Spirocyclic Benzopyran-Based Derivatives as New Anti-ischemic Activators of Mitochondrial ATP-Sensitive Potassium Channel

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

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

Received July 29, 2008

Heart mitochondrial ATP-sensitive potassium channels (mito-K_{ATP} channels) are deeply implicated in the self-defense mechanism of ischemic preconditioning. Therefore, exogenous molecules activating these channels are considered as a promising pharmacological tool to reduce the myocardial injury deriving from ischemia/reperfusion events. This paper reports the synthesis and pharmacological evaluation of original spiromorpholine- and spiromorpholone-benzopyran derivatives, with the aim to obtain selective activators of mito-K_{ATP} channels. Some compounds of this series showed appreciable cardioprotective effects on rat isolated and perfused hearts, submitted to ischemia/reperfusion cycles. The selective mito-K_{ATP} channel blocker 5-hydroxydecanoic acid antagonized the anti-ischemic activity, indicating a clear implication of this pharmacological target. Furthermore, these effects were not associated with significant hypotensive and vasorelaxing properties, which represent one of the main limiting factors for the clinical use of nonselective K_{ATP}-openers against myocardial ischemia.

Introduction

Single or multiple brief periods of ischemia/reperfusion processes protect the heart against a following and more prolonged ischemic insult. This phenomenon originally described by Murry et al.[1] is known as ischemic preconditioning (IPC[a]). IPC has been shown to reduce myocardial infarct size and also cardiac arrhythmias and ventricular fibrillation. An early protection of IPC appears immediately after the preconditioning stimulus and lasts 1 or 2 h. A more prolonged "second window" of protection (that lasts up to 72 h) is observed 12−24 h after the preconditioning stimulus.

The mechanism of IPC has been intensively investigated, and several triggers and mediators have been identified in this process. In particular, cardiac ATP-sensitive potassium channels (K_{ATP} channels) seem to be implicated in the cardioprotective effects and involved in the mechanism of preconditioning as both triggers and effectors of IPC.[2,3]

In the heart, K_{ATP} channels have been identified in both sarcolemmal and mitochondrial membranes (sarc- and mito-K_{ATP}, respectively).

The cardiac sarc-K_{ATP} channel is composed by an octameric architecture of four Kir6.2 (member of inward-rectifying potassium channel subfamily) subunits and four SUR2A (member of sulfonylurea receptor family) subunits.[4] Presently, the molecular composition of the cardiac mito-K_{ATP} channel has not been fully clarified, although recent reports suggest that they may be composed by Kir6.1, Kir6.2, and SUR2 subunits, while the SUR1 subunit seems to be excluded.[5]

Myocardial K_{ATP} channel gating is highly responsive to metabolic conditions: in normoxic conditions, K_{ATP} channels are preferentially in an inactivated state because they are inhibited by high levels of intracellular ATP, while in conditions of metabolic impairment, they are activated by decreased levels of intracellular ATP and increased levels of intracellular diphosphate nucleosides.[6,7] Hence, in physiological conditions, cardiac K_{ATP} channels are usually in a closed and inactivated state, whereas during myocardial ischemia or, more generally, in condition of metabolic stress, the fall in intracellular ATP concentration and/or an accumulation of ischemic metabolites increases the opening of this type of channels.[8]

The role of both sarcolemmal and mitochondrial K_{ATP} channels cardioprotection against myocardial ischemia/reperfusion injury has been intensively studied, and presently there is wide consensus that endogenous activation of mito-K_{ATP} channels is deeply involved in IPC.[3] The activation of these channels by exogenous molecules has been viewed as a promising approach to produce a "pharmacological preconditioning" able to mimick the endogenous IPC and, thus, to give myocardial cells an increased resistance against ischemia/reperfusion. Indeed, the administration of diazoxide, a K_{ATP} channel opener (KCO), at a dose able to induce the opening of mito-K_{ATP} channel but not sarc-K_{ATP} channel, shows clear cardioprotective effects. Moreover, the presence of 5-hydroxydecanoic acid (5-HD), a selective mito-K_{ATP} channel blocker, prevents the diazoxide-mediated protection against IPC,[9] thus confirming the general hypothesis that enhanced mitochondrial potassium influx induces cardioprotection.

Also many other different chemical classes of KCOs (Figure 1), including benzopyran derivatives such as cromakalim, thioformamides such as aprikalim, and cyanoguanidines such as pinacidil, induce cardioprotective effects, mainly attributable to activation of mito-K_{ATP}, but they are generally associated to sarc-K_{ATP}-mediated unsought systemic effects, such as reduction of peripheral resistance and marked hypotension, which repre-

* 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, Università di Pisa.

[‡] Dipartimento di Scienze Farmaceutiche, Università di Pisa.

[a] Abbreviations: IPC, ischemic preconditioning; RPP, rate pressure product; LVDP, left ventricular developed pressure; HR, heart rate.

10.1021/jm800956g CCC: $40.75
Published on Web 10/17/2008

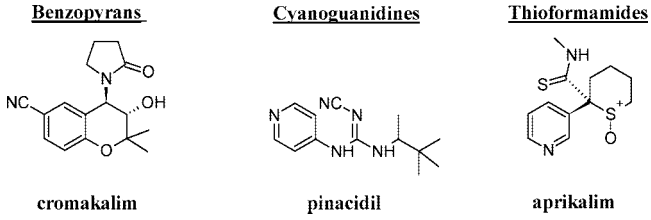


Figure 1. Structural classes of KCOs.

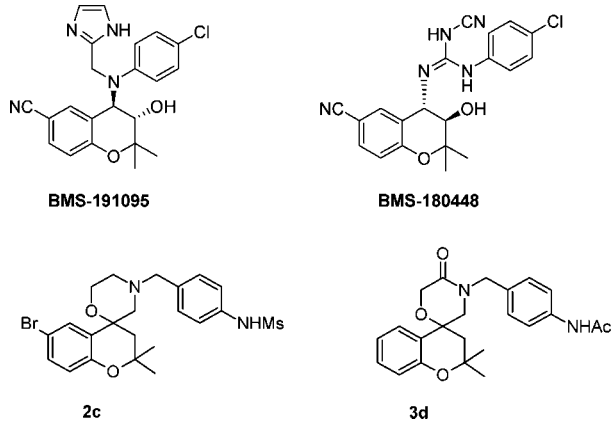


Figure 2. Mitochondrial K_{ATP} Channel Openers.

sent an insurmountable limit for their clinical use in cardioprotection against heart ischemia.

In recent years, the research was addressed to the development of new KCOs in order to find new compounds with higher selectivity toward specific targets including the cardiac mitochondrial K_{ATP} channels.[10] That of benzopyran KCOs represents the main chemical class studied; the two benzopyranyl-cianoguanidine derivatives BMS-180448 and BMS-191095 (Figure 2) showed a high cardioprotective activity linked to the mito-K_{ATP} channel activation and reduced vasorelaxing properties.[11,12] Starting from the hypothesis that the C4 substituent on benzopyran nucleus may have a relevant role in determining the selectivity of new benzopyran-type derivatives and from the observation that the 4-spiro-substitution have been scarcely investigated, we planned the synthesis of a limited number of 4-spiro-morpholine (A) and 4-spiro-morpholone (B) compounds (Figure 2) in order to evaluate their cardioprotective activity.[13] This preliminary work led us to identify new compounds endowed of a good cardioselectivity[13] and a pharmacological profile qualitatively similar to those exhibited by BMS-180448 and BMS-191095.[12]

With the aim to investigate more deeply this kind of spiro-like structures and the influence of some molecular modifications on their cardioprotective properties, we synthesized new spiromorpholines **1**−**2** and spiromorpholones **3**−**4** in which the substituent on the benzylic group directly linked to the nitrogen atom of both type of derivatives is a strong (bromine and trifluoromethyl) or a weak (acetamide and methanesulfonamide) electron-withdrawing group or an electron-donor (methoxy, methyl, amine) substituent.

Chemistry. The final compounds **1**−**4** were synthesized following the synthetic procedure shown in Scheme 1. Spiromorpholones **5**, **6**, obtained as previously described,[13] were submitted to a reaction with appropriate benzyl halide and NaH in DMF affording the *N*-benzyl-substituted compounds **3**, **4**. Compounds **1a**, **2a**, **1e**−**f**, **2e**−**f**, and **7**, **8** were obtained starting from spiromorpholones **3**, **4** or **5**, **6**, respectively, by reduction to the corresponding spiromorpholine derivatives with $LiAlH_4$ in the cases of compounds **1a**, **1e**−**f**, **7**, or with a borane−methyl sulfide complex for compounds **2a**, **2e**−**f**, and **8**. The subsequent reaction of spiromorpholine **7**, **8** with appropriate benzyl halide in the presence of K_2CO_3 afforded compounds **1g**−**h**, **2g**−**h**.

The methanesulfonamido derivatives **1c** and **3c** were synthesized from the corresponding amine by reaction with acetic anhydride and methanesulfonyl chloride following the experimental procedure previously described for compounds **3d** and **1d**,[13] respectively.

Results and Discussion

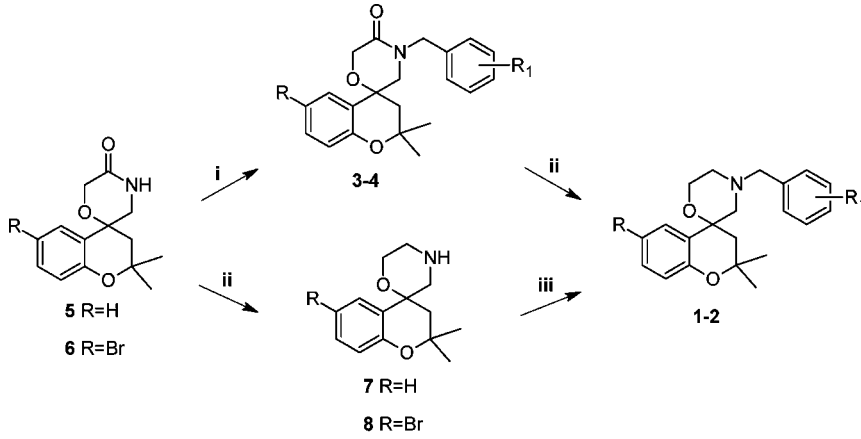
All the compounds synthesized (**1**−**4**) were tested as racemic mixtures at a dose of 40 mg kg^{1-} ip on Langendorff perfused rat hearts subjected to ischemia/reperfusion cycles (30 and 120 min, respectively). Two well-known K_{ATP} channel openers, diazoxide and cromakalim, were also tested as reference drugs at doses of 40 or 1 mg kg^{-1}, respectively. Diazoxide is a benzothiadiazine derivative widely considered to possess, at the tested dose, of a satisfactory degree of cardiac selectivity,[3] while cromakalim is a potent benzopyran-based K_{ATP} channel opener (and thus, from a structural point of view, is closer to the synthesized compounds), which exhibits both cardioprotective effects and marked hypotensive properties. For each compound, the resulting ischemic injury was quantified by evaluating functional and morphological parameters. In particular, the functional parameter of rate pressure product (RPP) recorded at the 120th min of reperfusion (RPP-120′) has been expressed as a percentage of RPP value recorded at the last minute of the preischemic period. This parameter was taken as indicator of the functional recovery of inotropism in the final stage of reperfusion. At the end of reperfusion, the treatment of the heart with triphenyltetrazolium chloride (TTC) made it possible to carry out a morphological comparison of the necrotic and healthy areas of the left ventricular tissue, colored white (or pale pink) and red, respectively, and then to calculate the ischemia-injured area as a % of the total area.

