Original 2-Alkylamino-6-halogenoquinazolin-4(3*H*)-ones and K_{ATP} Channel Activity

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A series of 6-substituted 2-alkylaminoquinazolin-4(3*H*)-ones structurally related to 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were synthesized and tested as putative K_{ATP} channel openers on isolated pancreatic endocrine tissue as well as on isolated vascular, intestinal, and uterine smooth muscle. Most of the 6-halogeno-2-alkylaminoquinazolin-4(3*H*)-ones were found to inhibit insulin release from pancreatic B-cells and to exhibit vasorelaxant properties. In contrast to their pyridothiadiazine dioxide isosteres previously described as more active on the endocrine than on the smooth muscle tissue, quinazolinones cannot be considered as tissue selective compounds. Biological investigations, including measurements of ^{86}Rb, ^{45}Ca efflux from pancreatic islet cells and measurements of vasodilator potency in rat aortic rings exposed to 30 or 80 mM KCl in the presence or the absence of glibenclamide, were carried out with 6-chloro- and 6-iodo-3-isopropylaminoquinazolin-4(3*H*)-ones. Such experiments showed that, depending on the tissue, these new compounds did not always express the pharmacological profile of pure K_{ATP} channel openers. Analyzed by X-ray crystallography, one example of quinazolinones appeared to adopt a double conformation. This only suggests a partial analogy between the 2-alkylaminoquinazolin-4(3*H*)-ones and the 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides. In conclusion, the newly synthesized quinazolinones interfere with insulin secretion and smooth muscle contractile activity. Most of the compounds lack tissue selectivity, and further investigations are required to fully elucidate their mechanism(s) of action.

Introduction

In the past few years, great interest has been focused on potassium channels that are sensitive to the intracellular levels of adenosine triphosphate[1] (K_{ATP} channels). Those channels open when the intracellular ATP concentration falls. They have been characterized in numerous cell types such as cardiac cells, pancreatic B-cells, skeletal and smooth muscle cells, and central neurons.[2] The role of ATP-sensitive potassium channels (K_{ATP} channels) is well understood in pancreatic B-cells where they have been shown to control the glucose-induced insulin secretion.[3,4] In the vascular tissue, K_{ATP} channels are involved in the control of muscle tone and contractility.[5]

Hypoglycaemic sulfonylureas such as glibenclamide or tolbutamide are generally reported as selective blockers of K_{ATP} channels.[2,3,6] By contrast, compounds such as pinacidil (**1**), diazoxide (**2**), cromakalim (**3**), or minoxidil sulfate (**4**) (Figure 1) are described as potassium channel (K_{ATP}) openers (PCOs).[4,7,8] PCOs exert mainly their biological activities by promoting membrane hyperpolarization, as a result of their properties to activate the K_{ATP} channels.[9] Diazoxide, used in clinical practice as an antihypertensive agent, has also an effect on the insulin releasing process. Its ability to activate vascu-

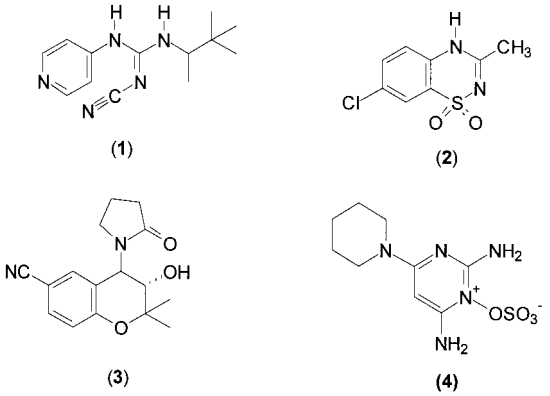

Figure 1. Chemical structure of pinacidil (**1**), diazoxide (**2**), cromakalim (**3**), and minoxidil sulfate (**4**).

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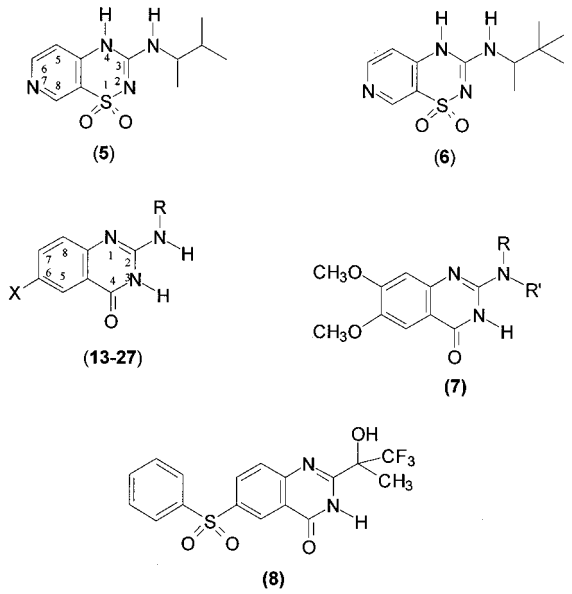


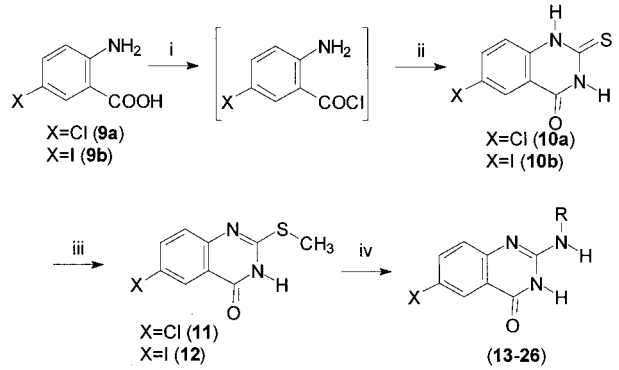
Figure 2. Chemical structure of 3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**5**) and 3-(3′,3′-dimethyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**6**), and general structure of 2-alkylamino-6-halogenoquinazolin-4(3*H*)-ones (**13**−**27**). This figure also includes the general structure of 6,7-dimethoxyquinazolin-4(3*H*)-ones (**7**) and a tertiary carbinol derivative (**8**).

lar[10] as well as pancreatic B-cell[11] K_{ATP} channels indicates a lack of tissue selectivity. In contrast, pinacidil and cromakalim exhibit a stronger activity on smooth muscle cells (relaxation) than on pancreatic B-cells (inhibition of insulin release).[11−13]

In previous reports, we described 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides bearing different aminoalkyl side chains in the 3-position.[14,15] Those derivatives might be regarded as hybrid compounds between diazoxide and pinacidil. Pharmacological investigations have indicated that the target site of compounds such as 3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 44, **5**) or 3-(3′,3′-dimethyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (BPDZ 62, **6**) was the ATP-sensitive potassium channel[16−18] (Figure 2). Whereas diazoxide did not express tissue selectivity, some of these original pyridothiadiazine dioxides (i.e., **5**) appeared to be more active on the endocrine pancreatic tissue (inhibition of insulin release) than on the vascular tissue (smooth muscle relaxation).[15,19]

Starting from these pyridothiadiazine dioxides, we decided to investigate original quinazolin-4(3*H*)-ones bearing a halogen atom in the 6-position and different short and branched alkylamino side chains in the 2-position (**13**−**27**) (Figure 2). Such compounds are expected to be analogues of pyridothiadiazine dioxides since a halogeno-substituted benzene ring is usually considered as a common isostere of a pyridine ring for which the nitrogen atom is located in the same position as the halogeno-substituted carbon atom. Moreover, it is generally accepted that the carbonyl group can be used as an isostere of the sulfonyl group. Furthermore, some 2-alkylaminoquinazolinones (**7**) have been reported to exhibit a hypotensive activity[20] and "ring closed" tertiary carbinol anilides (**8**) were claimed as K_{ATP} channel openers[21] (Figure 2).

Scheme 1[a]



13: X=Cl, R=$CH(CH_3)_2$
14: X=Cl, R=$CH(CH_3)CH_2CH_3$
15: X=Cl, R= $CH(CH_3)CH(CH_3)_2$
16: X=Cl, R=$CH_2C_6H_{11}$
17: X=Cl, R=$CH(CH_3)C_6H_{11}$ (R)
18: X=Cl, R=$CH(CH_3)C_6H_{11}$ (S)
19: X=Cl, R=$CH_2C_6H_5$
20: X=Cl, R=$CH(CH_3)C_6H_5$ (R)
21: X=Cl, R=$CH(CH_3)C_6H_5$ (S)
22: X=I, R=$CH(CH_3)_2$
23: X=I, R=$CH(CH_3)CH_2CH_3$
24: X=I, R=$CH(CH_3)CH(CH_3)_2$
25: X=I, R=$CH_2C_6H_{11}$
26: X=I, R=$CH_2C_6H_5$

[a] (i) $SOCl_2$; (ii) NH_4SCN, pyridine, acetone; (iii) CH_3I, NaOH, methanol/water; (iv) RNH_2.

The 2-alkylaminoquinazolin-4(3*H*)-ones synthesized were evaluated on different biological models: pancreatic B-cells, rat aorta rings, rat uterus, and guinea pig ileum. Particular attention was paid to their putative tissue selectivity. Comparison of the biological activity between the 6-halogeno-substituted quinazolinones and a few examples of nonsubstituted derivatives were also achieved.

