New Benzopyran-Based Openers of the Mitochondrial ATP-Sensitive Potassium Channel with Potent Anti-Ischemic Properties

Maria C. Breschi,[‡] Vincenzo Calderone,*[,‡] Alma Martelli,[‡] Filippo Minutolo,[#] Simona Rapposelli,*[,#] Lara Testai,[‡] Federica Tonelli,[#] and Aldo Balsamo[#]

Dipartimento di Scienze Farmaceutiche and Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno, 6, I-56126, Pisa, Italy

Received October 20, 2006

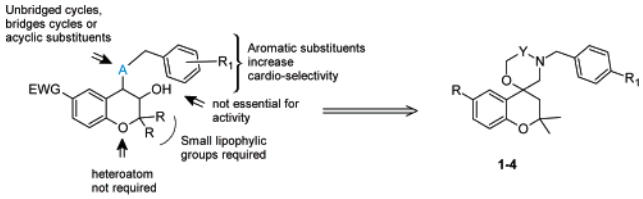
Abstract: This study was aimed at evaluating, on a limited number of benzopyran compounds, whether the insertion of an electron-rich spirocyclic substituent at the C4 carbon of the benzopyran molecular nucleus may improve the cardioprotective properties against ischemia. Some of the new compounds (**1b**, **2b**, and **4b**) exhibited interesting anti-ischemic properties without affecting significantly the blood pressure parameters.

Potassium channels are membrane proteins that selectively allow potassium ions to flow across the cell membrane. Among the different types of potassium channels, some of them, defined as ATP-sensitive (K_{ATP}), are mainly regulated by changes in the intracellular [ATP]/[ADP] ratio, thus associating the membrane potential with the metabolic state of the cell.

K_{ATP} channels are located in the sarcolemmal and inner mitochondrial membrane of the cells of many tissues, including heart, skeletal and smooth muscle, pancreatic β-cells, and neurons and are thus involved in many physiological processes, including hormone secretion, smooth muscle activity,[1] and neurotransmitter release.[2] In the heart, sarcolemmal and mitochondrial channels are involved in modulating myocardial functions, especially in ischemia or hypoxia conditions in which the activation of physiological cellular preservation systems may be important.

Various mechanisms of cardioprotection are known. One of them is the phenomenon of ischemic preconditioning (IPC), an endogenous mechanism whereby brief periods of ischemia (3−5 min) have been shown to increase the resistance of cardiomyocytes to injury induced by a subsequent prolonged period of ischemia, reducing the extent of necrosis in the damaged region. This phenomenon, which has not yet been completely clarified, is triggered by several processes, both receptor-mediated (with the activation of bradykinin, adenosine, and opioid systems)[3] and receptor-independent (due to the increase in NO and reactive oxygen species levels).[4]

To date, a key role in IPC seems to be played by mitochondrial K_{ATP} (mito-K_{ATP}) channels; mito-K_{ATP} channel openers, such as diazoxide, at concentrations that do not activate sarcolemmal K_{ATP} channel[5] have been widely shown to mimic IPC, producing pronounced cardioprotective effects. In addition, these pharmacological responses are blocked, or reduced, by the use of a selective mito-K_{ATP} channel blocking agent such as 5-hydroxydecanoic acid (5-HD).[5] However, the molecular structure of mito-K_{ATP} is still unknown, and the evidence for its involvement in cardioprotection is almost entirely of a pharmacological nature based on the relative selectivity for mito-K_{ATP} of the channel opener diazoxide and the selective inhibitor 5-HD. The possibility of triggering the above-mentioned physiological step involved in the IPC process through exogenous activators of the mito-K_{ATP} channel represents a rational basis for the development of highly interesting, innovative anti-ischemic drugs.[6,7]



Compd	Y	R	R_1
1a	CO	H	NHAc
1b	CO	Br	NHAc
2b	CO	Br	$NHSO_2Me$
3a	CH_2	H	NHAc
3b	CH_2	Br	NHAc
4b	CH_2	Br	$NHSO_2Me$

Figure 1. Left: graphical representation of SAR data for anti-ischemic properties of benzopyran derivatives, adapted with some modifications from Mannhold.[8] Right: general structure of the synthesized spiromorpholone- (**1a**,**b** and **2b)** and spiromorpholine KCOs (**3a**,**b** and **4b**).