The results of the pharmacological tests on Langendorf perfused rat hearts are reported in Tables 1 and 2, together with those obtained in the same type of test for the spiro-based benzopyran compounds (**1d**, **2c**,**d**, **3d**, **4c**,**d**) previously described.[13]

The "basic core" of spiromorpholine-derivative **1a**, devoid of substituents both on the morpholinic *N*-benzyl chain and on the C6 position of the benzopyran nucleus, showed a satisfactory anti-ischemic activity leading to a good inotropic recovery and a limited injured areas, with a profile comparable to or better than the two reference drugs cromakalim and diazoxide, respectively. The insertion on the *N*-benzyl-ring of electron-withdrawing groups such as $-CF_3$ (**1f**) and Br (**1g**), but also of other substituents such as methyl, acetamido, methanesulfonamido, led to a decrease of the pharmacological activity with the exception of the amino- (**1b**) and the *para*-methoxy-substituted- (**1h**) derivatives, which exhibited a cardioprotective activity slightly lower than that of **1a**. The shifting of -OMe group in position *ortho* (**1i**) or *meta* (**1l**) on the same benzyl ring did not afford a significant change in the pharmacological profile both in terms of inotropic recovery (RPP) and of injured areas (Ai/Atot).

The presence of a bromine atom in the 6 position of the benzopyran ring of **1a** led to compound **2a** showing a marked reduction of the cardioprotective effect when compared to **1a**. The insertion of electron-withdrawing groups seemed to be a positive requirement because the bromine-substituted derivative (**2g**) showed a good cardioprotective effect which resulted

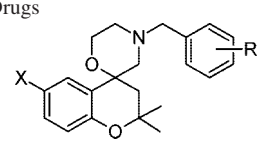
Scheme 1[a]



Compd	R	R_1	compd	R	R_1
1a, 3a	H	H	**2a, 4a**	Br	H
1e, 3e	H	4-Me	**2e, 4e**	Br	4-Me
1f, 3f	H	4-CF_3	**2f, 4f**	Br	4-CF_3
1g, 3g	H	4-Br	**2g, 4g**	Br	4-Br
1h, 3h	H	4-OMe	**2h, 4h**	Br	4-OMe
1i, 3i	H	2-OMe	**2i, 4i**	Br	2-OMe
1l, 3l	H	3-OMe	**2l, 4l**	Br	3-OMe

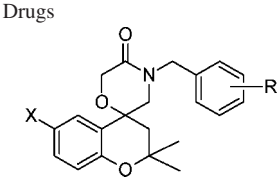
[a] Reagents and conditions: (i) benzylbromide, NaH, DMF, 2 h, rt; (ii) (a)$LiAlH_4$, THF, 1 h, reflux or b) $BH_3 \cdot SMe_2$, THF, mw, 30 min; (iii) K_2CO_3, MeCN, benzylhalide, 12 h, reflux.

Table 1. Functional (RPP-120′) and Morphological (% Ischemic Area vs Total Area) Parameters Recorded in Hearts Isolated from Rats Pretreated with the Vehicle, with the Spiromorpholine-Compounds, or with the Reference Drugs



compds	X	R	RPP 120′ (%) heart	A_i/A_{tot} (%)
vehicle			31 ± 4	37 ± 4
cromakalim			94 ± 17	25 ± 1
diazoxide			47 ± 9	28 ± 8
1a	H	H	77 ± 23	18 ± 3
1b	H	*p*-NH_2	58 ± 12	25 ± 2
1c	H	*p*-$NHSO_2Me$	23	35 ± 1
1d[13]	H	*p*-NHCOMe	17	50 ± 2
1e	H	*p*-Me	35 ± 18	47 ± 7
1f	H	*p*-CF_3	34 ± 8	31 ± 6
1g	H	*p*-Br	43 ± 12	35 ± 3
1h	H	*p*-OMe	61 ± 16	26 ± 2
1i	H	*o*-OMe	65 ± 28	34 ± 6
1l	H	*m*-OMe	38 ± 11	17 ± 3
2a	Br	H	42 ± 17	31 ± 3
2b	Br	*p*-NH_2	30 ± 7	39 ± 5
2c[13]	Br	*p*-$NHSO_2Me$	77 ± 19	14 ± 2
2d[13]	Br	*p*-NHCOMe	26	61 ± 2
2e	Br	*p*-Me	57 ± 12	29 ± 1
2f	Br	*p*-CF_3	84 ± 16	5 ± 2
2g	Br	*p*-Br	53 ± 10	16 ± 6
2h	Br	*p*-OMe	60 ± 7	10 ± 2
2i	Br	*o*-OMe	59 ± 25	30 ± 3
2l	Br	*m*-OMe	42 ± 22	29 ± 5

Table 2. Functional (RPP-120′) and Morphological (% Ischemic Area vs Total Area) Parameters Recorded in Hearts Isolated from Rats Pretreated with the Vehicle, with the Spiromorpholone Compounds, or with the Reference Drugs



compds	X	R	RPP 120′ (%) heart	A_i/A_{tot} (%)
vehicle			31 ± 4	37 ± 4
cromakalim			94 ± 17	25 ± 1
diazoxide			47 ± 9	28 ± 8
3a	H	H	35 ± 9	33 ± 3
3b[13]	H	*p*-NH_2	53 ± 9	19 ± 2
3c	H	*p*-$NHSO_2Me$	70 ± 24	23 ± 3
3d[13]	H	*p*-NHCOMe	62 ± 20	20 ± 4
3e	H	*p*-Me	44 ± 10	23 ± 2
3f	H	*p*-CF_3	2 ± 2	53 ± 10
3g	H	*p*-Br	80 ± 12	23 ± 5
3h	H	*p*-OMe	27 ± 6	29 ± 4
3i	H	*o*-OMe	36 ± 19	25 ± 5
3l	H	*m*-OMe	29 ± 16	41 ± 5
4a	Br	H	37 ± 15	43 ± 4
4b[13]	Br	*p*-NH_2	52 ± 12	22 ± 2
4c[13]	Br	*p*-$NHSO_2Me$	57 ± 19	13 ± 3
4d[13]	Br	*p*-NHCOMe	3	58 ± 4
4e	Br	*p*-Me	38 ± 3	22 ± 2
4f	Br	*p*-CF_3	47 ± 20	42 ± 6
4g	Br	*p*-Br	23 ± 19	47 ± 6
4h	Br	*p*-OMe	76 ± 19	16 ± 4
4i	Br	*o*-OMe	42 ± 17	28 ± 1
4l	Br	*m*-OMe	27 ± 18	29 ± 3

further improved in the trifluoromethyl-substituted derivative (**2f**). Generally, the insertion of electrondonor-groups in C4′ position of *N*-benzyl ring failed to lead substantial improvement with the only exception of compound **2h**, which exhibited a significant cardioprotective effect almost comparable to that previously observed for derivative **4c**.[13]

As regards the spiromorpholone compounds (**3**−**4**), differently from the "basic core" spiromorpholine derivative **1a**, its spiromorpholone analogue (**3a**) did not show any significant anti-ischemic activity. The insertion of CF_3 on the *N*-benzyl-ring (**3f**) increases the ischemic injury, while the bromine atom (**3g**) led to a good cardioprotective effect. The presence of other

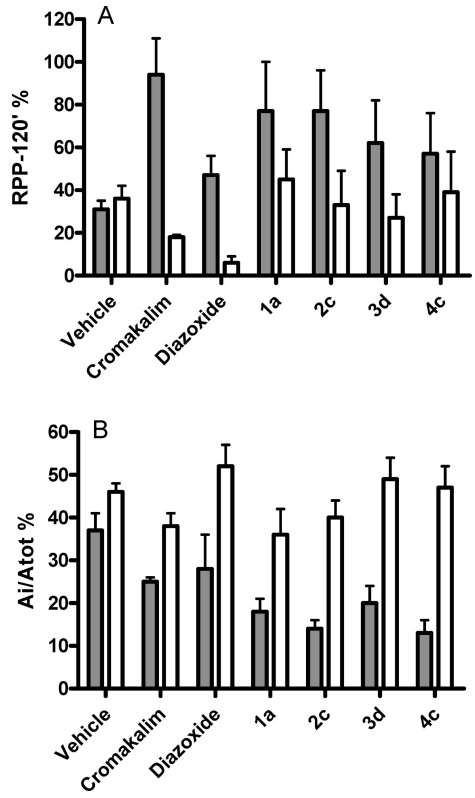


Figure 3. (A) Values of RPP-120′ (%) for selected compounds, recorded in the absence (gray columns) or in the presence (white columns) of the selective mitoKATP channel blocker 5-hydroxydecanoic acid (5-HD). (B) Values of A_i/A_{tot} (%) for selected compounds, recorded in the absence (gray columns) or in the presence (white columns) of 5-HD.

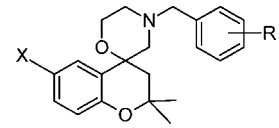
substituents such as amino- (**3b**), methanesulfonamido- (**3c**), or methyl-(**3e**) groups, as well as the previously synthesized acetamido-derivative (**3d**), led to compounds endowed of pharmacological activity. The pharmacological profile of the methoxy-substituted spiromorpholones (**3h**, **3i**, **3l**) was almost equivalent by that exhibited by **3a**.

In the limited series of 6-bromine-substituted spiromorpholones (**4a**−**l**), the presence of electronwithdrawing groups such as in compounds **4f** and **4g** was detrimental for the activity, while the amino-(**4b**) and the methyl-substituted (**4e**) compounds showed an increased cardioprotective activity, which, in terms of ischemic injury, was almost comparable to that previously observed for derivative **4c**.[13] A good pharmacological activity was also exhibited by the *para*-methoxy-spiromorpholone **4h**, while its *ortho*- (**4i**) and *meta*- (**4l**) analogues showed a decrease of activity.