Chemistry

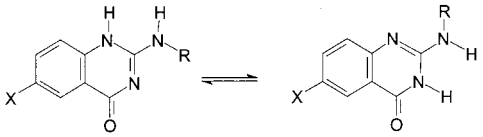
The chemical pathway giving access to the final products is reported in Scheme 1.

6-Halogeno-1,2-dihydro-2-thioxoquinazolin-4(3*H*)-ones **10a** and **10b** were obtained in a two-step reaction starting from 5-chloroanthranilic acid (**9a**) or 5-iodoanthranilic acid (**9b**). The two carboxylic acids **9a** and **9b** were first converted into a more reactive acid chloride by reaction with thionyl chloride. The latter acid chloride treated with ammonium isothiocyanate led, after spontaneous ring closure, to the expected derivatives **10a** and **10b**.

The synthesis of the key intermediates **11** and **12** (6-halogeno-2-methylsulfanylquinazolin-4(3*H*)-ones) was achieved by methylation with methyl iodide of the corresponding 6-halogeno-1,2-dihydro-2-thioxoquinazolin-4(3*H*)-ones **10a** and **10b** in an alkaline hydroalcoholic medium. In these experimental conditions, alkylation only occurred in the 2-position as a result of the presence of a negative charge located on the exocyclic sulfur atom.

6-Chloro- and 6-iodo-2-alkylaminoquinazolin-4(3*H*)-ones (**13**−**26**) were obtained by the reaction of the 6-halogeno-2-methylsulfanylquinazolin-4(3*H*)-ones **11** and **12** with an excess of the appropriate alkylamine. According to the alkylamine boiling point, the reaction was conducted at reflux or in an autoclave.

Table 1. Effects of 2-Alkylamino-6-halogenoquinazolin-4(3*H*)-ones on Rat Pancreatic B-Cells: Comparison with Pinacidil, Diazoxide, and 3-(3′-Methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**5**)



			% of residual insulin secretion ± SEM (*n*)[a]			
compd	X	R	50 μM	10 μM	1 μM	ee[b]
13	Cl	$CH(CH_3)_2$	6.6 ± 0.5 (18)	55.1 ± 3.5 (24)	80.7 ± 2.4 (24)	
14	Cl	$CH(CH_3)CH_2CH_3$	6.0 ± 0.6 (36)	53.2 ± 5.7 (15)	84.9 ± 4.9 (15)	
15	Cl	$CH(CH_3)CH(CH_3)_2$	10.2 ± 1.0 (26)	61.9 ± 6.0 (31)	81.3 ± 3.2 (32)	
16	Cl	$CH_2C_6H_{11}$	26.5 ± 2.5 (29)	90.1 ± 4.0 (12)	ND	
17	Cl	$CH(CH_3)C_6H_{11}$ (*R*)	16.5 ± 0.9 (16)	62.1 ± 3.9 (23)	ND	99.4
18	Cl	$CH(CH_3)C_6H_{11}$ (*S*)	8.9 ± 0.5 (16)	43.6 ± 2.0 (16)	ND	97.2
19	Cl	$CH_2C_6H_5$	82.5 ± 3.3 (31)	ND	ND	
20	Cl	$CH(CH_3)C_6H_5$ (*R*)	35.0 ± 2.7 (13)	ND	ND	97.4
21	Cl	$CH(CH_3)C_6H_5$ (*S*)	12.9 ± 0.9 (14)	76.9 ± 4.9 (16)	ND	98.0
22	I	$CH(CH_3)_2$	12.2 ± 0.9 (21)	90.9 ± 4.5 (24)	93.7 ± 4.1 (24)	
23	I	$CH(CH_3)CH_2CH_3$	11.2 ± 0.7 (28)	77.5 ± 4.8 (30)	94.9 ± 2.9 (31)	
24	I	$CH(CH_3)CH(CH_3)_2$	30.6 ± 1.8 (29)	87.7 ± 3.7 (31)	94.7 ± 5.6 (23)	
25	I	$CH_2C_6H_{11}$	10.0 ± 0.7 (15)	65.4 ± 3.9 (14)	ND	
26	I	$CH_2C_6H_5$	82.8 ± 4.5 (16)	ND	ND	
27	Br	$CH_2C_6H_5$	75.7 ± 3.6 (16)	ND	ND	
28	H	$CH(CH_3)_2$	45.6 ± 3.8 (28)	ND	ND	
29	H	$CH(CH_3)CH(CH_3)_3$	50.9 ± 3.1 (15)	ND	ND	
30	H	$CH_2C_6H_5$	61.8 ± 3.5 (24)	ND	ND	
Diazoxide[c]			28.8 ± 2.4 (21)	70.0 ± 3.6 (22)	78.9 ± 3.8 (21)	
Pinacidil[d]			92.1 ± 5.5 (21)	96.0 ± 4.2 (20)	97.8 ± 8.8 (13)	
5[e]			7.1 ± 0.6 (14)	26.8 ± 1.8 (21)	70.5 ± 4.4 (19)	

[a] % RIS: percentage of residual insulin release from pancreatic islets incubated in the presence of 16.7 mM glucose. [b] ee: enantiomeric excess; published results. [c] Published results: ref 14. [d] Published results: ref 17. [e] Published results: refs 14 and 19.

The synthesis of the 6-bromo-substituted and unsubstituted 2-alkylaminoquinazolin-4(3*H*)-ones (**27**−**30**) has been previously described.[22]

Results and Discussion

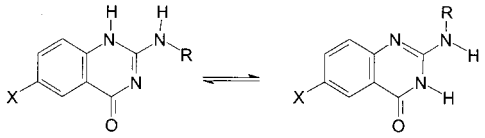
The different compounds reported in Table 1 were tested as inhibitors of insulin release from rat pancreatic islets incubated in the presence of an insulinotropic glucose concentration (16.7 mM). Results were expressed as the percentage of residual insulin secretion. A 90−95% inhibition (5−10% residual insulin secretion) may be considered as a full effect relative to the glucose-insensitive basal insulin release.[14] To define the tissue selectivity of those new compounds, we have also investigated their relaxant activity on different smooth muscles: rat aorta, rat uterus, and guinea pig ileum (Table 2). The effect of the compounds were compared with those of three reference molecules: pinacidil (a pyridyl-alkylcyanoguanidine), diazoxide (a benzothiadiazine dioxide), and **5** (a 3-(3′-methyl-2′-butylamino)-substituted pyridothiadiazine dioxide).

Effect on Pancreatic B-Cells. As observed in Table 1, except for the benzylamino-substituted compounds **19**, **26**, and **27**, most of the 2-alkylamino-6-halogenoquinazolin-4(3*H*)-ones were found to markedly inhibit the insulin releasing process at a 50 μM drug concentration. Some of them (**13**−**15**, **17**, **18, 21, 23, 25**), at a 10 μM concentration, were more potent than diazoxide (**13**, **14**, **18** vs diazoxide; $P < 0.05$) or at least equally potent as the reference drug (**15**, **17**, **21**, **23**, **25** vs diazoxide; $P > 0.05$). None of those derivatives, at this concentration, were found to be of comparable potency than **5**, the most potent drug on this model (**18** vs **5**; $P < 0.05$). As previously described, pinacidil exhibited a poor activity on endocrine pancreatic B-cells.[11,23] Substitution of the hydrogen atom in the 6-position by a chlorine or iodine atom enhanced the inhibitory activity of the drug on the endocrine tissue (compare **13** and **22** with **28** at 50 μM; $P < 0.05$). Comparison between the 6-chloro-substituted derivatives (**13** to **21**) and their 6-iodo counterparts (**22** to **26**) generally showed a weak difference of activity in favor of the first ones especially for compounds bearing a short branched hydrocarbon chain. The three examples of 6-halogeno-substituted 2-benzylaminoquinazolin-4(3*H*)-ones (**19**, **26**, **27**) were found to be equipotent and somewhat less active than the unsubstituted analogue (**30**) (**19**, **26**, **27** vs **30** at 50 μM; $P < 0.05$). At the lower concentration tested (10 μM), the 6-chloro-substituted compounds **13**, **14**, and **18** were the most potent quinazolinones.

Some optically active 6-chloro-substituted quinazolinones were prepared by introducing an alkylamino side chain in the 2-position bearing an asymmetric carbon atom. At a 50 μM concentration, the *S*-enantiomers **18** and **21** were found to be more potent than their *R*-counterparts **17** and **20**, respectively ($P < 0.05$). This finding was corroborated by experiments performed at a 10 μM concentration for **17** and **18** ($P < 0.05$).

Whatever the nature of the halogen atom in the 6-position (Cl: **13**, **14**, **15** or I: **22**, **23**, **24**), the enhancement of the size and the branching of the aliphatic side chain did not markedly modify the effects of the drugs on the insulin releasing process. These findings contrast with previous results showing that, for 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides, the best chain eliciting a potent biological activity was the 3-(3′-methyl-2′-butylamino) side chain (as that found in **5**[14,15]).

