiovasc. Res.* **1995**, *30*, 1−17. (c) Atwal, K. S. Pharmacology and Structure−Activity Relationships for K_{ATP} Modulators: Tissue-Selective K_{ATP} Openers. *J. Cardiovasc. Pharmacol.* **1994**, *24* (Suppl. 4), S12−S17. (d) Atwal, K. S. Advances in the Structure−Activity Relationships, Mechanism of Action, and Therapeutic Utilities of ATP-Sensitive Potassium Channel Openers. *Drug Dev. Res.* **1994**, *33*, 250−262.3.

(2) (a) For BMS-180448 (**2a**): Atwal, K. S.; Grover, G. J.; Ahmed, S. Z.; Ferrara, F. N.; Harper, T. W.; Kim, K. S.; Sleph, P. G.; Dzwonczyk, S.; Russell, A. D.; Moreland, S.; McCullough, J. R.; Normandin, D. E. Cardioselective anti-ischemic ATP-sensitive potassium channel openers. *J. Med. Chem.* **1993**, *36*, 3971−3974. (b) For BMS-191095 (**2b**): Rovnyak, G. C.; Ahmed, S. Z.; Ding, C. Z.; Dzwonczyk, S.; Ferrara, F. N.; Humpheries, 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-Sensitive Potassium Channel (K_{ATP}) Openers. 5. Identification of 4-(N-Aryl)-Substituted Benzopyran Derivatives with High Selectivity. *J. Med. Chem.* **1997**, *40*, 24−34.

(3) Atwal, K. S.; Grover, G. J. BMS-180448, A Glyburide-Reversible Cardioprotective Agent with Minimal Vasodilator Activity. *Cardiovasc. Drug Rev.* **1995**, *13*, 123−136.