K_{ATP} channel openers (KCOs) belong to a heterogeneous group of compounds of different chemical classes, including cyanoguanidines, thioformamides, and benzopyran derivatives. Some first-generation KCOs (cromakalim, nicorandil, pinacidil) showed cardioprotective effects, but their clinical use was limited because of many side effects, like those linked to their hypotensive properties.

In recent years many structural changes have been made, mainly on the nucleus of benzopyran KCOs,[8] in order to find new compounds with a higher selectivity toward specific targets, such as the K_{ATP} channel of pancreatic β-cells[9] or the cardiac mitochondrial K_{ATP} channel, such as the benzopyranylcyanoguanidine derivatives BMS-180448 and BMS-191095.[10,11] Until now, most chemical modifications on benzopyran KCOs have been studied at the C4 position (Figure 1), but a few cases[12] have regarded the restriction of the conformation of this substituent through its insertion into a spiro-like structure.

The absence of a detailed study of this kind of modification led us to explore it in depth, since the constrained structure due to the spirocyclic ring might give new compounds a higher selectivity toward a specific type of K_{ATP} channel and give useful information about steric requirements for their active interaction. In this work, some new spiromorpholone or spiromorpholine C4-substituted benzopyran derivatives were prepared in order to determine the effects of these structural modifications on the cardioprotective properties and on the activity of these new compounds on vascular smooth muscle and blood pressure.

The spiromorpholones **1a**,**b** and **2b** were synthesized following the synthetic procedure illustrated in Scheme 1. Chromanones **5a**,**b**, obtained from the appropriate 2-hydroxyacetophenone,[13] were subjected to nucleophilic addition with trimethylsilylcyanide (TMSCN) in the presence of ZnI_2 as the Lewis acid to afford the corresponding trimethylsilyl cyanohydrins (**6a**,**b**). Compounds **6a**,**b** were directly reduced to aminoalcohols **7a**,**b** with $LiAlH_4$ in accordance with the procedure of Amundsen and Nelson.[14] The subsequent reaction with chloroacetyl chloride of **7a**,**b** in a heterogeneous phase yielded the corresponding chloroacetamides **8a**,**b**. Base-catalyzed (*t*-BuOK) cyclization of **8a**,**b** gave the spiromorpholones **9a**,**b**,

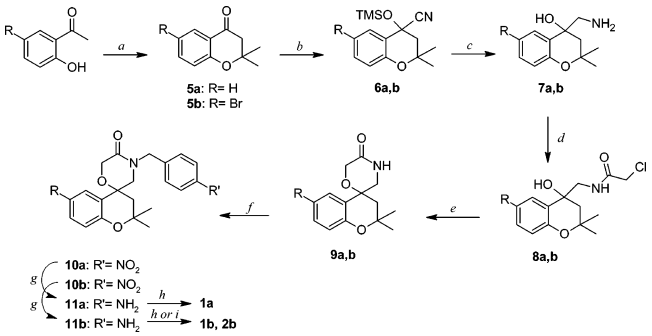
* To whom correspondence should be addressed. For V.C.: phone, +39-050-2219589; fax, +39-050-2219609; e-mail, calderone@farm.unipi.it. For S.R.: phone, +39-050-2219582; fax, +39-050-2219577; e-mail, rappsi@farm.unipi.it.

‡ Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie.

Dipartimento di Scienze Farmaceutiche.

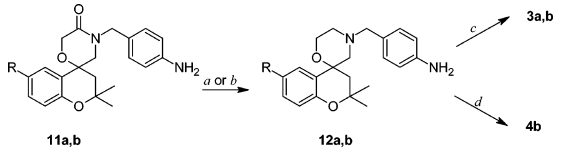
10.1021/jm061228l CCC: $33.50
Published on Web 11/29/2006

Scheme 1[a]



[a] (a) acetone, pyrrolidine; (b) TMSCN, ZnI_2; (c) $LiAlH_4$, THF; (d) chloroacetyl chloride, NaOH; (e) *t*-BuOK, toluene; (f) 4-substituted benzyl bromide, NaH, DMF; (g) $NH_2NH_2 \cdot H_2O$, $FeCl_3$, MeOH; (h) Ac_2O, acetone; (i) $MeSO_2Cl$, pyridine, dioxane.

