Acknowledgment. This research was made possible by grant CA47476 and contract NO1-CM-67699, both awarded by the National Cancer Institute, DHHS. We are grateful for the services of Bonita J. Norris and Vicky Croy, who performed the cytotoxicity testing at the Purdue Cell Culture Laboratory. We are grateful to Dr. Rudiger D. Haugwitz, Developmental Therapeutics Program, NCI, for suggesting the collaboration between our two research groups.

Registry No. **3a**, 61240-20-8; **3b**, 1530-38-7; **4a**, 123-11-5; **4b**, 591-31-1; **4c**, 135-02-4; **4d**, 2103-57-3; **4e**, 54439-75-7; **4f**, 100-52-7; **4g**, 104-88-1; **4h**, 1122-91-4; **4i**, 872-85-5; **4j**, 500-22-1; **4k**, 1121-60-4; **4l**, 555-16-8; **4m**, 120743-99-9; 4n, 120-21-8; **4o**, 120-14-9; **4p**, 7311-34-4; **4q**, 86-81-7; **4r**, 4460-86-0; **4s**, 830-79-5; **5a**, 134029-49-5; **5b**, 97399-87-6; **5c**, 134029-50-8; **5d**, 134029-51-9; **5e**, 134029-52-0; **5f**, 74809-44-2; **5g**, 134029-53-1; **5h**, 74809-51-1; **5i**, 134029-54-2; **5j**, 134029-55-3; **5k**, 134029-56-4; **5l**, 134029-57-5; **5m**, 134029-58-6; **5n**, 134029-59-7; **5o**, 134029-60-0; **5p**, 134029-61-1; **6a**, 134029-62-2; **6b**, 97399-88-7; **6c**, 134029-63-3; **6e**, 134054-80-1; **6f**, 74809-43-1; **6g**, 134029-64-4; **6h**, 74809-50-0; **6i**, 118160-75-1; **6j**, 134029-65-5; **6l**, 134029-66-6; **6m**, 134029-67-7; **6n**, 134029-68-8; **6o**, 116519-00-7; **6p**, 134029-69-9; **6q**, 3892-92-0; **6r**, 134029-70-2; **6s**, 89946-15-6; **6t**, 134029-71-3; **6u**, 61240-22-0; **6v**, 134029-72-4; **6w**, 129047-45-6; **6x**, 22887-34-9; **6y**, 83088-26-0; **6z**, 134029-73-5; **7a**, 1080-32-6; **7b**, 78055-65-9; **7c**, 134029-74-6; **8a**, 35450-81-8; **8b**, 108853-15-2; **8c**, 134029-75-7; **8f**, 133462-62-1; **8i**, 134029-76-8; **8j**, 20934-93-4; **8m**, 134029-77-9; **8n**, 134029-78-0; **8o**, 39499-95-1; **8p**, 39500-11-3; **8q**, 3892-93-1; **8r**, 134029-79-1; **8s**, 52961-91-8; **8t**, 117048-69-8; **8u**, 33284-74-1; **8v**, 134029-80-4; **8z**, 110997-90-5; **9a**, 100-07-2; **9b**, 3535-37-3; **9c**, 17213-57-9; **9d**, 4521-61-3; **10a**, 104-94-9; **10b**, 10272-07-8; **10c**, 24313-88-0; **11a**, 742-15-4; **11b**, 134029-81-5; **11c**, 134029-82-6; **11d**, 134029-83-7; **11e**, 134029-84-8; **11f**, 748-31-2; **12a**, 134029-85-9; **12b**, 134029-86-0; **12c**, 134029-87-1; **12d**, 134029-88-2; **12e**, 134054-81-2; **12f**, 134029-89-3.

Supplementary Material Available: Physical and spectral data of compounds **6b,c,e,g-j,s-y**, **8b,c,f,i,j,o-v**, **11b,d-f** and **12b,d-f** (6 pages). Ordering information is given on any current masthead page.

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Relaxant Activity of 6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-4-carboxamides and -thiocarboxamides and Their Analogues in Guinea Pig Trachealis

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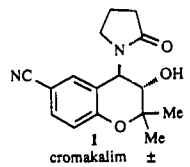
Structural modifications of the potassium channel activator cromakalim (1) are described in which the amide moiety at C-4 has been replaced by carboxamide and thiocarboxamide functions. Analogues in which the hydroxyl group at C-3 has been oxidized or removed are also disclosed. Such analogues display an interesting profile of smooth muscle relaxant activity in the guinea pig isolated trachea, not all of which appears to result from the opening of potassium channels, but few compounds retain useful in vivo activity. However, one compound in particular, 6-cyano-2,2-dimethyl-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide (**13**) was shown to be a potent potassium channel activator in vitro and to provide prolonged protection to guinea pigs from the respiratory effects of inhaled histamine.

Introduction

Interest in the potassium channel activators has increased markedly over the past few years, having gained particular momentum following the classification of additional potassium channel subtypes and the identification of a number of blockers with subtype specificity.[1] Another major contributor to this growth was the discovery of cromakalim (1), a specific potassium channel activator,[2]



1
cromakalim ±
BRL 38227 (3S,4R)

which is thought to exert its smooth muscle relaxant activity through the opening of ATP-sensitive potassium channels and consequent hyperpolarization.[3,4] While such activity was initially found in the smooth muscle of the vasculature, and thus demonstrated in models of hypertension, it is now recognized that potassium channel activators also have potential application for the treatment of other diseases involving smooth muscle, such as asthma and urinary incontinence.[1]

Particular support for the utility of potassium channel activators in respiratory disorders results from the detailed study of cromakalim in animal models[5] and preliminary

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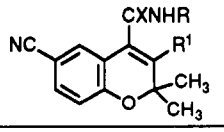
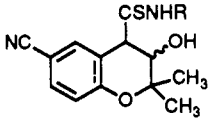
Table I. 6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-4-carboxamides and -thiocarboxamides