Table 2. Myorelaxant Activity of 6-Halogeno-2-alkylaminoquinazolin-4(3*H*)-ones on Rat Aorta, Rat Uterus, and Guinea Pig Ileum: Comparison with Pinacidil, Diazoxide, and 3-(3′-Methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**5**)



			myorelaxant activity on smooth muscles			
				rat uterus % residual contractile activity ± SEM (n)[b]		
compd	X	R	rat aorta IC_{50} ± SEM (n)[a]	50 μM	10 μM	guinea pig ileum IC_{50} ± SEM (n)[c]
13	Cl	$CH(CH_3)_2$	17.3 ± 5.9 (6)	61.0 ± 4.4 (4)	78.7 ± 7.0 (4)	74.1 ± 6.5 (3)
14	Cl	$CH(CH_3)CH_2CH_3$	13.8 ± 3.4 (6)	ND	ND	44.7 ± 7.3 (8)
15	Cl	$CH(CH_3)CH(CH_3)_2$	16.3 ± 4.2 (4)	ND	ND	59.2 ± 2.6 (4)
16	Cl	$CH_2C_6H_{11}$	2.2 ± 1.1 (4)	80.3 ± 0.5 (4)	86.0 ± 2.2 (4)	23.9 ± 3.7 (6)
17	Cl	$CH(CH_3)C_6H_{11}$ (*R*)	5.6 ± 1.3 (12)	90.7 ± 2.6 (4)	108 ± 2 (4)	8.2 ± 0.7 (5)
18	Cl	$CH(CH_3)C_6H_{11}$ (*S*)	2.1 ± 0.3 (4)	66.5 ± 8.4 (4)	102 ± 2 (4)	15.1 ± 1.7 (4)
19	Cl	$CH_2C_6H_5$	3.2 ± 0.4 (7)	98.1 ± 5.2 (4)	94.8 ± 9.4 (4)	13.3 ± 2.8 (4)
20	Cl	$CH(CH_3)C_6H_5$ (*R*)	2.8 ± 0.6 (4)	46.5 ± 6.5 (4)	120 ± 7 (4)	20.2 ± 2.5 (5)
21	Cl	$CH(CH_3)C_6H_5$ (*S*)	1.7 ± 0.1 (4)	44.9 ± 6.8 (8)	92.4 ± 8.3 (8)	13.2 ± 1.8 (5)
22	I	$CH(CH_3)_2$	12.1 ± 2.3 (12)	41.0 ± 2.3 (8)	77.6 ± 4.2 (8)	44.1 ± 5.4 (4)
23	I	$CH(CH_3)CH_2CH_3$	8.9 ± 3.3 (8)	ND	ND	23.6 ± 10.3 (3)
24	I	$CH(CH_3)CH(CH_3)_2$	4.1 ± 0.8 (6)	ND	ND	30.4 ± 4.5 (8)
25	I	$CH_2C_6H_{11}$	3.9 ± 1.2 (4)	97.9 ± 7.8 (4)	96.8 ± 7.4 (4)	26.0 ± 4.0 (4)
26	I	$CH_2C_6H_5$	1.4 ± 0.3 (4)	106 ± 5 (8)	106 ± 5 (8)	8.7 ± 1.7 (4)
27	Br	$CH_2C_6H_5$	7.6 ± 1.3 (5)	76.6 ± 5.3 (4)	76.8 ± 4.1 (4)	ND
28	H	$CH(CH_3)_2$	67.3 ± 11.4 (4)	ND	ND	ND
29	H	$CH(CH_3)CH(CH_3)_3$	12.4 ± 2.0 (4)	ND	ND	ND
30	H	$CH_2C_6H_5$	43.7 ± 6.5 (6)	47.4 ± 3.4 (4)	97.2 ± 6.2 (4)	ND
Diazoxide[d]			19.3 ± 1.5 (21)	76.3 ± 4.9 (4)	93.8 ± 2.2 (4)	>300 (7)
Pinacidil[d]			0.5 ± 0.1 (28)	35.5 ± 2.9 (4)	58.1 ± 4.4 (4)	9.2 ± 2.2 (18)
5[d]			154 ± 15 (8)	ND	ND	>300 (6)

[a] IC_{50}: drug concentration (μM) giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings. [b] Percentage of contraction (residual) of the rat uterine smooth muscle induced by 20 mU oxytocin in the presence of 10 and 50 μM of drugs. Contraction in the absence of drugs is referenced to 100%. [c] IC_{50}: drug concentration (μM) giving 50% relaxation of the electrically stimulated guinea pig ileum segments. [d] Published results: ref 29.

Effect on Smooth Muscle Cells. PCOs such as pinacidil, cromakalim, or diazoxide have been reported to exert myorelaxant activities on a variety of smooth muscles such as the vascular, the ileal, and the uterine smooth muscle.[24] To characterize the similarities between the newly synthesized quinazolinones and known PCOs, their activity on these three different tissues were investigated and compared with three reference drugs: diazoxide, pinacidil, and 3-(3′-methyl-2′-butylamino)-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**5**) (Table 2).

Effect on Rat Aorta. Pharmacological data clearly indicated that the 6-chloro-, 6-bromo-, and 6-iodo-2-alkylaminoquinazolin-4(3*H*)-ones (**13**−**27**) as well as the unsubstituted derivatives (**28**−**30**) were active on the vascular tissue and inhibited the KCl (30 mM)-induced contraction (Table 2). According to their potency, the drugs could be divided in three groups. The first group was a series of compounds (**16**−**21**, **23**−**27**) with marked vasorelaxing properties and exhibiting IC_{50} values below 10 μM. These compounds were much more potent than diazoxide but less potent than pinacidil. The second group consisted of compounds **13**−**15**, **22**, and **29** of intermediate activity but still more potent or equipotent to diazoxide. Finally, quinazolinones **28** and **30** were at least 2 to 3 times less potent than diazoxide.

In the 6-chloro- and 6-iodo-substituted series, compounds bearing a cycloalkylalkylamino- (**16**−**18**, **25**) or a arylalkylamino- (**19**−**21**, **26**) moiety in the 2-position were found of higher potency than most of the short branched 2-alkylamino-substituted derivatives (**13**−**15**, **22**, **23**). According to the results obtained with the 2-benzylamino-substituted (**19**, **26**, **27**, **30**) and the 2-isopropylamino-substituted quinazolinones (**13**, **22**, **28**), it clearly appeared that the introduction of a halogen atom in the 6-position increased the myorelaxant activity of the drugs (**13** and **22** > **28**, $P < 0.05$; **19**, **26** and **27** > **30**, $P < 0.05$). In the 6-chloro-substituted group (**13**−**21**), the enhancement of the size and the branching of the alkylamino side chain did not induce any important modification of the vasorelaxant activity. However, when substitution was made with iodine, the increasing branching of the chain appeared suitable for a higher myorelaxant activity (**24** > **23** > **22**). Comparison between compounds of the *R*-geometry (**17** and **20**) and their *S*-counterparts (**18** and **21**) indicated that the absolute configuration of the first carbon atom of the lateral chain seemed to be of minor importance for activity on the vascular tissue. An interesting observation was given by the finding that some of the most potent 2-alkylaminoquinazolin-4(3*H*)-ones (i.e., the benzylamino-substituted compounds **19**, **26**, **27**) were poorly active as inhibitors of the insulin releasing process. This could foreshadow a potential tissue selectivity for the vascular smooth muscle versus the pancreatic tissue.

Effect on Guinea Pig Ileum. The second model developed for the biological testing of these quinazolinones was the electrically stimulated guinea pig ileum (coaxial stimulation). Potassium channel openers such as pinacidil have been found to exert a myorelaxant activity on the gastrointestinal tract.[25,26] Since glib-

enclamide antagonized these relaxant effects of pinacidil, the presence of K_{ATP} channels was suggested.[8,24−26]

Our data revealed that most of the quinazolinones induced intestinal smooth muscle relaxation (Table 2). Among those, **17** and **26** (IC_{50} = 8.2 μM and 8.7 μM) exhibited a myorelaxant activity which was of the same order of magnitude than that expressed by pinacidil (IC_{50} = 9.2 μM). With IC_{50} values above 300 μM, **5** and diazoxide can be considered as inactive compounds. Comparison between the 6-chloro-substituted quinazolinones and their 6-iodo-substituted counterparts did not provide a clear and obvious difference in potency. However, the substitution of the chlorine atom in the 6-position by an iodine atom could, in some cases, slightly improve their myorelaxant activity on the gastrointestinal smooth muscle (compare **13** and **22**, $P < 0.05$; **14** and **23**, $P > 0.05$; **15** and **24**, $P < 0.05$). Likewise, the influence of the nature of the chiral carbon atom located on the hydrocarbon side chain was not clearly defined. Regarding the (*R*)- and (*S*)-1-cyclohexylethylamino-substituted molecules (**17** and **18**), the *R* absolute geometry seemed to be responsible for an improvement of the myorelaxant properties (IC_{50} **17** = 8.2 μM, IC_{50} **18** = 15.1 μM; **17** vs **18**, $P < 0.05$). Such an influence of stereochemistry was not confirmed with the (*R*)- and (*S*)-1-phenylethylamino-substituted derivatives **20** and **21**. Compounds **19** and **26** (2-benzylamino-quinazolin-4(3*H*)-ones) were again identified as potent myorelaxant drugs (aorta and ileum), being poorly active on the endocrine pancreatic tissue.