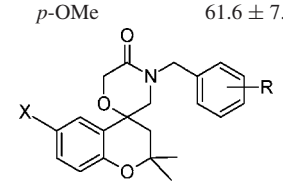
With regard to the definition of the mechanism of action, some compounds were selected and tested in the presence of the selective mito-K_{ATP} channel blocker 5-hydroxydecanoic acid (5-HD). In particular, the cardioprotective activity of **1a**, **2c**, **3d**, **4c** were almost completely abolished by this selective blocker (Figure 3), clearly indicating the involvement of mitochondrial K_{ATP} channel in the pharmacodynamic mechanism of cardioprotection. As expected, 5-HD abolished also the cardioprotective activity of diazoxide and cromakalim.

Vasorelaxing Properties. To investigate the selectivity profile, some compounds (**1a**, **1b**, **2c**, **2h**, **3c**, **3d**, **3g**, **4c**, **4h**) exhibiting a convincing cardioprotective effect and the reference drugs diazoxide and cromakalim were selected for studying their eventual vasorelaxing properties on in vitro vascular smooth muscle preparation. Also some spiromorpholine-derivatives (**3a**, **3f**, **4d**) and spiromorpholone-derivatives (**1c**, **1d**, **1f**, **2d**, **2f**, **4a**), which proved to be ineffective in cardioprotection, were tested in this experimental protocols.

Table 3. Parameters of Vasorelaxing Potency and Efficacy of Some Selected Spiromorpholone and Spiromorpholine Compounds and the Reference K_{ATP} Openers Recorded in Isolated Rat Aortic Rings



compds	X	R	E_{max} (%) aorta	pIC_{50} aorta
cromakalim			98.0 ± 1	7.01 ± 0.09
diazoxide			97.0 ± 2	4.72 ± 0.04
1a	H	H	85.9 ± 6.4	5.17 ± 0.12
1b[13]	H	*p*-NH_2	52.3 ± 4.1	4.55 ± 0.05
1c	H	p-$NHSO_2Me$	80.6 ± 8.2	4.81 ± 0.04
1d[13]	H	*p*-NHCOMe	76.6 ± 2.1	4.88 ± 0.03
1f	H	*p*-CF_3	59.9	4.64
2c[13]	Br	*p*-$NHSO_2Me$	98.1 ± 2.4	5.22 ± 0.02
2d[13]	Br	*p*-NHCOMe	99.8 ± 1.8	5.62 ± 0.03
2f	Br	*p*-CF_3	57.8 ± 11.0	4.75 ± 0.25
2h	Br	*p*-OMe	61.6 ± 7.3	4.46 ± 0.06



cromakalim			98.0 ± 1	7.01 ± 0.09
diazoxide			97.0 ± 2	4.72 ± 0.04
3a	H	H	55.3	4.55
3b[13]	H	*p*-NH_2	12.5 ± 5.7	NC
3c	H	*p*-$NHSO_2Me$	98.0 ± 6.1	5.53 ± 0.04
3d[13]	H	*p*-NHCOMe	56.5 ± 3.3	4.60 ± 0.03
3f	H	*p*-CF_3	101.2 ± 2.5	5.15 ± 0.03
3g	H	*p*-Br	89.2 ± 7.0	5.31 ± 0.09
4a	Br	H	65.8 ± 1.3	4.84 ± 0.03
4c[13]	Br	*p*-$NHSO_2Me$	86.8 ± 2.8	5.14 ± 0.03
4d[13]	Br	*p*-NHCOMe	69.7 ± 10.5	4.82 ± 0.07
4h	Br	*p*-OMe	91.8 ± 1.8	5.52 ± 0.03

As shown in Table 3, the tested compounds exhibited almost full or partial vasorelaxing effects on rat aortic rings, however, the potency values were quite modest and comparable to diazoxide, a "preferential" mito-K_{ATP} channel opener, and about 2 orders of magnitude lower than cromakalim, a potent non-selective mito/sarc-K_{ATP} channel opener.

Hypotensive Effects. The absence of hypotensive effects of some cardioprotective compounds was further confirmed by an in vivo approach. In particular compounds **1a**, **2c**, **2h**, **3g**, **4h**, and diazoxide were tested in the cardiac protocol and produced none or very modest influences on the systolic blood pressure, as previously observed for compounds **3b**, **3d**, and **4c**.[13] As expected, cromakalim caused a rapid and marked hypotensive response (Figure 4).

Conclusion

The experimental results reported in this work suggest that many spirocyclic benzopyran derivatives of this series are activators of mitochondrial K_{ATP} channel endowed of an appreciable degree of selectivity for this target and devoid of significant effects on blood pressure, which represent one of the main limiting factors for the cardioprotective therapeutic use of well-known K_{ATP} channel openers (i.e., cromakalim, pinacidil, etc).

The enlargement of the series of spirocyclic benzopyran derivatives, which in our previously work[13] emerged as

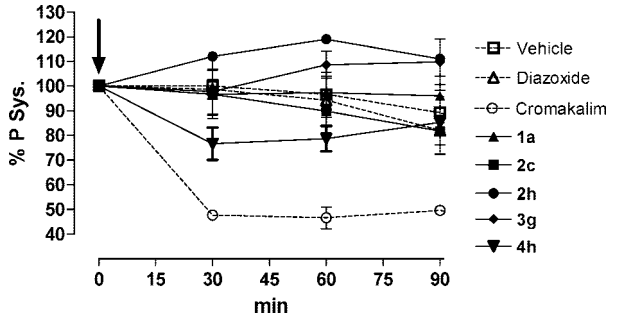


Figure 4. Effects of selected compounds and vehicle on the systolic blood pressure of normotensive animals. The arrow indicates the time of ip administration. Each point represents the mean value from five different experiments; the standard errors are also indicated.

potentially interesting class of anti-ischemic drugs, does not yet allow us to have an exhaustive definition of structure−activity relationships. However, the experimental results seems to indicate some considerations: (i) from the data available, it is not possible to define the role played by the bromine atom in 6-position in the cardioprotection of this series of spirocyclic-benzopyran derivatives; (ii) the presence of a sulfonamido-group on the *N*-benzyl portion could be globally viewed as a favorable requirement for the activity, as clearly emerged for compounds **2c**, **3c**, and **4c**; (iii) also the presence of an amino-group is generally well-accepted (**1b**, **3b**, **4b**); (iv) the presence of a methoxy-group, in particular in the *para* position of the *N*-benzyl ring, is broadly a positive structural feature; (v) the presence of strong electron-withdrawing groups, such as trifluoromethyl, often led to controversial results; in fact, in the case of **3f**, this kind of substitution led to an increased ischemic damage. On the contrary, compound **2f** was the most powerful cardioprotective agent of the whole series. Because of such a particular behavior of the CF_3-substituted compound, it will be subjected to further pharmacological investigations.

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Mass spectra were obtained on a Hewlett-Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040−0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063−0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F_{254}) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. The microwave-assisted procedures were carried out with a CEM Discover LabMate Microwave. Commercially available chemicals were purchased from Sigma-Aldrich. All hydrochloride salts were obtained by precipitation with Et_2O·HCl.

4′-Benzyl-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 1a. A solution of **3a** (698 mg; 2.07 mmol) in THF (3 mL) was added to a solution of $LiAlH_4$ 1 M in THF (315 mg, 8.30 mmol) cooled at 0 °C. The mixture was refluxed for 1 h, then water (0.6 mL) and NaOH 1 M (0.15 mL) was added, and the resulting suspension was filtrated. The solvent was evaporated, and the crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to give **1a** (446 mg, 1.24 mmol, 60% yield): mp 168−170 °C. ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 3H, Me), 1.36 (s, 3H, Me), 2.15 (d, 1H, J = 14.6 Hz, CH_2), 2.41−2.60 (m, 4H, CH_2), 2.71−2.77 (m, 1H, CH_2), 3.37 (d, 1H, J = 13.0 Hz, CH_2), 3.60 (d, 1H, J = 13.0 Hz, CH_2), 3.71−3.80 (m, 1H, CH_2), 3.90−4.02 (m,1H, CH_2), 6.77 (d, 1H, J = 8.1 Hz, Ar), 6.88−6.96 (m, 1H, Ar), 7.12−7.20 (m, 1H, Ar), 7.22−7.32 (m, 5H, Ar), 7.61 (d, 1H, J = 7.7 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 153.85, 131.49, 130.64, 130.38, 129.45, 127.51, 127.05, 121.21, 120.77, 118.56, 74.52, 70.02, 61.76, 58.33, 57.79, 52.41, 39.49, 28.27; 26.49. MS (*m*/*z*): 323 (M^+ 54%). Anal. ($C_{21}H_{25}NO_2$ HCl) C, H, N.

4′-(*N*-(4-Methansolfonamido)benzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinanane] 1c. Compound **1c** was prepared by reaction of 4′-(4-aminobenzyl)-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane][13] (389 mg, 1.15 mmol) in dry dioxane (12 mL) under nitrogen atmosphere to which was added dry pyridine (1.2 mL), and then the solution was put in an ice bath and methanesulfonyl chloride (0.10 mL, 1.38 mmol) was added dropwise. The reaction mixture was refluxed for 1 h and then acidified with diluted HCl and extracted with AcOEt. The organic layer was dried and concentrated under reduced pressure. The crude product was transformed to the hydrochloride salt and crystallized from *i*-PrOH to afford **1c** (113 mg, 0.25 mmol, 22% yield): mp 166−168 °C. ^{1}H NMR (DMSO): δ 1.25 (s, 6H, Me), 2.35 (d, 1H, J = 15.0 Hz, CH_2), 2.64 (d, 1H, J = 15.0 Hz, CH_2), 3.01 (s, 3H, Me), 3.22−3.32 (m, 2H, CH_2), 3.47−3.60 (m, 2H, CH_2), 3.85−4.10 (m, 2H, CH_2), 4.15−4.45 (m, 2H, CH_2), 6.77 (d, 1H, J = 8.0 Hz, Ar), 6.92−7.00 (m, 1H, Ar), 7.21−7.25 (m, 3H, Ar), 7.54−7.62 (m, 3H, Ar).^{13}C NMR ($CDCl_3$): δ 155.06, 141.68, 133.94, 131.57, 128.97, 124.56, 122.62, 121.87, 120.76, 119.21, 74.91, 71.03, 62.15, 60.73, 58.54, 52.35, 39.68, 39.53, 29.50, 25.24. MS (*m*/*z*): 416 (M+ 5%); 184 (100%). Anal. ($C_{22}H_{28}N_2O_4S$ HCl) C, H, N.