compd	R	X	R^1	% yield	mp, °C	formula	analysis	inhibition of spontaneous tone in guinea pig tracheal spirals[a]
	cromakalim							1.1 (0.6–1.9); 0.89 ● 0.02; 7
3	Me	S	OH	54	179.5–180.5	$C_{14}H_{14}N_2O_2S$	M^+	11.8 (4.8–28.9); 0.69 ● 0.10; 4
4	*t*-Bu	S	OH	77	166–167	$C_{17}H_{20}N_2O_2S$	C, H, N	4.35 (1.33–14.2); 0.93 ● 0.02; 4
5	Ph	S	OH	78	152–153[b]	$C_{19}H_{16}N_2O_2S$	C, H, N	0.25 (0.18–0.34); 0.99 ± 0.01; 6
6	Ph	O	OH	55	171–172	$C_{19}H_{16}N_2O_3$	C, H, N	0.095 (0.028–0.33); 0.98 ● 0.01; 5
7	*p*-FC_6H_4	S	OH	82	184–185[c]	$C_{19}H_{15}FN_2O_2S$	C, H, N	2.14 (0.37–12.27); 0.88 ± 0.09; 4
8	*p*-$MeOC_6H_4$	S	OH	4	169[d]	$C_{20}H_{18}N_2O_3S$	C, H, N	6.4 (2.6–15.7); 0.60 ● 0.03; 6
9	*p*-$MeOC_6H_4$	O	OH	33	158	$C_{20}H_{18}N_2OS$	C, H, N	1.2 (0.3–5.1); 0.94 ± 0.03; 4
13	Me	S	H	80	174.5–175	$C_{14}H_{14}N_2OS$	C, H, N	0.14 (0.08–0.24); 0.88 ● 0.06; 4
14	*t*-Bu	S	H	47	187	$C_{17}H_{20}N_2OS$	M^+	>20; 0; 2
15	Ph	O	H	10	233.5–234	$C_{19}H_{16}N_2O_2$	M^+	1.14 (0.73–1.78); 0.86 ± 0.04; 4

[a] IC_{50} in μM with 95% confidence limits; intrinsic activity ± SEM; number of determinations. [b] Resolidifies and melts at 255–260 °C. [c] Resolidifies and melts at 269–275 °C. [d] Resolidifies and melts at 225 °C.

Table II. 6-Cyano-3,4-dihydro-2,2-dimethyl-3-hydroxy-2*H*-1-benzopyran-4-thiocarboxamides



compd	R	stereochemistry	% yield	mp, °C	formula	analysis	inhibition of spontaneous tone in guinea pig tracheal spirals[a]
10a	Me	cis	27	170–171	$C_{14}H_{16}N_2O_2S$	C, H, N	5.92 (5.01–7.00); 0.84 ● 0.06; 4
10b	Me	trans	38	212–213	$C_{14}H_{16}N_2O_2S$	M^+	5.6 (2.0–15.5); 0.93 ● 0.03; 4
11a	*t*-Bu	cis	53	167.5–168	$C_{17}H_{22}N_2O_2S$	M^+	>20; 0.3; 2
11b	*t*-Bu	trans	19	215–216	$C_{17}H_{22}N_2O_2S$	M^+	11.1 (6.0–20.6); 0.79 ± 0.04; 4
12a	Ph	cis	*b*	201–202	$C_{19}H_{18}N_2O_2S$	H, N, C[c]	>20; 0.14; 4
12b	Ph	trans	*b*	90–93	$C_{19}H_{18}N_2O_2S$	M^+	3.4 (1.2–9.6); 0.82 ± 0.09; 4

[a] See footnote a of Table I. [b] See the text. [c] C: found, 66.98; required, 67.43.

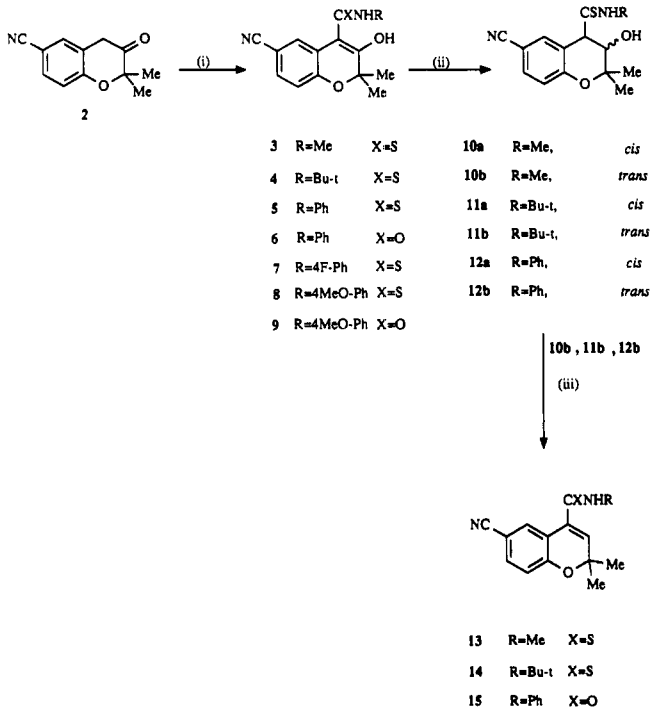
trials in man.[6] BRL 38227, the active 3*S*,4*R* enantiomer of cromakalim, is currently undergoing clinical evaluation in asthmatic patients.

In continuation of our investigation into the structural features of cromakalim crucial to airway relaxant activity,[7-10] we now report on modifications in which the amide function at position 4 is replaced by carboxamide and thiocarboxamide moieties.

Chemistry

The key compounds described in this paper were prepared as shown in Scheme I. Thus treatment of the

Scheme I[a]



[a] Reagents: (i) KO-*t*-Bu/RNCX/THF; (ii) KBH_4/MeOH; (iii) MsCl/Et_3N/CH_2Cl_2/KO-*t*-Bu (for **15**, $COCl_2$/Et_3N/KO-*t*-Bu).

enolate derived from 6-cyano-2,2-dimethyl-2*H*-1-benzopyran-3-one[11] (**2**) with isocyanates or isothiocyanates gave

(6) Williams, A. J.; Lee, T. H.; Cochrane, G. M.; Hopkirk, A.; Vyse, T.; Chiew, F.; Lavender, E.; Richards, D. H.; Owen, S.; Stone, P.; Church, S.; Woodcock, A. A. Attenuation of Nocturnal Asthma by Cromakalim. *Lancet* **1990**, *336*, 334–336.
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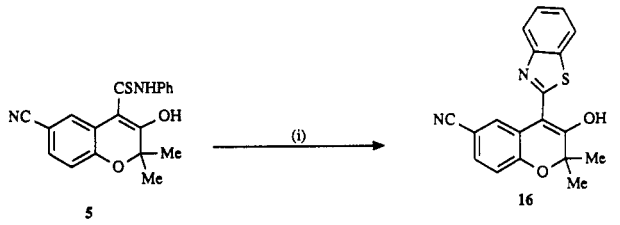
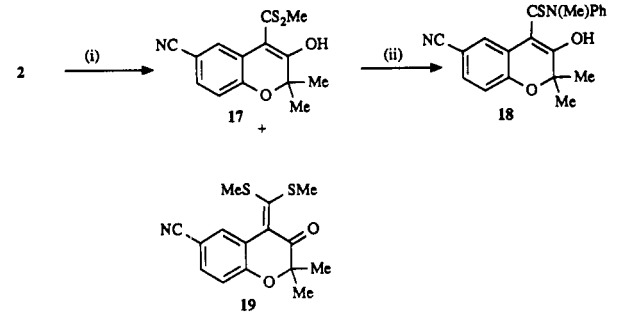
Scheme II[a]



[a] Reagents: (i) *m*-CPBA.