Scheme 2[a]



[a] (a) $LiAlH_4$, THF; (b) $BH_3 \cdot SMe_2$, microwave; (c) Ac_2O, acetone; (d) $MeSO_2Cl$, pyridine, dioxane.

which reacted with *p*-nitrobenzyl bromide and NaH in DMF to give the *N*-benzyl-substituted compounds **10a**,**b**.[15] The nitro derivatives were then reduced to the corresponding amines **11a**,**b** with hydrazine hydrate in the presence of a catalytic amount of ferric chloride and activated carbon. Treatment of **11a**,**b** with acetic anhydride afforded the corresponding acetamides **1a**,**b**, while methanesulfonylation of **11b** gave compound **2b**. Compounds **3a**,**b** and **4b** were obtained by reduction starting from spiromorpholones **11a**,**b** to the corresponding spiromorpholine derivatives **12a**,**b** with $LiAlH_4$ for **11a** or with a borane−methyl sulfide complex for **11b** (Scheme 2). The subsequent reaction of **12a**,**b** with acetic anhydride and methanesulfonyl chloride afforded the acetamido- (**3a**,**b**) and methanesulfonamido- (**4b**) compounds, respectively.

All the compounds synthesized (**1**−**4**) were tested as racemic mixtures at a dose of 40 mg kg^{-1} ip on Langendorff perfused rat hearts subjected to ischemia-reperfusion cycles (30 and 120 min). Furthermore, two well-known K_{ATP} channel openers, diazoxide and cromakalim, were also tested as reference drugs at doses of 40 or 1 mg kg^{-1}, respectively. Diazoxide is a benzothiadiazine derivative widely considered to possess a satisfactory degree of selectivity.[5,16] In contrast, cromakalim is a potent benzopyrane-based K_{ATP} channel opener (and is thus, from a structural point of view, closer to the compounds synthesized) that exhibits cardioprotective effects and marked hypotensive properties. For each compound, the resulting ischemic injury was quantified by evaluating functional, biochemical, and morphological parameters. In particular, the functional parameter at the 30th [RPP-(30)] or the 120th min of reperfusion [RPP-(120)] has been expressed as a percentage of the rate−pressure product (RPP) recorded during reperfusion, with respect to the percentage of the RPP value recorded at the last minute of the preischemic period. These parameters were taken as indicators of the functional recovery of inotropism in the early and final stages of reperfusion, respectively. The biochemical marker of ischemic injury was represented by the amount of lactate dehydrogenase (LDH) released by the heart in the perfusion fluid during the entire reperfusion time. At the end of reperfusion, the treatment of the heart with triphenyltetrazolium chloride (TTC) made it possible to carry out a

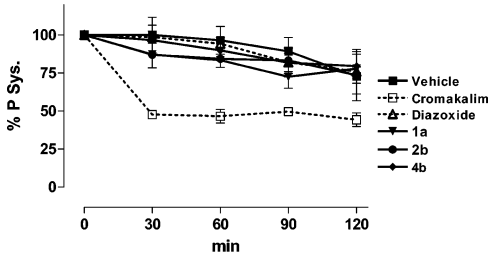


Figure 2. Changes in blood systolic pressure (expressed as a % of the basal level) following the intraperitoneal administration of the cardioprotective compounds synthesized (40 mg kg^{-1}), diazoxide (40 mg kg^{-1}), and cromakalim (1 mg kg^{-1}).

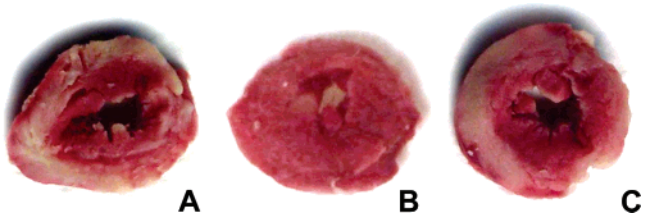


Figure 3. Representative pictures of left ventricle slices submitted to staining procedure with TTC. The pale areas represent the ischemia-injured tissue: (A) heart isolated from a rat pretreated with the vehicle; (B) heart isolated from a rat pretreated with compound **2b**; (C) heart isolated from a rat pretreated with 5-hydroxydecanoic acid and compound **2b**.