Effect on Rat Uterus. The newly synthesized 2-alkylamino-quinazolin-4(3*H*)-ones were evaluated on rat uterus as inhibitors of the contractile activity induced by oxytocin (Table 2). Pinacidil, the most potent compound, induced a dose-dependent myorelaxant activity. At a 10 μM concentration, the quinazolinones were found to be poorly active. At the higher concentration (50 μM), biological effects remained moderate, except for compounds **20**−**22** and **30**.

Taken as a whole, the pharmacological results indicate that the 2-alkylamino-quinazolin-4(3*H*)-ones were, in most cases, active on pancreatic B-cells as well as on smooth muscle cells. However, and according to the compound, the biological responses did not occur in the same range of concentration.

Two main mechanisms might be responsible for the inhibition of insulin release and for the myorelaxant activity. Indeed, such biological effects could result from the opening of ATP-sensitive K^+ channels or from the direct blocking of voltage-dependent Ca^{2+} channels. To detect the mechanism of action of the new drugs, additional pharmacological investigations were carried out on rat pancreatic islets and rat aorta.

Measurements Of ^{86}Rb and ^{45}Ca Outflow from Perifused Rat Pancreatic Islets. Figure 3 illustrates the effect of compound **15**, the isosteric analogue of the pyridothiadiazine dioxide **5**,[16] on ^{86}Rb outflow (^{42}K substitute) from prelabeled and perifused rat pancreatic islets. In the presence of 5.6 mM glucose in the basal medium, the addition of **15** (50 μM) provoked a rapid, sustained, and rapidly reversible increase in ^{86}Rb outflow rate (Figure 3, upper panel). When the same experiment was repeated in the presence of the hypoglycaemic sulfonylurea glibenclamide (10 μM) throughout, the stimulatory effect of compound **15** (50 μM) was totally abolished (Figure 3, upper panel). Experiments conducted with compound **13** also revealed a stimulatory effect on ^{86}Rb outflow from perifused rat pancreatic islets (data not shown).

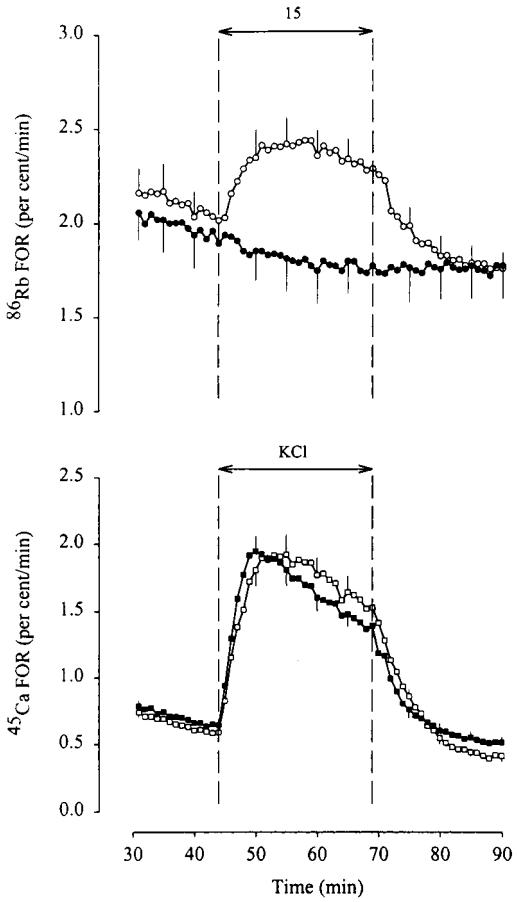


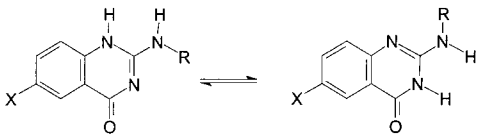
Figure 3. Upper panel: Effect of **15** (50 μM) on ^{86}Rb outflow from rat pancreatic islets perifused throughout in the absence (○) or presence (●) of glibenclamide (10 μM). Basal media contained 5.6 mM glucose. Mean values (±SEM) refer to 4−9 individual experiments. Lower panel: Effect of a rise in the extracellular concentration of K^+ from 5 to 50 mM on ^{45}Ca outflow from rat pancreatic islets perifused throughout in the absence (□) or presence (■) of 15 (50 μM). Basal media contained 2.8 mM glucose. Mean values (±SEM) refer to six individual experiments.

However, both compounds **15** and **13** were less potent than the reference compound **5** at enhancing ^{86}Rb outflow from prelabeled pancreatic islets.[16]

To further investigate the mechanism of action of compound **15**, we characterized its effect on the ^{45}Ca outflow response to high extracellular K^+ concentration. As previously reported,[27] a sudden rise in the extracellular concentration of K^+ (from 5 to 50 mM) induced an immediate and marked increase in ^{45}Ca outflow from prelabeled and perifused rat pancreatic islets (Figure 3, lower panel). Compound **15** (50 μM), when present in the basal medium, did not affect the cationic response to high K^+ (Figure 3, lower panel). Indeed, the integrated outflow of ^{45}Ca observed during exposure to 50 mM K^+ and corrected for basal value averaged 1.09 ± 0.10%/min in the absence and 0.99 ± 0.08%/min in the presence of compound **15** throughout ($P > 0.05$).

These radioisotopic experiments conducted on prelabeled and perifused rat pancreatic islets suggest that

Table 3. Effects of Selected 6-Halogeno-2-alkylaminoquinazolin-4(3*H*)-ones on the Contractile Activity of 30 mM and 80 mM K+-Depolarized Rat Aorta Incubated in the Absence or the Presence of 1 and 10 μM Glibenclamide: Comparison with Pinacidil and Diazoxide



			myorelaxant activity on rat aorta			
			IC_{50} 30 mM KCl ± SEM (n)[a]			
compd	X	R		1 μM glib.	10 μM glib.	IC_{50} 80 mM KCl ± SEM (*n*)[b]
13	Cl	$CH(CH_3)_2$	17.3 ± 5.9 (6)	ND	ND	34.3 ± 5.1 (8)
17	Cl	$CH(CH_3)C_6H_{11}$ (*R*)	7.6 ± 1.5 (8)	>30 (7)[c]	>30 (5)[c]	18.1 ± 4.4 (6)
22	I	$CH(CH_3)_2$	12.1 ± 2.3 (12)	37.3 ± 7.4 (6)	46.4 ± 7.4 (6)	37.2 ± 3.8 (7)
Diazoxide[d]			19.3 ± 1.5 (21)	85.8 ± 22.2 (6)	163 ± 41 (6)	>300 (6)
Pinacidil[d]			0.5 ± 0.1 (28)	10.4 ± 1.1 (8)	42.5 ± 2.8 (8)	16.0 ± 1.4 (8)

[a] IC_{50}: drug concentration (μM) giving 50% relaxation of the 30 mM KCl-induced contraction of rat aorta rings incubated in the absence of glibenclamide, in the presence of 1 μM glibenclamide (glib.), and in the presence of 10 μM glibenclamide. [b] IC_{50}: drug concentration (μM) giving 50% relaxation of the 80 mM KCl-induced contraction of rat aorta rings. [c] The compound precipitates at 100 μM. [d] Published results: ref 29.

the primary effect of compound **15** is to activate the K_{ATP} channels equipping the pancreatic B-cell plasma membrane. Indeed, the drug accelerated ^{86}Rb outflow from prelabeled rat pancreatic islets, an effect which can be interpreted as the result of an increase in the membrane K^+ permeability.[4] Moreover, this rise in ^{86}Rb outflow was completely abolished by glibenclamide, a hypoglycaemic sulfonylurea reported to close the K_{ATP} channels.[2,3,6] Last, the lack of effect of compound **15** on the increment in ^{45}Ca outflow evoked by high K^+ indicates that the drug does not directly interact at the level of voltage-sensitive Ca^{2+} channels.[27]

Eighty Millimolar KCl-Induced Contractions and Influence of Glibenclamide on the Contractile Responses of Rat Aorta. To further evaluate the possible PCOs properties of these new quinazolinones, vasorelaxant activities on K^+-depolarized rat aorta have been measured in different experimental conditions. Three drugs, the 6-chloro- and 6-iodo-2-isopropylaminoquinazolin-4(3*H*)-ones (**13**, **22**) and 6-chloro-2-[*R*-(1-cyclohexyl)ethylamino]quinazolin-4(3*H*)-one (**17**), have been selected. First, the myorelaxant potency of **13**, **17**, and **22** was examined versus diazoxide and pinacidil on rat aorta rings precontracted with a 80 mM KCl solution. Under such experimental conditions, the vasodilator efficiency of pure K^+ channel openers must theoretically be suppressed or at least significantly reduced. Conversely, drugs interfering directly at the level of Ca^{2+} channels, such as calcium entry blockers, are expected to exhibit identical myorelaxant activity on 30 and 80 mM KCl-precontracted aorta rings.[28]

As reported in Table 3, the myorelaxant activities of **13**, **17**, and **22** were 2 to 3 times less pronounced when a 80 mM KCl solution was used for eliciting aorta ring contraction (30 vs 80 mM KCl; $p < 0.05$). By contrast, the myorelaxant activity of pinacidil and diazoxide was more markedly reduced under these experimental conditions.