(4) Atwal, K. S.; Grover, G. J.; Ferrara, F. N.; Ahmed, S. Z.; Sleph, P. G.; Dzwonczyk, S.; Normandin, D. E. Cardioselective antiischemic ATP-sensitive potassium channel openers. 2. Structure−Activity Studies on Benzopyranyl Cyanoguanidines; Modification of the Benzopyran. *J. Med. Chem*. **1995**, *38*, 1966−1973.
(5) Atwal, K. S.; Grover, G. J.; Ahmed, S. Z.; Sleph, P. G.; Dzwonczyk, S.; Baird, A. J.; Normandin, D. E. Cardioselective antiischemic ATP-sensitive potassium channel openers 3. Structure−Activity Studies on Benzopyranyl Cyanoguanidines; Modification of the Cyanoguanidine Portion. *J. Med. Chem*. **1995**, *38*, 3238−3245.
(6) Rovnyak, G. C.; Ahmed, S. Z.; Ding, C. Z.; Dzwonczyk, S.; Ferrara, F. N.; Humpheries, 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 anti-ischemic ATP-sensitive potassium channel (K_{ATP}) openers. 5. Identification of 4-(N-Aryl)-Substituted Benzopyran Derivatives with High Selectivity. *J. Med. Chem*. **1997**, *40*, 24−34.
(7) Lee, N. H.; Muci, A. R.; Jacobsen, E. R. Enantiomerically Pure Epoxychromans via Asymmetric Catalysis. *Tetrahedron Lett.* **1991**, *32*, 5055.
(8) Atwal, K. S.; Ahmed S. Z.; O'Reilly, B. C. A Facile Synthesis of Cyanoguanidines from Thioureas. *Tetrahedron Lett*. **1989**, *30*, 7313−7316.
(9) Ding, C. Z. A Convenient Synthesis of 6-Substituted-2,2-Dimethyl-2H-1-Benzopyrans. *Synth. Comm*. **1996**, *26*, 4267−4273.
(10) Lei, H.; Stoakes, M. S.; Schwabacher, A. W. Palladium Catalyzed Preparation of Monoaryl and Diarylphosphinates from Methyl Phosphinate. *Synthesis* **1992**, 1255−60.
(11) Grover, G. J.; Newburger, J.; Sleph, P. G.; Dzwonczyk, S.; Taylor, S. C.; Ahmed, 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.
(12) Atwal, K. S.; Grover, G. J. Treatment of Myocardial Ischemia with ATP-Sensitive Potassium Channel (K_{ATP}) Openers. *Curr. Pharm. Des.* **1996**, *2*, 585−595.
(13) (a) Grover, G. J.; Dzwonczyk, S.; Sleph, P. G. Reduction of Ischemic Damage in Isolated Rat Hearts by the Potassium Channel Opener RP 52891. *Eur. J. Pharmacol*. **1990**, *69*, 571−581. (b) Sargent, C. A.; Smith, M. A.; Dzwonczyk, S.; Sleph, P. G.; Grover, G. J. Effect of Potassium Channel Blockade on the Anti-Ischemic Actions of Mechanistically Diverse Agents. *J. Pharmacol. Exp. Ther*. **1991**, *259*, 97−103.
(14) (a) Inagaki, N.; Gonoi, T.; Clement, J. P., IV; Namba, N.; Inazawa, J.; Gonzalez, G.; Aguilar-Bryan, L.; Seino, S.; Bryan, J. Reconstitution of IK_{ATP}: An Inward Rectifier Subunit Plus the Sulfonylurea receptor. *Science* **1995**, *270*, 1166. (b) Inagaki, N.; Gonoi, T.; Clement, J. P., IV; Wang, C.; Aguilar-Bryan, L.; Bryan J.; Seino, S. A Family of Sulfonylurea Receptors Determines the Pharmacological Properties of ATP-Sensitive K+ Channels. *Neuron* **1996**, *16*, 1011−1017.
(15) (a) Dickinson, K. E. J.; Bryson, C. C.; Cohen, R. B.; Rogers, L.; Green, D. W.; Atwal, K. S. Nucleotide Regulation and Characterization of Potassium Channel Opener Binding to Skeletal Muscle Membranes. *Mol. Pharmacol.* **1997**, *52*, 1473−1481. (b) Atwal, K. S.; Grover, G. J.; Lodge, N. J.; Normandin, D. E.; Traeger, S. C.; Sleph, P. G.; Cohen, R. B.; Bryson, C. C.; Dickinson, K. E. J. Binding of ATP-Sensitive Potassium Channel (K_{ATP}) Openers to Cardiac Membranes: Correlation of Binding Affinities with Cardioprotective and Smooth Muscle Relaxing Potencies. *J. Med. Chem*. **1998**, *41*, 271−275.
(16) Schwanstecher, M.; Sieverding, C.; Dorschner, H.; Gross, I.; Aguilar-Bryan. L.; Schwanstecher, C.; Bryan, J. Potassium Channel Openers Require ATP to bind to and act Through Sulfonylurea Receptors. *EMBO J*. **1998**, *17*, 5529−5535.
(17) (a) Ashwood, V. A.; Buckingham, R. E.; Cassidy, F.; Evans, J. M.; Faruk, E. A.; Hamilton, T. C.; Nash, D. J.; Stemp, G.; Willcock, K. Synthesis and Antihypertensive Activity of 4-(Cyclic amido)-2H-1-benzopyrans. *J. Med. Chem*. **1986**, *29*, 2194−2201. (b) Burrell, G.; Cassidy, F.; Evans, J. M.; Lightowler, D.; Stemp, G. Variation in the Aromatic Ring of Cromakalim: Antihypertensive Activity of Pyranopyridines and 6-Alkyl-2H-1-benzopyrans. *J. Med. Chem*. **1990**, *33*, 3023−3027. (c) Arch, J. R. S.; Buckle, D. R.; Carey, C.; P.-Dobrzanski, H.; Faller, A.; Foster, K. A.; H.-Frydrych, C. S. V.; Pinto, I. L.; Smith, D. G.; Taylor, S. G. Relaxant Activity of 6-Cyano-2,2-dimethyl-2H-1-benzopyran-4-carboxamides and -thiocarboxamides and Their Analogues in Guinea Pig Trachealis. *J. Med. Chem*. **1991**, *34*, 2588−2594. (d) Godfrey, J. D., Jr.; Mueller, R. H.; Sedergran, T. C.; Soundarajan, N.; Colandrea, V. J. Improved Synthesis of Aryl 1,1-Dimethylproparyl Ethers. *Tetrahedron Lett*. **1994**, *35*, 6405−6408.
(18) Debruin, K. E.; Tang, C-L. W.; Johnson, D. M.; Wilde, R. L. Kinetic facial Selectivity in Nucleophilic Displacements at Tetracoordinate Phosphorus: Kinetic and Stereochemistry in the Reaction of Sodium Ethoxide with O,S-Dimethyl Phenylphosphonothioate. *J. Am. Chem. Soc.* **1989**, *111*, 5871−5879.

JM990196H