Cardioselective Anti-Ischemic ATP-Sensitive Potassium Channel Openers. 3. Structure–Activity Studies on Benzopyranyl Cyanoguanidines: Modification of the Cyanoguanidine Portion

Karnail S. Atwal,* Gary J. Grover, Syed Z. Ahmed, Paul G. Sleph, Steven Dzwonczyk, Anne J. Baird, and Diane E. Normandin

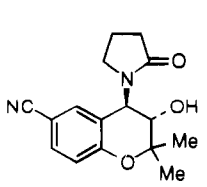
The Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000

Received May 3, 1995[⊗]

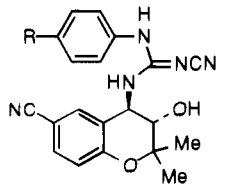
Structure–activity relationships for the cyanoguanidine portion of the lead cardiac selective ATP-sensitive potassium channel (K_{ATP}) opener (**3**) are described. The cyanoguanidine moity appears to be optimal since increasing or decreasing the distance between the aniline nitrogen and the pendant aromatic ring attenuates anti-ischemic potency/selectivity. Similarly, unfavorable results are obtained by replacement of the aniline nitrogen with other linkers (CH_2, S, O). Replacement of the phenyl ring with a methyl group diminishes cardiac selectivity. Constraining the urea moiety into a benzimidazolone or imidazolone ring retains anti-ischemic potency with significant improvement in cardiac selectivity. As shown by the ratio of vasorelaxant and anti-ischemic potencies, the cardiac selectivity in vitro varies over 3 orders of magnitude. These data are in agreement with previous results indicating that distinct structure–activity relationships exist for the anti-ischemic and vasorelaxant activities. Since the anti-ischemic effects of this series of compounds are abolished by pretreatment with structurally different K_{ATP} blockers (glyburide, sodium 5-hydroxydecanoate, meclofenamic acid), the mechanism for the anti-ischemic actions of these compounds still appears to involve the opening of K_{ATP}.

Introduction

In recent years a great deal of attention has been paid to the phenomenon of myocardial preconditioning, wherein the myocardium attains the ability to protect itself from ischemic damage following several short periods of ischemia.[1] Since it is a powerful and physiologically relevant mechanism used by the heart to withstand ischemic insult, its pharmacologic manipulation constitutes an attractive approach for the treatment of acute myocardial ischemia. Although the mechanism of myocardial preconditioning is not completely understood, evidence is mounting that the opening of an ATP-sensitive potassium channel (K_{ATP}) may be involved in mediating the cardioprotective effects of preconditioning in animal models[2] and man.[3] Further evidence for the role of K_{ATP} opening in cardioprotection has been provided by pharmacological studies demonstrating that K_{ATP} openers (e.g., cromakalim, **1**) can directly protect



Cromakalim (**1**)



2: BMS-180448 (R = Cl, chiral)
3: R = H (racemate)

the heart from an ischemic insult without contribution from vasodilatation.[4] However, the potent peripheral vasodilating properties of the first-generation compounds (**1**), which can result in underperfusion of the tissue already at risk, can limit their use for the treatment of acute myocardial ischemia. We have recently described a new class of K_{ATP} openers (BMS-180448, **2**) that are more selective for the ischemic myocardium compared to the first generation compounds (e.g., cromakalim, **1**).[5] These cardiac selective agents may have a higher window of safety for the treatment of acute myocardial ischemia.

In a previous publication we described structure–activity studies focusing on the benzopyran portion of the lead compound **3**.[6] This paper deals with structure–activity studies of the cyanoguanidine moiety of **3**. Our results support the existence of distinct structure–activity relationships for the anti-ischemic and vasorelaxant potencies of **3** and its congeners.

Chemistry

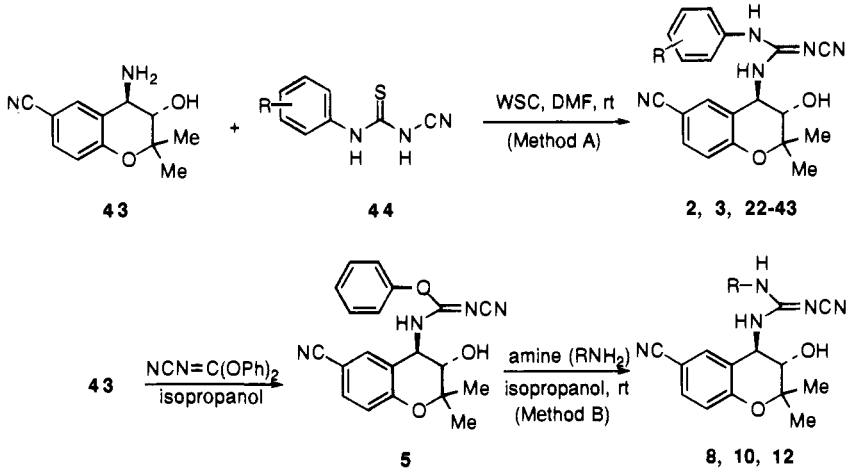
The cyanoguanidine analogs were prepared by two methods. Method A, used for the synthesis of compounds **3**, **22**–**42**, involves treatment of the amine (**43**) with *N*-cyano-*N*-arylthioureas (**44**) in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (water soluble carbodiimide, WSC) in dimethylformamide (Scheme 1, method A). The modest 50–60% yield of this step is due to incomplete reaction. Details of this method have been described.[5–7] Method B involves conversion of the amino alcohol (**43**) to a common intermediate (**5**) which is then reacted with the appropriate amine to provide the desired products (**8**, **10**, **12**) (Scheme 1).[8] Both racemic and chiral forms of benzopyranyl amine **43** are described.[5,9] The arythiourea employed in method A (Scheme 1) can be readily prepared by treatment of arylisothiocyanate with monosodium cyanamide.[7]

The acyl analog **7** was prepared from the amine **43** by treatment with phenylacetyl chloride in aqueous sodium bicarbonate. The acid analogs **32** and **33** were obtained by saponification of the esters **30** and **31**,

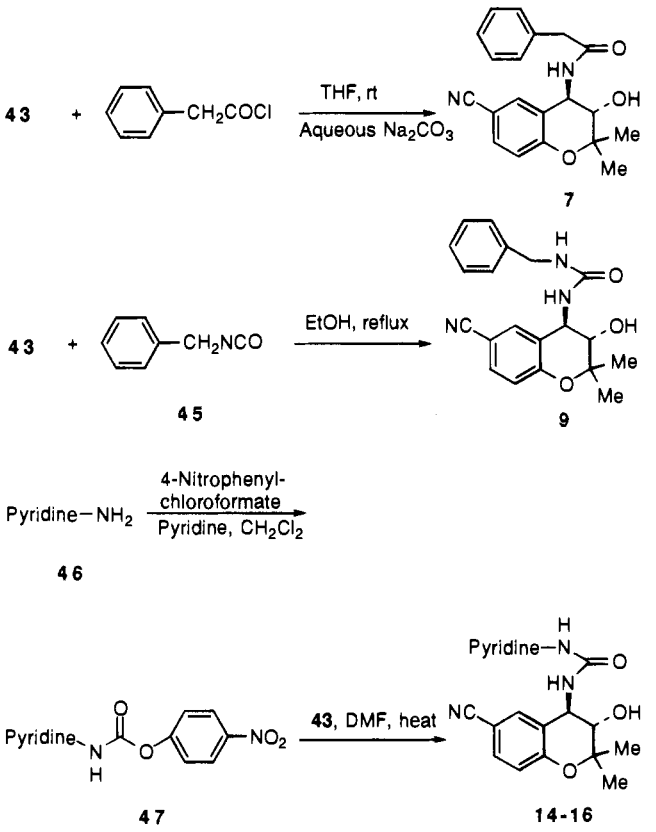
[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0022-2623/95/1838-3236$09.00/0

