methylamino)benzoyl)-L-glutamic acid (22b).

All free acids had correct elemental analyses (C, H, N, F) for the formulas listed in the table and NMR spectra consistent with the assigned structures.

Acknowledgment. This work was supported by grants from the Cancer Research Campaign and the Medical Research Council. We thank Professor M. Jarman for helpful discussions and Mr. M. H. Baker for determining the mass spectra.

Supplementary Material Available: 250-MHz NMR spectral data for N^{10}-propargyl (Table III) and N^{10}-methyl (Table IV) fluorine-substituted antifolate esters and acids in DMSO-d_6 (2 pages). Ordering information is given on any current masthead page.

Structure–Activity Studies of Potassium Channel Opening in Pinacidil-Type Cyanoguanidines, Nitroethenediamines, Thioureas, and Ureas

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Potassium channel opening activity for pinacidil-type cyanoguanidines, nitroethenediamines, thioureas, and ureas, has been assessed through simultaneous measurement of spontaneous contractile activity and stimulation of $^{86}Rb^+$ efflux from rat portal veins loaded with $^{86}Rb^+$. The good correlation between these two effects suggests that the vasodilator activity of the compounds is directly attributable to an increased opening of potassium channels. The resulting quantitative in vitro data has been used to analyze the structure–activity relationships for potassium channel opening, allowing the biological activity to be rationalized in terms of a pharmacophore involving a hydrogen-bond-acceptor element, a hydrogen-bond-donor element, and a lipophilic binding group. A model for the binding of pinacidil-related compounds to their potassium channel receptor has been developed, and compounds designed to test this model have been synthesized and tested. Prototropic equilibria are implicated as playing a fundamental role in determining the hydrogen-bonding ability of the compounds, and conformational changes in the receptor are invoked to explain disparities in the chiral recognition of lipophilic groups in different compounds.

Following the discovery that the antihypertensive agent pinacidil[1,2] (19; Pindac; Leo, Eli Lilly) acts like cromakalim[3] (BRL 34915, Beecham) via the opening of potassium channels[4,5] and the promising clinical trials with this latter compound in both cardiovascular indications[6,7] and asthma,[8] much interest has been aroused in this new approach to developing drugs for smooth muscle relaxation.

Potassium channels play an important role in controlling cellular membrane potential and hence, in the case of smooth muscle cells, contractility.[9,10] Pinacidil and cromakalim are thought to exert their biological effects by increasing the open probability (P_{open}) of plasmalemmal potassium channels in smooth muscle.[11-13] When these channels are in their open state, intracellular potassium effluxes along its electrochemical gradient, causing the cell membrane to become hyperpolarized until it approaches the potassium equilibrium potential. This hyperpolarization prevents depolarization of the membrane and thereby inhibits the opening of voltage-operated calcium channels (VOCs);[14] in addition the compounds inhibit other pathways leading to increased cytosolic calcium, involving mechanisms which in part may also depend on membrane potential.[12,13,15] With insufficient intracellular calcium, smooth muscle cells are unable to contract, resulting in their relaxation.

Most of the structure–activity studies reported to date for potassium channel openers have relied upon in vivo blood pressure lowering data.[16,17] Such data is often very

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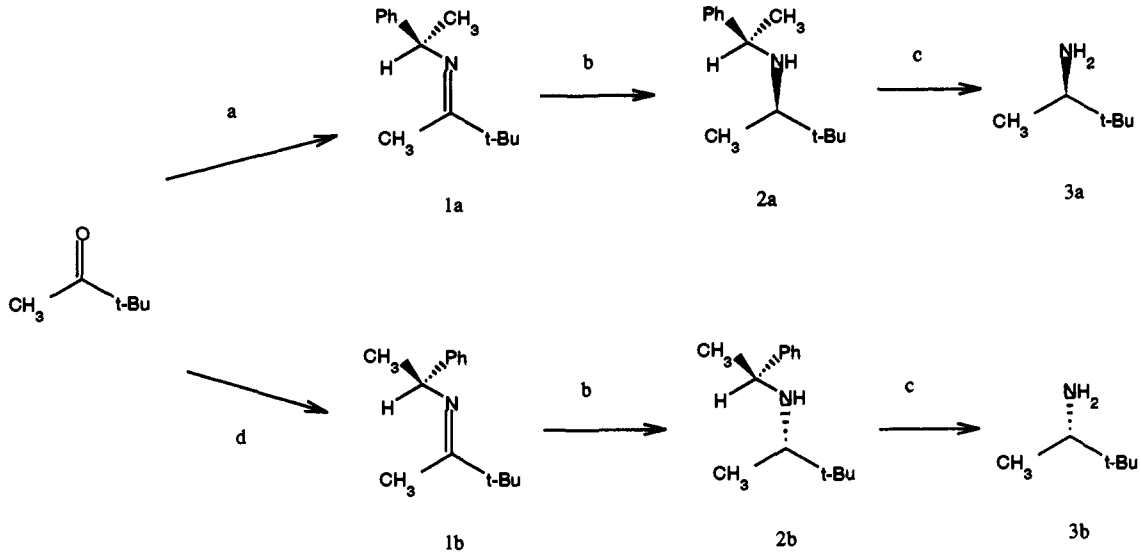
Scheme I[a]



[a] Reagents: (a) (*R*)-PhCHMeNH$_2$, *p*-TsOH, C_6H_6; (b) B_2H_6, THF; (c) Pd, H_2, EtOH; (d) (*S*)-PhCHMeNH$_2$, *p*-TsOH, C_6H_6.

unreliable for structure–activity studies, since it fails to take into account pharmacokinetic or metabolic factors, or even whether the blood pressure lowering effect is solely due to potassium channel opening. Blood pressure data is particularly unreliable for pinacidil-type compounds, since pinacidil itself is known to relax blood vessels by additional non potassium channel dependent mechanisms.[18-22] These additional mechanisms lack stereoselectivity and contribute toward vasorelaxation at concentrations much higher than those required to open potassium channels.[18] Thus recent reports indicate that racemic pinacidil, at micromolar (≥1 μM) concentrations, inhibits calcium release from intracellular stores and/or the refilling of these stores[21] and that at higher concentrations (>10 μM) it acts directly on the contractile apparatus to inhibit calcium-induced contractions.[22]

In view of these complications it is important to quantitatively assess the potency of presumed potassium channel openers through both their in vitro relaxant and potassium channel opening effects. This may be achieved through the simultaneous measurement of spontaneous activity and rubidium efflux from rat portal veins loaded with $^{86}Rb^+$, where the latter qualitatively reflects the change in potassium permeability of the smooth muscle cell membrane as induced by the potassium channel openers.[23] In this study we demonstrate that for the pinacidil class of compounds the inhibition of spontaneous myogenic activity in rat portal veins is directly correlated with the stimulation of $^{86}Rb^+$ efflux and hence potassium ion permeability. Consequently, we have been able to generate quantitative in vitro structure–activity data for potassium channel opening, analysis of which has enabled us to postulate a receptor-binding model for pinacidil-type compounds at the receptor responsible for opening these ion channels.

Synthetic Chemistry

Whereas previously reported preparations of the enantiomers of pinacidil (**19a,b**) have relied upon kinetic resolution of the D-(−)- and D-(+)-tartrates,[24] for this study the pure enantiomers of pinacidil and related compounds were synthesized from the corresponding (*R*)- and (*S*)-2-amino-3,3-dimethylbutanes (**3a,b**), prepared enantioselectively by utilizing (α)-phenethylamine as a chiral auxiliary (Scheme I).[25] Thus reaction of pinacolone with the (*R*)- and (*S*)-α-phenethylamines afforded the corresponding chiral *E*-imines (**1a,b**). Reduction of the imines with diborane in THF resulted in the addition of hydrogen to the azomethine double bond from the face opposite to that occupied by the bulky phenyl group, to give the (*R,R*)- and (*S,S*)-benzylamines (**2a,b**). Catalytic hydrogenolysis of the benzylamines afforded the optically pure (*R*)- and (*S*)-amines (**3a,b**), which were subsequently used in the preparation of the individual enantiomers of the required cyanoguanidines and diaminonitroethenes with enantiomeric excesses >99.3% (NMR).

Cyanoguanidines **19** and **29–32** were synthesized according to the method of Petersen et al.[2] as outlined in