Compounds **17** and **22** were also tested on 30 mM K^+-depolarized rat aorta rings incubated in the absence and in the presence of 1 or 10 μM glibenclamide, a blocker of K_{ATP} channels[2,3,6,13] (Table 3). For the two reference molecules as for **22**, a dose-dependent decrease of the myorelaxant efficiency was observed when increasing the glibenclamide concentration in the bathing medium. When calculating the ED_{50} ratio for experiments conducted in the absence and the presence of 10 μM glibenclamide, we found that the hypoglycaemic sulfonylurea displaced the concentration−response curve 3.8-fold for **22**, 8.4-fold for diazoxide, and 212.5-fold for pinacidil. For compound **17**, the myorelaxant activity was also found to be less pronounced in the presence of glibenclamide. Unfortunately, the IC_{50} values could not be calculated due to the poor solubility of the drug in physiological medium over 30 μM.

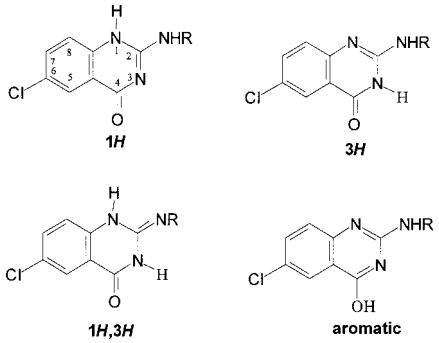


Figure 4. Possible tautomeric forms of the 6-substituted 2-alkylaminoquinazolin-4-ones.

All together, and compared to the reference molecules, such observations do not support the view that these quinazolinones act as pure potassium channel openers. Other mechanism(s) of action, including a direct interference at the level of voltage-sensitive Ca^{2+} channels, could mediate the vasorelaxant properties of such compounds.

Crystallography. According to the possible delocalization of the C═N double bond in the heterocyclic ring system and independently of the orientation of the hydrocarbon side chain, the 2-alkylaminoquinazolinones could theoretically exist in four different tautomeric forms: the 1*H*-, the 3*H*-, the 1*H*,3*H*-, and the aromatic form (Figure 4). The knowledge of the conformational aspects associated to this kind of ring system could help the identification of the most probable conformation adopted by the bioactive molecule in solution or during its interaction with its binding site. Assuming that

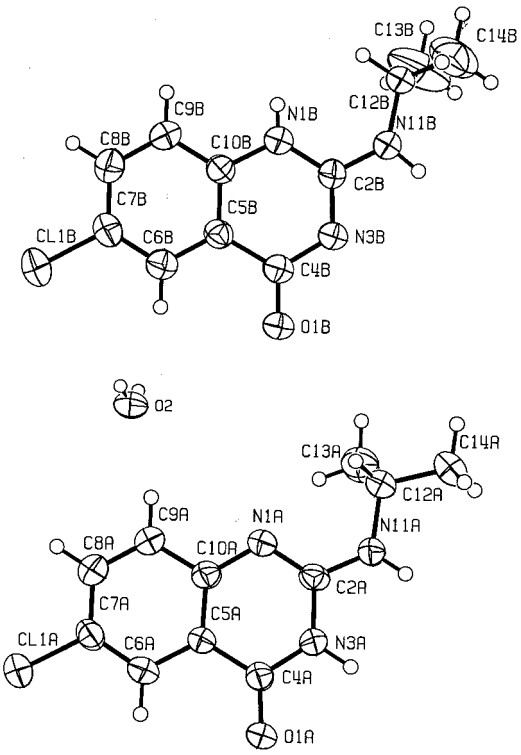


Figure 5. Molecular structure of **13** (molecule A/conformer II and molecule B/conformer I) with atom-labeling scheme. Thermal ellipsoids are shown at 50% probability levels. H atoms are drawn as small circles of arbitrary units.

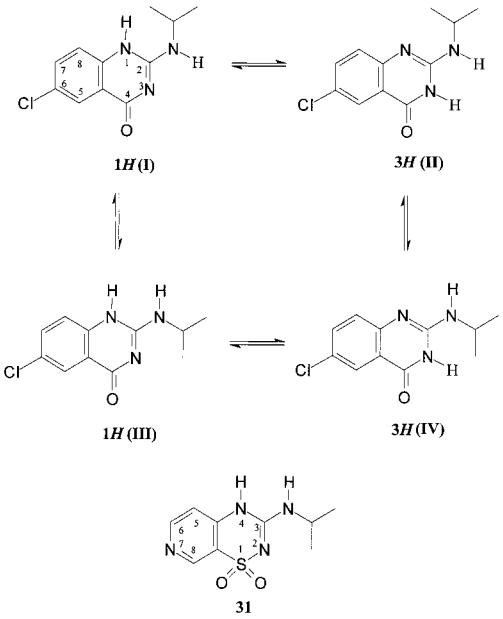


Figure 6. Four possible orientations of the hydrocarbon side chain of the 1*H*- and 3*H*-tautomeric forms of 6-chloro-2-isopropylaminoquinazolin-4-one: conformation 1*H* (I) and 3*H* (II) are found in the solid state. The figure also includes (bottom) the conformation of 3-isopropylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**31**) in the solid state.

crystallographic data on 2-alkylaminoquinazolinones can provide information to determine the most probable tautomer, we tried to establish the X-ray structure of 6-chloro-2-isopropylaminoquinazolin-4(3*H*)-one (**13**). The X-ray data of **13** were collected and compared to those of diazoxide[30] and 3-isopropylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxide (**31**).[15]

It was previously reported that, in the solid state, diazoxide and **31** adopted the same 4*H*-tautomeric form.[15] For both drugs, the N(2)−C(3) length was shorter than the N(4)−C(3) length, supporting the view that the C=N double bond of the thiadiazine ring was located in the 2,3-positions (numbering, see Figure 6). By contrast, X-ray analysis of **13** demonstrated that the drug was present, in the crystal, in two different tautomeric forms (Figure 5). Each asymmetric unit contains one conformer (I) where N(3)−C(2) (1.336 Å) is shorter than N(1)−C(2) (1.351 Å) (1*H*-tautomer), and a second conformer (II) where the N(3)−C(2) (1.381 Å) is longer than the N(1)−C(2) length (1.322 Å) (3*H*-tautomer) (numbering, see Figure 6). It must be emphasized that the H(N) hydrogen positions were located by Fourier-difference synthesis, and included in the refinement. The scheme of hydrogen bonds in the structure confirms the existence of the 1*H*- and the 3*H*-tautomers in the asymmetric unit. Moreover, the spatial orientation taken by the alkylamino side chain in each tautomer of **13** was different from that found in **31**. In the latter case, the two N−H hydrogen atoms located on the exocyclic and on the N(4) nitrogen atoms adopted a spatial orientation quite similar to that of the H atoms of the two cyanoguanidinic N−H groups of pinacidil in the solid state[31] (Figure 6).

The elemental analysis of **13** suggested the presence of a half water molecule of crystallization. This observation was corroborated by X-ray crystallography (Figure 5).

Although compound **13** does not present, in the solid state, the same side chain orientation as its pyridothiadiazine dioxide counterpart **31**, it cannot be excluded that, in solution, the drug adopts, at least in part, the 1*H*(III)-conformation (see Figure 6). Such a conformation could explain the pharmacological profile of **13** as a PCO structurally related to diazoxide and pyridothiadiazine dioxides.

Conclusions

6-Halogeno-substituted 2-alkylaminoquinazolin-4(3*H*)-ones expected to be structural analogues of 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides were synthesized and tested on distinct pharmacological models. Except the 2-benzylamino-substituted derivatives **19** and **26**, most of the new compounds were found to express a marked inhibitory activity on insulin release. On pancreatic B-cells, the 6-chloro-substituted molecules were generally more potent than the other compounds and, in this series, the *S*-enantiomers were found to be slightly more active than their *R*-counterparts. When investigated on three different smooth muscle preparations, most of the quinazolinones also showed myorelaxant activities.

Taken as a whole, these results suggest that most quinazolinones synthesized in the present study are not tissue selective in contradistinction to their pyridothiadiazine dioxides isosteres. Such isosteres were previously described as being more active on insulin secreting cells than on smooth muscle cells.[15]

Experiments conducted to characterize the mechanism of action of selected quinazolinones revealed that,

on pancreatic B-cells, compound **15** behaved as a K_{ATP} channel opener. This compound, however, was less potent than its pyridothiadiazine isostere (**5**[16]) to activate K_{ATP} channels and to inhibit insulin release. By contrast, the pharmacological profile of quinazolinones suggest that their vasorelaxant properties result from a more complex mechanism of action.

Last, X-ray crystallography analysis indicated that quinazolinone (**13**) appeared to adopt, in the solid state, a double conformation. The crystallographic data only suggested a partial analogy between the 3-alkylamino-4*H*-pyrido[4,3-*e*]-1,2,4-thiadiazine 1,1-dioxides and the 2-alkylaminoquinazolin-4(3*H*)-ones.