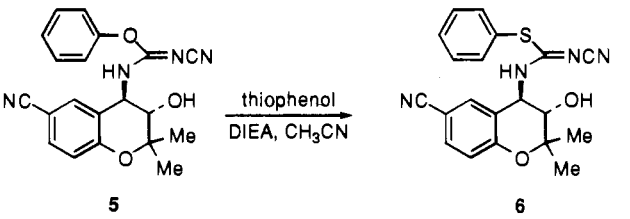
Scheme 1



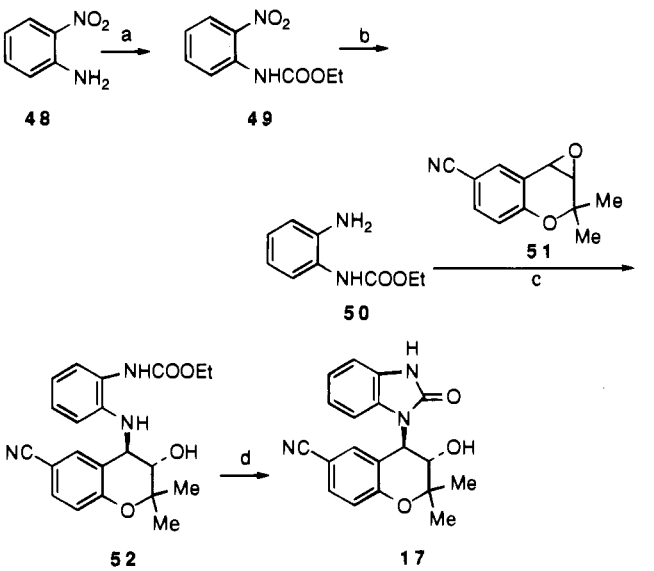
Scheme 2



Scheme 3



Scheme 4[a]



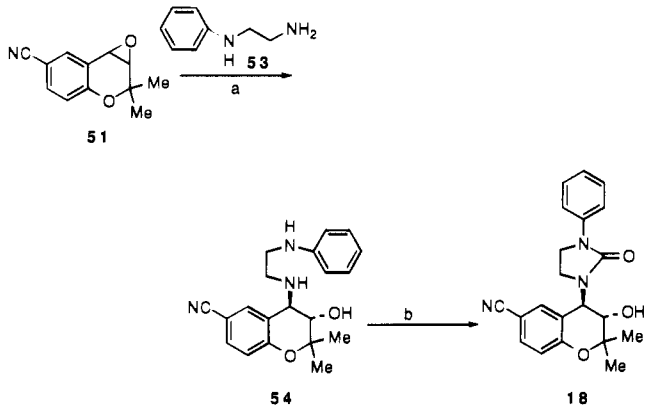
[a] Reagents: (a) Ethyl chloroformate, pyridine, 73%; (b) hydrogen, palladium hydroxide, ethanol, 65%; (c) magnesium perchlorate, acetonitrile, room temperature, 96%; (d) sodium methoxide, methanol, reflux, 60%.

respectively. The urea analog **9** was obtained in excellent yield by treatment of the amine **43** with benzylisocyanate **45** (Scheme 2). The pyridyl urea analogs **14–16** were obtained by reacting aminopyridines (**46**) with 4-nitrophenyl chloroformate followed by treatment of the resulting carbamate **47** with the amino alcohol **43** (Scheme 2).

The phenylthio analog (**6**) of **3** was prepared by treatment of **5** with thiophenol in the presence of diisopropylethylamine (Scheme 3). Synthesis of the conformationally restricted analog **17** is outlined in Scheme 4. 2-Nitroaniline (**48**) was converted to the carbamate **49** by reaction with ethyl chloroformate in pyridine. Catalytic hydrogenation of **49** provided the amine **50** which on treatment with the epoxide **51**[9] gave the penultimate intermediate **52**. Cyclization of **52** to the desired product **17** was accomplished by heating **52** with sodium methoxide in methanol. Compound **18** was prepared from the epoxide **51**[9] in two steps involving treatment of **51** with *N*-phenylethylenediamine (**53**) followed by cyclization of the resulting diamine **54** with 4-nitrophenyl chloroformate (Scheme 5).

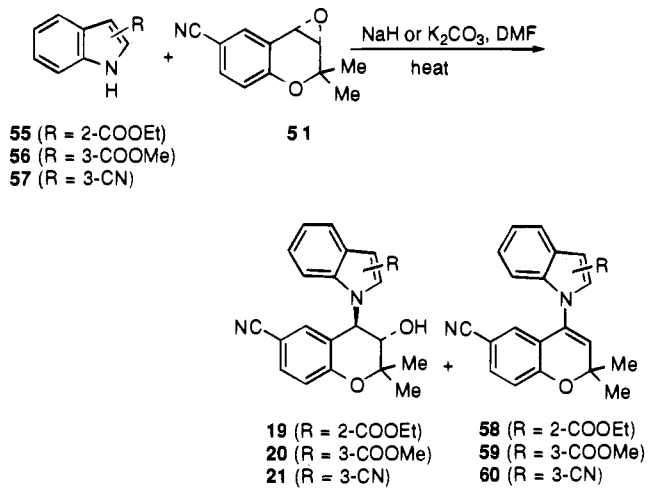
The indole analogs **19–21** were prepared in low yields by heating the epoxide **51**[9] with the appropriately substituted indole (**55–57**) in the presence of sodium hydride or potassium carbonate (Scheme 6). The major products of this reaction were the dehydrated compounds (**58–60**). In the case of the 2-carboxy analog **19**, we isolated a mixture of products which contained significant amounts of the carboxylic acid products, presumably obtained by saponification of the ester under the reaction conditions. Therefore, the crude

Scheme 5[a]



[a] Reagents: (a) ethanol, room temperature, 90%; (b) 4-nitrophenyl chloroformate, CH_2Cl_2, 0 °C, 40%.

Scheme 6



reaction mixture was treated with diazomethane, and the products (**19**, **58**) were separated by flash chromatography on silica gel. We often observed doubling of ^1H and ^{13}C NMR signals for compounds **17**–**21** due to restricted rotation around the C4–N bond. Heating in dimethyl sulfoxide up to 100–120 °C usually gave a time-averaged spectrum.

Results and Discussion

The vasorelaxant potencies were determined by measurement of IC_{50} values for relaxation of the methoxamine-contracted rat aorta, as described previously.[8] Anti-ischemic potencies *in vitro* were determined by measurement of EC_{25} values for 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.[11] The ratio of EC_{25} value for time to contracture and IC_{50} value for vasorelaxant potency indicates selectivity *in vitro* for the ischemic myocardium. Validity of these *in vitro* tests to predict anti-ischemic selectivity *in vivo* has been previously demonstrated by detailed studies *in vivo* with BMS-180448 (**2**).[12] BMS-180448 can protect the ischemic myocardium without effecting the peripheral hemodynamic status of the animals.[12]

Initial structure–activity studies evaluating the effects of modification of the cyanoguanidine moiety were carried out with racemic mixtures (**3**–**21**). Once we established that most of the the anti-ischemic activity resides in the 3*S*,4*R*-enantiomer, we restricted our studies to single enantiomers (**2**, **22**–**42**) for evaluating the effect of subtitution on the pendant aromatic ring. We evaluated both cyanoguanidine and urea analogs, depending upon synthetic accessibility. Previous studies have shown that the cyanoguanidine (**3**) and urea (**4**) analogs have similar anti-ischemic potencies (Table 1).[5,6]

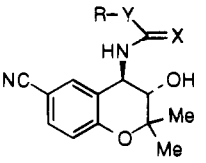
Replacement of the the aniline nitrogen of cyanoguanidine **3** with an oxygen (**5**) or sulfur (**6**) causes diminuition of anti-ischemic potency. Comparison between urea analog **4** and the acyl derivative **7** indicates that methylene replacement for the aniline nitrogen atom is also deleterious to anti-ischemic potency. These data are consistent with the requirement of an aniline nitrogen for optimal anti-ischemic potency. Insertion of a methylene unit (**8**, **9**) and a nitrogen atom (**10**) between the pendant phenyl ring and the urea nitrogen leads to diminished anti-ischemic potency (compare **3** vs **8** and **10**, **4** vs **9**). These data suggest that optimal anti-ischemic potency is achieved with a phenylurea/cyanoguanidine moiety.

Deletion of the aniline nitrogen provided the previously reported benzamide analog **11**[13] with potent vasorelaxant activity, yet having anti-ischemic potency similar to that of **4**. It is interesting to note how a small change in structure (**4** → **11**) can cause a 60-fold reduction in cardiac selectivity. This result suggests that the structure–activity relationships for the two activities are quite different. Replacment of the phenyl with a methyl group (**12**) retains anti-ischemic potency but not without the loss of cardiac selectivity. The bulky isoamyl replacement (**13**) for the phenyl ring of **3** yields a compound that is less potent than the lead compound **3**. These results suggest that the phenyl ring may be optimal for good anti-ischemic activity and cardiac selectivity. While both the 3- (**14**) and 2-pyridyl (**15**) analogs of phenylurea **4** have anti-ischemic potencies similar to that of **4**, the 4-pyridyl analog (**16**) is devoid of anti-ischemic activity. Thus, depending on the position of the heteroatom, the pendant phenyl ring can be replaced with an isosteric heterocycle.