4′-(4-Methylbenzyl)-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 1e. Compound **1e** was synthesized from **3e** (727 mg, 2.07 mmol) following the same procedure described above for the preparation of **1a**. The crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to give **1e** (333 mg, 0.89 mmol, 43% yield): mp 89−91 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 3H, Me), 1.35 (s, 3H, Me), 2.08−2.25 (m, 3H, CH_2, CH_2), 2.32 (s, 3H, Me), 2.39−2.75 (m, 3H, CH_2, CH_2), 3.33 (d, 1H, J = 12.8 Hz, CH_2), 3.54 (d, 1H, J = 12.8 Hz, CH_2), 3.65−3.77 (m, 1H, CH_2), 3.88−4.01 (m, 1H, CH_2), 6.75−6.79 (m, 1H, Ar), 6.87−6.95 (m, 2H, Ar), 7.08−7.22 (m, 4H, Ar), 7.59−7.63 (m, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 153.94, 131.47, 130.67, 130.16, 127.05, 124.34, 121.31, 120.80, 118.64, 74.63, 70.08, 61.72, 58.37, 57.81, 52.29, 39.56, 28.45, 26.40, 21.42. MS (*m*/*z*): 337 (M^+ 18%). Anal. ($C_{22}H_{27}NO_2$) C, H, N.

4′-(4-Trifluoromethylbenzyl)-2,2-dimethyl-2,3-dihydrospiro-[chromene-4,2′-[1,4]oxazinane] 1f. Compound **1f** was synthesized from **3f** (839 mg, 2.07 mmol) following the same procedure described above for the preparation of **1a**. The crude product was transformed to the hydrochloride salt and crystallized from *i*-PrOH to afford **1f** (222 mg, 0.52 mmol, 25% yield): mp 158−160 °C. ^{1}H NMR (CD_3OD): δ 1.29 (s, 3H, Me), 1.38 (s, 3H, Me), 2.04 (d, 1H, J = 15.3 Hz, CH_2), 2.73 (d, 1H, J = 15.3 Hz, CH_2), 3.22−3.65 (m, 4H, CH_2), 3.95−4.18 (m, 2H, CH_2), 4.46−4.64 (m, 2H, CH_2), 6.77−6.82 (m, 1H, Ar), 6.95−7.03 (m, 1H, Ar), 7.21−7.30 (m, 2H, Ar), 7.76−7.92 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$): δ 154.05, 132.08, 130.91, 126.87, 126.81, 126.70, 126.52, 122.94, 121.10, 120.90, 118.86, 74.66, 70.19, 61.46, 59.11, 57.79, 53.11, 39.67, 28.47, 26.43. MS (*m*/*z*): 391 (M^+ 15%); 159 (45%); 214 (100%). Anal. ($C_{22}H_{24}F_3NO_2$ HCl) C, H, N.

4′-(4-Bromobenzyl)-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 1g. To a solution of **7** (150 mg, 0.64 mmol) in MeCN (5 mL) was added K_2CO_3 (100 mg, 0.72 mmol) and 4-bromo-benzylbromide (160 mg, 0.64 mmol). The resulting mixture was refluxed for 12 h and then, after cooling, was filtered and the solvent evaporated. The crude product was transformed to the hydrochloride salt to yield **1g** (76 mg, 0.19 mmol, 30% yield): mp 182−184 °C. ^{1}H NMR ($CDCl_3$): δ 1.33 (s, 6H, Me), 2.51 (d, 1H, J = 15.2 Hz, CH_2), 2.86 (d, 1H, J = 15.2 Hz, CH_2), 2.95−3.03 (m, 2H, CH_2), 3.65−3.98 (m, 4H, CH_2), 4.51−4.65 (m, 2H, CH_2), 6.80−6.98 (m, 2H, Ar), 7.19−7.35 (m, 2H, Ar), 7.54 (d, 2H, J =

8.4 Hz, Ar), 7.63 (d, 2H, J = 8.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 153.93, 148.23, 147.19, 146.04, 133.15, 132.71, 130.76, 127.00, 120.82, 118.69, 74.54, 70.09, 61.12, 58.20, 57.80, 52.61, 39.56, 28.49, 26.36. MS (m/z): 402 (M^+ 34%). Anal. ($C_{21}H_{24}BrNO_2$ HCl) C, H, N.

4′-(4-Methoxybenzyl)-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 1h. Compound **1h** was synthesized from **7** (133 mg, 0.57 mmol) and 4-methoxy-benzylbromide (157 mg, 0.57 mmol) following the same procedure described above for the preparation of **1g**. The crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to give **1h** (355 mg, 0.91 mmol, 44% yield): mp 98−100 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 3H, Me), 1.36 (s, 3H, Me), 2.13 (d, 1H, J = 14.6 Hz, CH_2), 2.33−2.60 (m, 4H, CH_2), 2.70−2.75 (m, 1H, CH_2), 3.30 (d, 1H, J = 12.7 Hz, CH_2), 3.53 (d, 1H, J = 12.7 Hz, CH_2), 3.72−4.02 (m, 2H, CH_2), 3.79 (s, 3H, OMe), 6.76−6.96 (m, 4H, Ar), 7.13−7.26 (m, 3H, Ar), 7.62 (d, 1H, J = 1.6, 7.8 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 161.03, 153.93, 133.06, 130.67, 127.01, 121.33, 120.79, 119.18, 118.64, 114.80, 74.61, 70.08, 61.52, 58.24, 57.80, 55.44, 52.16, 39.52, 28.42, 26.43. MS (m/z): 353 (M^+ 36%). Anal. ($C_{22}H_{27}NO_3$ HCl) C, H, N.

4′-(2-Methoxybenzyl)-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 1i. Compound **1i** was synthesized from **7** (133 mg, 0.57 mmol) and 2-methoxy-benzylchloride (90 mg, 0.57 mmol) following the same procedure described above for the preparation of **1g**. The crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to give **1i** (296 mg, 0.76 mmol, 37% yield): mp 185−187 °C. ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 3H, Me), 1.36 (s, 3H, Me), 2.13 (d, 1H, J = 14.6 Hz, CH_2), 2.33−2.59 (m, 4H, CH_2, CH_2), 2.30 (d, 1H, J = 12.7 Hz, CH_2), 2.70−2.75 (m, 1H, CH_2), 3.53 (d, 1H, J = 12.7 Hz, CH_2), 3.72−4.01 (m, 2H, CH_2), 3.79 (s, 3H, OMe), 6.76−6.95 (m, 4H, Ar), 7.12−7.26 (m, 3H, Ar), 7.62 (d, 1H, J = 7.7 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 158.28, 153.98, 134.21, 132.15, 130.60, 126.85, 121.68, 121.41, 120.73, 118.64, 115.69, 111.14, 74.61, 69.99, 61.70, 57.88, 55.76, 55.00, 51.54, 39.45, 28.47, 26.27. MS (m/z): 353 (M^+ 58%). Anal. ($C_{22}H_{27}NO_3$ HCl) C, H, N.

4′-(3-Methoxybenzyl)-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 1l. Compound **1l** was synthesized from **7** (79 mg, 0.34 mmol) and 3-methoxy-benzylbromide (70 mg, 0.34 mmol) following the same procedure described above for the preparation of **1g**. The crude product was transformed to the hydrochloride salt and crystallized from *i*-PrOH to yield **1l** (323 mg, 0.83 mmol, 40% yield): mp 141−143 °C. ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H, Me), 1.35 (s, 3H, Me), 2.63 (d, 1H, J = 15.0 Hz, CH_2), 2.83 (d, 1H, J = 15.0 Hz, CH_2), 2.94−3.08 (m, 2H, CH_2), 3.65−3.99 (m, 4H, CH_2, CH_2), 3.84 (s, 3H, OMe), 4.55−4.69 (m, 2H, CH_2), 6.82 (d, 1H, J = 8.1 Hz, Ar), 6.90−6.98 (m, 2H, Ar), 7.14−7.35 (m, 4H, Ar), 7.43−7.56 (m, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 160.19, 153.89, 130.67, 130.44, 128.93, 127.05, 123.32, 121.28, 120.79, 118.58, 116.58, 116.25, 74.57, 70.04, 61.85, 58.44, 57.82, 55.71, 52.58, 39.54, 28.23, 26.63. MS (m/z): 353 (M^+ 58%). Anal. ($C_{22}H_{27}NO_3$ HCl) C, H, N.

4′-Benzyl-6-bromo-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 2a. A solution of **4a** (179 mg, 0.43 mmol) in THF (3 mL) was added to a solution of $BH_3 \cdot SMe_2$ 2 M in THF (0.15 mL, 1.72 mmol). The resulting mixture was heated for 30 min by microwave irradiation at 70 °C and with a power of 50 W, and then water was added and the solvent evaporated. The aqueous phase was acidified with HCl 1 N, neutralized with NaOH 1 N, and extracted with AcOEt. The organic layer was dried and the solvent was evaporated. The crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to give **2a** (88 mg, 0.20 mmol, 47% yield): mp 134−136 °C. ^{1}H NMR (DMSO): δ 1.23 (s, 3H, Me), 1.25 (s, 3H, Me), 2.40 (d, 1H, J = 15.0 Hz, CH_2), 2.63 (d, 1H, J = 15.0 Hz, CH_2), 3.05−3.27 (m, 2H, CH_2), 3.56−3.66 (m, 2H, CH_2), 3.88−4.08 (m, 2H, CH_2), 4.22−4.46 (m, 2H, CH_2), 6.74 (d, 1H, J = 8.7 Hz, Ar), 7.33−7.51 (m, 4H, Ar), 7.67−7.75 (m, 3H, Ar). ^{13}C NMR ($CDCl_3$): δ 153.10, 133.60, 131.55, 130.51, 129.93, 129.55, 127.41, 123.44, 120.55, 112.68, 75.21, 69.95, 62.03, 58.31, 57.99, 52.60, 39.36, 28.09, 26.70. MS (m/z): 402 (M^+ 61%). Anal. ($C_{21}H_{24}BrNO_2$ HCl) C, H, N.

4′-(4-Methylbenzyl)-6-bromo-2,2-dimethyl-2,3-dihydrospiro-[chromene-4,2′[1,4]oxazinane] 2e. Compound **2e** was synthesized from **4e** (185 mg, 0.43 mmol) following the same procedure described above for the preparation of **2a**. The crude residue was transformed into the hydrochloride salt to give **2e** (63 mg, 0.14 mmol, 33% yield): mp 135−137 °C. ^{1}H NMR (CD_3OD): δ 1.28 (s, 3H, Me), 1.35 (s, 3H, Me), 2.02 (d, 1H, J = 15.0 Hz, CH_2), 2.37 (s, 3H, Me), 2.68 (d, 1H, J = 14.8 Hz, CH_2), 3.16−3.58 (m, 4H, CH_2), 3.96−4.16 (m, 2H, CH_2), 4.29−4.47 (m, 2H, CH_2), 6.74 (d, 1H, J = 8.7 Hz, Ar), 7.21−7.40 (m, 3H, Ar), 7.46 (d, 2H, J = 7.7 Hz, Ar), 7.35 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 153.12, 140.71, 133.59, 131.49, 130.20, 129.93, 124.28, 123.52, 120.55, 112.70, 75.19, 69.95, 61.76, 58.19, 57.91, 52.32, 39.36, 28.25, 26.50, 21.42. MS (m/z): 416 (M^+ 49%). Anal. ($C_{22}H_{26}BrNO_2$ HCl) C, H, N.