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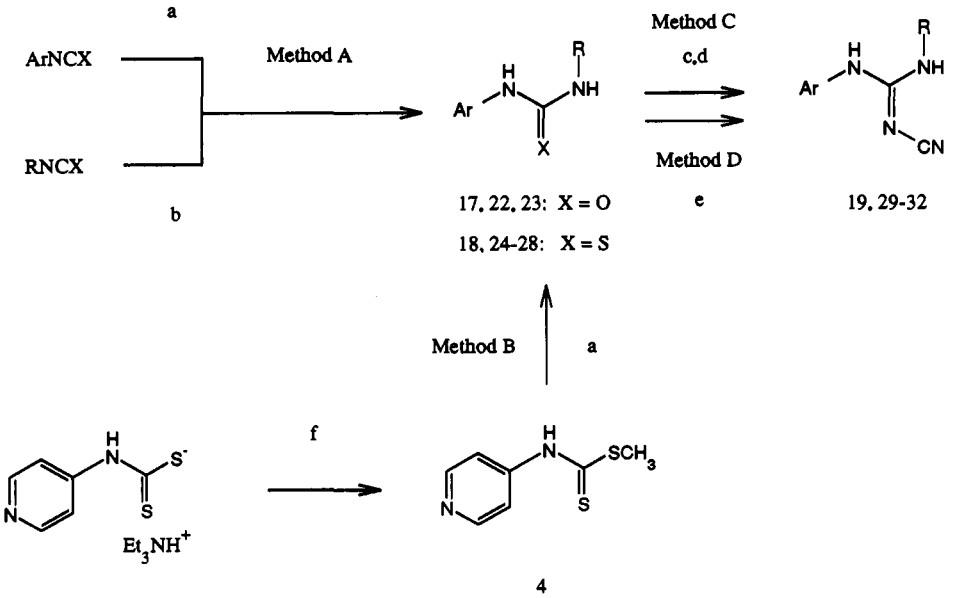
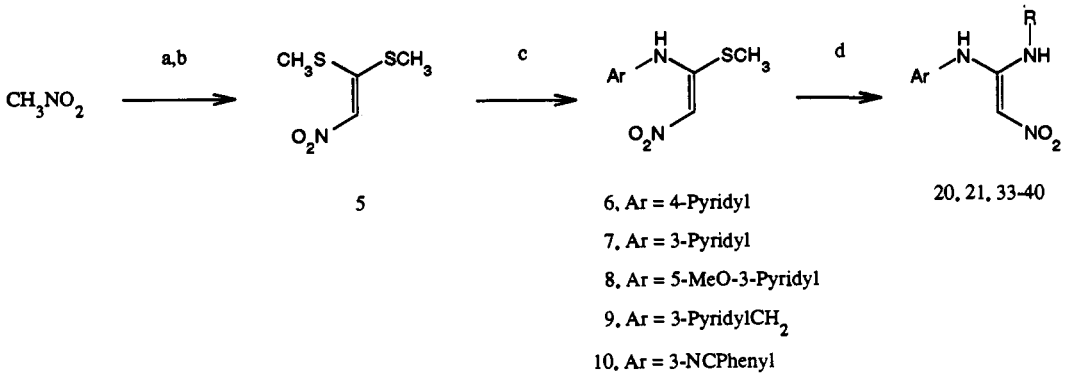
Scheme II[a]



[a] Reagents: (a) RNH_2, EtOH; (b) $ArNH_2$, EtOH; (c) PPh_3, CCl_4, Et_3N, CH_2Cl_2; (d) H_2NCN, i-Pr_2NEt; (e) H_2NCN, DCC, i-Pr_2NEt; (f) MeI, EtOH.

Scheme III[a]



[a] Reagents: (a) CS_2, KOH, i-Pr_2NEt; (b) Me_2SO_4, KOH; (c) $ArNH_2$, EtOH; (d) RNH_2, EtOH.

Scheme II. The intermediate ureas and thioureas were prepared by the addition of the relevant amines to the corresponding isocyanates or isothiocyanates (method A), with the exception of the 4-pyridylthioureas, **18a** and **18b**, which, due to the instability of 4-pyridyl isothiocyanate, were prepared via carbamodithioic ester **4**[26] (method B). Generally, the ureas and thioureas were then converted into the desired cyanoguanidines via the intermediate carbodiimides, prepared by the method of Appel,[27] involving reaction with triphenylphosphine and carbon tetrachloride in the presence of triethylamine, and these were directly reacted with cyanamide (method C). However, for thioureas **18a** and **18b**, direct activation by dicyclohexylcarbodiimide with in situ addition of cyanamide proved advantageous (method D).[28]

Nitroethenediamines **20**, **21**, and **33–40** were prepared by the method of Niemers et al.,[29] utilizing a Michael addition of the relevant amine to the *E*-*N*-aryl-1-(methylthio)-2-nitroethenamines (**6–10**; Table II), with concomitant elimination of methanethiol (Scheme III, method E). Although this reaction was facile for unhindered amines, those having bulky, tertiary-alkyl groups α to the amine failed to react even under forcing conditions.

Cyanoamides **41** and **42** were synthesized using the method of Kupchik and Hanke,[30] utilizing the reaction of bis(triphenylstannyl)diimide with thioamides **12** and **14**, which were readily prepared by reacting the corresponding amides with Lawesson's reagent (Scheme IV). Thiazolines **43** and **44** were synthesized according to the route shown in Scheme V. Thus for example, addition of amino alcohol **15**, obtained by lithium aluminum hydride reduction of DL-*tert*-leucine, to 3-pyridyl isothiocyanate afforded *N*-(hydroxyethyl)thiourea **27**. This, when treated with methanesulfonyl chloride, furnished target compound **43**, via intramolecular S-alkylation by the intermediate me-

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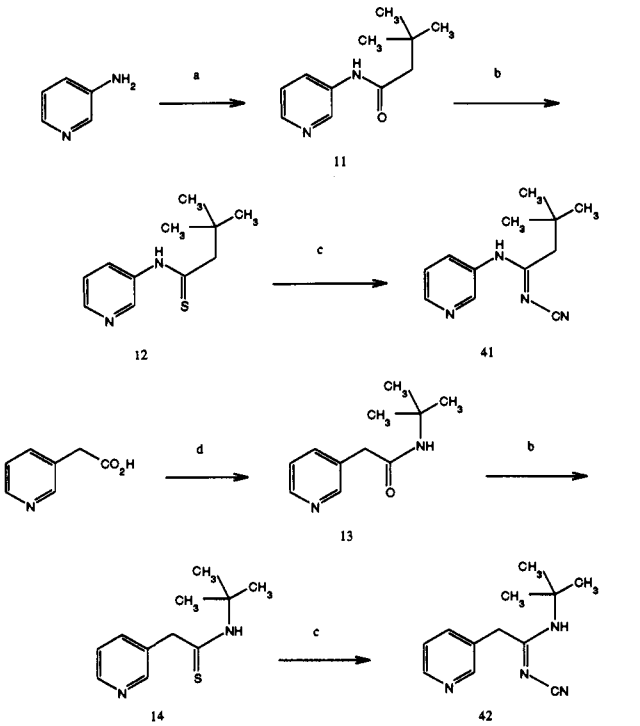
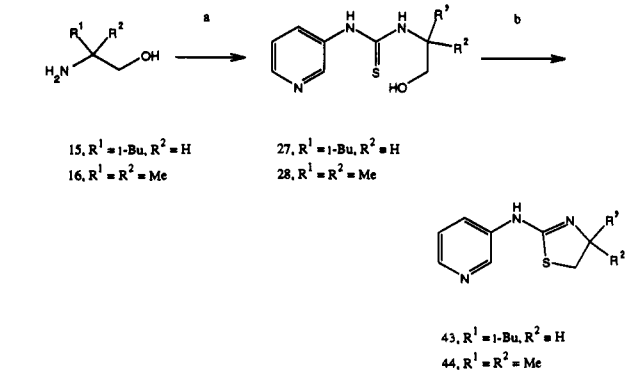
Scheme IV[a]



[a] Reagents: (a) t-$BuCH_2COCl$, Et_3N, CH_2Cl_2; (b) Lawesson's reagent, toluene; (c) $Ph_3PSnN{=}C{=}NSnPPh_3$, EtOH; (d) t-$BuNH_2$, $(PhO)_2PON_3$, Et_3N, CH_2Cl_2.

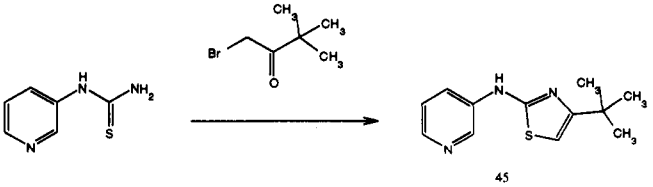
Scheme V[a]



15, R^1 = t-Bu, R^2 = H
16, R^1 = R^2 = Me
27, R^1 = t-Bu, R^2 = H
28, R^1 = R^2 = Me
43, R^1 = t-Bu, R^2 = H
44, R^1 = R^2 = Me

[a] Reagents: (a) 3-pyridyl isothiocyanate, EtOH; (b) $MeSO_2Cl$, Et_3N, CH_2Cl_2.

Scheme VI

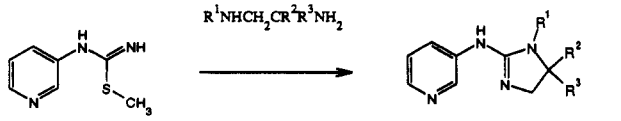


sylate. Thiazole **45** was prepared via the Hantzsch synthesis, involving S-alkylation of 3-pyridylthiourea with the α-bromo ketone followed by in situ intramolecular condensation and dehydration (Scheme VI). Imidazolines **46–48** were obtained from the reaction of N-3-pyridinylcarbamimidothioic acid, methyl ester, with the corresponding 1,2-diaminoethanes (Scheme VII).

Potassium Channel Opening Studies

The portal vein of rats is a spontaneously active vessel, where some smooth muscle cells (pacemaker cells) spontaneously generate bursts of action potentials which

Scheme VII



46, R^1 = R^2 = H, R^3 = t-Bu
47, R^1 = H, R^2 = R^3 = Me
48, R^1 = CHMeEt, R^2 = R^3 = H

propagate over large parts of the vein.[31,32] Potassium channel opening drugs, such as cromakalim and pinacidil, inhibit this depolarization thereby reducing spontaneous electrical activity and thus myogenic activity, leading to a decrease in the duration, frequency, and amplitude of the spontaneous contractions (Figure 1).[4,5,33,34]

In this study compounds were evaluated in rat portal veins for their effect on both $^{86}Rb^+$ efflux and spontaneous myogenic activity. Figure 1 illustrates the effect of increasing concentrations of (*R*)- and (*S*)-pinacidil (**19a** and **19b**, respectively) on these parameters. The (*R*)-enantiomer **19a** was 23 times more potent than the (*S*)-enantiomer **19b** in inhibiting spontaneous activity, and 30 times more potent in stimulating $^{86}Rb^+$ efflux, representing eudismic ratios similar to those reported for the enantiomers of pinacidil in other systems.[35] The results from the simultaneous measurements of $^{86}Rb^+$ efflux and spontaneous activity in rat portal vein, on either phenomenon under identical conditions, with pinacidil and the structurally related compounds reported in this study, are presented in Table III. The regression analysis between the concentrations required for stimulation of $^{86}Rb^+$ efflux by 15%, expressed as pEC_{15} ($-\log EC_{15}$), and the inhibition of spontaneous activity by 50%, expressed as pIC_{50} ($-\log IC_{50}$), is shown in Figure 2. A good linear correlation was found for the double-logarithmic plot ($r^2 = 0.95$, $p < 0.0001$, $n = 25$). From the slope value of 0.77 ± 0.05 the potencies for the two effects approximately follow a proportional relationship on the linear concentration scale. Thus it appears that the $^{86}Rb^+$ efflux and inhibition of mygenic activity are not independent events, with both either having a common cause or one being the cause of the other. Therefore the most plausible interpretation of this data is that the increase in K^+ permeability, as measured by stimulation of $^{86}Rb^+$ efflux, is the cause of the inhibition of spontaneous myogenic activity.