Table 1. Functional (RPP-30′ and RPP-120′), Biochemical (LDH), and Morphological (% Ischemic Area vs Total Area) Parameters Recorded in Hearts Isolated from Rats Pretreated with the Vehicle, with the Synthesized Compounds or with the Reference Drugs, in the Absence or in the Presence of the Selective mito-K_{ATP} Blocker 5-Hydroxydecanoic Acid

compd	RPP−30′ %	RPP−120′ %	LDH U/g, 120 min	% ischemic area vs total area
vehicle	23 ± 12	23 ± 6	10.6 ± 1.0	35 ± 7
(±)-**1a**	79 ± 14	62 ± 20	6.0 ± 1.3	20 ± 4
(±)-**1b**	2 ± 1	3 ± 1	12.4 ± 0.9	58 ± 4
(±)-**2b**	75 ± 24	57 ± 19	3.0 ± 1.7	13 ± 3
(±)-**3a**	12 ± 3	17 ± 5	16.0 ± 1.7	50 ± 2
(±)-**3b**	15 ± 4	26 ± 4	11.3 ± 1.2	61 ± 2
(±)-**4b**	89 ± 11	77 ± 19	6.9 ± 3.8	14 ± 2
(±)-**1a** + 5-HD	16 ± 7	27 ± 11	13.5 ± 4.2	49 ± 5
(±)-**2b** + 5-HD	52 ± 16	39 ± 19	11.7 ± 2.0	47 ± 5
(±)-**4b** + 5-HD	17 ± 2	33 ± 16	13.4 ± 1.3	40 ± 1
(±)-cromakalim	99 ± 1	84 ± 16	5.5 ± 0.7	25 ± 1
diazoxide	80 ± 20	47 ± 3	5.7 ± 3.6	22 ± 6
diazoxide + HD	13 ± 7	6 ± 3	14.5 ± 5.4	52 ± 5
(±)cromakalim + HD	11 ± 2	18 ± 1	8.6 ± 3.0	38 ± 3

morphological comparison of the necrotic and healthy areas of the left ventricular tissue, colored white (or pale pink) and red, respectively (some representative examples are shown in Figure 3), and then to calculate the ischemia-injured area as a percentage of the total area.

As indicated by the data shown in Table 1, global ischemia induced severe damage in control hearts, which exhibited a low level (23%) of postischemic functional recovery, high levels of LDH released (>10 U g^{-1}), and the clear presence of necrotic areas (35%). As expected, the two reference drugs diazoxide and cromakalim, administered at a dose of 40 and 1 mg/kg, respectively, led to a significant improvement of these parameters, as shown by an increase in the postischemic functional recovery and a clear reduction in LDH release and in the necrotic areas. Among the compounds synthesized, **1a**, **2b**, and **4b** (administered at the same dose as diazoxide) exhibited a cardioprotective behavior that was qualitatively and quantitatively similar to (and in some cases more satisfactory than) that

Table 2. Parameters of Vasorelaxing Potency and Efficacy of the Synthesized Compounds and the Reference K_{ATP} Openers Recorded in Isolated Rat Aortic Rings

compd	pIC_{50}	E_{max}, %
(±)-**1a**	4.60 ± 0.03	57 ± 3
(±)-**1b**	4.82 ± 0.07	70 ± 11
(±)-**2b**	5.14 ± 0.03	87 ± 3
(±)-**3a**	4.88 ± 0.03	77 ± 2
(±)-**3b**	5.62 ± 0.03	99 ± 1
(±)-**4b**	5.22 ± 0.02	98 ± 2
(±)-cromakalim	7.01 ± 0.09	98 ± 1
diazoxide	4.72 ± 0.04	97 ± 2

exhibited by the reference drugs. In contrast, **1b**, **3a**, and **3b** did not show any cardioprotective effects.