4′-(4-Trifluoromethylbenzyl)-6-bromo-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′[1,4]oxazinane] 2f. Compound **2f** was synthesized from **4f** (208 mg, 0.43 mmol) following the same procedure described above for the preparation of **2a.** The crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to give **2f** (106 mg, 0.21 mmol, 50% yield): mp 107−109 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 3H, Me), 1.32 (s, 3H, Me), 2.56 (d, 1H, J = 15.2 Hz, CH_2), 2.83 (d, 1H, J = 15.2 Hz, CH_2), 2.96−3.03 (m, 3H, CH_2, CH_2), 3.68−3.74 (m, 1H, CH_2), 3.89−4.00 (m, 2H, CH_2), 4.53−4.76 (m, 2H, CH_2), 6.70 (d, 1H, J = 8.7 Hz, Ar), 7.31 (dd, 1H, J = 2.3, 8.7 Hz, Ar), 7.46 (d, 1H, J = 2.2 Hz, Ar), 7.70 (d, 2H, J = 7.8 Hz, Ar), 7.97 (d, 2H, J = 7.8 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 153.14, 133.70, 132.11, 130.04, 126.63, 126.49, 126.32, 125.80, 123.26, 120.64, 112.74, 75.10, 70.00, 61.23, 58.79, 58.00, 53.00, 39.54, 28.33, 26.40. MS (m/z): 470 (M^+ 23%). Anal. ($C_{22}H_{23}BrF_3NO_2$ HCl) C, H, N.

4′-(4-Bromobenzyl)-6-bromo-2,2-dimethyl-2,3-dihydrospiro-[chromene-4,2′-[1,4]oxazinane] 2g. Compound **2g** was synthesized from **8** (216 mg, 0.76 mmol) and 4-bromo-benzylbromide (190 mg, 0.76 mmol) following the same procedure described above for the preparation of **1g**. The crude product was transformed into the hydrochloride salt and crystallized from *i*-PrOH to obtain **2g** (497 mg, 0.93 mmol, 45% yield): mp 168−170 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 3H, Me), 1.32 (s, 3H, Me), 2.56 (d, 1H, J = 15.2 Hz, CH_2), 2.80 (d, 1H, J = 15.2 Hz, CH_2), 2.89−3.04 (m, 2H, CH_2), 3.66−3.99 (m, 4H, CH_2), 4.50−4.67 (m, 2H, CH_2), 6.70 (d, 1H, J = 8.8 Hz, Ar), 7.31 (dd, 1H, J = 2.3, 8.8 Hz, Ar), 7.46 (d, 1H, J = 2.3 Hz, Ar), 7.56 (d, 2H, J = 8.4 Hz, Ar), 7.67 (d, 2H, J = 8.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 153.14, 133.70, 133.22, 132.79, 129.91, 126.45, 125.21, 123.32, 120.66, 112.76, 75.16, 69.99, 61.19, 58.44, 57.88, 52.67, 39.40, 28.33, 26.49. MS (m/z): 481 (M^+ 57%). Anal. ($C_{21}H_{23}Br_2NO_2$ HCl) C, H, N.

4′-(4-Methoxybenzyl)-6-bromo-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′[1,4]oxazinane] 2h. Compound **2h** was synthesized from **8** (178 mg, 0.57 mmol) following the same procedure described above for the preparation of **1g**. The crude residue was transformed into the hydrochloride salt to give **2h** (152 mg, 0.32 mmol, 57% yield): mp 108−110 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 3H, Me), 1.32 (s, 3H, Me), 2.61 (d, 1H, J = 15.2 Hz, CH_2), 2.75−2.90 (m, 2H, CH_2), 3.03 (d, 1H, J = 12.5 Hz, CH_2), 3.66 (d, 1H, J = 12.5 Hz, CH_2), 3.78−4.00 (m, 3H, CH_2), 3.81 (s, 3H, OMe), 4.51−4.63 (m, 2H, CH_2), 6.71 (d, 1H, J = 8.8 Hz, Ar), 6.93 (d, 2H, J = 8.6 Hz, Ar), 7.32 (dd, 1H, J = 2.4, 8.8 Hz, Ar), 7.44 (d, 1H, J = 2.4 Hz, Ar), 7.63 (d, 2H, J = 8.6 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 153.14, 133.59, 133.10, 129.87, 128.87, 123.54, 120.59, 119.09, 117.42, 112.70, 75.23, 69.99, 61.68, 58.19, 57.95, 55.49, 52.27, 39.38, 28.23, 26.61. MS (m/z): 432 (M^+ 31%). Anal. ($C_{22}H_{26}BrNO_3$ HCl) C, H, N.

4′-(2-Methoxybenzyl)-6-bromo-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′[1,4]oxazinane] 2i. Compound **2i** was synthesized from **8** (178 mg, 0.57 mmol) and 2-methoxy-benzylchloride (90 mg, 0.57 mmol) following the same procedure described above for the preparation of **1g**. The crude residue was transformed into the hydrochloride salt and crystallized from *i*-PrOH to afford **2i** (100 mg, 0.21 mmol, 26% yield): mp 158−160 °C. ^{1}H NMR ($CDCl_3$):

δ 1.33 (s, 3H, Me), 1.36 (s, 3H, Me), 2.61(d, 1H, J = 15.0 Hz, CH_2), 2.85 (d, 1H, J = 15.0 Hz, CH_2), 2.95−3.15 (m, 2H, CH_2), 3.58−4.03 (m, 4H, CH_2), 3.85 (s, 3H, OMe), 4.35−4.60 (m, 2H, CH_2), 6.72 (d, 1H, J = 8.8 Hz, Ar), 6.94 (d, 1H, J = 8.2 Hz, Ar), 7.03−7.11 (m, 1H, Ar), 7.29−7.47(m, 3H, Ar), 7.86−7.90 (m, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 157.95, 152.81, 132.15, 131.13, 130.42, 128.31, 126.94, 125.98, 120.37, 119.60, 112.36, 110.61, 75.21, 70.06, 63.47, 61.32, 56.40, 55.60, 53.69, 40.74, 29.13, 27.00. MS (*m*/*z*): 432 (M+, 31%). Anal. ($C_{22}H_{26}BrNO_3$ HCl) C, H, N.

4′-(3-Methoxybenzyl)-6-bromo-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′[1,4]oxazinane] 2l. Compound **2l** was synthesized from **8** (134 mg, 0.43 mmol) and 3-methoxy-benzylbromide (67 mg, 0.43 mmol) following the same procedure described above for the preparation of **1g**. The crude residue was transformed to the hydrochloride salt to give **2l** (66 mg, 0.14 mmol, 41% yield): mp 141−143 °C. ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 3H, Me), 1.33 (s, 3H, Me), 2.64−3.09 (m, 4H, CH_2), 3.66−3.99 (m, 4H, CH_2), 3.84 (s, 3H, OMe), 4.53−4.68 (m, 2H, CH_2), 6.70 (d, 1H, J = 8.8 Hz, Ar), 6.96 (dd, 1H, J = 1.5, 8.2 Hz, Ar), 7.16−7.36 (m, 3H, Ar), 7.45−7.48 (m, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 160.10, 153.21, 133.25, 131.35, 129.89, 129.75, 123.52, 121.23, 120.16, 114.36, 113.20, 112.79, 75.28, 69.73, 61.95, 58.30, 57.97, 55.46, 52.50, 39.49, 28.05, 26.88. MS (*m*/*z*): 432 (M^+ 31%). Anal. ($C_{22}H_{26}BrNO_3$ HCl) C, H, N.

4′-Benzyl-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3a. To a stirred solution of NaH (0.27 g, 13.00 mmol, 60% dispersion in mineral oil) in dry DMF (10 mL) was added **5** (990 mg, 4.00 mmol) under N_2 atmosphere. After 30 min, the reaction mixture was cooled at 0 °C and benzyl bromide (854 mg, 5.00 mmol) was added. The reaction mixture was allowed to warm at 25 °C and stirred for 1 h before being quenched with water and extracted with AcOEt. The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product was purified by trituration with Et_2O to give **3a** (1.44 g, 3.48 mmol, 87% yield): mp 79−81 °C. ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, Me), 1.32 (s, 3H, Me), 1.71 (d, 1H, J = 14.6 Hz, CH_2), 2.23 (d, 1H, J = 14.6 Hz, CH_2), 3.07 (d, 1H, J = 12.4 Hz, CH_2), 3.8 (d, 1H, J = 12.4 Hz, CH_2), 4.18−4.46 (m, 3H, CH_2, CH_2N), 5.05 (d, 1H, J = 14.0 Hz, CH_2N), 6.78−6.94 (m, 2H, Ar), 7.16−7.39 (m, 7H, Ar). ^{13}C NMR ($CDCl_3$): δ 166.60, 153.83, 136.19, 130.25, 129.00, 128.89, 128.14, 127.36, 121.97, 120.80, 118.31, 74.30, 69.29, 63.79, 55.42, 49.94, 40.07, 28.80, 26.38. MS *m*/*z*: 337 (M^+ 17%). Anal. ($C_{21}H_{23}NO_3$) C, H, N.

4′-(*N*-(4-Methansolfonamidobenzyl))-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3c. Compound **3c** was synthesized from 4′-(4-aminobenzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one (405 mg, 1.15 mmol)[13] following the same procedure described above for the preparation of **1c**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (3:7) to give **3c** (113 mg, 0.26 mmol, 23% yield): mp 83−85 °C. ^{1}H NMR ($CDCl_3$): δ 1.19 (s, 3H, Me), 1.33 (s, 3H, Me), 1.73 (d, 1H, J = 14.6 Hz), 2.28 (d, 1H, J = 14.6 Hz, CH_2), 3.01 (s, 3H, Me), 3.07 (d, 1H, J = 12.4 Hz, CH_2), 3.79 (d, 1H, J = 12.4 Hz, CH_2), 4.29 (d, 1H, J = 17.0 Hz, CH_2), 4.34 (d,1H, J = 14.2 Hz, CH_2N), 4.42 (d, 1H, J = 17.0 Hz, CH_2), 4.85 (d, 1H, J = 14.2 Hz, CH_2N), 6.78−6.94 (m, 2H, Ar), 7.17−7.27 (m, 5H, Ar), 7.31−7.37 (m, 1H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 166.50, 154.86, 136.47, 131.66, 130.42, 129.73, 127.42, 121.67, 120.92, 120.21, 118.39, 74.36, 65.64, 63.78, 55.62, 45.10, 40.60, 39.60, 29.22, 28.10. MS (*m*/*z*): 430 (M^+, 17%), 184 (100%). Anal. ($C_{22}H_{26}N_2O_5S$) C, H, N.