A problem with this hypothesis is the fact that all of the compounds increase $^{86}Rb^+$ permeability only at concen-

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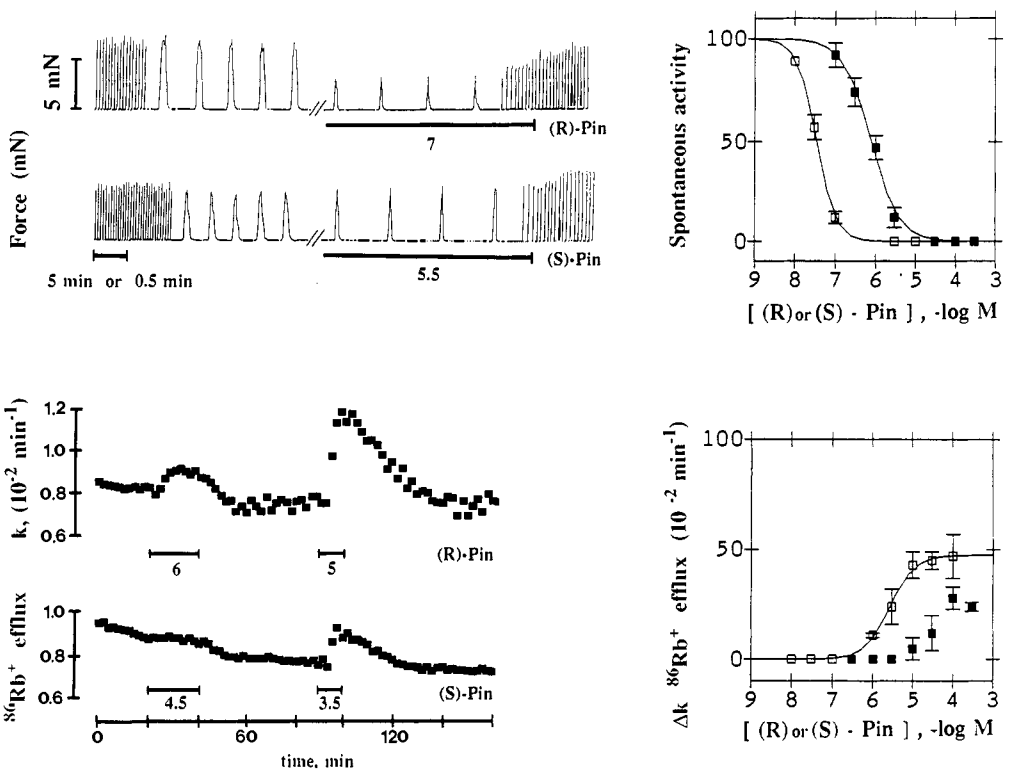

Figure 1. Effects of (*R*)-pinacidil (**19a**) and (*S*)-pinacidil (**19b**) on spontaneous activity and $^{86}Rb^+$ efflux in rat portal vein. Top left: original traces from two vessels showing slow and fast recording of spontaneous contractions under control conditions, recorded after 15 min perfusion with **19a** [(*R*)-Pin, 0.10 μM, concentration indicated as -log M] or **19b** [(*S*)-Pin, 30 μM], and the reversal of this effect by subsequent washing out of the drugs. Integrated spontaneous activity was reduced after 15 min with **19a** (0.1 μM) and **19b** (30 μM) from 1.03 to 0.14 mN/min and from 0.82 to 0.20 mN/min, respectively; the preparations recovered after washout to 0.92 and 0.85 mN/min. Note the differential effect of **19a** and **19b** on the duration, frequency, and amplitude of the contractions. Top right: concentration dependence of the effect on integrated spontaneous activity (□, **19a**; ■, **19b**). The data were fitted to the Hill equation yielding the following parameters (**19a**/**19b**): IC_{50} = 34 ± 1/785 ± 56 nM; Hill coefficients 1.8 ± 0.1/1.2 ± 0.1. The data are means ± SEM from four to six observations. Bottom left: original traces showing the effect of **19a** at 1.0 μM (perfused for 20 min, as indicated by the bar) and 10 μM (10 min) and **19b** at 30 μM (20 min) and 300 μM (10 min), on the rate constant, *k*, of $^{86}Rb^+$ efflux (concentrations are indicated as -log M). Bottom right: concentration dependence of the stimulation of the rate of $^{86}Rb^+$ efflux. The data for **19a** were fitted to the Hill equation giving a midpoint of 2.7 ± 0.2 μM, a maximum increase of 46 ± 1%, and a Hill coefficient of 1.4 ± 0.1. Since the maximum effect of **19b** is unclear (the value at 300 μM may be an artifact), the data were not fitted. The eudismic ratio for a 15% increase in $^{86}Rb^+$ efflux is 30. Data points are means SEM of four to six observations.

trations which are 100 times greater than those required for them to antagonize spontaneous activity (see Figure 2). Cromakalim shows a similar discrepancy, and double isotope experiments have shown the effect to be only partially due to a decreased permeability of the potassium channel toward $^{86}Rb^+$ as compared to $^{42}K^+$.[23] This discrepancy may be explained by a preferential action of the potassium channel openers on pacemaker cells, which results in an inhibition of the spontaneous discharges without producing a measurable increase in tracer efflux. The action on the majority of the smooth muscle cells in the preparation, accompanied by the observable increase in tracer efflux, only then occurs at higher concentrations.[14,36] Whatever the explanation for this discrepancy, it is similar for all potassium channel openers at a particular potassium channel in a particular tissue, and should therefore have no influence on structure–activity studies based upon results obtained under similar experimental conditions. Therefore, it can be safely assumed that the compounds under evaluation in this study are indeed potassium channel openers and that their activities may be quantified either directly through their pEC_{15} value for stimulation of $^{86}Rb^+$ efflux or through their pIC_{50} value for inhibition of spontaneous activity.

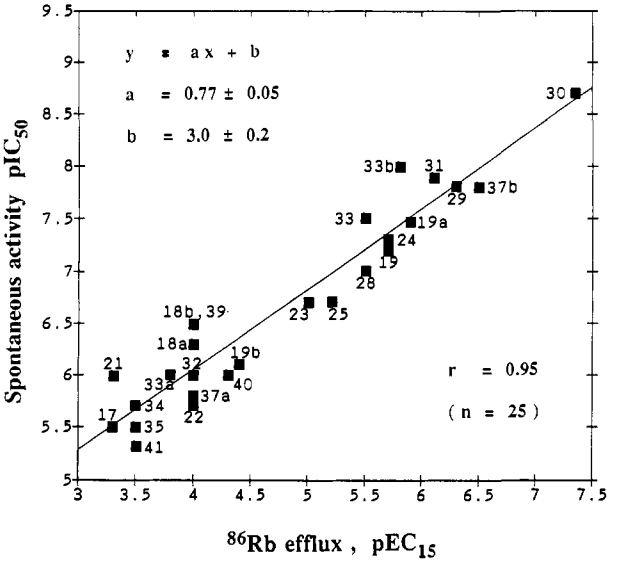

Figure 2. Comparison of the potencies of the pinacidil-type compounds in inhibiting spontaneous activity by 50% (pIC_{50}) and stimulating $^{86}Rb^+$ efflux by 15% (pEC_{15}) in rat portal vein.