In conclusion, the newly synthesized quinazolinones inhibit the glucose-induced insulin release and depress the contractile activity of vascular and gastrointestinal smooth muscle. However, and compared to their pyridothiadiazine dioxides isosteres, these compounds lack tissue selectivity. Further investigation is required to fully elucidate the mechanism(s) of action of this new family of compounds.

Experimental Section

Chemistry. The reactions were monitored by TLC using precoated aluminum-backed plates (Kieselgel HF_{254} type 60, Merck). Melting points are uncorrected and were determined on a Büchi-Tottoli capillary apparatus. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1750 FT-spectrophotometer using Spectrum for Windows version 1.5. The ^{1}H NMR spectra were taken on a Brucker AW-80 (80 MHz) in DMSO-d_6. Chemical shifts are reported in δ units (ppm) with HMDS as an internal standard. The abbreviations s = singlet, d = doublet, t = triplet, m = multiplet, and b = board are used throughout. Elemental analyses were realized on a Carlo-Erba EA 1108 elemental analyzer.

6-Chloro-1,2-dihydro-2-thioxoquinazolin-4(3*H*)-one (10a). A suspension of 5-chloroanthranilic acid (**9a**) (10 g, 0.058 mol) in thionyl chloride (40 mL) was heated at reflux for 1.5 h. After removal of the excess of thionyl chloride under reduced pressure, the residue was treated three times by dissolution in CH_2Cl_2 (20 mL) and concentration. The crude acid chloride was dissolved in acetone (10 mL), and this solution was added, under stirring, to a solution of ammonium isothiocyanate (4.5 g, 0.060 mol) in acetone (20 mL). The reaction mixture was stirred at room temperature for 30 min. The resulting precipitate was collected by filtration and dissolved in 10% w/v NaOH in water (50 mL). The solution was treated with charcoal and filtered. The filtrate was conducted to pH 2 with 2 N HCl, and the final product (**9**), which precipitated, was collected by filtration, washed with water, and dried (yield 6.54 g, 53%). Mp: >300 °C. IR (KBr): 3078 (N−H), 1707 (C=O), 1619, 1562, 1462 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 7.30 (d, 1H, 8-*H*), 7.70 (d, 1H, 7-*H*), 7.75 (s, 1H, 5-*H*), 12.60 (bs, 1H, N*H*). Anal. ($C_8H_5ClN_2OS$): C, H, N, S.

6-Iodo-1,2-dihydro-2-thioxoquinazolin-4(3*H*)-one (10b). The title compound was obtained from 5-iodoanthranilic acid (**9b**) (15 g, 0.057 mol) by following the same experimental conditions as for 6-chloro-1,2-dihydro-2-thioxoquinazolin-4(3*H*)-one (**9**) (yield 13.17 g, 76%). Mp: >300 °C. IR (KBr): 3105 (N−H), 1698 (C=O), 1613, 1552, 1459 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 7.10 (d, 1H, 8-*H*), 7.90 (d, 1H, 7-*H*), 8.05 (s, 1H, 5-*H*), 12.55 (bd, 1H, N*H*). Anal. ($C_8H_5IN_2OS$): C, H, N, S.

6-Chloro-2-methylsulfanylquinazolin-4(3*H*)-one (11). To a solution of **10a** (5 g, 0.023 mol) in 1% w/v NaOH in water (50 mL) were added methyl iodide (5 mL) and methanol (50 mL). The reaction mixture was stirred at room temperature for 1 h. The resulting suspension was adjusted to pH 7 with 1 N HCl, and methanol was removed under reduced pressure. After cooling, the title product, which was in suspension, was collected by filtration, washed with water, and dried (4.8 g, yield 90%). Mp: 219−221 °C. IR (KBr): 3154 (N−H), 1673 (C=O), 1605, 1581, 1465 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 2.45 (s, 3H, SCH_3), 7.45 (d, 1H, 8-*H*), 7.70 (dd, 1H, 7-*H*), 7.85 (d, 1H, 5-*H*). Anal. ($C_9H_7ClN_2OS$): C, H, N, S.

6-Iodo-2-methylsulfanylquinazolin-4(3*H*)-one (12). The title compound was obtained from 6-iodo-1,2-dihydro-2-thioxoquinazolin-4(3*H*)-one (**10b**) (7 g, 0.023 mol) by following the same experimental conditions as described for 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (yield 4.61 g, 63%). Mp: 253−255 °C. IR (KBr): 3149 (N−H), 1677 (C=O), 1580, 1547, 1457 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 2.45 (s, 3H, SCH_3), 7.25 (d, 1H, 8-*H*), 7.90 (d, 1H, 7-*H*), 8.15 (s, 1H, 5-*H*), 12.40 (bs, 1H, N*H*). Anal. ($C_9H_7IN_2OS$): C, H, N, S.

6-Chloro-2-isopropylaminoquinazolin-4(3*H*)-one Hemihydrate (13). A solution of 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) in isopropylamine (7 mL) was heated in an autoclave at 150 °C for 5 h. The excess of amine was removed under reduced pressure and the residue was suspended in water (20 mL). The insoluble material was dissolved by addition of a 10% w/v aqueous solution of NaOH. After treatment with activated charcoal and filtration, the filtrate was adjusted to pH 5 with formic acid. The title compound was collected by filtration, washed with water, and dried (yield 0.36 g, 67%). Mp: 227−229 °C. IR (KBr): 3240 (N−H), 1671 (C=O), 1623, 1596, 1475 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 1.10 (d, 6H, CH(CH_3)$_2$), 4.00 (m, 1H, C*H*(CH$_3$)$_2$), 6.20 (m, 1H, N*H*CH(CH$_3$)$_2$), 7.20 (d, 1H, 8-*H*), 7.50 (dd, 1H, 7-*H*), 7.70 (d, 1H, 5-*H*), 10.80 (bs, 1H, N*H*). Anal. ($C_{11}H_{12}ClN_3O\cdot{}^1/_2H_2O$): C, H, N.

6-Chloro-2-*R,S*-(2-butylamino)quinazolin-4(3*H*)-one Monohydrate (14). The title compound was obtained as described for **13** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) and 2-butylamine (10 mL) (yield 0.33 g, 55%). Mp: 200−202 °C. IR (KBr): 3238 (N−H), 1672 (C=O), 1623, 1599, 1474 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.80 (t, 3H, CH(CH$_3$)CH$_2$CH_3), 1.05 (d, 3H, CH(CH_3)CH$_2$CH$_3$), 1.45 (q, 2H, CH(CH$_3$)CH_2CH$_3$), 3.80 (m, 1H, C*H*(CH$_3$)CH$_2$CH$_3$), 6.10 (bd, 1H, N*H*CH(CH$_3$)), 7.15 (d, 1H, 8-*H*), 7.50 (dd, 1H, 7-*H*), 7.70 (d, 1H, 5-*H*), 10.65 (bs, 1H, N*H*). Anal. ($C_{12}H_{14}ClN_3O\cdot H_2O$): C, H, N.

6-Chloro-2-*R,S*-(3-methyl-2-butylamino)quinazolin-4(3*H*)-one Monohydrate (15). The title compound was obtained as described for **13** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) and 3-methyl-2-butylamine (10 mL), except that the reaction mixture was heated at 120 °C for 15 h (yield 0.35 g, 56%). Mp: 207−208 °C. IR (KBr): 3233 (N−H), 1675 (C=O), 1623, 1600, 1474 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.80 (d, 6H, CH(CH$_3$)-CH(CH_3)$_2$), 1.00 (d, 3H, CH(CH_3)CH(CH$_3$)$_2$), 1.70 (m, 1H,CH-(CH$_3$)C*H*(CH$_3$)$_2$), 3.80 (m, 1H, C*H*(CH$_3$)CH(CH$_3$)$_2$), 6.10 (b, 1H, N*H*CH(CH$_3$)), 7.20 (d, 1H, 8-*H*), 7.50 (dd, 1H, 7-*H*), 7.70 (d, 1H, 5-*H*), 10.80 (bs, 1H, N*H*). Anal. ($C_{13}H_{16}ClN_3O\cdot H_2O$): C, H, N.

6-Chloro-2-[(cyclohexyl)methylamino)]quinazolin-4(3*H*)-one (16). A solution of 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.4 g, 1.8 mmol) and cylcohexylamine (0.4 mL) in 3-chlorotoluene (4 mL) was heated under reflux for 1.5 h. After cooling, the reaction mixture was supplemented with cyclohexane (20 mL). The crude precipitate was collected by filtration, washed with cyclohexane and dried. This precipitate was suspended in a mixture of water and ethanol (1:1), and a 10% w/v solution of NaOH in water was added until a near complete solubilization occurred. After treatment with charcoal and filtration, the filtrate was adjusted to pH 4−5 with 2 N HCl. The precipitate was collected by filtration, washed with water, and dried (yield 0.26 g, 50%). Mp: 278−279 °C. IR (KBr): 3234 (N−H), 1675 (C=O), 1623, 1549, 1474 (C=N, C=C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.65−1.90 (m, 11H, C_6H_{11}), 3.10 (m, 2H, C$H_2C_6H_{11}$), 6.20 (b, 1H, N*H*C$H_2C_6H_{11}$), 7.10 (d, 1H, 8-*H*), 7.45 (dd, 1H, 7-*H*), 7.70 (d, 1H, 5-*H*), 10.70 (bs, 1H, N*H*). Anal. ($C_{15}H_{18}ClN_3O$): C, H, N.