To evaluate the effect of the urea conformation on biological activity, we prepared the conformationally restrained analogs **17** and **18** of **4** (Table 2). Both compounds **17** and **18** retain most of the anti-ishcemic potency of **4** with significant improvement in cardiac selectivity. These data further exemplify the differential effects of structural changes on anti-ischemic and vasorelaxant activities. Retention of anti-ischemic potency by the constrained analogs **17** and **18** provided the opportunity to evaluate surrogates for the cyclic phenylurea moiety. Those efforts led to the identification of 2- and 3-substituted indole analogs **19**–**21** (Table 2).

While the 2-carbomethoxy indole analog **19** has anti-ischemic potency and cardiac selectivity comparable to the fused analog **17**, the 3-carbomethoxy analog **20** is more potent than **17** as an anti-ischemic and vasorelxant agent. These data imply that location of the carbomethoxy group can have a large impact on cardiac selectivity, predominantly by effecting vasorelaxant

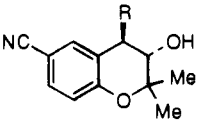
Table 1. Vasorelaxant and Anti-Ischemic Potencies of Benzopyranyl Cyanoguanidine Potassium Channel Openers (all Compounds Racemates)

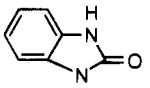
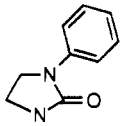
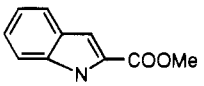
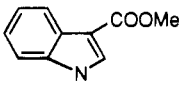
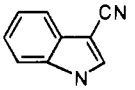


compd	R	X	Y	time to contracture,[a] EC_{25}, μM or % increase at 10 μM	vasorelaxant potencies,[b] IC_{50}, μM (95% CI)	ratio EC_{25}/IC_{50}
3	C_6H_5	NCN	NH	11.0	1.4 (0.98, 1.93)	7.9[c]
4	C_6H_5	O	NH	5.1	0.8 (0.51, 1.28)	6.4[c]
5	C_6H_5	NCN	O	68.8	>100	
6	C_6H_5	NCN	S	>30	4.9 (3.2, 7.5)	
7	C_6H_5	O	CH_2	22.2	1.8 (0.9, 2.5)	12.3
8	$C_6H_5CH_2$	NCN	NH	>30	2.4 (1.6, 3.5)	
9	$C_6H_5CH_2$	O	NH	>30	5.6 (3.9, 8.2)	
10	$C_6H_5NH_2$	NCN	NH	4%	3.5 (2.4, 5.2)	
11	C_6H_5	O		11.8	0.03 (0.023, 0.039)	393
12	Me	NCN	NH	13.3	0.2 (0.13, 0.32)	66.5
13	1,1-Me_2Pr	NCN	NH	20.1	29.8 (23.2, 38.3)	0.7[c]
14	3-pyridinyl	O	NH	4.2	0.5 (0.35, 0.74)	8.4
15	2-pyridinyl	O	NH	2.6	0.44 (0.34, 0.60)	5.9
16	4-pyridinyl	O	NH	1%	8.5 (4.2, 17.1)	

[a] Anti-ischemic potency was determined by measurement of EC_{25}, concentration necessary for increase in time to contracture by 25%, in the globally ischemic rat hearts. Time to contracture was defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg. Each value is an average of three or four determinations and is within approximately ±20%. [b] Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine contracted rat aorta. IC_{50} is presented as a mean with 95% confidence interval in parentheses, $n = 4$ or higher. [c] Data presented previously in ref 5.

Table 2. Vasorelaxant and Anti-Ischemic Potencies of Benzopyranyl Cyanoguanidine Potassium Channel Openers (all Compounds Racemates)

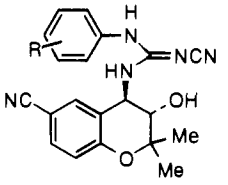


compd	R	time to contracture[a] EC_{25}, μM	vasorelaxant potencies,[b] IC_{50}, μM (95% C I)	ratio EC_{25}/IC_{50}
17		11.7	41.0 (23.0, 73.0)	0.28
18		10.0	23.7 (13.0, 43.1)	0.42
19		8.1	33.1 (19.0, 58.6)	0.24
20		3.2	0.5 (0.3, 0.87)	6.4
21		25.5	2.2 (1.7, 2.9)	11.5

[a] Anti-ischemic potency was determined by measurement of EC_{25}, concentration necessary for increase in time to contracture by 25%, in the globally ischemic rat hearts. Time to contracture was defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg. Each value is an average of three or four determinations and is within approximately ±20%. [b] Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine contracted rat aorta. IC_{50} is presented as a mean with 95% confidence interval in parentheses, $n = 4$ or higher.

potency (compare **19** vs **20**). Cyano replacement (**21**) for the carbomethoxy group of **20** is harmful to both anti-ischemic and vasorelaxant potencies. The lower anti-ischemic activity of the cyano analog **21** relative to **20** indicates that the carbonyl group is important for the anti-ischemic activity of the indole analogs **19** and **20**; it might be serving as a hydrogen bond acceptor, a role presumably played by the urea carbonyl of **17**. The biological activities of **19**–**21** support that the fused phenylurea moiety of **17** can be replaced with other surrogates such as 2- and 3-substituted indoles. Retention of anti-ischemic potencies by compounds **17**–**20** also

Table 3. Vasorelaxant and Anti-ischemic Potencies of Aromatic Substituted Benzopyranyl Cyanoguanidine Potassium Channel Openers (all Compounds Chiral with 3*S*,4*R*-Stereochemistry) and Cromakalim (1)



compd	R	time to contracture,[a] EC_{25}, μM, or % increase at 10 μM	vasorelaxant potencies,[b] IC_{50}, μM (95% CI)	ratio EC_{25}/IC_{50}
2	4-Cl	2.5	1.8 (0.78, 4.25)	1.4
22	H	6.1	1.3 (0.95, 1.89)	4.7
23	3-Cl	1.4	0.6 (0.39, 0.89)	2.3
24	3-NO_2	5.0	0.67 (0.43, 1.1)	7.5
25	4-NO_2	6.2	0.30 (0.17, 0.48)	20.6
26	3-CF_3	5.3	1.6 (0.9, 2.7)	3.3
27	4-CF_3	13.4	8.1 (5.3, 1.24)	1.4
28	3-$COCH_3$	17%	8.4 (5.9, 12.1)	
29	4-$COCH_3$	52.0	21.0 (15.2, 28.9)	2.5
30	3-$COOCH_3$	13%	4.1 (2.9, 5.9)	
31	4-$COOCH_3$	8%	18.4 (14.4, 23.6)	
32	3-COOH	13%	>100	
33	4-COOH	8%	>100	
34	4-OMe	5.3	32.5 (24.6, 39.1)	0.16
35	3-Me	2.7	1.0 (0.54, 1.9)	2.7
36	4-Me	1.6	2.7 (1.8, 3.8)	0.6
37	3-SMe	8.2	1.4 (0.75, 2.6)	5.6
38	4-SMe	6%	6.3 (2.6, 1.5)	
39	3,4-Cl_2	12.9	4.6 (2.9, 0.74)	2.8
40	3-F, 4-Cl	11%	1.66 (1.0, 2.7)	
41	3,4-Me_2	2.5	5.1 (3.0, 8.5)	0.49
42	3,4-$CH_2CH_2CH_2$	3.5	41.3 (32.4, 52.5)	0.08
1, Cromakalim		8.9	0.032 (0.021, 0.049)	278.1[c]

[a] Anti-ischemic potency was determined by measurement of EC_{25}, concentration necessary for increase in time to contracture by 25%, in the globally ischemic rat hearts. Time to contracture was defined as the time necessary during total global ischemia to increase end diastolic pressure by 5 mmHg. Each value is an average of three determinations and is within approximately ±20%. [b] Vasorelaxant potency was assessed by measurement of IC_{50} for inhibition of methoxamine contracted rat aorta. IC_{50} is presented as a mean with 95% confidence interval in parentheses, $n = 4$ or higher. [c] Data presented previously in ref 5.

indicates that the protons on the urea nitrogens are not mandatory for anti-ischemic activity.