In order to investigate the potential role of the mito-K_{ATP} channel in cardioprotective mechanisms, the effective compounds were tested in the presence of 5-hydroxydecanoic acid (5-HD), a selective blocker of this channel type. The effects of **1a** and **4b** and those of the reference drugs were almost completely abolished by 5-HD, suggesting that their anti-ischemic properties may be due to the activation of the mito-K_{ATP} channels. For the parameters recorded in hearts treated with **2b** in the presence of 5-HD, although the parameter of inotropic recovery was not significantly influenced by the antagonist, the levels of LDH and the percentage of necrotic areas proved to be significantly enhanced, indicating that 5-HD played at least a partial role of antagonism toward the cardioprotective effects of **2b**.

As reported above, one of the main problems in the use of KCOs for the pharmacological prevention of ischemic injury is linked to the lack of selectivity toward the mitochondrial channel with respect to those expressed in the plasmalemma of myocardiocytes and of vascular smooth muscle cells. Consequently, in this work, **1**−**4** and the reference drugs diazoxide and cromakalim were tested in vitro for their vasorelaxing effects on rat aorta preparations contracted with KCl solution. Results showed that all the compounds synthesized exhibited vasodilator effects with full (**1b**, **3b**, **4b**) or partial (**1a**, **2b**, **3a**) efficacy (E_{max}) and with modest levels of potency, with pIC_{50} ranging from 4.60 and 5.62, thus showing a pharmacodynamic profile similar to that of diazoxide (pIC_{50} = 4.72). As expected, cromakalim showed full vasorelaxing efficacy, with high levels of potency (pIC_{50} = 7.01) (Table 2).

In order to perform a more direct evaluation of the effects produced by the new compounds showing cardioprotective properties (**1a**, **2b**, and **4b**) and by the reference drugs diazoxide and cromakalim on the haemodynamic parameters in vivo, these derivatives were administered to male normotensive rats and the systolic blood pressure was recorded for 60 min. As shown in Figure 2, **1a**, **2b**, **4b**, and diazoxide (administered at doses that produced cardioprotective effects) did not show any significant effects on the systolic blood pressure. In contrast, cromakalim (administered at a dose that produced cardioprotective effects) caused a rapid, dramatic, and long-lasting fall of the blood pressure.

This study was designed to evaluate, on a limited number of benzopyran compounds, whether the insertion of an electron-rich spirocyclic substituent at the C4 carbon of the molecular nucleus present in some potent but scarcely selective KCOs may improve the pharmacological properties of compounds of this type, in particular as far as selectivity is concerned. Although the overall data obtained with the new compounds synthesized do not allow us to advance any hypothesis about the role of the substituents on the 4-spiroheterocyclic benzopyran derivatives in their pharmacological activity, because of the limited number of compounds studied, they indicate that in some cases this type of substitution may effectively lead to promising compounds possessing anti-ischemic properties at doses devoid of significant hypotensive effects, with a pharmacological profile qualitatively similar to those exhibited by BMS-180448 and BMS-191095.[11] Further development of new structural analogues of the most effective compounds reported in this study and their optical resolution will make it possible to gain a better understanding of the structure−activity relationships of this new class of cardioprotective benzopyran-based K_{ATP} openers.

Supporting Information Available: Chemical and pharmacological experimental procedures, ^1H NMR, ^{13}C NMR, and MS data, histograms concerning the pharmacological data shown in Table 1, and results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Quayle, J. M.; Standen, N. B. K_{ATP} channels in vascular smooth muscle. *Cardiovasc. Res.* **1994**, *28* (6), 797−804.

(2) Amoroso, S.; Schmidt-Antomarchi, H.; Fosset, M.; Lazdunski, M. Glucose, sulfonylureas, and neurotransmitter release: role of ATP-sensitive K^+ channels. *Science* **1990**, *247*, 852−854.

(3) Gross, E. R.; Gross, G. J. Ligand triggers of classical preconditional and postconditioning. *Cardiovasc. Res.* **2006**, *70*, 212−221.