Discussion

Structure–Activity Findings. A number of conclusions regarding the structure–activity requirements for the

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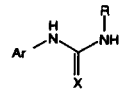
Table I. Physical Data for Pinacidil-Related Ureas, Thioureas, Cyanoguanidines, and Nitroethenediamines



17-40

no.	Ar	R	X	synthesis method[a]	% yield[b]	recryst solvent	mp, °C	$[\alpha]_D^{20}$ [c]	formula[d]	analysis[e]
17	4-pyridyl	CH_2CMe_3	O	A	25	EtOH–ether	251–252		$C_{11}H_{17}N_3O$·HCl	C,H,N,Cl,O
18a	4-pyridyl	(*R*)-$CHMeCMe_3$	S	B	96	acetone–ether	212–213	−35.9	$C_{12}H_{19}N_3S$·HCl	C,H,N,Cl,S
18b	4-pyridyl	(*S*)-$CHMeCMe_3$	S	B	87	acetone–pentane	215–217	+32.4	$C_{12}H_{19}N_3S$·HCl	C,H,N,Cl,S
19a	4-pyridyl	(*R*)-$CHMeCMe_3$	NCN	D	65	acetone–ether	164–165	−147.9[f]	$C_{13}H_{19}N_5$	C,H,N
19b	4-pyridyl	(*S*)-$CHMeCMe_3$	NCN	D	75	acetone–ether	135–136	+144.9[g]	$C_{13}H_{19}N_5$	C,H,N
20	4-pyridyl	CH_2CMe_3	*E*-$CHNO_2$	E	63	benzene–cyclohexane	149–151		$C_{12}H_{19}N_4O_2$·$0.2H_2O$	C,H,N,O
21	4-pyridyl	(±)-$CHMeCMe_3$	*E*-$CHNO_2$	E	30	CH_2Cl_2–ether	148–150[h]		$C_{13}H_{20}N_4O_2$	C,H,N
22	3-pyridyl	CH_2CMe_3	O	A	43	toluene	107–108[i]		$C_{11}H_{17}N_3O$	C,H,N,O
23	3-pyridyl	CMe_3	O	A	35	CH_2Cl_2–pentane	145–147[j]		$C_{10}H_{15}N_3O$	C,H,N
24	3-pyridyl	CH_2CMe_3	S	A	67	toluene	138–139[k]		$C_{11}H_{17}N_3S$	C,H,N,S
25	3-pyridyl	(±)-$CHMeCMe_3$	S	A	64	CH_2Cl_2–ether	124–125		$C_{12}H_{19}N_3S$	C,H,N
26	3-pyridyl	CMe_2Et	S	A	37	CH_2Cl_2–ether	133–135[l]		$C_{11}H_{17}N_3S$	C,H,N,S
27	3-pyridyl	(±)-$CH(CH_2OH)CMe_3$	S	A	94	EtOH–ether	181–182		$C_{12}H_{19}N_3OS$·HCl	C,H,N,Cl,S
28	3-pyridyl	CMe_2CH_2OH	S	A	78	EtOH–toluene	125–127		$C_{10}H_{15}N_3OS$	C,H,N,S
29	3-pyridyl	CH_2CMe_3	NCN	C	63	EtOH–toluene	214–215[m]		$C_{12}H_{17}N_5$	C,H,N
30	3-pyridyl	(±)-$CHMeCMe_3$	NCN	C	91	$CHCl_3$–ether	163–165[n]		$C_{13}H_{19}N_5$	C,H,N
31	3-pyridyl	CMe_2Et	NCN	C	92	EtOH–ether	185–187[o]		$C_{12}H_{17}N_5$	C,H,N
32	3-pyridyl	CMe_3	NCN	C	55	EtOH–ether	204–206[p]		$C_{11}H_{15}N_5$	C,H,N
33	3-pyridyl	CH_2CMe_3	*E*-$CHNO_2$	E	78	CH_2Cl_2–pentane	186–187		$C_{12}H_{19}N_4O_2$	C,H,N
34	3-pyridyl	(±)-$CHMeCMe_3$	*E*-$CHNO_2$	E	76	CH_2Cl_2–hexane	175–177[q]		$C_{13}H_{20}N_4O_2$	C,H,N
34a	3-pyridyl	(*R*)-$CHMeCMe_3$	*E*-$CHNO_2$	E	36	CH_2Cl_2–pentane	194–195	−195.7	$C_{13}H_{20}N_4O_2$·$0.2H_2O$	C,H,N,O
34b	3-pyridyl	(*S*)-$CHMeCMe_3$	*E*-$CHNO_2$	E	41	EtOAc–pentane	197–198	+195.9	$C_{13}H_{20}N_4O_2$	C,H,N,O
35	3-pyridyl	$CH_2CH_2CMe_3$	$CHNO_2$[r]	E	22	CH_2Cl_2–ether	132–134		$C_{13}H_{20}N_4O_2$	C,H,N
36	3-pyridyl	c-C_6H_{11}	$CHNO_2$[r]	E	69	EtOH–CH_2Cl_2	180–182		$C_{13}H_{19}N_4O_2$·$0.2H_2O$	C,H,N,O
37	3-pyridyl	$CHMe_2$	*E*–$CHNO_2$	E	26	EtOH–CH_2Cl_2	178–179		$C_{10}H_{14}N_4O_2$	C,H,N
38a	5-methoxy-3-pyridyl	(*R*)-$CHMeCMe_3$	*E*-$CHNO_2$	E	37	CH_2Cl_2–ether	170–171	−169.3	$C_{14}H_{22}N_4O_3$	C,H,N,O
38b	5-methoxy-3-pyridyl	(*S*)-$CHMeCMe_3$	*E*-$CHNO_2$	E	27	CH_2Cl_2–ether	167–168	+169.4	$C_{14}H_{22}N_4O_3$	C,H,N,O
39	3-pyridyl-CH_2	CH_2CMe_3	$CHNO_2$[r]	E	76	EtOH–ether	139–140		$C_{13}H_{20}N_4O_2$	C,H,N,O
40	3-NCC_6H_4	(±)-$CHMeCMe_3$	$CHNO_2$[r]	E	39	acetone	212–214		$C_{15}H_{20}N_4O_2$	C,H,N

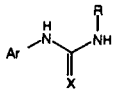
[a] Refers to general method of synthesis as shown in Schemes II and III and detailed in the text. [b] Yields are based on the final step of the indicated synthetic method and are not optimized. [c] All optical rotations were determined in EtOH (c = 1.00). [d] Empirical formula with water of hydration. [e] All compounds were fully characterized spectroscopically (^{1}H NMR; MS) and gave analytical results for the indicated elements within ±0.4% of the calculated values. [f] Reference 24, $[\alpha]_D^{20}$ −135°. [g] Reference 24, $[\alpha]_D^{20}$ +135°. [h] Reference 29, mp 110 °C. [i] Reference 1, mp 107–108 °C. [j] Reference 1, mp 146–146.5 °C. [k] Reference 1, mp 139–139.5 °C. [l] Reference 1, mp 134.5–135.5 °C. [m] Reference 2, mp 214–215 °C. [n] Reference 2, 167–168 °C. [o] Reference 2, mp 184–186 °C. [p] Reference 2, mp 204–205 °C. [q] Reference 29, mp 182 °C. [r] Stereochemistry not definable by NOE.

Table II. Physical Data for *E*-*N*-Aryl-1-(methylthio)-2-nitroethenamines (6–10)

no.	Ar	% yield[a]	recryst solvent	mp, °C	formula	analysis[b]
6	4-pyridyl	47	EtOH–ether	142–147[c]	$C_8H_9N_3O_2S$	
7	3-pyridyl	29	EtOH–ether	134–136	$C_8H_9N_3O_2S$	C,H,N,S
8	5-methoxy-3-pyridyl	31	EtOH–ether	165–167	$C_9H_{11}N_3O_3S$	C,H,N,S
9	3-pyridyl-CH_2	62	EtOH–ether	130–131	$C_9H_{11}N_3O_2S$	C,H,N,S
10	3-NCC_6H_4	23	$CHCl_3$–ether	149–151	$C_{10}H_9N_3O_2S$	C,H,N,S

[a] Yields are not optimized. [b] All compounds were fully characterized spectroscopically (^1H NMR; MS) and gave analytical results for the indicated element within ±0.4% of the calculated values. [c] Reference 29; crude material which, due to purification problems, was used directly.

Table III. Ionization Constants and Biological Activity in Rat Portal Vein of Pinacidil-Related Ureas, Thioureas, Cyanoguanidines, and Nitroethenediamines (17–40), Cyanoamidines (41–42; Scheme IV), and Cyclic Analogues (43–48; Schemes V–VII)



17-40

no.	Ar	R	X	pK_{a1}[a]	pK_{a2}[b]	pEC_{15}[c]	pIC_{50}[d]
17	4-pyridyl	CH_2CMe_3	O	5.46	>14	3.3	5.5
18a	4-pyridyl	(*R*)-$CHMeCMe_3$	S	nd[e]	nd	4.0	6.3
18b	4-pyridyl	(*S*)-$CHMeCMe_3$	S	4.89	12.99	4.0	6.5
19 (pinacidil)	4-pyridyl	(±)-$CHMeCMe_3$	NCN	4.81	11.35	5.7	7.2
19a	4-pyridyl	(*R*)-$CHMeCMe_3$	NCN	nd	nd	5.9	7.47
19b	4-pyridyl	(*S*)-$CHMeCMe_3$	NCN	nd	nd	4.4	6.11
20	4-pyridyl	CH_2CMe_3	*E*-$CHNO_2$	nd	nd	<3.5	5.0
21	4-pyridyl	(±)-$CHMeCMe_3$	*E*-$CHNO_2$	3.96	8.48	3.3	6.0
22	3-pyridyl	CH_2CMe_3	O	3.74	>14	4.0	5.7
23	3-pyridyl	CMe_3	O	nd	nd	nd	5.7
24	3-pyridyl	CH_2CMe_3	S	3.08	13.11	5.0	6.7
25	3-pyridyl	(±)-$CHMeCMe_3$	S	2.97	13.24	5.7	7.3
26	3-pyridyl	CMe_2Et	S	nd	nd	5.2	6.7
27	3-pyridyl	(±)-$CH(CH_2OH)CMe_3$	S	nd	nd	<3.5	5.5
29	3-pyridyl	CH_2CMe_3	NCN	2.83	11.82	5.5	7.0
30	3-pyridyl	(±)-$CHMeCMe_3$	NCN	2.70	12.04	6.3	7.8
30a	3-pyridyl	(*R*)-$CHMeCMe_3$	NCN	nd	nd	nd	7.0[f]
30b	3-pyridyl	(*S*)-$CHMeCMe_3$	NCN	nd	nd	nd	5.2[f]
31	3-pyridyl	CMe_2Et	NCN	2.61	12.18	7.4	8.7
32	3-pyridyl	CMe_3	NCN	nd	nd	6.1	7.9
33	3-pyridyl	CH_2CMe_3	$CHNO_2$[g]	nd	8.86	4.0	6.0
34	3-pyridyl	(±)-$CHMeCMe_3$	*E*-$CHNO_2$	2.28	9.03	5.5	7.5
34a	3-pyridyl	(*R*)-$CHMeCMe_3$	*E*-$CHNO_2$	nd	nd	3.8	6.0
34b	3-pyridyl	(*S*)-$CHMeCMe_3$	*E*-$CHNO_2$	nd	nd	5.8	8.0
35	3-pyridyl	$CH_2CH_2CMe_3$	$CHNO_2$[g]	nd	nd	3.5	5.7
36	3-pyridyl	c-C_6H_{11}	$CHNO_2$[g]	nd	nd	3.5	5.5
37	3-pyridyl	$CHMe_2$	*E*-$CHNO_2$	nd	nd	<3.5	4.5
38a	5-methoxy-3-pyridyl	(*R*)-$CHMeCMe_3$	*E*-$CHNO_2$	nd	nd	4.0	5.8
38b	5-methoxy-3-pyridyl	(*S*)-$CHMeCMe_3$	*E*-$CHNO_2$	1.86	8.75	6.5	7.8
39	3-pyridyl-CH_2	CH_2CMe_3	$CHNO_2$[g]	nd	nd	<3.5	4.7
40	3-NCC_6H_4	(±)-$CHMeCMe_3$	$CHNO_2$[g]	–	8.88	4.0	6.5
41			NCN	2.23	11.29	4.3	6.0
42			NCN	2.71	>14	3.5	5.3
43				3.89	nd	nd	4.7
44				nd	nd	nd	4.5
45				3.57	nd	nd	6.0
46				2.03[h]	8.10[i]	nd	5.5
47				nd	8.20[i]	nd	<5
48				nd	nd	<3.0	4.0