6-Chloro-2-[*R*-(1-cyclohexyl)ethylamino]quinazolin-4(3*H*)-one Monohydrate (17). The title compound was obtained as described for **16** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) and *R*-1-

cyclohexylethylamine (0.5 mL). The reaction mixture was heated at reflux for 3 h (yield 0.32 g, 45%). Mp: 242−244 °C. IR (KBr): 3360 (N−H), 1660 (C═O), 1628, 1521, 1462 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.75−1.90 (bm, 11H, C_6H_{11}), 1.00 (d, 3H,CH(CH_3)C_6H_{11}), 3.85 (m, 1H, C*H*(CH_3)-C_6H_{11}), 6.15 (bd, 1H, N*H*CH(CH_3)C_6H_{11}), 7.15 (d, 1H, 8-*H*), 7.50 (d, 1H, 7-*H*), 7.75 (s, 1H, 5-*H*), 10.65 (bs, 1H, N*H*). Anal. ($C_{16}H_{20}ClN_3O\cdot H_2O$): C, H, N.

6-Chloro-2-[*S*-(1-cyclohexyl)ethylamino]quinazolin-4(3*H*)-one Monohydrate (18). The title compound was obtained as described for **16** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.4 g, 1.8 mmol) and *S*-1-cyclohexylethylamine (0.5 mL). The reaction mixture was heated at reflux for 3 h (yield 0.31 g, 55%). Mp: 233−236 °C. IR (KBr): 3265 (N−H), 1660 (C═O), 1628, 1521, 1463 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.75−1.90 (bm, 11H, C_6H_{11}), 1.00 (d, 3H,CH(CH_3)C_6H_{11}), 3.85 (m, 1H, C*H*(CH_3)-C_6H_{11}), 6.35 (bd, 1H, N*H*CH(CH_3)C_6H_{11}), 7.20 (d, 1H, 8-*H*), 7.50 (d, 1H, 7-*H*), 7.75 (s, 1H, 5-*H*). Anal. ($C_{16}H_{20}ClN_3O\cdot H_2O$): C, H, N.

6-Chloro-2-benzylaminoquinazolin-4(3*H*)-one (19). The title compound was obtained as described for **16** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) and benzylamine (0.5 mL). The reaction mixture was heated at reflux for 1.5 h (yield 0.36 g, 57%). Mp: 254−256 °C. IR (KBr): 3357 (N−H), 1666 (C═O), 1625, 1520, 1462 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 4.45 (d, 2H, C$H_2C_6H_5$), 6.80 (m,1H, N*H*CH_2), 7.05−7.40 (m, 6H,C_6H_5 + 8-*H*) 7.45 (dd, 1H, 7-*H*), 7.70 (d, 1H, 5-*H*). Anal. ($C_{15}H_{12}ClN_3O$): C, H, N.

6-Chloro-2-[*R*-(1-phenyl)ethylamino]quinazolin-4(3*H*)-one Hemihydrate (20). The title compound was obtained as described for **16** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) and *R*-1-(phenyl)ethylamine (0.5 mL). The reaction mixture was heated at reflux for 1.5 h (yield 0.27 g, 40%). Mp: 233−235 °C. IR (KBr): 3260 (N−H), 1677 (C═O), 1626, 1572, 1472 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 1.40 (d, 3H, CH(CH_3)C_6H_5), 5.10 (bm, 1H, C*H*(CH_3)C_6H_5), 6.80 (bd, 1H, N*H*CH(CH_3)), 7.10−7.60 (m, 7H, C_6H_5 + 8-*H* + 7-*H*), 7.70 (d, 1H, 5-*H*). Anal. ($C_{16}H_{14}ClN_3O\cdot {}^1/_2H_2O$): C, H, N.

6-Chloro-2-[*S*-(1-phenyl)ethylamino]quinazolin-4(3*H*)-one Monohydrate (21). The title compound was obtained as described for **16** starting from 6-chloro-2-methylsulfanylquinazolin-4(3*H*)-one (**11**) (0.5 g, 2.2 mmol) and *S*-1-(phenyl)ethylamine (0.5 mL). The reaction mixture was heated at reflux for 1.5 h (yield 0.30 g, 43%). Mp: 222−224 °C. IR (KBr): 3262 (N−H), 1675 (C═O), 1630, 1548, 1471 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 1.40 (d, 3H, CH(CH_3)C_6H_5), 5.10 (bm, 1H, C*H*(CH_3)C_6H_5), 6.80 (bd, 1H, N*H*CH(CH_3)), 7.10−7.50 (m, 6H, C_6H_5 + 8-*H*), 7.60 (dd, 1H, 7-*H*), 7.70 (bd, 1H, 5-*H*). Anal. ($C_{16}H_{14}ClN_3O\cdot H_2O$): C, H, N.

6-Iodo-2-isopropylaminoquinazolin-4(3*H*)-one (22). A solution of 6-iodo-2-methylsulfanylquinazolin-4(3*H*)-one (**12**) (0.5 g, 1.6 mmol) in isopropylamine (7 mL) was heated in an autoclave at 150 °C for 5 h. The excess of amine was removed under reduced pressure, and the residue was triturated with water (35 mL). The resulting precipitate was dissolved in a 10% w/v aqueous solution of NaOH. After clarification with activated charcoal and filtration, the filtrate was adjusted to pH 5 with formic acid. The title compound was collected by filtration, washed with water, dried, and recrystallized from hot methanol (yield 0.21 g, 41%). Mp: 258−260 °C. IR (KBr): 3255 (N−H), 1681 (C═O), 1625, 1591, 1466 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 1.10 (d, 6H, CH(CH_3)$_2$), 4.00 (m, 1H, C*H*(CH_3)$_2$), 6.10 (bs, 1H, N*H*CH(CH_3)$_2$), 7.00 (bd, 1H, 8-*H*), 7.70 (bd, 1H, 7-*H*), 8.05 (bs, 1H, 5-*H*), 10.6 (bs, 1H, N*H*). Anal. ($C_{11}H_{12}IN_3O$): C, H, N.

6-Iodo-2-*R*,*S*-(2-butylamino)quinazolin-4(3*H*)-one Monohydrate (23). The title compound was obtained as described for **22** starting from 6-iodo-2-methylsulfanylquinazolin-4(3*H*)-one (**12**) (0.5 g, 1.6 mmol) and 2-butylamine (10 mL) (yield 0.25 g, 44%). Mp: 235−236 °C. IR (KBr): 3264 (N−H), 1679 (C═O), 1632, 1546, 1467 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, 3H, CH(CH_3)CH_2CH_3), 1.10 (d, 3H, CH(CH_3)CH_2-CH_3), 1.50 (m, 2H, CH(CH_3)C$H_2$$CH_3$), 4.00 (m, 1H, C*H*($CH_3$)$CH_2$-$CH_3$), 7.20 (d, 1H, 8-*H*), 7.50 (bm, 1H, N*H*CHCH$_3$), 7.85 (d, 1H, 7-*H*), 8.10 (s, 1H, 5-*H*). Anal. ($C_{12}H_{14}IN_3O\cdot H_2O$): C, H, N.

6-Iodo-2-*R*,*S*-(3-methyl-2-butylamino)quinazolin-4(3*H*)-one (24). A solution of 6-iodo-2-methylsulfanylquinazolin-4(3*H*)-one (**12**) (0.5 g, 1.6 mmol) in 3-methyl-2-butylamine (7 mL) was heated in an hermetically closed autoclave at 140 °C for 3 h. The excess of amine was removed by distillation under reduced pressure. The crude product was mixed with fresh amine (5 mL) and heated in an autoclave at 140 °C for 6 h. The excess reactant was removed by distillation under reduced pressure. The residue was triturated with water (20 mL). The resulting suspension was supplemented with a 10% w/v aqueous solution of NaOH until dissolution. After clarification with activated charcoal and filtration, the solution was adjusted to pH 5 with formic acid. The title compound was collected by filtration, washed with water, and dried (yield 0.25 g, 45%). Mp: 219−221 °C. IR (KBr): 3397 (N−H), 1691 (C═O), 1625, 1598, 1466 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.85 (d, 6H, CH(CH_3)CH(CH_3)$_2$), 1.00 (d, 3H, CH(CH_3)CH-(CH_3)$_2$), 1.65 (m,1H, CH(CH_3)C*H*(CH_3)$_2$), 3.80 (m,1H, C*H*(CH_3)-CH(CH_3)$_2$), 6.10 (bd, 1H, N*H*CH(CH_3)), 6.95 (d, 1H, 8-*H*), 7.70 (dd, 1H, 7-*H*), 8.00 (d, 1H, 5-*H*), 10.60 (bs, 1H, N*H*). Anal. ($C_{13}H_{16}IN_3O$): C, H, N.