(4) Garlid, K. D.; Puddu, P. E.; Pasdois, P.; Costa, A. D.; Beauvoit, B.; Criniti, A.; Tariosse, L.; Diolez, P.; Dos Santos, P. Inhibition of cardiac contractility by 5-hydroxydecanoate and tetraphenylphosphonium ion: a possible role of mitoKATP in response. *Am. J. Physiol.: Heart Circ. Physiol.* **2006**, *291*, 2067−2074.

(5) Garlid, K. D.; Paucek, P.; Yarov-Yarovoy, B.; Murray, H. N. M.; Darbenzio, R. B.; D'Alonzo, A. J.; Lodge, N. J.; Smith, M. A.; Grover, G. J. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP sensitive potassium channels. Possible mechanism of cardioprotection. *Circ. Res.* **1997**, *81*, 1072−1082.

(6) Liu, Y.; Sato, T.; Seharaseyon, J.; Szewczyk, A.; O'Rourke, B.; Marban, E. Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. *Ann. N.Y. Acad. Sci.* **1999**, *874*, 27−37.

(7) Testai, L.; Rapposelli, S.; Calderone, V. Cardiac ATP-sensitive potassium channels, a potential target for an anti-ischaemic pharmacological strategy. *Cardiovasc. Haematol. Agents Med. Chem.*, in press.

(8) Mannhold, R. Structure−activity relationships of K(ATP) channel openers. *Curr. Top. Med. Chem.* **2006**, *6* (10), 1031−1047.

(9) Nielsen, F. E.; Ebdrup, S.; Jensen, A. F.; Ynddal, L.; Bodvarsdottir, T. B.; Stidsen, C.; Worsaae, A.; Boonen, H. C.; Arkhammar, P. O.; Fremming, T.; Wahl, P.; Korno, H. T.; Hansen, J. B. New 3-alkylamino-4*H*-thieno-1,2,4-thiadiazine 1,1-dioxide derivatives activate ATP-Sensitive potassium channels of pancreatic beta cells. *J. Med. Chem.* **2006**, *49* (14), 4127−4139.

(10) Atwal, K. S.; Ferrara, F. N.; Ding, C. Z.; Grover, G. J.; Sleph, P. G.; Dzwonczyk, S.; Baird, A. J.; Normandin, D. E. Cardioselective antiischemic ATP-sensitive potassium channel openers. 4. Structure−activity studies on benzopyranylcyanoguanidines: replacement of the benzopyran portion. *J. Med. Chem.* **1996**, *39*, 304−313.

(11) Grover, G. J.; D'Alonzo, A. J.; Garlid, K. D.; Bajgar, R.; Lodge, N. J.; Sleph, P. G.; Darbenzio, R. B.; Hess, T. A.; Smith, M. A.; Paucek, P.; Atwal, K. S. Pharmacologic characterization of BMS-191095, a mitochondrial KATP opener with no peripheral vasodilator or cardiac action potential shortening activity. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 1184−1192.

(12) Gadwood, R. C.; Kamdar, B. V.; Cipkus Dubray, L. A.; Wolfe, M. L.; Smith, M. P.; Watt, W.; Mizsak, S. A.; Groppit, V. E. Synthesis and biological activity of spirocyclic benzopyran imidazolone potassium channel openers. *J. Med. Chem.* **1993**, *36*, 1480−1487.

(13) Kabbe, H. J. A simple synthesis of 4-chromanones. *Synthesis* **1978**, *12*, 886−887.

(14) Amundsen, L. H.; Nelson, L. S. Reduction of nitriles to primary amines with lithium aluminum hydride. *J. Am. Chem. Soc.* **1951**, *73*, 242−244.

(15) Battistini, L.; Rassu, G.; Pinna, L.; Zanardi, F.; Casiraghi, G. Diastereoselective synthesis of a novel lactam peptidomimetic exploiting vinylogous Mannich addition of 2-(silyloxy)furan reagents. *Tetrahedron: Asymmetry* **1999**, *10*, 765−773.

(16) Korge, P.; Honda, H. M.; Weiss, J. N. Protection of cardiac mitochondria by diazoxide and protein kinase C: implications for ischemic preconditioning. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 3312−3317.

JM061228L