Scheme III[a]



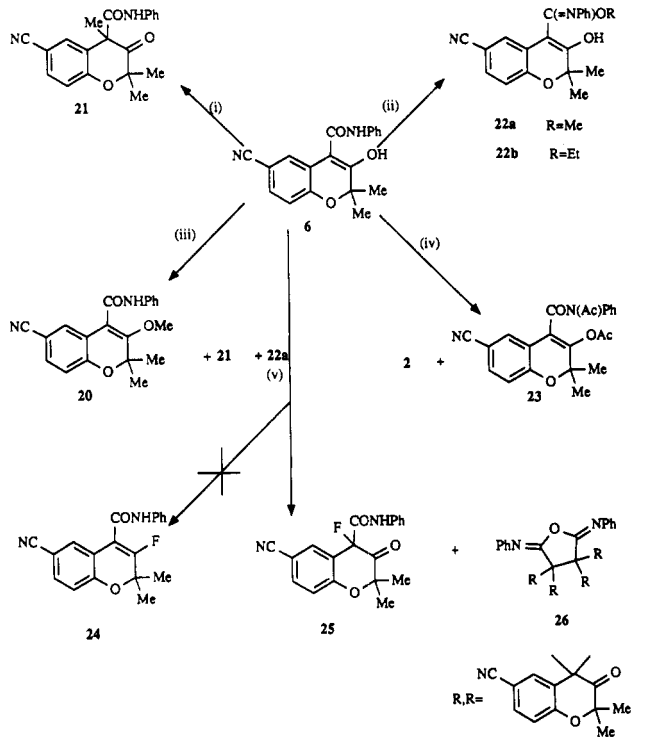
[a] Reagents: (i) KO-*t*-Bu/CS_2/MeI; (ii) PhNHMe.

the β-keto amides and β-keto thioamides **3–9** in moderate to good yields (Table I). Reduction of **3–5** with potassium borohydride in methanol gave the β-hydroxy amides **10–12** as mixtures of cis and trans isomers (Table II). Whereas the isomers of the methyl (**10**) and *tert*-butyl (**11**) derivatives were readily separated by chromatography on silica, only the pure cis isomer **12a** was isolable in this manner. The trans isomer **12b**, however, was obtained free from cis contaminant on treatment of the mixture of cis/trans isomers with 1,5-diazabicyclo[4.3.0]nonane (DBN). Reaction of the trans isomers of **10** and **11** with methanesulfonyl chloride and triethylamine in dichloromethane gave the corresponding mesylates, which, after treatment with potassium *tert*-butoxide, provided benzopyrans **13** and **14** (Table I). Surprisingly, the corresponding trans phenyl analogue **12** gave only complex reaction products with methanesulfonyl chloride under these conditions, but treatment with phosgene and triethylamine in chloroform, followed by reaction of the crude product with potassium *tert*-butoxide, gave a low yield of amide **15**, which presumably resulted by oxidation of the corresponding thioamide by phosgene itself or by hydroperoxide contaminant present in the potassium *tert*-butoxide.

Attempted oxidation of thioanilide **5** to the corresponding amide with *m*-chloroperbenzoic acid gave instead benzothiazole **16** (Scheme II) in 68% yield. Similar reactions have been reported with thioanilides using other oxidizing agents.[12]

Owing to the inability of the isocyanate method to yield tertiary amides, a different synthetic route was used to prepare this type of compound (Scheme III). Thus, trapping of the enolate derived from **2** with carbon disulfide followed by iodomethane gave a mixture of dithioester **17** and ketene dithioacetal **18**. Separation of these and treatment of the dithioester with *N*-methylaniline gave tertiary amide **19** in 61% yield.

Scheme IV[a]



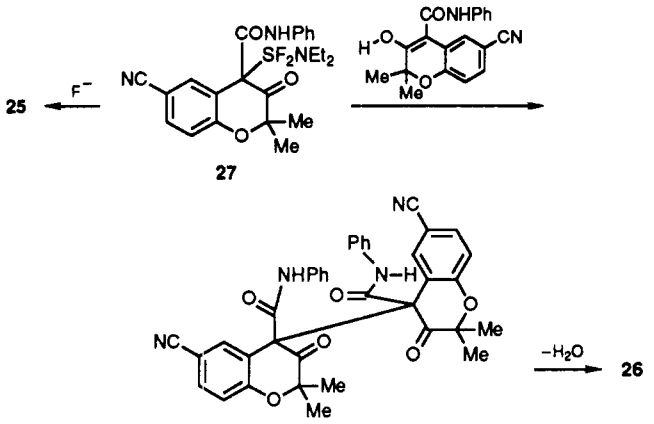
[a] Reagents: (i) KO-*t*-Bu/MeI/DMSO; (ii) CH_2N_2/Et_2O; (iii) Me_3OBF_4/CH_2Cl_2; (iv) Et_3N/AcCl; (v) DAST/CH_2Cl_2.

Because of the possibility of an interesting dual mechanism of action for β-keto thioamide **5** (see later), a number of derivatives of this system have been prepared. Of particular interest were those derivatives which would stabilize the β-keto amide in either the keto or enol form and thus would provide information regarding which of these tautomers, if either, contributed more to the auxiliary mechanism of action. Attempted etherification of anilide **6** by treatment of the sodium or potassium enolate with methyl sulfate or iodomethane in tetrahydrofuran gave complex mixtures, while treatment of the potassium enolate with iodomethane in dimethyl sulfoxide gave the C-methylated derivative **21** (Scheme IV) in 54% yield. No O-alkylated material was isolated from these reactions, nor was it obtained in those cases where silver tetrafluoroborate/18-crown-6 were added to the reaction. Treatment of anilide **6** with 2 equiv of diazomethane in methanol–ether (1:9) gave a 9:1 mixture of the methyl and ethyl imidates **22a** and **22b** in 12% combined yield together with recovered starting material, but no enol ether. Insertion of diazomethane into an imidate ether bond has been documented.[13] Use of a large excess of diazomethane (12 equiv) resulted only in an increased amount of the imidate mixture (65%) with a ratio of methyl:ethyl imidates of 2:3. Treatment of **6** with 1.1 equiv of trimethyloxonium tetrafluoroborate in dichloromethane, however, gave 13% of the required methyl ether **20**, together with 48% of C-methylated derivative **21** and 24% of methyl imidate **22a**.

Treatment of **6** with acetyl chloride–triethylamine in an attempt to form the enol acetate gave instead diacetylated material **23** in 12% yield together with ketone **2**, with no monoacetylated material being isolated. Ketone **2** presumably arises via elimination of phenyl isocyanate from the starting material.