Since the pendant aryl ring was found to be important for anti-ischemic potency and cardiac selectivity, we investigated substitution on the pendant phenyl ring. For ease of chemical synthesis, we concentrated on the lead cyanoguanidine analog **3**. Previous studies have shown that most of the anti-ischemic activity of **3** resides in the 3*S*,4*R*-enantiomer (**22**) (Table 3).[5] Therefore aromatic substitution was explored on the single enantiomer **22**. Since the unsubstituted pendant phenyl ring of **22** is rapidly hydroxylated at the 4-position *in vivo*,[5] we concentrated on making additional analogs of **23** by substituting at the 3- and 4-positions to suppress hydroxylation.

As shown by the anti-ischemic potencies of compounds **2**, **23**–**27**, electron-withdrawing groups at the 3- and 4-positions of the pendant aromatic ring are tolerated for anti-ischemic activity (Table 3). The bulky substituents such as the methyl ketone (**28**, **29**) and methyl ester (**30**, **31**) cause reduction of anti-ischemic potency. The 3-substituted compounds **23**, **24**, and **26** are qualitatively more potent than their 4-substituted analogs **2**, **25**, and **27**, respectively. The lack of anti-ischemic potency in **32** and **33** indicates that a carboxylic acid is not compatible with anti-ischemic activity. As demonstrated by the anti-ischemic potency of the methoxy analog **34**, electron-releasing groups are tolerated for anti-ischemic potency. With the exception of the 4-thiomethyl ether (**38**), neutral lipophilic substituents (**35**–**37**) in general are beneficial to anti-ischemic activity (compare **35**–**37** with **22**). These results indicate that the substituent on the pendant aryl ring may occupy a lipophilic pocket. Comparison of **37** and **38** indicates that the thiomethyl ether is preferred at the 3-position of the pendant aryl ring. The 3,4-dihalo analogs (**39**, **40**) are less potent than their monosubstituted analogs (**2**, **23**). The lower anti-ischemic potencies of the dihalo analogs **39** and **40** appear to be due to electronic rather than steric reasons as the 3,4-dimethyl (**41**) and the indanyl (**42**) analogs are comparable to the monomethyl analogs **35** and **36**. The indanyl analog **42** (BMS-183867) is the most selective compound of this series. It is over 3 orders of magnitude more selective for the ischemic myocardium than the reference agent cromakalim (**1**).

The cardiac selectivity index, calculated by the ratio of vasorelaxant and anti-ischemic potencies, varies over 3 orders of magnitude, being 298 for the reference agent cromakalim (**1**) and 0.08 for the most selective compound (**42**) of this series. A correlation graph between vasorelaxant and anti-ischemic potencies using the data from this (Tables 1–3) and the two previously published papers in this series (46 compounds) is shown in Figure 1. Clearly, there is no correlation ($r < 0.009$) between the two potencies. These results show that distinct structure–activity relationships exist for anti-ischemic and vasorelaxant acticvities. We have no clear explanation for these differences at the present time.

We entertained the notion that the differences in

Table 4. Physical Properties of Cyanoguanidine **3** and Its Analogs

compd	molecular formula	microanalysis	physical characterization	mp, °C[a]
2	see ref 5			
3	see ref 5			
4	see ref 5			
5	$C_{20}H_{18}N_4O_3$	C, H, N	colorless solid	186–8 (A)
6	$C_{20}H_{18}N_4O_2S$	C, H, N, S	colorless solid	191–2 (B)
7	$C_{20}H_{20}N_2O_3$	C, H, N	colorless solid	204–5 (C)
8	$C_{21}H_{21}N_5O_2$	C, H, N	colorless solid	188–9 (D)
9	$C_{20}H_{21}N_3O_3$	C, H, N	colorless solid	147–8 (B)
10	$C_{20}H_{20}N_6O_2 \cdot 0.44H_2O$	C, H, N	colorless solid	188–90 (E)
11	see ref 13			
12	see ref 7			
13	see ref 5			
14	$C_{18}H_{18}N_4O_3 \cdot 1.53H_2O$	C, H, N	colorless solid	225–7 (F)
15	$C_{18}H_{18}N_4O_3 \cdot 0.66H_2O$	C, H, N	colorless solid	212–4 (E)
16	$C_{18}H_{18}N_4O_3 \cdot 0.72H_2O$	C, H, N	colorless solid	227–8 (F)
17	$C_{19}H_{17}N_3O_3$	C, H, N	colorless solid	255–6 (B)
18	$C_{21}H_{21}N_3O_3$	C, H, N	colorless solid	205–6 (G)
19	$C_{22}H_{20}N_3O_4$	C, H, N	colorless solid	198–200 (B)
20	$C_{22}H_{20}N_2O_4$	C, H, N	colorless solid	211–3 (B)
21	$C_{21}H_{17}N_3O_2 \cdot 0.3H_2O$	C, H, N	colorless solid	175–80 (B)
22	see ref 5			
23	$C_{20}H_{18}ClN_5O_2 \cdot 0.27H_2O$	C, H, N, Cl	colorless solid	216–8 (F)
24	$C_{20}H_{18}N_6O_4 \cdot 0.07H_2O$	C, H, N	colorless solid	214–6 (H)
25	$C_{20}H_{18}N_6O_4 \cdot 0.1H_2O$	C, H, N	colorless solid	165–70 (H)
26	$C_{21}H_{18}F_3N_5O_2$	C, H, N	colorless solid	205–8 (H)
27	$C_{21}H_{18}F_3N_5O_2$	C, H, N	colorless solid	209–10 (H)
28	$C_{22}H_{21}N_5O_3 \cdot 0.44H_2O$	C, H, N	light yellow solid	155–8 (H)
29	$C_{22}H_{21}N_5O_3 \cdot 0.37H_2O$	C, H, N	light yellow solid	170–2 (H)
30	$C_{22}H_{21}N_5O_4$	C, H, N	colorless solid	213–5 (H)
31	$C_{22}H_{21}N_5O_4$	C, H, N	colorless solid	228–9 (H)
32	$C_{21}H_{19}N_5O_4 \cdot 0.61H_2O$	C, H, N	colorless solid	210–4 (G)
33	$C_{21}H_{19}N_5O_4 \cdot 0.79H_2O$	C, H, N	colorless solid	211–4 (B)
34	$C_{21}H_{21}N_5O_3$	C, H, N	colorless solid	206–7 (H)
35	$C_{21}H_{21}N_5O_2 \cdot 0.25C_4H_{10}O$	C, H, N	colorless solid	213–4 (H)
36	$C_{21}H_{21}N_5O_2 \cdot 0.40H_2O$	C, H, N	colorless solid	146–50 (H)
37	$C_{21}H_{21}N_5O_2S \cdot 0.2C_4H_{10}O$	C, H, N	colorless solid	142–5 (H)
38	$C_{21}H_{21}N_5O_2S \cdot 0.17H_2O$	C, H, N	colorless solid	193–208 (H)
39	$C_{20}H_{17}Cl_2N_5O_2 \cdot 0.37H_2O$	C, H, N	colorless solid	168–70 (H)
40	$C_{20}H_{17}ClFN_5O_2$	C, H, N	colorless solid	161–2 (H)
41	$C_{21}H_{23}N_5O_2 \cdot 0.11C_4H_{10}O$	C, H, N	colorless solid	152–3 (H)
42	$C_{23}H_{23}N_5O_2 \cdot 0.32H_2O$	C, H, N	colorless solid	148–55 (H)

[a] Solvent for crystallization: A, isopropyl ether–chloroform; B, isopropyl ether; C, chloroform; D, isopropyl ether; E, isopropyl ether–dichloromethane; F, ethyl acetate; G, ethyl ether–ethyl acetate; H, trituration with ethyl ether.