6-Iodo-2-[(cyclohexyl)methylamino]quinazolin-4(3*H*)-one (25). A solution of 6-iodo-2-methylsulfanylquinazolin-4(3*H*)-one (**12**) (0.8 g, 2.5 mmol) and cylcohexylamine (1.6 mL) in *m*-chlorotoluene (8 mL) was heated at reflux for 1.5 h. After cooling, the reaction mixture was supplemented with cyclohexane (20 mL). The crude precipitate was collected by filtration, washed with cyclohexane and dried. The solid was dissolved in a hydromethanolic (1:1) solution of 5% w/v NaOH. After treatment with activated charcoal and filtration, the solution was adjusted to pH 5 with formic acid. (yield 0.38 g, 40%). Mp: 269−271 °C. IR (KBr): 3364 (N−H), 1689 (C═O), 1625, 1523, 1459 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 0.75−1.90 (bm, 11H, C_6H_{11}), 3.10 (t, 2H, C$H_2C_6H_{11}$), 6.40 (bt, 1H, N*H*C$H_2C_6H_{11}$), 6.95 (d, 1H, 8-*H*), 7.70 (bd, 1H, 7-*H*), 8.05 (bs, 1H, 5-*H*), 10.40 (bs, 1H, N*H*). Anal. ($C_{15}H_{18}IN_3O$): C, H, N.

6-Iodo-2-benzylaminoquinazolin-4(3*H*)-one (26). The title compound was obtained as described for **25** starting from 6-iodo-2-methylsulfanylquinazolin-4(3*H*)-one (**12**) (0.8 g, 2.5 mmol) and benzylamine (1.5 mL). The reaction mixture was heated at reflux for 1.5 h (yield 0.42 g, 45%). Mp: 274−276 °C. IR (KBr): 3424 (N−H), 1691 (C═O), 1627, 1599, 1465 (C═N, C═C, N−H). ^{1}H NMR (DMSO-d_6) δ: 4.50 (m, 2H, C$H_2C_6H_5$), 6.80 (m,1H, N*H*CH_2), 7.00 (d,1H, 8-*H*), 7.50 (m, 5H, C_6H_5), 7.70 (bd, 1H, 7-*H*), 8.10 (bs, 1H, 5-*H*), 10.90 (bs, 1H, N*H*). Anal. ($C_{15}H_{12}IN_3O$): C, H, N.

Determination of the Optical Purity. Four 6-chloroquinazolin-4-ones bearing an asymmetric carbon atom on the side chain in the 2-position (**17**, **18**, **20**, **21**) were obtained by reaction of 6-chloro-2-methylsulfanylquinazolin-4-one (**11**) with pure *R*- and *S*-(1-cyclohexyl)ethylamine or (1-phenyl)ethylamine commercially available from Sigma-Aldrich. To control the absence of epimerization reactions, the purity of the final products was assessed by high-pressure liquid chromatography on a Merck-Hitachi apparatus (L6000 pump, L4000 UV detector, and T2000 integrator) equipped with a CHIRALCEL OD-R column. Chromatographic conditions were as follows: mobile phase, 50 mM KPF_6 in water/acetonitrile (in %: 40/60 for **17**, **18** and 45/55 for **20**, **21**); flow rate, 0.5 mL/min; temperature, 25 °C; sample concentration, 0.2 mg/mL; injection volume, 5.0 μL; detection, UV at 225 nm. Results expressed as the enantiomeric excesses were calculated from the following equation: for the *R*-isomer: ee % = [(peak surface of *R* − peak surface of *S*)/(peak surface of *R* + peak surface of *S*)]; for the *S*-isomer: ee % = [(peak surface of *S* − peak surface of *R*)/(peak surface of *R* + peak surface of *S*)]. Results reported are the means of two individual determinations.

Biological assays. Measurements of Insulin Release from Incubated Rat Pancreatic Islets. All experiments were performed with islets isolated from fed Wistar rats (180−

220 g). Groups of 10 islets, each derived from the same batch of islets, were preincubated for 30 min at 37 °C in 1 mL of a physiological salt medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialyzed albumin and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). The islets were incubated at 37 °C for a further 90 min in 1 mL of the same medium containing 16.7 mM glucose and, in addition, the quinazolinone derivatives. The release of insulin was measured radioimmunologically using rat insulin as a standard.[32]

Measurements of Contractile Activity in Rat Aorta. All experiments were performed in aorta removed from Fed Wistar rats (body weight 250−300 g). A section of the aorta was cleared of adhering fat and connective tissue and was cut into transverse rings (3−4 mm long). The endothelium was removed by rubbing the intimal surface with forceps. The segments were suspended under 2 g of tension by means of steel hooks in an organ bath containing 20 mL of Krebs-bicarbonate-buffered solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5. The physiological solutions were maintained at 37 °C and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The isometric contractions of the aortic rings were measured with a force-displacement transducer. After 60 min of equilibration, the rings were exposed to 30 mM or 80 mM KCl. When the tension had stabilized, the drugs were added at increasing concentrations until maximal relaxation. The same experiments were repeated in the presence of glibenclamide throughout (1 and 10 μM). The relaxation response was expressed as the percentage of the contractile response to KCl. The IC_{50} value was graphically assessed for each dose response curve as the concentration evoking 50% inhibition of the plateau induced by KCl. Results were expressed as the means (± SEM) of 4−12 experiments.

Measurements of Contractile Activity in Guinea Pig Ileum. Adult guinea pigs (body weight 300−400 g) were stunned and bled. Segments of the ileum (4 cm long) were removed at least 10 cm from the caecum. They were set up under an initial load of 1 g in a Krebs-bicarbonate-buffered solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5, maintained at 37 °C and gassed with a mixture of O_2 (95%) and CO_2 (5%). Coaxial stimulation was carried out as described by Paton[33] with rectangular pulses of 0.5 ms duration, 0.1 Hz, 5−25 V. Muscle contractions were recorded isometrically. The inhibitory effects of drugs were assessed on electrically induced contractions by adding increasing concentrations until maximal effect. Results were expressed as percentage of control responses (measured during 5 min before adding the drug). IC_{50} values (means ± SEM) were graphically assessed.

Measurements of Contractile Activity in Rat Uterus. Fed Wistar rats (body weight 150−200 g) were treated the day before killing with diethylstilboestrol dipropionate [i.m. injection of 0.1 mL/100 g of a 1 mg/mL oily solution of diethylstilboestrol dipropionate (Sigma)]. The rats were anesthetized and then sacrified. The two uterine horns were removed, cleared of adhering fat and connective tissue, and separated. Each horn was superfused with a Tyrode solution (in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.1, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6) and bubbled continuously with a mixture of O_2 (95%) and CO_2 (5%). The superfusate was maintained at 37 °C. Injections of 20 mU oxytocin (200 μL of a 0.1 U/mL solution of the hormone in 0.9% NaCl) in the superfusion channel were repeated at 10 min intervals until the recorded contractions (AUC) were constant. The mean of the three last injections gave the 100% of the contractile response to oxytocin. For each drug concentration added in the medium, injection of 20 mU oxytocin was repeated at least three times. The contractile responses recorded (mean of three AUC) were expressed as a percentage of the reference value (contractile response to oxytocin in the absence of drug).

Measurements of ^{86}Rb and ^{45}Ca Outflow from Perifused Rat Pancreatic Islets. The media used for incubating, washing, and perifusing the islets consisted of a physiological salt medium supplemented with 0.5% (w/v) dialyzed albumin and gassed with O_2 (95%) and CO_2 (5%). Groups of 100 islets were incubated for 60 min in a medium containing 16.7 mM glucose and either ^{86}Rb (0.15−0.25 mM; 50 μCi/mL) or ^{45}Ca (0.02−0.04 mM; 100 μCi/mL). After incubation, the islets were washed three times and then placed in a perifusion chamber. The perifusate was delivered at a constant rate (1 mL/min). From the 31st to the 90th min, the effluent was continuously collected over successive periods of 1 min each. An aliquot of the effluent (0.6 mL) was used for scintillation counting. At the end of the perifusion, the radioactive content of the islets was also determined. The outflow of ^{86}Rb or ^{45}Ca was expressed as a fractional outflow rate (% of instantaneous islets content/min; FOR). The validity of ^{86}Rb as a substitute for ^{42}K has been previously established.[11,17]

Crystal Structure Determination of 13. Crystal was grown by slow evaporation from methanol. Diffraction data were measured at room temperature using a Stoe-Siemens AED four-circle diffractometer. Final cell dimensions were obtained by a least-squares fit to the automatically centered setting for 30 reflections ($51.23 < 2\theta < 79.70°$). Two reference reflections monitored during each experiment showed no significant variation. Intensity data were corrected for Lorentz polarization effects and for absorption (semiempirical method, ψ scan). Space group assignment was suggested by cell geometry and average values of the normalized structure factors; choice was confirmed by successful refinement.

The structure was solved by direct methods (SHELXS-97[34]). The non-hydrogen atoms were refined anisotropically (SHELXL-97[35]). H atoms have been included in the refinement as riding atoms at calculated positions except those involved in hydrogen bonds of which positions were refined. There were two independent molecules in the asymmetric unit. The final difference Fourier map had no significant features. Atomic scattering factors were taken from ref 36. The thermal ellipsoid views of **13** (molecules A and B) was undertaken with program ORTEP-III.[37]

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Supporting Information Available: Crystal data, final atomic positional parameters, bond distances and angles, atomic thermal parameters, hydrogen coordinates, torsion angles, and hydrogen bonds for compound **13** (Tables 1−7). This material is available free of charge via the Internet at http://pubs.acs.org.

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