(11) Patent to SmithKline Beecham, EP 366,273, 1990.
(12) Metzer, J. V. Thiazoles and their Benzo Derivatives. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Rees, C. W., Eds.; Pergamon Press: Oxford, 1984; Vol. 6, p 322.
(13) Pizey, J. S. *Synthetic Reagents*; Ellis Horwood Ltd.: Chichester, UK, 1974; Vol. 2, Chapter 2, p 78.

Scheme V



It was anticipated that treatment of **6** with diethylamidosulfur trifluoride (DAST) would give the C-3 difluoro derivative, which would eliminate hydrogen fluoride to give vinyl fluoride **24**. In the event this material was not isolated, the reaction proceeding either at ambient temperature or at −78 °C in dichloromethane to give a low yield (4.5%) of C-4 fluoride **25** and 27% of bis-spirotetrahydrofuran **26**. Both products presumably arise via intermediate **27** (Scheme V). Displacement of the SF_2NEt_2 moiety by fluoride would give rise to **25**, while displacement with the enolate derived from a second molecule of β-keto amide **6** followed by ring closure and dehydration would furnish **26**. The favored C-alkylation by DAST in this case and the presence of large amounts of C-methylation in the alkylation reactions of **6** probably reflect the greater stability of the anionic intermediate at C-4, through cross-conjugation with two adjacent carbonyl centers and with the cyanophenyl moiety.

Results and Discussion

As part of an ongoing program to evaluate the potential of potassium channel activators in respiratory disease, we have recently reported the airway smooth muscle relaxant activity of a series of dihydropyran[7] and indane[9] derivatives related to cromakalim. As an extension of this work we have explored the effect of structural modification at the C-4 position of cromakalim, since the work of ourselves and others has shown this position to tolerate significant structural change without undue reduction in potency.[3,7,9,14,15] We now describe the relaxant activity and SAR of a series of 6-cyanobenzopyrans which carry carboxamide and thiocarboxamide functionalities at this position. The cyano substituent at C-6 was chosen since we have previously demonstrated that this is one of several substituents which confer a high degree of potency to the parent system.[7] In addition to 3-hydroxy derivatives **10–12** and benzopyrans **13–15**, particular interest lay in the corresponding 3-keto compounds **3–9** (Scheme I). Compounds **3–9** represent direct analogues of the 3-keto derivative **28** of cromakalim and, since they exist either totally or predominantly in the enol form, can be considered hybrids of cromakalim and its derived 3-alkene **29**.[3] Ketone **28** is unusual in that, unlike most modifications at position 3,

Table III. Inhibition of Spontaneous Tone in Guinea Pig Isolated Tracheal Spirals

compd	inhibition of spontaneous tone in guinea pig tracheal spirals[a]
16	1.36 (0.33–5.64); 0.96 ± 0.01; 4
19	>20
20	>20
21	>20
22a	0.95 (0.71–1.27); 0.82 ± 0.06; 4
23	1.35 (0.45–4.12); 0.95 ± 0.02; 5
25	0.44 (0.26–0.77); 0.95 ● 0.05; 6

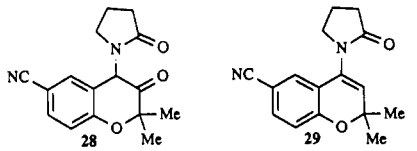
[a] See footnote a of Table I.

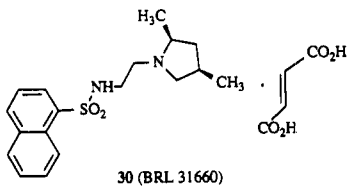
Table IV. Inhibition of Histamine-Induced Bronchospasm in Conscious Guinea Pigs

compd	dose, mg kg^{-1} (po)	time between dose and challenge, min	% prolongation of collapse time ± SEM (n)[a]
cromakalim	5	60	174 ± 17 (18)
5	20	15	63 ± 41 (3)
		30	14 ± 14 (3)
		60	10 ● 7 (3)
6	10	15	−6 ● 11 (3)
		60	−14 ± 15 (3)
13	5	60	12 ● 10 (3)
	10	60	9 ± 4 (3)
	20	30	244 ± 36 (6)
	20	60	230 ● 29 (6)
	20	90	175 ± 46 (6)
	20	120	187 ± 43 (6)
15	20	60	34 ● 26 (3)
16	20	60	−1 ± 5 (3)
22a	5	60	1 ● 12 (3)
23	20	60	5 ± 2 (3)
25	10	60	10 ● 20 (3)

[a] Mean time to collapse for control animals was 75 ± 3 s, (n = 30).

it has been shown to retain the potassium channel opening properties of cromakalim.[10]





30 (BRL 31660)

Comparing *N*-methyl, *tert*-butyl, and phenyl thioamides **3–5**, respectively (Table I), it is evident that potency as relaxants of spontaneous tone in guinea pig isolated trachealis increased in the order Me ≤ *t*-Bu < Ph, and **5** was evaluated further in vivo. Unlike cromakalim,[5] however, oral administration (20 mg kg^{-1}) of **5** elicited only a transient protection of guinea pigs from the respiratory effects of histamine (Table IV). In an attempt to block potential metabolism of **5** the *p*-fluoro and *p*-methoxy compounds, **7** and **8**, respectively, were prepared, but both showed a marked drop in in vitro potency compared to the unsubstituted compound **5** and were not evaluated further. Analogue **6**, in which the sulfur atom of **5** was replaced by oxygen, was also a potent relaxant of trachealis in vitro, but activity was again reduced somewhat when the aromatic ring was substituted by a *p*-methoxy group (**9**). In

(14) Bergmann, R.; Gericke, R. Synthesis and Antihypertensive Activity of 3-(1,2-Dihydro-2-oxo-1-pyridyl)-2*H*-1-benzopyrans and Related Compounds, New Potassium Channel Activators. *J. Med. Chem.* **1990**, *33*, 492–504.

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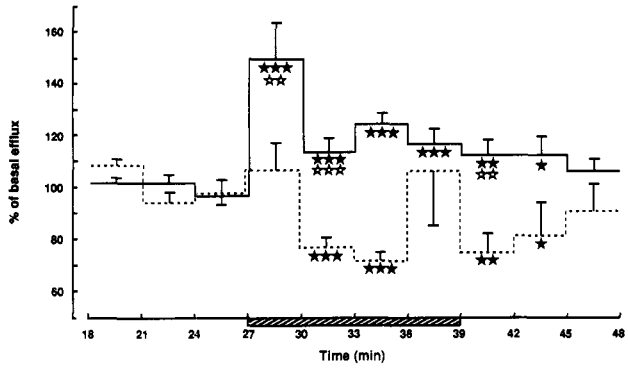

Figure 1. Stimulation of $^{42/43}K$ efflux from guinea pig isolated trachealis by 10 μM compound **5** (—) and 10 μM compound **5** in the presence of BRL 31660 (- -). The period when the compounds were present is indicated by the hatched area on the time axis. ★ indicates statistical significance relative to the percentage of radioactivity effluxing in the 9 min before addition of compounds (100% level), ☆ a significant difference between the percentage of basal efflux in the absence and presence of BRL 31660. $P <$ 0.05, 0.01, and 0.001 are indicated by one, two, or three symbols, respectively.

contrast to **5**, however, **6** was ineffective in vivo after oral administration (Table IV).