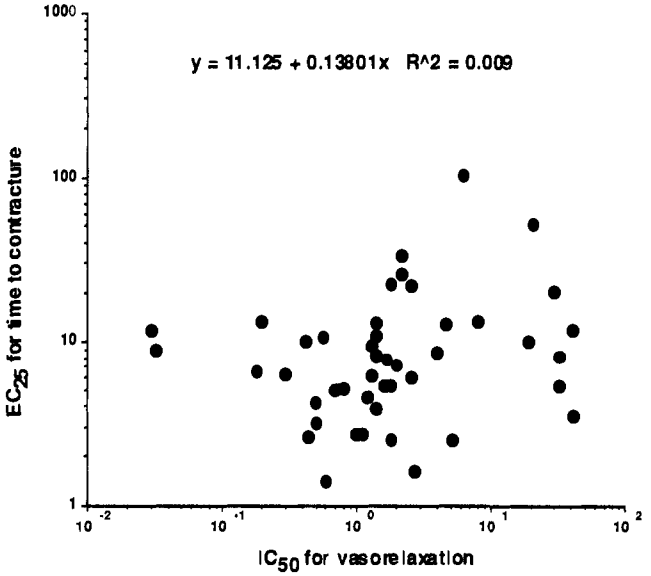


Figure 1. Correlation between anti-ischemic (measured by EC_{25} values for increase in time to contracture) and vasorelaxant (measured by IC_{50} values for relaxation of methoxamine contracted rat aorta) potencies. Data for 45 compounds (for which IC_{50} and EC_{25} were determined) taken from Tables 1 and 2 and two previous papers (refs 5 and 6).

structure–activity relationships may be due to different mechanisms of action in the two tissues (smooth muscle and heart). Previous studies have shown that the vasorelaxant and anti-ischemic effects of this series of compounds are inhibited by the K_{ATP} blocker glyburide, indicating that K_{ATP} opening is involved in their mechanism of action.[5,12] To further support a K_{ATP} opening mechanism for the anti-ischemic actions of these compounds, we studied the effect of two structurally different blockers of K_{ATP} (glyburide, sodium 5-hydroxydecanoate) on the cardioprotective effects of **13** (BMS-189365) as measured by the increase in time to contracture in isolated perfused rat hearts. We chose to study compound **13** since previous studies by other workers have shown that cardioprotective effects of this compound in rabbit hearts are not affected by cotreatment with glyburide.[14] The concentrations of the blockers used, while having no effect of their own on the time to the onset of contracture, have been previously shown to inhibit the cardioprotective effects of a variety of structurally different K_{ATP} openers (**1**, **2**) in rat hearts.[12,15] In this study we also included the anti-inflammatory agent meclofenamic acid, which has previously been shown to inhibit the cardioprotective effects of K_{ATP} openers such as cromakalim (**1**), presumably by blocking K_{ATP}.[16]

The effect of K_{ATP} blockers on the increase in time to contracture induced by 30 μM of **13** (BMS-189365) is shown in Figure 2. All three compounds abolished the

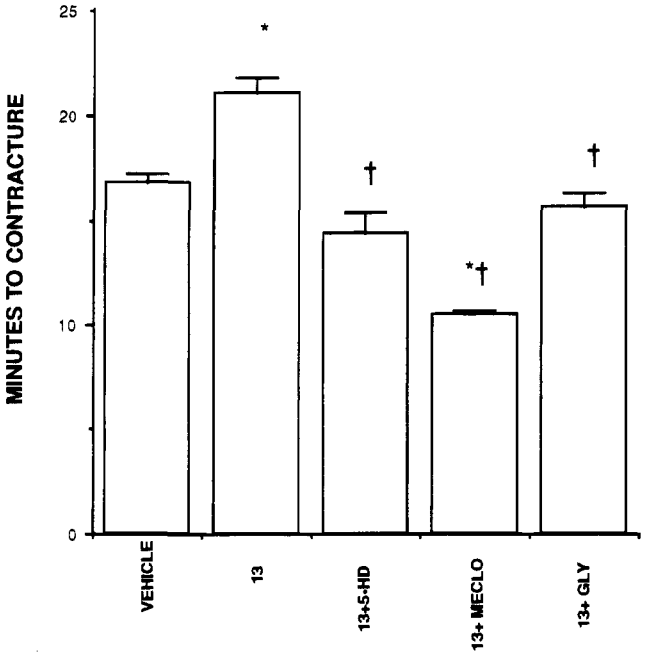


Figure 2. The effect of 5-hydroxydecanoate (5-HD, 100 μM), glyburide (GLY, 0.1 μM), and meclofenamic acid (MECLO, 5.0 μM) on the increase in time to contracture by 30 μM BMS-189365 (**13**) in isolated perfused rat hearts. All three agents abolish the increase in time to contracture induced by **13**. * = Significantly different from vehicle treated hearts; † = significantly different from hearts treated with BMS-189365 (**13**).

increase in time to contracture seen with **13**. Further, the combination of blockers and compound **13** is slightly proischemic (significant for meclofenamic acid), as shown by the decrease in time to the onset of contracture compared to the vehicle-treated hearts (Figure 2). Similar proischemic effects for combinations of K_{ATP} openers and blockers have previously been demonstrated (e.g., cromakalim).[12,15] Thus, the profile of anti-ischemic activity of **13** is typical of first-generation K_{ATP} openers in the isolated perfused rat hearts. We have previously shown that the cardioprotective effects of **2** and **3** are abolished by pretreatment with glyburide and sodium 5-hydroxydecanoate.[5,12] Taken together, these data indicate that the mechanism of anti-ischemic action of **3** and its congeners is in some fashion related to the opening of K_{ATP} in rat hearts.

We have no clear explanation as to why the cardioprotection afforded by **13** in rabbit hearts is not abolished by glyburide.[14] The protocol employed for assessing cardioprotection in rabbit hearts is different from that used in the present studies. Studies in rabbits employed assessment of infarct size as an index of myocardial injury following iv administration of test agents. A number of variables, including delivery of glyburide to the target organ, could affect the conclusion of those in vivo studies. In our studies we used isolated rat hearts, a more controlled model wherein the test agent is directly perfused into the myocardium. This protocol eliminates variables associated with the delivery of the test agent to the heart. Thus considering these differences in the two models, the exact reasons for the lack of interaction of glyburide with compound **13** in rabbit hearts are not clear. One can speculate that the mechanism of action of **13** may be different in the rat and rabbit hearts or that the K_{ATP} is modulated by compound **13** in a species specific manner.

Conclusion

Our results show that urea and cyanoguanidine moieties are optimal for anti-ischemic potency. The distance between the urea carbonyl and the pendant aryl ring has a dramatic affect on cardiac selectivity. The urea moiety can be constrained with retention of anti-ischemic potency and gain in cardiac selectivity. The cyclic phenylurea in the constrained analog **17** can be replaced with indole surrogates, indicating that the requirements in this region of the molecule are flexible. The substituents in the pendant aryl ring of **3** are more sensitive to steric than electronic parameters. The cardiac selectivity, as shown by the ratio of vasorelaxant and anti-ischemic potencies, varies over 3 orders of magnitude. These data suggest that the structural requirements for the anti-ischemic and vasorelaxant activies of K_{ATP} openers are quite distinct. Abolition of the anti-ischemic effects of **3** and its congeners with K_{ATP} blockers suggests that the anti-ischemic effects of these compounds are mediated *via* K_{ATP} opening. At the present time, we have no clear explanation for the cardiac selectivity of these compounds.

Experimental Section

Chemistry. Method A: Illustrated by the Synthesis (3*S*)-*trans*-*N*-(3-Chlorophenyl)-*N*″-cyano-*N*′-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-guanidine (23). The solution containing *N*′-(3-chlorophenyl)-*N*-cyanothiourea sodium salt (1.26 g, 5.96 mmol, prepared by the treatment of 3-chlorophenyl isothiocyanate with monosodium cyanamide)[7] and (3*S*)-*trans*-4-amino-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile[5] (1.0 g, 4.59 mmol) in dimethylformamide (5 mL) under argon was treated with 1-(3-(dimethylamino)propyl)-2-ethylcarbodiimide hydrochloride (1.17 g, 5.96 mmol). The reaction was stirred at room temperature for 2 h and partitioned between 5% citric acid and ethyl acetate. The aqueous phase was reextracted with ethyl acetate, and the combined extracts were washed with water, sodium bicarbonate, and brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated and the residue was purified by flash chromatography on silica gel (ethyl acetate–hexanes 80:20) to yield a colorless solid (1.0 g, 55%): ^{1}H NMR (DMSO-d_6) δ 9.42 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.61 (d, J = 8.8 Hz, 1 H), 7.31 (m, 3 H), 6.91 (d, J = 8.8 Hz, 1 H), 5.90 (br s, 1 H), 4.98 (t, J = 9.0 Hz, 1 H), 3.69 (m, 1 H), 1.41, 1.18 (s, 3 H each); ^{13}C NMR (DMSO-d_6) δ 159.0, 156.3, 139.3, 133.1, 132.7, 130.5, 124.5, 124.3, 123.1, 121.9, 119.1, 117.9, 116.8, 102.7, 80.4, 71.0, 52.0, 26.6, 18.6; IR (KBr) 1609, 1575, 1490 cm^{-1}.