[a] Negative logarithm of equilibrium constant for first proton dissociation of cation [HMH^+] into uncharged molecule [HM]. [b] Negative logarithm of equilibrium constant for dissociation of uncharged molecule [MH] into anion [M^-]. [c] Negative logarithm of the concentration causing a 15% increase in $^{86}Rb^+$ efflux from rat portal vein. [d] Negative logarithm of the concentration causing a 50% inhibition of spontaneous activity in rat portal vein. [e] Not determined. [f] Reference 35; negative logarithm of the concentration causing a 50% relaxation of noradrenaline-contracted dog cephalic vein. [g] Stereochemistry not definable by NOE. [h] Negative logarithm of equilibrium constant for first proton dissociation from dication [HMH^{2+}] into monocation [MH^+]. [i] Negative logarithm of equilibrium constant for second proton dissociation from cation [MH^+] into uncharged molecule [M].

in vitro opening of potassium channels, caused by the pinacidil-related compounds included in this study, are revealed by the data presented in Table III. Primarily, the data demonstrates that in addition to the cyanoguanidine series of compounds (X = NCN), to which pinacidil (19) belongs, potent activity also resides within both the nitroethenediamine (X = $CHNO_2$) and thiourea (X = S) series, but not with the ureas (X = O). In view of the potency of some of the thioureas (24–26), a direct receptor interaction involving the cyano and nitro groups of the former two series of compounds can probably be excluded, and consequently a possible key to a receptor binding model for these compounds is an understanding of why the ureas should be relatively inactive. The reason for this

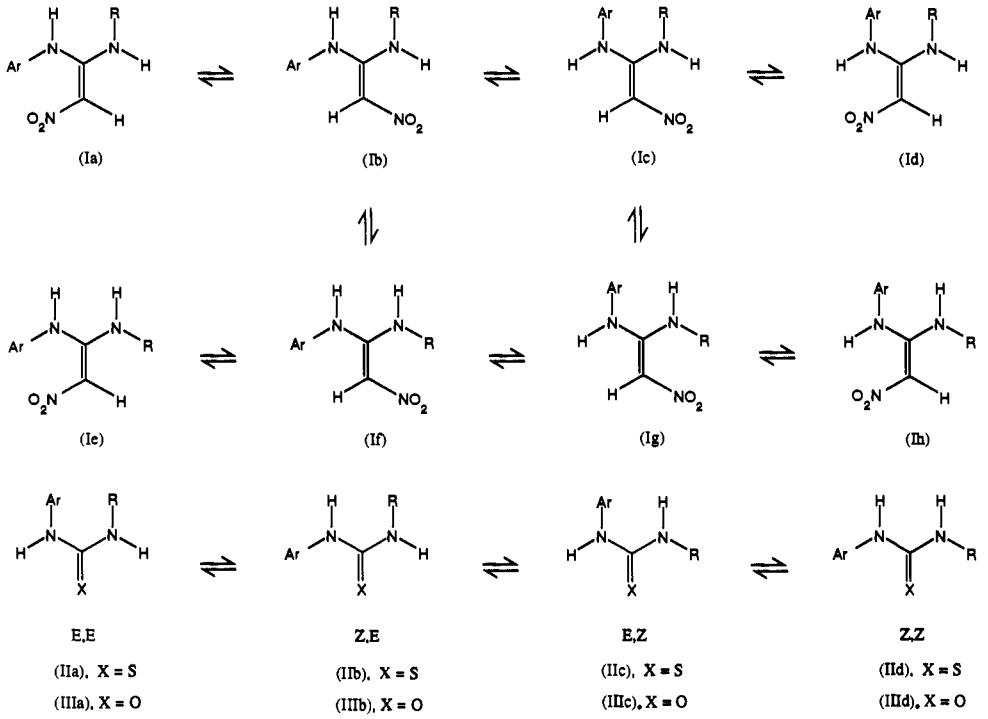


Figure 3. Conformational isomerism of nitroethenediamines (**I**), thioureas (**II**), and ureas (**III**).

inactivity is not immediately obvious on structural grounds since the cyanoguanidine, nitroethendiamine, thiourea, and urea functionalities are all planar structures with almost identical C–N bond lengths and bond angles.[37]

Nitroethenediamines have the potential to exhibit considerable configurational and conformational isomerism. Thus, due to tautomeric equilibria between the nitroenamine and iminonitronic acid forms,[38] resulting in restricted rotation about both sp^2-carbon to nitrogen bonds, the nitroethenediamines possess eight possible conformations (**Ia–h**, Figure 3). However, due to internal steric hindrance, those conformations in which the substituents are eclipsed are energetically disfavored with respect to the two staggered conformations **Ib** and **Ih**. In accordance with this, NMR studies (DMSO-d^6, 25 °C) of the 3- and 4-pyridylnitroethenediamines showed strong NOE enhancements between the ethene proton and the two proximal pyridine protons (e.g. the singlet at δ 6.09 with the doublet of doublets at δ 7.31 and doublet at δ 8.07 in **38b**), indicating that the compounds predominantly exist in conformation **Ib**. In contrast, for the precursor (methylthio)nitroethenamines (Scheme III) NOE enhancement was observed between the ethene proton and the thiomethyl protons (e.g. the singlet at δ 6.80 with the doublet of doublets at δ 7.45 and doublet at δ 8.17 in **8**). This stereochemistry is such that the compounds predominantly exist in the configuration most stabilized by intramolecular hydrogen bonding between the nitro group and an amino group, with the alkylamino H bond being preferred over the arylamino H bond in the nitroethenediamines.

Cyanoguanidines, thioureas, and ureas also have the propensity for conformational isomerism due to restricted rotation about their carbon–nitrogen bonds (Figure 3). However, whereas certain nitroethenediamine conformations can be stabilized by intramolecular hydrogen bonding, this is not possible for these other groups and consequently the relative populations of their different conformations is governed largely by steric interactions. Thus the cyanoguanidines exist predominantly, as the cyanimino tautomer, in a mixture of two staggered *E,Z*-conformations (analogous to the nitroethenediamine conformations **Ib** and **Ih**). In contrast *N*-aryl-*N'*-alkylthioureas (**IIa–d**), due to the absence of the third, very sterically demanding substituent, can additionally exist in the *Z,Z*-conformation (**IId**), although the staggered *Z,E*- and *E,Z*-conformations (**IIb** and **IIc**) are normally favored, with the population of conformation **IIc** decreasing in favor of conformation **IIb** (analogous to nitroethenediamine conformation **Ib**) as the bulk of the *N*-alkyl substituent increases.[39,40] Although *N,N'*-disubstituted ureas show similar conformational behavior to that of their thiourea analogues, the *Z,Z*-conformation (**IIId**) is strongly favored and the increase in the proportion of staggered conformation **IIIb** with increasing alkyl bulk is less marked, due to the smaller size of the oxygen atom compared to that of sulfur.[41,42] Consequently, the relative inactivity of the ureas may be explained on conformational grounds, since only the cyanoguanidines, nitroethenediamines, and thioureas can readily adopt similar, relatively low-energy staggered

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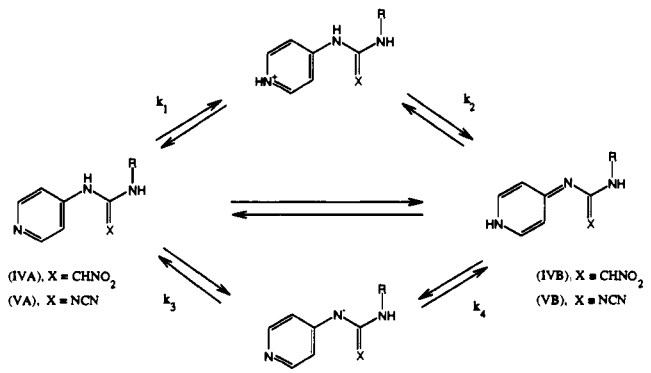

Figure 4. Some prototropic equilibria of 4-pyridylnitroethenediamines (IV) and 4-pyridylcyanoguanidines (V).

conformations (Figure 3; type **Ib** in the case of the former two series, and the *Z,E*-conformation **IIb** for the *N*-aryl-*N'*-alkylthioureas) which could correspond to the active conformation at the receptor. The function of the active conformation would then be to hold the pyridyl and alkyl groups in regions of space enabling favorable receptor interactions. Indirect support for this comes from the SAR discussed below, which suggests that the alkyl and pyridyl groups both interact highly specifically with discreet binding sites within the receptor and therefore their relative positions, as governed by the conformation of the linking group, are an important determinant for activity.