4′-(4-Methylbenzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3e. Compound **3e** was synthesized from **5** (990 mg, 4.00 mmol) and 4-methyl-benzylbromide (924 mg, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by trituration with Et_2O to afford **3e** (256 mg, 0.80 mmol, 20% yield): mp 107−109 °C. ^{1}H NMR ($CDCl_3$): δ 1.13 (s, 3H, Me), 1.32 (s, 3H, Me), 1.71(d, 1H, J = 14.6 Hz, CH_2), 2.24 (d, 1H, J = 14.6 Hz, CH_2), 2.33 (s, 3H, Me), 3.05 (d, 1H, J = 12.5 Hz, CH_2), 3.77 (d, 1H, J = 12.5 Hz, CH_2), 4.22 (d, 1H, J = 14.2 Hz, CH_2N), 4.28 (d, 1H, J = 17.3 Hz, CH_2), 4.40 (d, 1H, J = 17.3 Hz, CH_2), 4.97 (d, 1H, J = 14.2 Hz, CH_2N), 6.79 (d, 1H, J = 8.2 Hz, Ar), 6.86−6.94 (m, 1H, Ar), 7.11−7.26 (m, 5H, Ar), 7.36 (d, 1H, J = 7.7 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.40, 153.65, 137.85, 132.89, 130.24, 129.47, 128.91, 127.34, 121.70, 120.77, 118.18, 74.23, 69.13, 63.69, 55.07, 49.46, 39.60, 28.67, 26.10, 21.26. MS *m*/*z*: 351 (M^+ 15%). Anal. ($C_{22}H_{25}NO_3$) C, H, N.

4′-[(4-Trifluoromethyl)benzyl]-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3f. Compound **3f** was synthesized from **5** (990 mg, 4.00 mmol) and 4-(trifluoromethyl-)benzylbromide (1.19 g, 5.00 mmol) as described for the preparation of **3a**. The crude product was purified by trituration with Et_2O to obtain **3f** (1.41 g, 3.48 mmol, 87% yield): mp 85−87 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (s, 3H, Me), 1.33 (s, 3H, Me), 1.71 (d, 1H, J = 14.6 Hz, CH_2), 2.28 (d, 1H, J = 14.6 Hz, CH_2), 3.05 (d, 1H, J = 12.4 Hz, CH_2), 3.82 (d, 1H, J = 12.4 Hz, CH_2), 4.26−4.47 (m, 3H, CH_2, CH_2N), 4.97 (d, 1H, J = 14.5 Hz, CH_2N), 6.81 (dd, 1H, J = 1.1, 8.2 Hz, Ar), 6.87−6.95 (m, 1H, Ar), 7.18−7.23 (m, 1H, Ar), 7.35 (dd, 1H, J = 1.5, 7.8 Hz, Ar), 7.43 (d, 2H, J = 8.1 Hz, Ar), 7.62 (d, 2H, J = 8.1 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 167.47, 154.41, 140.87, 131.03, 129.78, 127.85, 126.50, 125.98, 122.28, 121.49, 120.95, 119.03, 74.84, 69.89, 64.34, 56.63, 50.37, 40.76, 29.74, 26.63. MS *m*/*z*: 405 (M^+ 5%), 159 (47%). Anal. ($C_{22}H_{22}F_3NO_3$) C, H, N.

4′-(4-Bromobenzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3g. Compound **3g** was synthesized from **5** (900 mg, 4.00 mmol) and 4-bromo-benzylbromide (1.30 g, 5.00 mmol) as described above for **3a**. The crude product was purified by trituration with Et_2O to yield **3g** (633 mg, 1.52 mmol, 38% yield): mp 134−136 °C. ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 3H, Me), 1.33(s, 3H, Me), 1.72 (d, 1H, J = 14.6 Hz, CH_2), 2.27 (d, 1H, J = 14.6 Hz, CH_2), 3.04 (d, 1H, J = 12.4 Hz, CH_2), 3.79 (d, 1H, J = 12.4 Hz, CH_2), 4.29−4.44 (m, 3H, CH_2N), 4.87 (d, 1H, J = 14.4 Hz, CH_2N), 6.80−6.95 (m, 2H, Ar), 7.16−7.22 (m, 3H, Ar), 7.35 (dd, 1H, J = 1.5, 7.9 Hz, Ar), 7.48 (d, 2H, J = 8.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.69, 153.78, 135.16, 132.03, 130.55, 130.30, 127.20, 122.19, 121.74, 120.79, 118.35, 74.21, 69.23, 63.67, 55.73, 49.47, 40.16, 29.13, 26.04. MS *m*/*z*: 416 (M^+ 46%). Anal. ($C_{21}H_{22}BrNO_3$) C, H, N.

4′-(4-Methoxybenzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3h. Compound **3h** was synthesized from **5** (900 mg, 4.00 mmol) and 4-methoxybenzyl chloride (783 mg, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by trituration with Et_2O to give **3h** (294 mg, 0.80 mmol, 20% yield): mp 105−107 °C. ^{1}H NMR ($CDCl_3$) δ: 1.15 (s, 3H, Me), 1.32 (s, 3H, Me), 1.70 (d, 1H, J = 14.6 Hz, CH_2), 2.24 (d, 1H, J = 14.6 Hz, CH_2), 3.06 (d, 1H, J = 12.4 Hz, CH_2), 3.75 (d, 1H, J = 12.4 Hz, CH_2), 3.80 (s, 3H, OMe), 4.15−4.43 (m, 3H, CH_2, CH_2N), 4.93 (d, 1H, J = 14.3 Hz, CH_2N), 6.77−6.94 (m, 4H, Ar), 7.15−7.39 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 166.40, 159.44, 153.63, 130.24, 129.44, 128.62, 127.96, 127.30, 121.66, 120.75, 118.18, 74.21, 69.09, 63.61, 55.40, 54.95, 49.10, 39.60, 28.69, 26.12. MS *m*/*z*: 367 (M^+ 47%). Anal. ($C_{22}H_{25}NO_4$) C, H, N.

4′-(2-Methoxybenzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 3i. Compound **3i** was synthesized from **5** (900 mg, 4.00 mmol) and 2-methoxybenzyl bromide (1.00 g, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by flash column chromatography eluting hexane/AcOEt (1:1) to give **3i** (441 mg, 1.20 mmol, 30% yield) as an oil. ^{1}H NMR ($CDCl_3$): δ 1.15 (s, 3H, Me), 1.33 (s, 3H, Me), 1.77 (d, 1H, J = 14.5 Hz, CH_2), 2.25 (d, 1H, J = 14.5 Hz, CH_2), 3.11 (d, 1H, J = 12.6 Hz, CH_2), 3.79 (s, 3H, OMe), 3.82 (d, 1H, J = 12.6 Hz, CH_2), 4.26 (d, 1H, J = 17.4 Hz, CH_2), 4.38 (d, 1H, J = 17.4 Hz, CH_2), 4.48 (d, 1H, J = 14.2 Hz, CH_2N), 4.94 (d, 1H, J = 14.2 Hz, CH_2N), 6.78−6.98 (m, 4H, Ar), 7.17−7.41 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$) δ: 166.49, 157.79, 153.67, 131.04, 130.16, 129.42, 127.43, 123.97, 121.86, 120.86, 120.75, 118.15, 110.55, 74.26, 69.17, 63.72, 55.42, 53.56, 44.08, 39.61, 28.60, 26.27. MS *m*/*z*: 367 (M^+ 22%). Anal. ($C_{22}H_{25}NO_4$) C, H, N.

4′-(3-Methoxybenzyl)-2,2-dimethyl-2,3-dihydro-5′*H*-spiro-[chromene-4,2′-[1,4]oxazinan]-5′-one 3l. Compound **3l** was synthesized from **5** (900 mg, 4.00 mmol) and 3-methoxybenzyl chloride (783 mg, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by flash column chromatography eluting hexane/AcOEt (7:3) to give **3l** (793 mg, 2.16 mmol, 54% yield) as an oil. ^{1}H NMR ($CDCl_3$): δ 1.14 (s, 3H, Me), 1.33 (s, 3H, Me), 1.76 (d, 1H, J = 14.5 Hz, CH_2), 2.26 (d, 1H, J = 14.5 Hz, CH_2), 3.08 (d, 1H, J = 12.4 Hz, CH_2), 3.79 (d, 1H, J = 12.4 Hz, CH_2), 3.80 (s, 3H, OMe), 4.24 (d, 1H, J = 14.3 Hz, CH_2N), 4.29 (d, 1H, 17.4 Hz, CH_2), 4.41 (d, 1H, J = 17.4 Hz, CH_2), 4.99 (d, 1H, J = 14.3 Hz, CH_2N), 6.79−6.94 (m, 5H, Ar), 7.17−7.30 (m, 2H, Ar), 7.37 (dd, 1H, J = 1.6, 7.9 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.51, 160.03, 153.67, 131.48, 130.29, 129.89, 127.34, 121.66, 121.19, 120.80, 118.24, 114.43, 113.58, 74.25, 69.15, 63.69, 55.40, 55.20, 49.74, 39.69, 28.65, 26.21. MS *m*/*z*: 367 (M^+ 45%). Anal. ($C_{22}H_{25}NO_4$) C, H, N.

4′-Benzyl-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro-[chromene-4,2′-[1,4]oxazinan]-5′-one 4a. Compound **4a** was synthesized from **6** (1.30 g, 4.00 mmol) and benzyl bromide (854 mg, 5.00 mmol) following the same procedure described above for the preparation of **3a**. The crude product was purified by trituration with Et_2O to give **4a** (1.11 g, 2.68 mmol, 67% yield): mp 127−129 °C. ^{1}H NMR ($CDCl_3$): δ 1.06 (s, 3H, Me), 1.29 (s, 3H, Me), 1.70 (d, 1H, J = 14.5 Hz, CH_2), 2.20 (d, 1H, J = 14.5 Hz, CH_2), 3.06 (d, 1H, J = 12.5 Hz, CH_2), 3.75 (d, 1H, J = 12.5 Hz, CH_2), 4.24 (d, 1H, J = 14.3 Hz, CH_2N), 4.28 (d, 1H, J = 17.4 Hz, CH_2), 4.41 (d, 1H, J = 17.4 Hz, CH_2), 5.03 (d, 1H, J = 14.3 Hz, CH_2N), 6.68 (d, 1H, J = 8.8 Hz, Ar), 7.26−7.33 (m, 6H, Ar), 7.49 (d, 1H, J = 2.2 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.27, 152.91, 135.99, 133.19, 130.20, 129.30, 128.96, 128.22, 127.11, 120.13, 112.87, 74.81, 69.11, 63.74, 55.13, 49.83, 39.54, 28.47, 26.34. MS *m*/*z*: 417 (M^+ 22%); 91 (100%). Anal. ($C_{21}H_{22}BrNO_3$) C, H, N.