Attempts to enhance the oral in vivo effectiveness of amides **5** and **6** by N-, O-, or C-methylation resulted in compounds **19–21**, respectively, which had little in vitro activity at concentrations below 20 μM. In contrast, 4-fluoro compound **25** and diacetate **23** retained their in vitro activity (Table III), but neither compound afforded protection in vivo (Table IV). It is of interest that cyclic thioimidate **16**, which maintains the key pharmacophore of **5**, also relaxes guinea pig trachealis (Table III), although it too is without oral activity in vivo (Table IV).

Thioamide **5** is an unusual relaxant of guinea pig trachealis in that it did not evoke the steep concentration–response curve usually observed with cromakalim and other potassium channel activators.[5,16] Moreover, the responses in this preparation were incompletely inhibited by the potassium channel blocker BRL 31660, **30**.[17] Thus, 10 μM concentrations of **30** only resulted in a small rightward shift of the concentration–response curve of **5**, with a resultant dose ratio of 3.4 compared to greater than 100 for compound **1**. Nonetheless, **5** (10 μM) did elicit efflux of $^{42/43}K$ from prelabeled guinea pig trachealis and this was completely prevented by prior incubation of the tissue with the potassium channel blocker **30** (Figure 1). While some of these data are consistent with **5** having potassium channel activator activity, there is strong evidence also for the presence of an auxiliary effect. One possibility is that some of the relaxant activity of **5** is exerted through the inhibition of cyclic nucleotide phosphodiesterases (PDE), a known mechanism of smooth-muscle relaxation.[18] However, since it has been shown to possess only a small inhibitory effect on PDE III, IV, and Va isoenzymes at 100 μM concentrations (28, 57, and 30% inhibition, respectively[19]), this is unlikely. Alternatively, since spontaneous tone in guinea pig trachealis is mediated through prostaglandins, and is known to be inhibited by cyclooxygenase inhibitors,[20,21] it is possible that the inhibition of this enzyme contributes to the in vitro activity of **5**. Support for the latter argument is obtained from the structural similarity of **5** to known inhibitors of prostaglandin synthesis.[22]

Saturation of the 3,4-double bond to give β-hydroxy amides **10–12** produced some interesting results. Thus, unlike the unsaturated precursors **3–5**, potency was less dependent on the nature of the amide substitution, and the trans compounds showed similar IC_{50} values in guinea pig trachealis (Table II). Moreover, **12b** was some 10-fold less potent than the corresponding β-keto amide **5**, a result which contrasts markedly with that found with cromakalim and its 3-keto analogue.[10] The cis analogues did not appear to follow any obvious pattern in that **11a** and **12a** were poor inhibitors of spontaneous tone at concentrations <20 μM, whereas the activity of **10a** showed little difference from that of the trans isomer. Like **5**, **12b** showed a small nonspecific inhibition of phosphodiesterases III, IV, and Va at 100 μM concentrations (68, 0, and 11%, respectively[19]), although this is unlikely to have accounted for its in vitro activity, and its relaxant effects were not appreciably blocked by compound **30**. These data suggest that **10–12** are relatively poor potassium channel activators and they were not evaluated further.

Of considerably greater interest was anhydro derivative **13**, which in contrast to its precursor **12b**, was a potent smooth-muscle relaxant with an IC_{50} value of 0.14 μM (Table I). The activity of **13** differs markedly from that of the corresponding 3-hydroxyalkene **3**, which had only weak activity in vitro. Further evaluation of **13** confirmed that the relaxant activity was blocked by 10 μM concentrations of compound **30** (dose ratio >38), suggesting that its activity was at least in part mediated through the opening of potassium channels. Compound **13** was also shown to possess weak phosphodiesterase inhibitory activity (IC_{50} = 64 μM against PDE IV, but less than 20% inhibition against PDE III and Va at 100 μM[19]), although this is unlikely to have contributed to its activity. Evaluation in vivo against the respiratory effects of histamine in the guinea pig showed that **13** was a potent bronchodilator in this model, providing lasting protection when administered orally at 20 mg kg^{-1} (Table IV). It is of interest that the in vitro activity of *tert*-butyl analogue **14** did not improve relative to trans alcohol **11b** and, indeed, showed no significant relaxant activity at 20 μM (Table I). Unfortunately, the corresponding phenyl thioamide was not available, but the effectiveness of amido analogue **15** suggests that the phenyl moiety is not detrimental to smooth muscle relaxant activity in this series.

Experimental Section

Melting points were determined with a Buchi melting point apparatus and were recorded uncorrected. The structures of all compounds were consistent with their IR and ^1H NMR spectra, which were determined with a Perkin-Elmer 298 spectrophotometer and a Varian EM390 90-MHz or a Jeol GX270 270-MHz spectrometer, respectively. Mass spectra were recorded with a

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VG-micromass 70-70F spectrometer using electron-impact techniques. Where represented by elemental symbols, the analyses of these elements fall within ±0.4% of the calculated value. All organic extracts were dried over $MgSO_4$ and samples were chromatographed on silica in all instances.

General Preparation of 3-Hydroxybenzopyran-4-carboxamides and -thiocarboxamides 3–9 Illustrated by 6-Cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-thiocarboxamide (5). 6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-3-one[11] (300 mg, 1.49 mmol) in THF (2 mL) was added to a solution of KO-*t*-Bu (167 mg, 1.5 mmol) in THF (5 mL) at 0 °C under N_2 and the solution was stirred at room temperature for 0.75 h. Phenyl isothiocyanate (0.18 mL, 1.49 mmol) in THF (2 mL) was added and the solution was stirred for a further 3 h. The reaction mixture was quenched with HCl (2 M, 50 mL) and extracted with $CHCl_3$, and the dried organic layers were concentrated to an orange solid (603 mg). Chromatography (CH_2Cl_2) gave **5** (388 mg, 78%) as a white solid: mp 152–153 °C (resolidifies and then melts at 255–260 °C); ^1H NMR ($CDCl_3$) δ 1.51 (6 H, s, C-2Me), 7.01 (1 H, d, J = 8.5 Hz, C-8H), 7.35–7.69 (7 H, m, C-5, C-7H + Ph), 8.73 (1 H, exchangeable s, NH), 13.62 (1 H, exchangeable s, OH). Anal. ($C_{19}H_{16}N_2O_2S$) C, H, N.