The activity of the compounds within the cyanoguanidine, nitroethenediamine, and thiourea series is considerably influenced by the aryl ring, with the 3-pyridyl derivatives being invariably more active in vitro (and in vivo[2]) than the corresponding 4-pyridyl analogues (**30** > **19**; **33** > **20**; **34** > **21**; **25** > **18b**) and the 3-cyanophenyl analogue (**34** > **40**). It is difficult to rationalize these activities purely on the basis of either a lipophilic or a π-interaction involving the electron-deficient aryl ring. Much of the observed structure–activity can be explained on the basis of a highly directional receptor interaction at the 3-pyridyl nitrogen, with the weaker activity of the 4-pyridyl compounds being due to an inappropriate orientation of the nitrogen toward the receptor. However, this is unlikely to be the only determinant of activity since it does not explain either the relative weak activity of cyanoamidines **41** (pIC_{50} = 6.0) and **42** (pIC_{50} = 5.3), compared to that of *N-tert*-butylcyanoguanidine **32** (pIC_{50} = 7.9), or the potency of the 3-cyanophenyl derivative **40** (pIC_{50} = 6.5).

The pyridine group, in addition to contributing a nitrogen atom, the basicity of which is dependent upon the ring substituents, also affects the acidity of the adjacent NH group, with the degree of influence depending upon the position of substitution. In the case of the 4-substituted pyridines this leads to the system of prototropic equilibria shown in Figure 4, where aminopyridine species **IVA** and **VA** coexist with iminopyridine species **IVB** and **VB**. Although such equilibria are also possible for the 3-pyridyl compounds, the zwitterionic species corresponding to **IVB** and **VB** are of much higher energy since they cannot be stabilized by tautomerism to a neutral imino species, and consequently they are far less predominant. Direct evidence for this behavior comes from the spectroscopic properties of the compounds. Thus, whereas the IR spectra of the 4-pyridylnitroethenediamines exhibited a series of broad bands in the 3300–2300 cm^{-1} region, in contrast the corresponding 3-pyridyl compounds showed only sharp absorptions in the range 3195–2850 cm^{-1}. Similarly in the case of the cyanoguanidines, whereas both the 4-pyridyl (**19**) and 3-pyridyl (**30**) compounds showed single, sharp ν(C≡N) absorptions at 2171 and 2179 cm^{-1} (KBr disk), consistent with them predominating as the cyanoimino tautomer,[37] compound **19**, in contrast to **30**, exhibited a series of broad bands between 3300 and 2500 cm^{-1}. Furthermore, in both the nitroethenediamine and cyanoguanidine series, at ambient temperature the NMR spectra ($CDCl_3$ and DMSO-d_6) of the 4-pyridyl compounds showed only broad absorptions with little fine structure for the aromatic protons, whereas the corresponding 3-pyridyl derivatives had sharp absorptions with well-resolved fine couplings. Low-temperature NMR studies of the 4-pyridyl compounds revealed new peaks, arising from the presence of two molecular species in slow exchange. Thus for example, the NMR spectrum of compound **19**, in $CDCl_3$ at 300 K, showed two broad doublets corresponding to the 4-pyridyl AB quartet at δ 7.18 and 8.57, whereas at 223 K, in addition to the original AB quartet at δ 7.15 and 8.53, a second, less intense AB quartet occurred at δ 7.40 and 8.38, with the two species being present in a population ratio of approximately 8:1. These observations are consistent with the 4-pyridylamino compounds **IVA** (X = $CHNO_2$) and **VA** (X = NCN) existing in equilibrium with their corresponding imino tautomers, **IVB** and **VB** (Figure 4), with tautomerization being fast at ambient temperature on the NMR time scale, resulting in the recording of a time-averaged signal.

Close precedence for this behavior is found with 3- and 4-hydroxypyridine, the former having a phenolic structure whereas the latter exists as 4-pyridone,[43] and the methanesulfonamidopyridines, in which the 3-substituted compound exists mainly in the sulfonimido form whereas the 4-derivative predominates in the imino form.[44]

As a consequence of these prototropic equilibria, the measured pK_a's of the 4-pyridyl compounds relate to the macroscopic constants K_{a1} and K_{a2}, which are composites of the individual microscopic ionization constants from both the amino and imino forms, such that the first dissociation constant $K_{a1} = k_1 + k_2$ and the second dissociation $K_{a2} = k_3 + k_4$ (Figure 4).[45] In contrast the pK_a data for the 3-pyridyl compounds relate more directly to the protonation of the pyridine ring (pK_{a1}) and proton loss from the pyridine NH (pK_{a2}). It can be seen from the pK_a data in Table II that on traversing the series urea → thiourea → cyanoguanidine → nitroethenediamine, basicity decreases for both the 4-pyridyl (pK_{a1}: **17** > **18b** > **19** > **21**) and 3-pyridyl (pK_{a1}: **22** > **25** > **30** > **34**) compounds. This trend reflects an increase in inductive electron-withdrawing ability, which is also manifested by an increase in acidity, urea → thiourea → cyanoguanidine → nitroethenediamine, for both the 4-pyridyl (pK_{a2}: **17** > **18b** > **19** > **21**) and 3-pyridyl (pK_{a2}: **22** > **25** > **30** > **34**) series of compounds. As indicated by the difference in potency between cyanoguanidine **32** (pIC_{50} = 7.9) and cyanoamidine **42** (pIC_{50} = 5.3), the pyridylamino group makes an important contribution toward activity. In contrast to simple alkylamines, activated NH groups can act as hydrogen-bond donors and, as has been shown for phenols,[46] their hydrogen-bonding ability should increase

(43) Voegeli, U.; von Philipsborn, W. ^{13}C and ^{1}H NMR Spectroscopic Studies on the Structure of *N*-Methyl-3-pyridone and 3-Hydroxypyridine. *Org. Magn. Res.* **1973**, *5*, 551–559.

(44) Jones, R. A.; Katritsky, A. R. Potentially Tautomeric Pyridines. Part III. 2-, 3-, and 4-Methanesulphonamidopyridine. *J. Chem. Soc.* **1961**, 378–384.

(45) The ionization constant for the loss of a proton from the alkyl-NH site is also a component of K_{a2}. However, since dissociation from the Aryl-NH site is far more favorable due to greater stabilization of the anion, the former contribution may be ignored in these discussions.

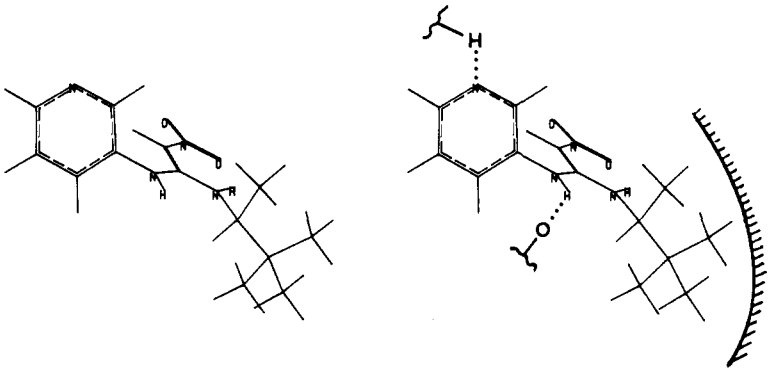

Figure 5. Stereo plot of the calculated minimum-energy conformation of **34b**, showing postulated receptor-binding interactions.

in parallel with their acidity. Consequently, the hydrogen-bond-donating ability, particularly for the 3-pyridyl compounds, should increase across the series urea → thiourea → cyanoguanidine → nitroethenediamine. Since potency increases in a similar rank order, activity may be related to the acidity of the NH proton and consequently to its ability/availability to behave as a hydrogen-bond donor group. Indirect support for such a hydrogen-bond interaction comes from the 4-pyridyl compounds, which despite their greater acidity (pK_{a2}: **18b** > **25**; **19** > **30**; **21** > **34**) are less active than the corresponding 3-pyridyl compounds, presumably due to the population of imino tautomers **IVB** and **VB** (Figure 4) not having an appropriate hydrogen-bond-donor site. Precedent for the cyanoguanidine group being an effective intramolecular hydrogen bond donor is found in IR studies of cimetidine.[47]

If the only receptor interaction of the pyridylamino portion of the molecules was such a hydrogen-bond-donor interaction, the activity of (3-cyanophenyl)nitroethenediamine **40** (pK_{a2} = 8.88, pIC_{50} = 6.5) should be more comparable to that of the corresponding 3-pyridyl compound **34** (pK_{a2} = 9.03, pIC_{50} = 7.5). This relative inactivity is not due to the additional bulk of the cyano group, since the receptor can clearly accommodate the methoxy group of compound **38b**, but is consistent with a second receptor interaction at the pyridine nitrogen. Considering the weak basicity of the compounds and the fact that decreasing pK_{a1} further, as in 5-methoxypyridine **38b** (pK_{a1} = 1.86; pIC_{50} = 7.8) compared to that of the unsubstituted analogue **34b** (pK_{a2} = 2.28, pIC_{50} = 8.0), has little effect on potency, it is unlikely that the pyridine nitrogen interacts with the receptor as a pyridinium cation in a Coulombic interaction. Consequently, the nitrogen probably acts as a hydrogen-bond acceptor, as discussed above, with the interaction being less favorable for the 4-pyridyl compounds due to either an inappropriate geometry, or again, the population of imino tautomers **IVB** and **VB** (Figure 4).