4′-(4-Methylbenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 4e. Compound **4e** was synthesized from **6** (1.30 g, 4.00 mmol) and 4-methyl benzyl bromide (925 mg, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (1:1) to give **4e** (310 mg, 0.72 mmol, 18% yield): mp 80−82 °C. ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, Me), 1.30 (s, 3H, Me), 1.68 (d, 1H, J = 14.6 Hz, CH_2), 2.20 (d, 1H, J = 14.6 Hz, CH_2), 2.34 (s, 3H, Me), 3.04 (d, 1H, J = 12.6 Hz, CH_2), 3.72 (d, 1H, J = 12.6 Hz, CH_2), 4.21−4.44 (m, 3H, CH_2, CH_2N), 4.93 (d, 1H, J = 14.3 Hz, CH_2N), 6.68 (d, 1H, J = 8.6 Hz, Ar), 7.16−7.20 (m, 4H, Ar), 7.25−7.31 (m, 1H, Ar), 7.48 (d, 1H, J = 2.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.18, 152.83, 137.99, 133.19, 132.75, 130.20, 129.60, 128.93, 123.88, 120.09, 112.83, 74.79, 69.06, 63.69, 54.95, 49.46, 39.32, 28.51, 26.12, 21.31. MS *m*/*z*: 430 (M^+ 35%). Anal. ($C_{22}H_{24}BrNO_3$) C, H, N.

4′-[(4-Trifluoromethyl)benzyl]-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 4f. Compound **4f** was synthesized from **6** (1.30 g, 4.00 mmol) and 4-(trifluoromethyl)benzyl bromide (1.20 g, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by trituration with Et_2O to afford **4f** (1.16 g, 2.40 mmol, 60% yield): mp 105−107 °C. ^{1}H NMR ($CDCl_3$): δ 1.16 (s, 3H, Me), 1.31 (s, 3H, Me), 1.69 (d, 1H, J = 14.6 Hz, CH_2), 2.25 (d, 1H, J = 14.6 Hz, CH_2), 3.05 (d, 1H, J = 12.4 Hz, CH_2), 3.77 (d, 1H, J = 12.4 Hz, CH_2), 4.29 (d, 1H, J = 17.6 Hz, CH_2), 4.42 (d, 1H, J = 17.6 Hz, CH_2), 4.44 (d, 1H, J = 14.6 Hz, CH_2N), 4.92 (d, 1H, J = 14.6 Hz, CH_2N), 6.70 (d, 1H, J = 8.6 Hz, Ar), 7.29 (dd, 1H, J = 2.5, 8.9 Hz, Ar), 7.41−7.47 (m, 3H, Ar), 7.63 (d, 2H, J = 8.1 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.51, 152.85, 133.31, 130.06, 129.13, 127.69, 126.00, 125.92, 123.65, 120.24, 112.87, 74.72, 71.77, 69.04, 63.61, 55.71, 49.59, 39.58, 28.85, 25.92. MS *m*/*z*: 484 (M^+ 25%), 159 (100%). Anal. ($C_{22}H_{21}BrF_3NO_3$) C, H, N.

4′-(4-Bromobenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 4g. Compound **4g** was synthesized from **6** (1.30 g, 4.00 mmol) and 4-bromo-benzylbromide (1.25 g, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by trituration with Et_2O/hexane to give **4g** (515 mg, 1.04 mmol, 26% yield): mp 83−85 °C. ^{1}H NMR ($CDCl_3$): δ 1.18 (s, 3H, Me), 1.31 (s, 3H, Me), 1.69 (d, 1H, J = 14.6 Hz, CH_2), 2.24 (d, 1H, J = 14.6 Hz, CH_2), 3.04 (d, 1H, J = 12.4 Hz, CH_2), 3.74 (d, 1H, J = 12.4 Hz, CH_2), 4.23−4.49 (m, 3H, CH_2, CH_2N), 4.83 (d, 1H, J = 14.5 Hz, CH_2N), 6.70 (d, 1H, 8.8 Hz, Ar), 7.16−7.34 (m, 4H, Ar), 7.45−7.50 (m, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 166.29, 152.88, 133.54, 133.26, 132.31, 130.23, 127.58, 125.62, 123.41, 120.50, 112.90, 74.11, 69.30, 63.54, 55.65, 49.40, 39.89, 29.05, 26.12. MS *m*/*z*: 495 (M^+ 36%). Anal. ($C_{21}H_{21}Br_2NO_3$) C, H, N.

4′-(4-Methoxybenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 4h. Compound **4h** was synthesized from **6** (1.30 g, 4.00 mmol) and 4-methoxybenzyl chloride (783 mg, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (1:1) to afford **4h** (660 mg, 1.48 mmol, 37% yield): mp 111−113 °C. ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, Me), 1.29 (s, 3H, Me), 1.67 (d, 1H, J = 14.7 Hz, CH_2), 2.19 (d, 1H, J = 14.7 Hz, CH_2), 3.04 (d, 1H, J = 12.6 Hz, CH_2), 3.70 (d, 1H, J = 12.6 Hz, CH_2), 3.79 (s, 3H, OMe), 4.21 (d, 1H, J = 14.3 Hz, CH_2N), 4.24 (d, 1H, J = 17.4 Hz, CH_2), 4.37 (d, 1H, J = 17.4 Hz, CH_2), 4.89 (d, 1H, J = 14.3 CH_2N), 6.68 (d, 1H, J = 8.8 Hz, Ar), 6.84−6.90 (m, 2H, Ar), 7.18−7.30 (m, 3H, Ar), 7.47 (d, 1H, J = 2.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.25, 159.77, 153.02, 133.20, 130.33, 130.24, 128.14, 124.28, 120.17, 114.52, 112.92, 74.88, 69.24, 63.81, 55.51, 55.11, 49.32, 39.96, 28.82, 26.30. MS *m*/*z*: 446 (M^+ 39%). Anal. ($C_{22}H_{24}BrNO_4$) C, H, N.

4′-(2-Methoxybenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 4i. Compound **4i** was synthesized from **6** (1.30 g, 4.00 mmol) and 2-methoxybenzyl bromide (1.00 g, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (1:1) to afford **4i** (375 mg, 0.84 mmol, 21% yield): mp 48−50 °C. ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, Me), 1.30 (s, 3H, Me), 1.74 (d, 1H, J = 14.6 Hz, CH_2), 2.22 (d, 1H, J = 14.6 Hz, CH_2), 3.10 (d, 1H, J = 12.8 Hz, CH_2), 3.72−3.84 (m, 1H, CH_2), 3.80 (s, 3H, OMe), 4.25 (d, 1H, J = 17.4 Hz, CH_2), 4.38 (d, 1H, J = 17.4 Hz, CH_2), 4.47 (d, 1H, J = 14.3 Hz, CH_2N), 4.93 (d, 1H, J = 14.3 Hz, CH_2N), 6.69 (d, 1H, J = 8.6 Hz, Ar), 6.85−7.00 (m, 2H, Ar), 7.24−7.35 (m, 3H, Ar), 7.50 (d, 1H, J = 2.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.41, 157.63, 152.33, 133.07, 131.00, 129.87, 129.56, 127.87, 123.46, 120.54, 120.19, 112.50, 110.21, 74.68, 68.99, 63.63, 55.78, 53.21, 44.33, 39.57, 28.80, 25.97. MS *m*/*z*: 446 (M^+ 38%). Anal. ($C_{22}H_{24}BrNO_4$) C, H, N.

4′-(3-Methoxybenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-5′*H*-spiro[chromene-4,2′-[1,4]oxazinan]-5′-one 4l. Compound **4l** was synthesized from **6** (1.30 g, 4.00 mmol) and 3-methoxybenzyl bromide (1.00 g, 5.00 mmol) following the same procedure described above for **3a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (7:3) to obtain **4l** (571 mg, 1.28 mmol, 32% yield) as an oil. ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, Me), 1.31 (s, 3H, Me), 1.74 (d, 1H, J = 14.6 Hz, CH_2), 2.23 (d, 1H, J = 14.6 Hz, CH_2), 3.06 (d, 1H, 12.4 Hz, CH_2), 3.72−3.80 (m, 1H, CH_2), 3.80 (s, 3H, OMe), 4.23 (d, 1H, J = 14.2 Hz, CH_2N), 4.28 (d, 1H, J = 17.4 Hz, CH_2), 4.41 (d, 1H, J = 17.4 Hz, CH_2), 4.97 (d, 1H, J = 14.2 Hz, CH_2N), 6.69 (d, 1H, J = 8.8 Hz, Ar), 6.83−6.88 (m, 3H, Ar), 7.22−7.32 (m, 2H, Ar), 7.49 (d, 1H, J = 2.4 Hz, Ar). ^{13}C NMR ($CDCl_3$): δ 166.52, 160.12, 152.45, 133.26, 131.09, 130.41, 129.68, 127.64, 120.55, 120.23, 114.16, 113.88, 112.49, 74.50, 69.04, 63.53, 55.30, 55.21, 49.60, 39.81, 28.55, 25.14. MS *m*/*z*: 446 (M^+ 51%). Anal. ($C_{22}H_{24}BrNO_4$) C, H, N.

2,2-Dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 7. Compound **7** was synthesized as described for the preparation of compound **1a**, starting from **5** (700 mg, 2.83 mmol) giving **7** (349 mg, 1.50 mmol, 53% yield). ^{1}H NMR ($CDCl_3$): δ 1.37 (s, 3H, Me), 1.41 (s, 3H, Me), 2.11 (d, 1H, J = 14.7 Hz, CH_2), 2.58 (d, 1H, J = 14.7 Hz, CH_2), 2.78 (d, 1H, J = 12.2 Hz,

CH_2), 2.86−3.12 (m, 2H, CH_2), 3.23 (d, 1H, J = 12.2 Hz, CH_2), 3.65−3.73 (m, 1H, CH_2), 3.85−3.98 (m, 1H, CH_2), 6.82 (d, 1H, J = 8.1 Hz, Ar), 6.90−6.98 (m, 1H, Ar), 7.15−7.23 (m, 1H, Ar), 7.57 (dd, 1H, J = 1.7,7.8 Hz, Ar). Anal. ($C_{14}H_{19}NO_2$) C, H, N.

6-Bromo-2,2-dimethyl-2,3-dihydrospiro[chromene-4,2′-[1,4]oxazinane] 8. A solution of **6** (219 mg, 0.67 mmol) in THF (3 mL) was added to a solution of $BH_3 \cdot SMe_2$ 2 M in THF (205 mg, 2.70 mmol). The resulting mixture was heated for 30 min by microwave irradiation at 100 °C and with a power of 150 W, and then water was added and the solvent evaporated. The aqueous phase was acidified with HCl 1 N, neutralized with NaOH 1 N and extracted with AcOEt. The organic layer was dried, and the solvent was evaporated. The crude product was transformed into the hydrochloride salt to give **8** (540 mg, 1.55 mmol, 88% yield). ^{1}H NMR ($CDCl_3$): δ 1.37 (s, 3H, Me), 1.45 (s, 3H, Me), 2.45 (d, 1H, J = 14.7 Hz, CH_2), 2.63 (d, 1H, J = 14.7 Hz, CH_2), 3.20−3.42 (m, 4H, CH_2), 3.92 (d, 1H, J = 13.2 Hz, CH_2), 4.16−4.30 (m, 1H, CH_2), 6.74 (d, 1H, J = 8.6 Hz, Ar), 7.34 (dd, 1H, J = 2.3, 8.6 Hz, Ar), 7.51 (d, 1H, J = 2.3 Hz, Ar). Anal. ($C_{14}H_{18}BrNO_2$) C, H, N.