General Preparation of 3,4-Dihydro-3-Hydroxybenzopyran-4-thiocarboxamides 10–12 Illustrated by *cis*- and *trans*-6-Cyano-3,4-dihydro-2,2-dimethyl-3-hydroxy-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide (10a,b). Potassium borohydride (81 mg, 1.50 mmol) was added portionwise to a solution of 6-cyano-2,2-dimethyl-3-hydroxy-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide (**3**; 375 mg, 1.37 mmol) in methanol (10 mL) and the solution was stirred for 1.5 h. The reaction mixture was poured into water, extracted with $CHCl_3$, dried, filtered, and concentrated to an oil which was chromatographed. Elution with $CHCl_3$ gave the cis isomer **10a** (102 mg, 27%) as a white solid: mp 170–171 °C; ^1H NMR ($CDCl_3$) δ 1.29 (3 H, s, C-2Me), 1.52 (3 H, s, C-2Me), 3.25 (3 H, d, J = 5 Hz, NMe), 3.33 (1 H, br exchangeable s, OH), 4.07 (1 H, dd, J = 5, 3 Hz, collapses to d with D_2O, C-3H), 4.51 (1 H, d, J = 3 Hz, C-4H), 6.92 (1 H, d, J = 8.5 Hz, C-8H), 7.43–7.47 (2 H, m, C-5, 7H), 8.23 (1 H, br exchangeable s, NH); MS found M^+ 276.0927 ($C_{14}H_{16}N_2O_2S$ requires 276.0932). Further elution gave the trans isomer **10b** (140 mg, 38%) as a white solid: mp 212–213 °C; ^1H NMR ($CDCl_3$) δ 1.21 (3 H, s, C-2Me), 1.53 (3 H, s, C-2Me), 3.28 (3 H, d, J = 5 Hz, collapses to a singlet with D_2O, NMe), 3.31 (1 H, exchangeable s, OH), 4.02 (2 H, br, collapses to 2 d, J = 10 Hz with D_2O, C-3, C-4H), 6.92 (1 H, d, J = 8.5 Hz, C-8H), 7.29 (1 H, d, J = 2 Hz, C-5H), 7.45–7.53 (1 H, dd, J = 8.5, 2 Hz, C-7H, + 1 H, b, exchangeable, NH). Anal. ($C_{14}H_{16}N_2O_2S$) C, H, N. For the phenyl derivative **12** chromatography provided the cis isomer followed by fractions containing both cis and trans derivatives. These were treated with DBN (6 mg) in THF under N_2 and the solution was stirred for 3 h. The reaction was quenched with water and the product was isolated by extraction into ether followed by preparative HPLC (MeOH–H_2O, 7:3, reverse phase) to give the pure trans isomer.

6-Cyano-2,2-dimethyl-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide (13). Methanesulfonyl chloride (0.16 mL, 2 mmol) was added to a solution of *trans*-6-cyano-3,4-dihydro-2,2-dimethyl-3-hydroxy-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide (**10b**; 500 mg, 1.81 mmol) and triethylamine (0.33 mL, 2.36 mmol) in CH_2Cl_2 (20 mL) and the solution was stirred for 4.5 h. The reaction was quenched with water, extracted with CH_2Cl_2, dried, and concentrated to a solid (708 mg) which was chromatographed. Elution with $CHCl_3$ gave ***trans*-6-cyano-3,4-dihydro-2,2-dimethyl-3-[(methylsulfonyl)oxy]-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide** (560 mg, 88%) as a white solid: mp 160.5–161 °C; ^1H NMR ($CDCl_3$) δ 1.33 (3 H, s, C-2Me), 1.53 (3 H, s, C-2Me), 3.13 (3 H, s, SO_2Me), 3.26 (3 H, d, J = 5 Hz, NMe), 4.29 (1 H, d, J = 8 Hz, C-4H), 5.43 (1 H, d, J = 8 Hz, C-3H), 6.95 (1 H, d, J = 8.5 Hz, C-8H), 7.39 (1 H, d, J = 2 Hz, C-5H), 7.49 (1 H, d, J = 8.5, 2 Hz, C-7H), 7.70–7.72 (1 H, b, NH). To this compound (280 mg, 0.79 mmol) in THF (20 mL) at 0 °C under N_2 was added KO-*t*-Bu (99 mg, 0.88 mmol) and the solution was stirred for 3 h. The reaction mixture was poured into water, extracted with ether, dried, concentrated, and chromatographed. Elution with $CHCl_3$ gave **13** (164 mg, 80%) as a white solid: mp 174.5–175 °C; ^1H NMR ($CDCl_3$) δ 1.49 (6 H, s, C-2Me), 3.28 (3 H, d, J = 5 Hz, NMe), 5.86 (1 H, s, C-3H), 6.85 (1 H, d, J = 8.5 Hz, C-8H), 7.41 (1 H, dd, J = 8.5, 2 Hz, C-7H), 7.71 (1 H, d, J = 2 Hz, C-5H + 1 H, b, NH). Anal. ($C_{14}H_{14}N_2OS$) C, H, N, S. *tert*-Butyl analogue **14** was prepared similarly.

6-Cyano-2,2-dimethyl-*N*-phenyl-2*H*-1-benzopyran-4-thiocarboxamide (15). Phosgene (1 equiv of a solution in toluene) was added to a solution of *trans*-6-cyano-3,4-dihydro-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-thiocarboxamide (**12b**; 1.75 g, 5.18 mmol) and triethylamine (1.44 mL, 10.5 mmol) in $CHCl_3$ (120 mL) at 0 °C and the solution was stirred for 16 h. The reaction mixture was poured into water and the organic phase was separated, dried, and concentrated. The residue was dissolved in THF (100 mL) and KO-*t*-Bu (610 mg, 5.4 mmol) was added. The solution was stirred for 3 h, poured into water, extracted with EtOAc, dried, concentrated, and chromatographed. Elution with hexane–EtOAc (7:3) gave **15** (170 mg, 10%) as a white solid: mp 233.5–234 °C; ^1H NMR ($CDCl_3$) δ 1.5 (6 H, s, C-2Me), 6.41 (1 H, s, C-3H), 7.0 (1 H, d, J = 8 Hz, C-8H), 7.10 (1 H, m, Ar), 7.3 (2 H, m, Ar), 7.65 (3 H, m, Ar + C-7H), 7.89 (1 H, d, J = 2 Hz, C-5H), 10.32 (1 H, s, NH); MS found M^+ 304.1219 ($C_{19}H_{16}N_2O_2$ requires 304.1212).