The third region of the molecules having a profound effect on potassium channel opening activity is the alkyl group. Although there is no obvious relationship between activity and either bulk or lipophilic descriptors for the alkyl group, as observed by Moriguchi and co-workers,[16] the size and shape of R is critical for activity. Thus in the nitroethenediamine series, optimum activity is observed for the (*S*)-enantiomers **34b** (pIC_{50} = 8.0) and **38b** (pIC_{50} = 7.8), and since distomer **34a** (pIC_{50} = 6.0) is only equipotent with the corresponding desmethyl analogue **33** (R = CH_2CMe_3), chiral recognition of the α-methyl group makes a highly significant contribution to receptor binding. Paradoxically, in the cyanoguanidine series the more active enantiomers are those having the opposite stereochemistry, **19a** and **30a** (R = (*R*)-$CHMeCMe_3$),[18,35] but despite this enantioselectivity achiral compounds **31** and **32**, where R represent CMe_2Et and CMe_3, respectively, have equal or better activity than compound **30** (and consequently to **30a**). These findings are consistent with an interaction with a binding site having limited bulk tolerance and within which a strong lipophilic interaction is possible for a suitably oriented ethyl or methyl group. Thus although compounds having R = CMe_2Et and CMe_3 can readily interact with the lipophilic binding site, in the case of compounds where R = $CHMeCMe_3$, the receptor can only accommodate the α-methyl group in addition to the CMe_3 group, when it is in a particular orientation, *R* in the case of the cyanoguanidines and *S* for the nitroethenediamines. Since there is no significant difference in activity between (*R*)- and (*S*)-thioureas **18a** and **18b**, the observed enantioselectivity may be related to the additional bulk of the $CHNO_2$ and NCN portions of the molecule. These disparate geometry requirements for activity between different enantiomeric pairs of compounds may then be explained by the receptor undergoing conformational changes in order to accommodate either $CHNO_2$ or NCN moieties, and bind the different pharmacophore elements. This could involve different compounds being recognized by different receptor conformations, or a stepwise binding, where individual binding elements of the drug bind sequentially as the receptor adopts suitable conformations. In the case of the latter "zipperlike" model,[48] the structure–activity data for the pinacidil-type compounds studied here would be consistent with the pyridine nitrogen and the pyridylamino group initially interacting with the receptor, which then undergoes conformational changes until it is able to bind to the lipophilic alkyl group.

Receptor Binding Model. From the above discussions it is apparent that the activity of these pinacidil-type compounds can be rationalized in terms of three potassium channel, receptor-binding elements: a hydrogen-bond-donating site, flanked by a hydrogen-bond-accepting site, and a lipophilic site. Although the relative spatial relationship between these fundamental binding sites, as governed by the geometry of the linking group, is important for these substances, as demonstrated by compounds **19a** and **34b** where different receptor enantioselectivity

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(47) Mitchell, R. C. An Infrared Study of Intramolecular Hydrogen-Bonding in the Histamine H_2-Receptor Antagonists, Burimamide, Metiamide, Cimetidine and Related Compounds. *J. Chem. Soc., Perkin 2* **1980**, 915–918.

(48) Burgen, A. S. V.; Roberts, G. C. K.; Feeney, J. Binding of Flexible Ligands to Macromolecules. *Nature* **1975**, *253*, 753–755.

operates, a precise geometry pharmacophore model is not relevant, because the receptor probably has a flexible geometry, which allows different structures to be accommodated through dynamic receptor–ligand interactions. This binding model is illustrated for compound **34** (Figure 5), the structure of which was built using SYBYL and energy minimized using the MAXIMIN2 force field.[49] In this model, the nitroethenediamine group adopts the same staggered configuration as that indicated by the NMR studies, with the preferred rotamer of the $CHMeCMe_3$ group being such that the α-methyl group and α-hydrogen atom are staggered with respect to the 3-pyridylamino proton. In order to minimize steric interactions between the ethene proton and the 2- and 4-pyridyl hydrogens, the molecule adopts a conformation having an angle of 79° between the plane of the nitroethenediamine group and that of the pyridine ring.[50] Consequently, the position of the pyridine nitrogen, when hydrogen bonded to the receptor in the active conformation, is located somewhere on the surface of two arcs taken from the circumference of the cone described by rotation about the pyridine–NH bond, with the two missing surfaces being due to the disfavored conformations. On the basis of such a ligand–receptor binding model the two hydrogen bonds would need to be quite strong in order to account for the potency of compounds such as **31**,[51] and therefore the orientation of both the NH moiety and the pyridine ring toward their receptor-binding sites should be near optimal in the active conformations.

Conformationally Restricted Analogues. In an attempt to test the soundness of the above binding model, a series of conformationally restricted compounds were prepared and tested for potassium channel opening activity. In the design of these compounds, the 3-pyridylamino portion of the molecule was kept constant, while the central planar portion was replaced with a heterocyclic framework capable of bearing alkyl groups to probe the position of the lipophilic binding site. Initially we investigated thiazolidines **43** and **44**, which, in addition to having a suitably placed, weakly-acidic proton, possess a rigid 2-aminothiazolidine framework, shown by X-ray and NMR studies to be planar due to endo–exo tautomerism of the carbon–nitrogen double bond.[52,53] Although neither **43** nor **44** showed significant activity, thiazole analogue **45** (pIC_{50} = 6.0), in which the position of the *tert*-butyl is modified through attachment to a sp^2-carbon, had sufficient potassium channel opening activity to suggest that such heterocyclic variations could lead to active compounds. As a second approach the imidazoline ring system was employed. In these compounds (**46**–**48**) the aminoimidazolidine ring system is sufficiently basic for the protonated species to predominate at physiological pH, and this cation could then provide the hydrogen-bond-donor interaction with the receptor. Furthermore, compound **48**, enabled us to probe the region of space occupied by the lipophilic binding group in the nitroethenediamine minimum-energy conformations. However, these latter three compounds were less active than **45**, suggesting that compounds in which the central portion is protonated cannot be accommodated within the receptor binding site. Consequently, although these conformationally restricted compounds were relatively inactive, their activities are not incompatible with the postulated binding model.

Conclusion

In summary, we have shown that for pinacidil-type cyanoguanidines, nitroethenediamines, and thioureas, there is a good correlation between the inhibition of spontaneous myogenic activity and the stimulation of $^{86}Rb^+$ efflux in rat portal vein, which is attributable to the compounds increasing the open probability of potassium channels. The resulting quantitative in vitro data has been used to analyze the structure–activity relationships for potassium channel opening, allowing the biological activity to be rationalized in terms of a pharmacophore involving a hydrogen-bond-acceptor element, a hydrogen-bond-donor element, and a lipophilic binding group. Conformational changes in the receptor have been invoked to explain disparities in the chiral recognition of lipophilic groups in different compound classes.

Experimental Section

Chemistry. General Procedures. Reagents, starting materials, and solvents were purchased from common commercial suppliers and were used as received or distilled from an appropriate drying agent. Reactions requiring anhydrous reaction conditions were performed under an atmosphere of argon. Evaporations were carried out on a rotary evaporator at bath temperatures <50 °C and under an appropriate vacuum. Unless stated otherwise, column chromatography was carried out at medium pressure using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–400 mesh ASTM) with the solvent system indicated. Melting points were determined with a Buchi-Tottoli apparatus in open capillary tubes and are uncorrected. Optical rotations were determined on a Perkin-Elmer Type 24 polarimeter. Proton NMR spectra were routinely recorded at ambient temperature using a Brucker Spektrospin WH 360 (360 MHz), in either $CDCl_3$ or DMSO-d_6 with Me_4Si as an internal standard; chemical shifts are reported in δ values (ppm) relative to internal Me_4Si, and J values are reported in hertz. Where elemental analyses are indicated by the symbols of the elements, they were performed by the analytical department at Sandoz and results obtained were within ±0.4% of the theoretical values.

(*R*)-α-Methyl-*N*-(1,2,2-trimethylpropylidene)benzenemethanamine (1a). A mixture of pinacolone (75.0 g, 750 mmol), (*R*)-(+)-1-phenylethylamine (45.5 g, 375 mmol), and *p*-toluenesulfonic acid (0.6 g) in dry benzene (750 mL) was heated under reflux for 70 h with the liberated water being removed azeotropically. The solvent was evaporated and the residue was purified by column chromatography on basic alumina (500 g of activity grade 1, 10% EtOAc in hexane) followed by vacuum distillation to give **1a** (55.2 g, 72%) as a colorless oil: bp 116–118 °C (13 mmHg); $[\alpha]^{20}_D$ +28.7° (*c* = 1.066, EtOH) [lit.[25] $[\alpha]^{23}_D$ +27.5° (*c* = 1.54, EtOH)]; NMR ($CDCl_3$) δ 1.13 (s, 9, *t*-Bu), 1.36 (d, 3, CH*Me*), 1.78 (s, 3, N=CMe), 4.57 (q, 1, C*H*Me), 7.19 (ddd, 1, *p*-ArH), 7.30 (ddd, 2, *m*-ArH), and 7.40 (dd, 2, o-ArH).