Method B: Illustrated by the Preparation of (*trans*)-*N*″-Cyano-*N*′-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N*-(phenylmethyl)guanidine (8). A. (*trans*)-4-[[(Cyanoimino)phenoxymethyl]amino]-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (5). A suspension of (*trans*)-4-amino-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (1.0 g, 5.00 mmol)[9] in 2-propanol was treated with diphenylcyanocarbonimidate (1.1 g, 5.0 mmol) and triethylamine (0.6 g, 6.6 mmol). The reaction mixture was stirred at room temperature for 7 h and concentrated in vacuo, and the residue was triturated with isopropyl ether to provide the title compound (1.2 g, 66%): ^{1}H NMR ($CDCl_3$) δ 7.95 (s, 1 H), 7.4 (m, 7H), 6.8 (dd, J = 9.0 and 4.0 Hz, 1 H), 4.9 (br s, 1 H), 3.8 (d, J = 10.0 Hz, 1 H), 1.4, 1.1 (s, 3 H each); ^{13}C NMR ($CDCl_3$) δ 164.0, 157.0, 151.0, 134.0, 132.0, 130.0, 129.8, 127.0, 122.0, 121.8, 120.5, 119.0, 118.5, 115.0, 104.0, 80.0, 72.0, 54.0, 26.0, 18.0.

B. (*trans*)-*N*″-Cyano-*N*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-*N*′-(phenylmethyl)-guanidine (8). A suspension of (*trans*)-4-[[(cyanoimino)-phenoxymethyl]amino]-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (0.5 g, 1.4 mmol) in 2-propanol

(3 mL) was treated with benzylamine (0.5 mL). The reaction mixture was allowed to stir at room temperature for 20 h and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with hexane–ethyl acetate (3:7) and the product was crystallized from acetonitrile–isopropyl ether to give a white solid (310 mg, 59%): ^{1}H NMR ($CDCl_3$) δ 7.7 (m, 1 H),7.5 (dd, J = 2.0 and 9.0 Hz, 1 H), 7.4 (m, 6 H), 6.86 (d, J = 9.0 Hz, 1 H), 5.8 (s, 1 H), 4.8 (m, 1 H), 4.5 (d, J = 5.0 Hz, 2 H), 3.7 (dd, J = 6.0 and 4.0 Hz, 1 H), 1.41, 1.19 (s, 3 H each); ^{13}C NMR ($CDCl_3$) δ 158.7, 154.5, 136.8, 130.7, 126.5, 125.2, 125.0, 123.0, 116.0, 101.0, 78.6, 42.6, 24.8, 16.9.

(*trans*)-*N*″-Cyano-*N*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)carbamimidothioic Acid Phenyl Ester (6). A suspension of (*trans*)-4-[[(cyanoimino)phenoxymethyl]amino]-3,4-dihydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**5**) (1.0 g, 2.8 mmol) in isopropyl alcohol (6 mL) was treated with diisopropylethylamine (0.4 g, 3.3 mmol) and thiophenol (0.36 g, 3.3 mmol). The reaction was allowed to stir at room temperature for 20 h and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with hexane–ethyl acetate (1:1) and the product crystallized from isopropyl alcohol-ether to give a colorless solid (0.5 g, 48%): ^{1}H NMR ($CDCl_3$) δ 7.7 (m, 2 H),7.5 (m, 3 H), 7.3 (d, J = 9.0 Hz, 1 H), 7.17 (s, 1 H), 6.7 (d, J = 8.0 Hz, 1 H), 5.48 (s, 1 H), 4.9 (m, 1 H), 3.5 (d, J = 10.0 Hz, 1 H), 1.38 (s, 3 H), 1.18 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 171.7, 171.2, 156.6, 136.2, 133.4, 132.3, 131.8, 131.7, 131.1, 124.6, 121.5, 118.7, 114.6, 104.0, 80.4, 77.5, 76.0, 73.0, 60.4, 54.2, 26.3, 21.0, 18.8, 14.2.

(*trans*)-*N*-(6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)benzeneacetamide (7). To a solution of (*trans*)-4-amino-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile hydrochloride (1.0 g, 3.9 mmol)[9] in 20% aqueous tetrahydrofuran (25 mL) was added phenylacetyl chloride (0.91 g, 5.9 mmol) (0.8 mL) dropwise. The pH of the reaction mixture was maintained between 8.5 and 9.0 by simultaneous addition of 25% aqueous sodium carbonate solution. After completion of addition, the reaction mixture was stirred for 1 h more. It was then diluted with ethyl acetate (200 mL) and the layers were separated. Organic layer was washed with water, dried and concentrated in vacuo and the residue crystallized from chloroform to give a colorless solid (1.1 g, 84%): ^{1}H NMR (DMSO-d_6) δ 8.55 (d, J = 8.0 Hz, 1 H), 7.6 (dd, J = 1.0 and 9.0 Hz, 1 H),7.35 (m, 6 H), 6.95 (d, J = 9.0 Hz, 1 H), 5.7 (d, J = 6.0 Hz, 1 H), 4.85 (t, J = 10.0 and 9.0 Hz, 1 H), 3.6 (m, 3 H), 1.4 (s, 3 H), 1.2 (s, 3 H); ^{13}C NMR (DMSO-d_6) 171.5, 156.5, 136.5, 132.8, 129.3, 128.5, 126.8, 125.4, 119.1, 118.1, 103.0, 80.6, 71.3, 48.8, 43.0, 26.8, 19.1.

(*trans*)-1-(6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-3-(phenylmethyl)urea (9). A suspension of (*trans*)-4-amino-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (1.0 g, 4.6 mmol)[9] in ethanol (4 mL) under argon was treated with benzyl isocyanate (0.6 g, 4.6 mmol) and the reaction was heated at reflux temperature for 4 h. The reaction was then concentrated in vacuo and the residue was triturated with isopropyl ether to give (*trans*)-1-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-3-(phenylmethyl)urea (**9**) (1.3 g, 81%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 7.60 (d, J = 2.4, 1 H), 7.52 (s, 1 H), 7.35 (m, 3 H), 6.90 (d, J = 8.8 Hz, 1 H), 6.59 (t, J = 5.9 and 6.4 Hz, 1 H), 6.50 (d, J = 8.2 Hz, 1 H), 5.67 (d, J = 5.9 Hz, 1 H), 4.64 (t, J = 9.3 and 8.8 Hz, 1 H), 4.3 (m, 2 H), 3.52 (dd, J = 3.5 and 5.9 Hz,1 H), 1.40 (s, 3 H), 1.17 (s, 3 H); ^{13}C NMR (DMSO) 158.8, 156.2, 140.8, 132.7, 132.3, 128.2, 126.9, 126.6, 119.1, 117.8, 102.5, 80.3, 71.7, 49.5, 43.0, 26.5, 18.8.

(*trans*)-1-(6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-(3-pyridinyl)urea (14). A. 3-Pyridinylcarbamic Acid 4-Nitrophenyl Ester (47). The solution containing 3-aminopyridine (5.0 g, 5.3 mmol) in methylene chloride (40 mL) at 0 °C under argon was treated with 4-nitrophenyl chloroformate (10.7 g, 5.3 mmol) in methylene chloride (40 mL) followed by pyridine (4.2 g, 5.3 mmol). The cooling bath was removed and the reaction mixture was stirred at room temperature for 24 h. The solid that precipitated out was filtered and washed with methylene chloride to give **47** (13.0 g, 94%) as a light yellow solid which was used in the next step without further purification: ^{1}H NMR (DMSO-d_6) δ 8.14 (d, J = 7.1 Hz, 2 H), 8.02 (d, J = 1.8 Hz, 1 H), 7.9 (m, 1 H), 7.5 (m, 2 H), 6.98 (d, J = 7.0 Hz, 2 H).

B. (*trans*)-1-(6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-(3-pyridinyl)urea (14). A solution of (*trans*)-4-amino-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**43**) (1.0 g, 4.6 mmol)[9] in acetonitrile (20 mL) under argon was treated with title A compound (1.8 g, 6.9 mmol) and the reaction was heated at 80°C for 4 h. The solid was filtered off and the filtrate was concentrated in vacuo. The residue was diluted with ethyl acetate and washed with water, saturated sodium bicarbonate solution, and brine. The solvent was removed in vacuo and the residue was triturated with ethyl acetate to give (*trans*)-1-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-3-pyridylurea (**14**) as a colorless solid (1.4 g, 90%): ^{1}H NMR (DMSO-d_6) δ 9.0 (s, 1 H), 8.42 (d, J = 4.7 Hz, 1 H), 8.25 (d, J = 8.2 Hz, 1 H), 7.80 (m, 1 H), 7.79 (m, 2 H),7.38 (d, J = 8.8 Hz, 1 H), 6.95 (d, J = 8.8 Hz, 1 H), 4.74 (t, J = 8.8 Hz, 1 H), 3.65 (d, J = 9.4Hz,1 H), 1.44, 1.21 (s, 3 H each); ^{13}C NMR (DMSO-d_6) δ 156.3, 155.3, 139.5, 135.8, 132.6, 132.3, 130.8, 126.4, 125.5, 119.1, 117.9, 102.7, 80.4, 71.2, 49.5, 26.5, 18.9.