4-(2-Benzothiazolyl)-6-cyano-2,2-dimethyl-2*H*-1-benzopyran-3-ol (16). *m*-Chloroperbenzoic acid (*m*-CPBA; 385 mg, 1.79 mmol) was added to a solution of 6-cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-thiocarboxamide (**5**; 500 mg, 1.49 mmol) in CH_2Cl_2 (20 mL) at 0 °C under N_2. The solution was stirred for 72 h, after which time a further 100 mg (0.45 mmol) of *m*-CPBA was added. After a further hour the reaction mixture was poured into $NaHCO_3$ solution (50 mL) and extracted with CH_2Cl_2. The organic phase was dried, concentrated, and chromatographed ($CHCl_3$) to give **16** (340 mg, 68%) as a white solid: mp 186–187 °C; ^1H NMR ($CDCl_3$) δ 1.53 (6 H, s, C-2Me), 7.03 (1 H, d, J = 8.5 Hz, C-8H), 7.4 (2 H, m, Ar), 7.5 (2 H, m, Ar), 7.75 (1 H, dd, J = 8.5, 2 Hz, C-7H), 7.89 (1 H, d, J = 2 Hz, C-5H), 15.47 (1 H, bs, OH). Anal. ($C_{19}H_{14}N_2O_2S$) C, H, N, S.

6-Cyano-2,2-dimethyl-3-hydroxy-*N*-methyl-*N*-phenyl-2*H*-1-benzopyran-4-thiocarboxamide (19). 6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-3-one (**2**; 250 mg, 1.24 mmol) was added slowly to a solution of KO-*t*-Bu (150 mg, 1.37 mmol) in dry THF (10 mL) at 0 °C and the solution was stirred for 1 h before the addition of carbon disulfide (0.23 mL, 3.72 mmol) in THF (2 mL). After a further 3 h at 0 °C, iodomethane (0.23 mL, 3.72 mmol) was added and the solution was stirred for 1 h then poured into water and extracted with EtOAc. The dried organic phase was concentrated and the residue was chromatographed (hexane–EtOAc, 9:1) to give **methyl 6-cyano-2,2-dimethyl-3-hydroxy-2*H*-1-benzopyran-4-carbodithioate** (**17**; 188 mg, 51%) as a white solid [mp 83–84 °C; ^1H NMR ($CDCl_3$) δ 1.5 (6 H, s, C-2Me), 2.7 (3 H, s, SMe), 7.0 (1 H, d, J = 8 Hz, C-8H), 7.4 (1 H, dd, J = 8, 2 Hz, C-7H), 8.15 (1 H, d, J = 2 Hz, C-5H), 14.6 (1 H, s, OH); MS found M^+ 291.0393 ($C_{14}H_{13}NO_2S_2$ requires 291.0388)] followed by dithioacetal **18** (56 mg, 15%) as an oil [^1H NMR ($CDCl_3$) δ 1.4 (6 H, s, C-2Me), 2.15 (3 H, s, SMe), 2.57 (3, H, s, SMe), 7.05 (1 H, d, J = 8 Hz, C-8H), 7.45 (1 H, dd, J = 8, 2 Hz, C-7H), 8.2 (1 H, d, J = 2 Hz)].

A solution of methyl 6-cyano-2,2-dimethyl-3-hydroxy-2*H*-1-benzopyran-4-carbodithioate (**17**; 291 mg, 1.0 mmol) in *N*-methylaniline (4 mL) was heated under reflux for 12 h. After cooling, the solution was poured into HCl (2 M, 20 mL) and extracted with EtOAc. The combined organic extracts were washed with water, dried, concentrated, and chromatographed. Elution with EtOAc–hexane (1:9) gave **19** as a yellow solid (212 mg, 61%): mp 104–107 °C (ether–hexane); ^1H NMR (DMSO-d_6) δ 0.64 (3 H, s, C-2Me), 1.33 (3 H, s, C-2Me), 3.75 (3 H, s, NMe), 6.75 (1 H, d, J = 8 Hz, C-8H), 7.28 (5 H, m, Ph), 7.37 (1 H, dd, J = 8, 1.5 Hz, C-7H), 7.47 (1 H, d, J = 1.5 Hz, C-5H), 10.01 (1 H, exchangeable s, OH). Anal. ($C_{20}H_{18}N_2O_2S$) C, H, N.

6-Cyano-3-oxo-*N*-phenyl-2,2,4-trimethyl-2*H*-1-benzopyran-4-carboxamide (21). 6-Cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (**6**; 500 mg, 1.5 mmol) in DMSO (5 mL) was added to a solution of KO-*t*-Bu (170 mg, 1.52 mmol) in DMSO (10 mL) at 0 °C under N_2 and left to stir for 0.75 h before the addition of iodomethane (0.5 mL, 8.1 mmol). The reaction mixture was stirred at room temperature for 24 h, poured into water, and extracted with EtOAc. The combined organic phases were washed with water and brine, dried, con-

centrated, and chromatographed. Elution with $CHCl_3$ gave **21** as a white solid (298 mg, 54%): mp 126–127 °C; ^1H NMR ($CDCl_3$) δ 1.35 (3 H, s, C-2Me), 1.63 (3 H, s, C-2Me), 1.88 (3 H, s, C-4Me), 7.25 (7 H, m, Ar + C-8H + NH), 7.67 (1 H, dd, J = 8, 2 Hz, C-7H), 7.69 (1 H, d, J = 2 Hz, C-5H). Anal. ($C_{20}H_{18}H_2O_3$) C, H, N.

Methyl 6-Cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-imidate (22a). Diazomethane [formed from Diazald (217 mg, 1.0 mmol) and aqueous KOH (58 mg, 1.0 mmol in 1.2 mL) in ethanol (14 mL)–ether (1.4 mL)] was passed into a solution of 6-cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (**6**; 160 mg, 0.5 mmol) in methanol (1 mL)–ether (9 mL) and the solution was left to stand for 24 h. The reaction mixture was diluted with CH_2Cl_2 dried, concentrated, and chromatographed. Elution with CH_2Cl_2 gave **22a** together with its ethyl homologue **22b** in the ratio of 9:1 as an oil (200 mg, 12%): ^1H NMR ($CDCl_3$) δ (for **22a**) 1.45 (6 H, s, C-2Me), 3.71 (3 H, s, OMe), 6.98 (1 H, d, J = 8.25 Hz, C-8H), 7.34 (6 H, m, Ph + C-7H), 7.91 (1 H, d, J = 1.9 Hz, C-5H), 13.3 (1 H, b, exchangeable s, OH); (for **22b**) 1.38 (3 H, t, J = 7 Hz, CH_3CH_2), 1.51 (6 H, s, C-2Me), 3.94 (2 H, q, J = 7 Hz, CH_3CH_2), 6.91 (1 H, d, J = 8.25 Hz, C-8H), 7.34 (6 H, m, Ph + C-7H), 8.02 (1 H, d, J = 1.9 Hz, C-5H), 13.4 (1 H, b, exchangeable s, OH).