Pharmacological Procedures. All the experimental procedures were carried out following the guidelines of the European Community Council Directive 86-609.

In Vitro Cardiac Protocols. Adult male Wistar rats (260−350 g) were treated with an ip injection (about 0.3 mL) of 40 mg/kg with diazoxide (40 mg/kg), 1−4 (40 mg/kg), cromakalim (1 mg/kg). or vehicle (DMSO).

After 2 h, all the animals were anaesthetised with sodium pentobarbital (100 mg/kg ip) and heparinized (100 UI ip) to prevent blood clotting. To verify the effective selectivity toward the mitochondrial K_{ATP} channels, in another series of experiments, a selective mito-K_{ATP} blocker was administered at a dose of 10 mg/kg, 20 min before the administration of the tested compounds. After the opening of the chest, the hearts were quickly excised and placed in a 4 °C Krebs solution (composition mM: $NaHCO_3$ 25.0, NaCl 118.1, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2 \cdot 2H_2O$ 1.6, KH_2PO_4 1.2, glucose 11.5) equilibrated with 95% O_2 5% CO_2, to stop the contraction and reduce oxygen consumption. Rapidly, the ascending aorta was cannulated and hearts mounted on a Langendorff apparatus, then the perfusion with Krebs solution (thermostatted at 37 °C and continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2) was started at constant pressure (70−80 mmHg). The above procedure was executed within 2 min. A water-filled latex balloon connected to a pressure transducer (Bentley Trantec, model 800) was introduced into the left ventricle via the mitral valve and the volume was adjusted to achieve a stable left ventricular end-diastolic pressure of 5−10 mmHg during initial equilibration. The heart rate (HR) and left ventricular developed pressure (LVDP) were continuously monitored by a computerized Biopac system (California) and the parameter of rate pressure product (RPP) was calculated as RPP = HR × LVDP. Hearts showing severe arrhythmia or unstable LVDP and HR values, during the preischemic phase, were discarded.

After a 30 min equilibration pre-ischemic period, the hearts were subjected to 30 min of global ischemia (no flow). At the end of the ischemic period, the hearts were reperfused for a period of 120 min. At the end of the reperfusion period, the hearts were removed from the Langendorff apparatus and the left ventricle was cut in 2 mm large slices, which were immersed in a 10% aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 20 min and then in a 20% aqueous solution of formaldehyde. After 24 h, the ventricular slices were photographed and analyzed in order to highlight the necrotic areas due to the ischemic process (visible as white or light pink color) and the healthy areas (visible as strong red due to the TCC reaction).

Data Analysis. To obtain the functional parameter of cardiac inotropism at the final stages of reperfusion, the RPP recorded at the 120th min of reperfusion was calculated and expressed (RPP-120′ %) as a percentage of the preischemic RPP, recorded at the last min of perfusion. Furthermore, the ischemic areas were evaluated planimetrically and expressed as a percentage of the whole area of the slices of left ventricle (Ai/Atot %). All the values are expressed as mean ± standard error for 6−8 different experiments.

In Vitro Vascular Protocols. The effects of the compounds were tested on isolated thoracic aortic rings of male normotensive Wistar rats (250−350 g).

After a light ether anesthesia, the rats were sacrificed by cervical dislocation and bleeding.

The aortas were immediately excised and freed of extraneous tissues, and the endothelial layer was removed by gently rubbing the intimal surface of the vessels with a hypodermic needle. Five millimeter wide aortic rings were suspended, under a preload of 2 g, in 20 mL organ baths containing Tyrode solution (composition of saline in mM: NaCl 136.8; KCl 2.95; $CaCl_2$ 1.80; $MgSO_4 \cdot 7H_2O$ 1.05; NaH_2PO_4 0.41; $NaHCO_3$ 11.9; glucose 5.5), thermostatted at 37 °C and continuously gassed with a mixture of O_2 (95%) and CO_2 (5%). Changes in tension were recorded by means of an isometric transducer (Grass FTO3), connected with a preamplifier (Buxco Electronics) and with a software of data acquisition (BIOPAC Systems Inc., MP 100). After an equilibration period of 60 min, endothelium removal was confirmed by the administration of acetylcholine (ACh) (10 μM) to KCl (20 mM)-precontracted rings. A relaxation <10% of the KCl-induced contraction was considered to be indicative of an acceptable lack of the endothelial layer, while the organs showing a relaxation ≥10% (i.e., significant presence of the endothelium) were discarded.

From 30 to 40 min after the confirmation of the endothelium removal, the aortic preparations were contracted by a single concentration of KCl (20 mM), and when the contraction reached a stable plateau, 3-fold increasing concentrations of the test substances (from 10 nM to 100 μM) were added.

Preliminary experiments showed that the KCl (20 mM)-induced contractions remained in a stable tonic state for at least 40 min.

Data Analysis. The vasorelaxing efficacy was evaluated as the maximal vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by 20 mM KCl. When the limit concentration of 100 μM (the highest concentration that could be administered) of the tested compounds did not reach the maximal effect, the parameter of efficacy represented the vasorelaxing response, expressed as a percentage (%) of the contractile tone induced by 20 mM KCl, evoked by this limit concentration.

The parameter of potency was expressed as pIC_{50}, calculated as negative logarithm of the molar concentration of the test compounds, evoking a 50% reduction of the contractile tone induced by 20 mM KCl. The pIC_{50} could not be calculated for those compounds showing an efficacy parameter lower than 50%. The parameters of efficacy and potency were expressed as means ± standard error for 5−10 experiments. Student t test was selected for statistical analysis, and $P < 0.05$ was considered to be indicative of a significant statistical differences. Experimental data were analyzed by a computer fitting procedure (software: GraphPad Prism 4.0).

In Vivo Protocols. The effects of the compounds on blood pressure were also tested on male 10-week-old normotensive Wistar rats (250 g).

To establish a homogeneity of treatment with the in vitro cardiac protocol, the animals were heparinized (100 UI ip) and then anesthetised with sodium pentobarbital (60 mg/kg). After the administration of the anesthetic drug, the animal tails were exposed to a 20 min of irradiation with an IR lamp to determine a vasodilation of the tail-vessel, permitting recording of the basal systolic blood pressure with the "tail-cuff" method by a BP recorder (Ugo Basile 58500).

Then, the examined substances, such as diazoxide, **1a**, **2c**, **2h**, **3g**, **4h**, and the vehicle (DMSO), were administered by an intraperitoneal injection to different groups of five rats each, at a dose of 40 mg/kg. Cromakalim was tested at the dose of 1 mg/kg. Starting from the administration of the tested compounds, the systolic blood pressure values were recorded, as described above, for 90 at 30 min intervals, (during this period, when required a maintenance dose of 10 mg/kg ip of sodium pentobarbital was administered).

Data Analysis. The values of systolic blood pressure, recorded after the drug administration, were expressed as a percentage of the basal ones.

Materials. The substances used in the pharmacological experimental protocols were KCl (Carlo Erba) dissolved (2 M) in Tyrode solution, acetylcholine chloride (Sigma) dissolved (0.1 M) in EtOH 95%, and further diluted in twice-distilled water. Sodium pentobarbital (Sessa) and 5-hydroxydecanoic acid (Sigma) were both dissolved in twice-distilled water. Heparin Vister was purchased by Pfizer as injectable preparation. All the synthesized compounds were dissolved in DMSO and, when required, further diluted in Tyrode solution.

All the solutions were freshly prepared immediately before the pharmacological experimental procedures. For the in vitro vascular experiments, previous experiments showed a complete ineffectiveness of the administration of the vehicles.

Supporting Information Available: Elemental analyses of the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Murry, C. E.; Jennings, R. B.; Reimer, K. A. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **1986**, *74*, 1124–1136.

(2) Wang, Y.; Haider, H. K.; Ahmad, N.; Ashraf, M. Mechanisms by which K_{ATP} channel openers produce acute and delayed cardioprotection. *Vasc. Pharmacol.* **2005**, *42*, 253–264.

(3) O'Rourke, B. Evidence for mitochondrial K^+ channels and their role in cardioprotection. *Circ. Res.* **2004**, *94*, 420–432.

(4) Yokoshiki, H.; Sunagawa, M.; Seki, T.; Sperelakis, N. ATP-sensitive K^+ channels in pancreatic, cardiac, and vascular smooth muscle cells. *Am. J. Physiol. Cell Physiol.* **1998**, *274*, C25–C37.

(5) Von Cuong, D.; Kim, N.; Joo, H.; Joum, J. B.; Choung, J. Y.; Lee, Y.; Park, W. S.; Kim, E.; Park, J. S.; Han, S. Subunit composition of ATP-sensitive potassium channels in mitochondria of rat hearts. *Mitochondrion* **2005**, *5*, 121–133.

(6) O'Rourke, F.; Soons, K.; Flaumenhauft, R.; Watras, J.; Baio-Larue, C.; Matthews, E.; Feinstein, M. B. Ca^{2+} release by inositol 1,4,5-trisphosphate is blocked by the K^+-channel blockers apamin and tetrapentylammonium ion, and a monoclonal antibody to a 63 kDa membrane protein: reversal of blockade by K^+ ionophores nigericin and valinomycin and purification of the 63 kDa antibody-binding protein. *Biochem. J.* **1994**, *300*, 673–683.

(7) Fujita, A.; Kurachi, Y. Molecular aspects of ATP-sensitive K^+ channels in the cardiovascular system and K^+ channels openers. *Pharmacol. Ther.* **2000**, *85*, 39–53.

(8) Kane, G. C.; Liu, X. K.; Yamada, S.; Olson, T. M.; Terzic, A. Cardiac K_{ATP} channels in health and disease. *J. Mol. Cell. Cardiol.* **2005**, *38*, 937–943.

(9) 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.

(10) Coghlan, M. J.; Carroll, W. A.; Gopalakrishnan, M. Recent developments in the biology and medicinal chemistry of potassium channel modulators: update from a decade of progress. *J. Med. Chem.* **2001**, *44*, 1627–1653.

(11) Atwall, 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.

(12) 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.

(13) Breschi, M. C.; Calderone, V.; Martelli, A.; Minutolo, F.; Rapposelli, S.; Testai, L.; Tonelli, F.; Balsamo, A. New benzopyran-based openers of the mitochondrial ATP-sensitive potassium channel with potent anti-ischemic properties. *J. Med. Chem.* **2006**, *49*, 7600–7602.

JM800956G