(3*S*)-*trans*-3-[[[(6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)amino](cyanoimino)methyl]amino]benzoic Acid (32). A solution of (3*S*)-*trans*-3-[[[(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)amino](cyanoimino)methyl]amino]benzoic acid methyl ester (**30**) (0.5 g, 1.2 mmol) in methanol (10 mL) under argon was treated with lithium hydroxide (0.4 g, 9.6 mmol). The reaction was stirred at room temperature for 24 h and then concentrated in vacuo. The residue was diluted with 10% sodium hydroxide solution (100 mL) and extracted with ethyl acetate (2 × 100 mL). The organic layer was discarded and the aqueous layer was acidified with 2 N hydrochloric acid and extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, and the solvent was evaporated. The residue was crystallized from ethyl acetate–ethyl ether to yield the desired product (0.43 g, 89%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 9.4 (s, 1 H), 7.9 (s, 1H), 7.70 (d, J = 7.6 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2 H), 6.91 (d, J = 8.2 Hz, 1 H), 6.0 (s, 1 H), 4.93 (t, J = 8.8 and 9.4 Hz 1 H), 3.70 (m, 1 H), 1.41, 1.19 (s, 3 H each); ^{13}C NMR (DMSO-d_6) δ 166.9, 159.1, 156.3, 138.0, 132.7, 129.1, 127.8, 125.4, 124.6, 124.2, 119.0, 117.9, 116.8, 102.7, 80.4, 52.1, 26.6, 18.7.

(*trans*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-(2,3-dihydro-2-oxo-1*H*-benzimidazol-1-yl)-2*H*-1-benzopyran-6-carbonitrile (17). A. (2-Nitrophenyl)carbamic Acid Ethyl Ester (49). To the solution of 2-nitroaniline (**48**) (6.9 g, 50.0 mmol) in pyridine (6 mL) and dichloromethane (25 mL) at 0 °C under argon was slowly added ethyl chloroformate (7.3 mL, 75.0 mmol). The cooling bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was poured into 2 N hydrochloric acid and extracted with ethyl acetate. The combined extracts were washed with water, saturated sodium bicarbonate solution, and brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated to yield a yellow solid (7.7 g, 73%): ^{1}H NMR ($CDCl_3$) δ 8.55 (d, J = 8.0 Hz, 1 H), 8.2 (d, J = 8.0 Hz, 1 H), 7.6 (t, J = 8.0 Hz, 1 H), 7.1 (t, J = 7.5 Hz, 1 H), 4.25 (q, J = 6.0 Hz, 2 H), 1.3 (t, J = 6.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 153.0, 135.8, 135.4, 125.7, 122.1, 120.5, 63.6, 14.3.

B. (2-Aminophenyl)carbamic Acid Ethyl Ester (50). The solution of (2-nitrophenyl)carbamic acid ethyl ester (**49**) (2.0 g, 9.5 mmol) in absolute ethanol (25 mL) was hydrogenated at atmospheric pressure in the presence of 10% palladium hydroxide on carbon catalyst (200 mg). The catalyst was filtered off using a Celite pad and the filtrate was evaporated. The residue was triturated with isopropyl ether to give a colorless solid (**50**) (820 mg, 48%). ^{1}H NMR ($CDCl_3$) δ 7.2 (d, J = 7.0 Hz, 1 H), 7.0 (t, J = 6.5 Hz, 1 H), 6.7 (m, 3 H), 4.2 (q, J = 6.0 Hz, 2 H), 3.75 (br s, 2 H), 1.3 (t, J = 6.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 158.2, 140.0, 126.3,124.9, 124.0, 119.3, 117.3, 61.3, 14.4.

C. (*trans*)-[2-[(6-Cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-pyran-4-yl)amino]phenyl]carbamic Acid Ethyl Ester (52). The reaction mixture containing (2-aminophenyl)carbamic acid ethyl ester (**50**) (900 mg, 5.0 mmol), 6-cyano-3,4-dihydro-2,2-dimethyl-3,4-epoxy-2*H*-1-benzopyran (**51**) (1.0 g, 5.0 mmol),[9] and magnesium perchlorate (1.12 g, 5.0 mmol) in acetonitrile (5.0 mL) was stirred under argon at room temperature for 48 h. The reaction mixture was diluted with ethyl acetate and washed with water, saturated sodium bicarbonate solution, and brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated to give a colorless amorphous solid (1.82 g, 96%): ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1 H), 7.34 (dd, J = 8.8 and 2.3 Hz, 1 H), 7.0 (m, 2 H), 6.77 (d, J = 8.2 Hz, 2 H), 6.62 (m, 2 H), 6.5 (br s, 1 H), 4.35 (d, J = 8.7 Hz, 2 H), 4.24 (br s, 1 H), 4.1 (q, J = 7.0 Hz, 2 H), 3.64 (d, J = 8.8 Hz, 1 H), 1.4 (s, 3 H), 1.2 (s, 3 H), 1.18 (t, J = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 156.6, 155.9, 142.9, 132.6, 128.0, 127.3, 126.5, 124.9, 122.8, 119.2, 118.2, 118.0, 113.5, 103.5, 80.0, 72.5, 61.88, 54.7, 26.7, 19.2, 14.3.

D. (*trans*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-(2,3-dihydro-2-oxo-1*H*-benzimidazol-1-yl)-2*H*-1-benzopyran-6-carbonitrile (17). To a solution of (*trans*)-[2-[(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-pyran-4-yl)amino]-phenyl]carbamic acid ethyl ester (**52**) (1.15 g, 3.0 mmol) in methanol (6 mL) was added a sodium methoxide–methanol solution (2 mL of 4.4 M solution), and the resulting reaction mixture was heated at reflux temperature for 8 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The resulting solution was washed with 10% citric acid, sodium bicarbonate solution, and brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated and the residue was purified by flash chromatography (20% acetone in dichloromethane). The product was crystallized from isopropyl alcohol to yield a colorless solid (605 mg, 60%): ^{1}H NMR (DMSO-d_6) δ 7.6 (dd, J = 8.4 and 1.8 Hz, 1 H), 7.55, 7.38 (d, J = 8.8 Hz, 1 H), 7.55, 7.40 (AB q, J = 8.8 Hz, 1 H), 7.18 (s, 1 H), 7.06 (m, 2 H), 6.95 (t, J = 8.1 Hz, 1 H), 6.77 (t, J = 7.6 Hz, 1 H), 6.16 (d, J = 6.6 Hz, 1 H), 5.90 (d, J = 6.3 Hz, 1 H), 5.41, 5.13 (d, J = 10.0 Hz, 1 H), 4.45, 4.07 (dd, J = 9.5 and 5.8 Hz, 1 H), 1.45, 1.43 (s, 3 H each). Doubling of some signals is noted due to the presence of two rotamers in solution.

(*trans*)-3,4-Dihydro-3-hydroxy-2,2-dimethyl-4-(2-oxo-3-phenyl-1-imidazolidinyl)-2*H*-1-benzopyran-6-carbonitrile (18). A solution of 6-cyano-3,4-dihydro-2,2-dimethyl-3,4-epoxy-2*H*-1-benzopyran (1.0 g, 5.0 mmol)[9] in ethanol (10 mL) was treated with phenylethylenediamine (0.74 g, 5.4 mmol) under argon and allowed to stir at room temperature for 24 h. The reaction mixture was concentrated in vacuo to give a colorless solid (**54**) (1.67 g, 5.0 mmol) which was dissolved in dichloromethane (20 mL) and cooled to 0 °C under argon. The reaction mixture was treated with 4-nitrophenyl chloroformate (1.3g, 6.4 mmol) in methylene chloride (10 mL) followed by the addition of triethylamine (0.65 g, 6.4 mmol). The reaction was allowed to stir at room temperature for 16 h, diluted with dichloromethane, and washed with 10% hydrochloric acid and water. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The product was crystallized from ethyl acetate–ether to give the title compound (0.7 g, 39%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 7.52 (m, 7 H), 7.24 (t, J = 7.6 Hz, 1 H), 7.02 (d, J = 8.7 Hz, 1 H), 5.30 (d, J = 10.5 Hz,1 H), 3.88 (d, J = 5.8 Hz, 1 H), 3.87 (m, 1 H), 3.57 (m, 1 H), 3.27 (m, 1 H), 1.71 (s, 3 H), 1.45 (s, 3 H); ^{13}C NMR (DMSO) δ 159.0, 157.5, 139.6, 133.1, 131.8, 128.8, 123.0, 120.9, 119.0, 118.7, 117.7, 104.0, 80.4, 69.7, 52.7, 42.5, 36.8, 26.7, 18.5.