6-Cyano-2,2-dimethyl-3-methoxy-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (20). 6-Cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (**6**; 150 mg, 0.47 mmol) in CH_2Cl_2 (5 mL) at room temperature was treated with trimethyloxonium tetrafluoroborate (75 mg, 0.51 mmol) and diisopropylethylamine (0.1 mL, 1.0 mmol). The reaction mixture was stirred at room temperature for 4 h, poured into water, and extracted with CH_2Cl_2. The combined organic phases were washed with $NaHCO_3$ and water, dried, concentrated, and chromatographed. Elution with hexane–EtOAc (5:1) gave imidate **22a** (38 mg, 24%) followed by trimethyl ketone **21** (74 mg, 48%) and then methyl ether **20** as a white solid (21 mg, 13%): mp 137 °C; ^1H NMR ($CDCl_3$) δ 1.51 (6 H, s, C-2Me), 3.97 (3 H, s, OMe), 6.88 (1 H, d, J = 8.25 Hz, C-8H), 7.22 (1 H, m, Ar), 7.37 (3 H, m, Ar), 7.64 (3 H, m, Ar), 7.71 (1 H, b, NH). MS found M^+ 334.1319 ($C_{20}H_{18}N_2O_3$ requires 334.1317).

3-Acetoxy-*N*-acetyl-6-cyano-2,2-dimethyl-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (23). 6-Cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (**6**; 500 mg, 1.5 mmol) in CH_2Cl_2 (25 mL) at room temperature was treated with triethylamine (0.23 mL, 3 mmol), and acetyl chloride (0.12 mL, 1.68 mmol) and the solution was stirred for 24 h. The reaction mixture was poured into $NaHCO_3$ solution and extracted with $CHCl_3$, and the dried organic phase was concentrated and chromatographed. Elution with $CHCl_3$–EtOAc (85:15) gave ketone **2**, followed by **23** as a white solid (78 mg, 12%): mp 146–147 °C; ^1H NMR ($CDCl_3$) δ 1.31 (6 H, s, C-2Me), 2.31 (3 H, s, NCOMe), 2.38 (3 H, s, OCOMe), 6.84 (1 H, dd, J = 6.6, 2.2 Hz, C-8H), 7.1 (2 H, m, Ar), 7.4 (5 H, m, Ar). MS found M^+ 404.1372 ($C_{23}H_{20}N_2O_5$ requires 404.1372).

6-Cyano-2,2-dimethyl-4-fluoro-3-oxo-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (25). Diethylamidosulfur trifluoride (DAST; 0.26 mL, 1.97 mmol) was added slowly to a solution of 6-cyano-2,2-dimethyl-3-hydroxy-*N*-phenyl-2*H*-1-benzopyran-4-carboxamide (**6**; 336 mg, 1.0 mmol) in CH_2Cl_2 (15 mL) at 0 °C and the solution was allowed to warm to room temperature and left to stir for 24 h. The reaction mixture was poured into water and extracted with CH_2Cl_2, and the dried organic phases were concentrated and chromatographed. Elution with CH_2Cl_2 gave **25** as a white solid (16 mg, 5%) [mp 184–185 °C; ^1H NMR ($CDCl_3$) δ 1.51 (3 H, s, C-2Me), 1.71 (3 H, s, C-2Me), 7.22 (2 H, m, Ar), 7.35 (2 H, m, Ar), 7.69 (2 H, m, Ar + C-8H), 7.72 (1 H, dd, J = 8.5, 1.9 Hz, C-7H), 7.77 (1 H, d, J = 1.9 Hz, C-5H), 8.22 (1 H, d, J = 4.7 Hz, NH); MS found M^+ 338.1067 ($C_{19}H_{15}FN_2O_3$ requires 338.1067)] followed by the bis-spiro derivative **26** as a white solid (88 mg, 29%) [mp 214 °C; ^1H NMR ($CDCl_3$) δ 0.83 (6 H, s, C-2Me), 1.14 (6 H, s, C-2Me), 7.1 (2 H, dd, J = 7.4, 1.4 Hz, C-7H), 7.22 (6 H, m, Ar), 7.36 (4 H, m, Ar), 7.67 (4 H, m, C-5 + C-8H); MS found M^+ 620.2051 ($C_{28}H_{28}N_4O_5$ requires 620.2060)].

Relaxation of Guinea Pig Isolated Tracheal Spirals. Guinea pig tracheal spiral strips were suspended under isometric conditions in oxygenated Krebs solution. Tension was allowed to develop spontaneously and was maintained at 2 g. Compounds were added in a cumulative fashion up to a maximum concentration of 20 μM and the inhibitory effects were calculated as a percentage of the relaxation induced by isoprenaline (10^{-3} M) added at the end of the experiment. The IC_{50} value of each compound was that concentration which produced 50% of the response to isoprenaline as measured from the concentration–response curve, and was generally (apart from those compounds which had IC_{50} values of >15 μM) a geometric mean of three or more determinations. The intrinsic activity (IA) for each compound was calculated as the ratio of its maximum relaxant activity over that produced by isoprenaline and expressed as an arithmetic mean.

Bronchoconstriction in Conscious Guinea Pigs. Male guinea pigs (400–460 g) were dosed orally with the compound or the vehicle and placed in a Perspex chamber of ca. 8-L capacity. At various times subsequent to dosing, the animals were challenged for 4 min with a histamine aerosol generated over 20 s from a 5 mM solution of histamine diphosphate and a Monaghan 675 ultrasonic nebulizer (power setting 7). The time from introduction of the aerosol to collapse was recorded, with those animals not collapsing within the 4-min observation time being considered to be fully protected and allotted a time of 240 s for the purpose of calculating means ± SE.

Potassium Efflux Experiments. Tracheae excised from male Dunkin–Hartley guinea pigs (300–600 g) were cleaned of adhering fat and connective tissue and opened by longitudinal section. The trachealis muscle was dissected free from cartilage and divided into three segments, and each segment was randomly assigned to the various treatment groups. Following a preincubation at 37 °C in 2.5 mL of Krebs solution bubbled with 95% O_2–5% CO_2, tissues were loaded with $^{42/43}K$ (37–74 MBq/L) at 37 °C in 2.5 mL of Krebs solution for 60 min. Efflux was followed by transferring tissues through a sequence of 17 washing samples at 37 °C, with a residence time in each wash of 3 min. When present, compound **5** was added to tubes 11–14. At the end of the efflux period, the tissues were blotted and the radioactivity in both tissues and washing samples was measured by γ-counting. Efflux was calculated as a rate coefficient (fractional loss of radioactivity from the tissue per minute, expressed as a percentage) and is expressed as a percentage of the mean efflux occurring in the three tubes immediately prior to the addition of the compound.

Acknowledgment. We thank Ms. P. N. Newson, Ms. D. Shaw, Mr. J. F. Taylor, and Mr. J. Ward for technical assistance and Dr. K. J. Murray and Mr. B. J. Connolly for the phosphodiesterase work.