1-(6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-1*H*-indole-3-carboxylic Acid Methyl Ester (20) and 1-(6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-1*H*-indole-3-carboxylic Acid Methyl Ester (59). To the solution of 6-cyano-3,4-dihydro-2,2-dimethyl-3,4-epoxy-2*H*-1-benzopyran (**51**) (1.15 g, 5.71 mmol)[9] and indole-3-carboxylic acid methyl ester (**56**) (1.0 g, 5.71 mmol) in dimethylformamide (5.0 mL) under argon was added finely ground potassium carbonate (1.93 g, 14.0 mmol). The reaction mixture was heated at 90 °C for 4 h and cooled to ambient temperature. It was diluted with ethyl acetate and filtered. The filtrate was washed with water, 10% citric acid, and brine and dried over anhydrous magnesium sulfate. The solvent was evaporated to yield a colorless foam. It was purified by flash chromatography (5% ethyl acetate in hexanes) to give 1-(6-cyano-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-1*H*-indole-3-carboxylic acid methyl ester (**59**) (990 mg, 50%): ^{1}H NMR (DMSO-d_6) δ 8.3 (s, 1 H), 8.21 (d, J = 6.4 Hz, 1 H), 7.80 (dd, J = 8.8 and 2.4 Hz, 1 H), 7.35 (m, 3 H), 7.18 (d, 8.2 Hz, 1 H), 6.90 (d, J = 1.7 Hz, 1 H), 6.42 (s, 1 H), 3.93 (s, 3 H), 1.68 (s, 6 H); ^{13}C NMR (DMSO-d_6) 164.5, 156.9, 136.9, 135.5, 135.2, 130.9, 128.7, 126.7, 126.3, 123.8, 122.7, 121.4, 119.8, 118.8, 118.4, 111.6, 108.4, 103.7, 79.3, 51.3, 27.9. Further elution of the column provided the more polar *trans*-1-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)-1*H*-indole-3-carboxylic acid methyl ester (**20**) (660 mg, 28%). ^{1}H NMR and ^{13}C NMR spectroscopy show this compound to be a mixture of rotamers. The synthesis of **19** and **21** was carried out in a similar manner except that sodium hydride was used as a base for **19** and the crude product mixture was esterified with diazomethane.

Biological Assays. EC_{25} values for increasing time to contracture were determined in isolated, perfused, globally ischemic rat hearts. To compare the anti-ischemic vs peripheral vasodilator potencies, we determined IC_{50} values for relxation of the methoxamine contracted rat aorta. Experimental details of both methods are described.[6]

References

(1) Parratt, J. R. Protection of the Heart by Ischemic Preconditioning: Mechanism and Possibilities for Pharmacological Exploitation. *Trends Pharmacol. Sci.* **1994**, *15*, 19–25.

(2) (a) Gross, G. J.; Auchampach, J. A. Blockade of ATP-Sensitive Potassium Channels Prevents Myocardial Preconditioning in Dogs. *Circulation Res.* **1990**, *70*, 223–233. (b) Auchampach, J. A. Grover, G. J.; Gross, G. J. Blockade of Ischemic Preconditioning in Dogs by the Novel ATP-dependent Potassium Channel Antagonist Sodium 5-Hydroxydecanoate. *Cardiovas. Res.* **1992**, *26*, 1054–1062. (c) Yao, Z; Gross, G. J. The ATP-Dependent Potassium Channel: An Endogenous Cardioprotective mechanism. *J. Cardiovas. Pharmacol.* **1994**, *24*, S28-S34. (d) Yao, Z.; Mizumura, T.; Mei, D. A.; Gross, G. J. Activation of KATP-Channels May Play a Role in the "Memory" of Ischemic Preconditioning (PC) in Anesthetized Dogs. *Circulation* **1994**, *90* (4 Part 2), I108.

(3) Tomai, F.; Crea, F.; Gaspardone, A.; Versaci, F.; De Paulis, R.; Pentadepeppo, A.; Chiariello, L.; Gioffre, P. Ischemic Preconditioning During Coronary Artery Angioplasty is Prevented by Glybenclamide, a Selective ATP-Sensitive K+ channel Blocker. *Circulation* **1994**, *90*, 700–705.

(4) (a) Grover, G. J.; McCullough, J. R.; Henry, D. E.; Conder, M. L.; Sleph, P. G. Anti-i-Ischemic Effects of the Potassium Channel Activator Pinacidil and Cromakalim and the Reversal of these Effects with the Potassium Channel Blocker Glyburide. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 98. (b) Auchampach, J. A.; Maruyama, M.; Cavero, I.; Gross, G. J. Pharmacological Evidence for a Role of ATP-Dependent Potassium Channels in Myocardial Stunning. *Circulation* **1992**, *86*, 311.

(5) 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.

(6) Atwal, K. S.; Grover, G. J.; Ferrara, F. N; Ahmed, S. Z.; Sleph, P. G.; Dzwonczyk, S.; Normandin, D. E. Cardioselective anti-ischemic ATP-sensitive potassium channel openers. 2. Structure–activity Studies on Benzopyranyl Cyanoguanidines; Modification of the Benzopyran. *J. Med. Chem.* 1995, *38*, 1966–1973.

(7) Atwal, K. S.; Ahmed S. Z.; O'Reilly, B. C. A Facile Synthesis of Cyanoguanidines from Thioureas. *Tetrahedron Lett.* **1989**, *30*, 7313–7316.

(8) Atwal, K. S.; Moreland, S.; McCullough, J. R.; Ahmed, S. Z.; Normandin, D. E. Benzopyranyl-Cyanoguanidine Potassium Channel Openers. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 87–90.

(9) (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)-2*H*-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-2*H*-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-2*H*-1-benzopyran-4-carboxamides and -thiocarboxamides and Their Analogs in Guinea Pig Trachealis. *J. Med. Chem.* **1991**, *34*, 2588–2594.

(10) Atwal, K. S.; Moreland, S.; McCullough, J. R.; O'Reilly, B. C.; Ahmed, S. Z.; Normandin, D. E. Aryl Cyanoguanidine Potassium Channel Openers. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 83–86.

(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) Grover, G. J.; McCullough, J. R.; D'Alonzo, A. J.; Sargent, C. S.; Atwal, K. S. Cardioprotective Profile of the Cardiac-Selective ATP-Sensitive Potassium Channel Opener BMS-180448. *J. Cardiovasc. Pharmacol.* **1994**, *25*, 40–50.

(13) Ashwood, V. A.; Cassidy, F; Evans, J. M.; Gagliardi, S.; Stemp, G. Synthesis and Antihypertensive Activity of Pyran Oxygen and Amide Nitrogen Replacement Analogues of the Potassium Channel Activator Cromakalim. *J. Med. Chem.* **1991**, *34*, 3261–3267.

(14) Toombs, C. F.; Moore, T. F.; Shebuski, R. J. Cardioprotection with U-89232 is not Reversible with Glibenclamide: Evidence of a Novel Anti-ischemic Agent Derived from Cromakalim. *Pharmacology* **1994**, *49*, 96–104.

(15) (a) Grover, G. J.; Sargent, C. S.; McCullough, J. R.; Atwal, K. S. In vitro Anti-ischemic Profile of Activity of a Novel Cardioselective K_{ATP} Opener, BMS-180448. *The Pharmacologist* **1993**, *35*: 178. (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.

(16) Grover, G. J.; D'Alonzo, A. J.; Sleph, P. G.; Dzwonczyk, S.; Hess, T. A.; Darbenzio, R. B. The Cardioprotective and Electrophysiological Effects of Cromakalim are Attenuated by Maclofenamate Through a Cyclooxygenase Independent Mechanism. *J. Pharmacol. Exp. Ther.* **1994**, *269*, 536–540.

JM950321Z