(*S*)-α-Methyl-*N*-(1,2,2-trimethylpropylidine)benzenemethanamine (1b), prepared from (*S*)-(−)-1-phenylethylamine using the above procedure, was obtained as a colorless oil: bp 110–112 °C (12 mmHg); $[\alpha]^{20}_D$ −28.9° (*c* = 1.026, EtOH) [lit.[25] $[\alpha]^{23}_D$ −27.3° (*c* = 1.46, EtOH)].

(*R*,*R*)-α-Methyl-*N*-(1,2,2-trimethylpropyl)benzenemethanamine (2a). A solution of **1a** (12.2 g, 60 mmol) in dry THF (150 mL) at 2 °C was treated with a solution of borane–tetrahydrofuran complex (120 mL of 1.0 M) and stirred at room temperature for 5 h. The mixture was evaporated to dryness to give a residue which was treated with EtOH (300 mL) and heated under reflux for 1 h. The mixture was again evaporated to dryness

(49) SYBYL version 5.3, Tripos Associates, Inc., St. Louis, MO.
(50) The pinacidil enantiomer **30a** and the analogue **31**, built with SYBYL using bond parameters for the cyanoguanidine moiety derived from an X-ray crystal structure of cimetidine, gave very similar energy-minimized structures to that of **34b**, having angles between the planes of the cyanoguanidine group and that of the pyridine ring of 52° and 54°, respectively.
(51) Farmer, P. S. Bridging the Gap between Bioactive Peptides and Nonpeptides: Some Perspectives in Design. *Drug Des.* **1980**, *10*, 119–143.
(52) Petrovic, D.; Ribar, B.; Argay, G.; Kalman, A.; Nowacki, W. 2-Phenyliminothiazolidine. *Acta Crystallogr.* **1977**, *B33*, 106–108.
(53) Toth, G.; Almasy, A. Isomerization and Conjugation Characteristics of the C=N Double Bond—^{15}N NMR Studies of Tautomerism in 2-Phenylamino-2-thiazoline and Its Thiazine Analogue. *Org. Magn. Res.* **1982**, *19*, 219–221.

to give a residue which was purified by column chromatography on basic alumina (activity grade 1, hexane) to give **2a** (9.4 g, 74%) as a colorless oil: $[\alpha]^{20}_D$ −2.14° (*c* = 0.981, EtOH); NMR ($CDCl_3$) δ 0.80 (br s, 1, NH), 0.85 (d, 3, BuCH*Me*), 0.90 (s, 9, *t*-Bu), 1.28 (d, 3, ArCH*Me*), 2.30 (q, 1, BuC*H*), 3.77 (q, 1, ArC*H*), 7.22 (ddd, 1, *p*-ArH), 7.31 (ddd, 2, *m*-ArH), and 7.35 (dd, 2, o-ArH).

(*S*,*S*)-α-Methyl-*N*-(1,2,2-trimethylpropyl)benzenemethanamine (2b), prepared from **1b** using the above procedure, was obtained as a colorless oil: $[\alpha]^{20}_D$ +1.58° (*c* = 1.076, EtOH).

***rac*-2-Amino-3,3-dimethylbutanol (15).** DL-*tert*-leucine (4.00 g, 30 mmol) was added in small portions to an ice-cooled, stirred suspension of lithium aluminum hydride (3.8 g, 100 mmol) in dry THF (150 mL). The mixture was heated at 50 °C for 1 h and then cooled to 0 °C and treated dropwise with a saturated aqueous solution of Na_2SO_4 (8 mL). The precipitated salts were filtered off and washed with hot MeOH (50 mL). The filtrate and washings were combined, and the solvent was evaporated off to give a residue which was purified by column chromatography (silica gel, 0.5% NH_4OH, 4.5% EtOH in CH_2Cl_2) to give **15** (1.8 g, 51%) as a pale yellow oil: NMR ($CDCl_3$) δ 0.90 (s, 9, *t*-Bu), 1.94 (br s, 3, NH_2 and OH), 2.51 (dd, 1, C*H*NH_2), 3.21 (t, 1, C*H*OH), and 3.71 (dd, 1, C*H*OH).

Method A. *rac*-*N*-3-Pyridinyl-*N*′-[2,2-dimethyl-1-(hydroxymethyl)propyl]thiourea, Hydrochloride (27). A mixture of 3-pyridyl isothiocyanate[54] (0.92 g, 6.7 mmol) and **15** (0.79 g, 6.7 mmol) in EtOH (25 mL) was stirred for 2 h at 20 °C. The solvent was evaporated and the residue was purified by column chromatography (silica gel, eluent 0.5% NH_4OH, 4.5% EtOH in CH_2Cl_2) to give the free base (1.6 g, 94%) as a colorless oil. To further characterize the base it was dissolved in EtOH (10 mL), treated with EtOH/HCl (excess), evaporated to dryness, and recrystallized from EtOH–ether to give hydrochloride salt **27** (0.90 g, 46%) as a pale yellow crystalline solid: mp 181–182 °C; NMR (DMSO-d_6) δ 0.98 (s, 9, *t*-Bu), 3.53 (dd, *J* = 5.9, 38.6, 1, OCH), 3.64 (dd, *J* = 4.0, 38.6, 1, OCH), 4.30 (m, *J* = 4.0, 5.9, 1, *t*-BuCH), 7.95 (dd, 1, 5-PyrH), 8.54 (d, 1, 4-PyrH), 8.61 (dd, 1, 6-PyrH), 8.67 (br s, 1, NH), 9.54 (d, 1, 2-PyrH), and 11.44 (br s, 1, NH). Anal. ($C_{12}H_{19}N_3OS$·HCl) C, H, N, Cl, S.

Method B. (i) *N*-4-Pyridinylcarbamodithioic Acid, Methyl Ester (4). A solution of *N*-4-pyridinylcarbamodithioic acid, triethylammonium salt[55] (56.6 g, 208 mmol), in EtOH (700 mL) was treated with methyl iodide (14.0 mL, 225 mmol) and stirred for 5 h at room temperature. The solution was evaporated to dryness and the residue was purified by column chromatography (silica gel, 0.2% NH_4OH, 1.8% EtOH in CH_2Cl_2) to give **4** (32.8 g, 85%) as a yellow crystalline solid: mp 144–146 °C (lit.[54] 142–144 °C); NMR ($CDCl_3$) δ 2.64 (s, 3, Me), 7.89 (AB q, 2, β-PyrH), and 8.51 (AB q, 2, α-PyrH).

(ii) (*R*)-*N*-4-Pyridinyl-*N*′-(1,2,2-trimethylpropyl)thiourea, Hydrochloride (18a). A solution of **2a** (5.10 g, 25 mmol) in EtOH (250 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (0.8 g). The calculated amount of H_2 was taken up in 5 h. The solution containing (*R*)-1,2,2-trimethylpropylamine (**3a**) was filtered, treated with **4** (4.60 g, 25 mmol), and heated at 40 °C for 24 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel, 0.5% NH_4OH, 4.5% EtOH in CH_2Cl_2) to give the free base (5.70 g, 96%) as a yellow crystalline solid; mp 138–140 °C. To further purify the base it was dissolved in EtOH (50 mL), treated with EtOH/HCl (excess), evaporated to dryness, and recrystallized from acetone–Et_2O to give hydrochloride salt **18a** (5.5 g, 80%) as a pale yellow crystalline solid: mp 212–213 °C; $[\alpha]^{20}_D$ −35.9° (*c* = 1.02, EtOH); NMR ($CDCl_3$) δ 1.01 (s, 9, *t*-Bu), 1.17 (d, 3, CH*Me*), 4.41 (m, 1, C*H*Me), 8.18 (d, 2, β-PyrH), 8.53 (d, 1, N*H*Me), 8.71 (d, 2, α-PyrH), 11.84 (s, 1, PyrNH), and 12.7 (br s, 1, NH^+). Anal. ($C_{12}H_{19}N_3S$·HCl) C, H, N, Cl, S.

(*S*)-*N*-4-Pyridinyl-*N*′-(1,2,2-trimethylpropyl)thiourea, Hydrochloride (18b), prepared from **2b** by the above procedure, was obtained as pale yellow crystalline solid: mp 215–217 °C; $[\alpha]^{20}_D$ +32.4° (c = 1.095, EtOH). Anal. ($C_{12}H_{19}N_3S$·HCl) C, H, N, Cl, S.

Method C. *N*-Cyano-*N*′-(1,1-dimethylpropyl)-*N*″-3-pyridinylguanidine (31). A mixture of **26** (3.8 g, 17 mmol), triphenylphosphine (6.6 g, 25 mmol), triethylamine (3.0 mL, 22 mmol), and CCl_4 (3.0 mL, 31 mmol) in dry CH_2Cl_2 (60 mL) was heated under reflux in an argon atmosphere for 4 h. The mixture was evaporated to dryness and the residue was triturated with Et_2O (3 × 100 mL). The combined extracts were dried (K_2CO_3), and the solvent was evaporated to give the crude carbodiimide which was treated with cyanamide (1.0 g, 24 mmol) and *N*-ethyldiisopropylamine (1.0 mL, 6 mmol) and heated at 35 °C for 18 h. The mixture was purified by column chromatography (silica gel, 5% EtOH in CH_2Cl_2) and recrystallized from EtOH–Et_2O to give **31** (3.6 g, 91%) as a colorless crystalline solid: mp 185–187 °C (lit.[2] mp 184–186 °C); NMR (DMSO-d_6) δ 0.81 (t, 3, CH_2Me), 1.28 (s, 6, CMe_2), 1.71 (q, 2, CH_2Me), 6.9 (br s, 1, NH), 7.35 (dd, 1, 5-PyrH), 7.50 (m, 1, 4-PyrH), 8.27 (dd, 1, 6-PyrH), 8.37 (d, 1, 2-PyrH), and 9.1 (br s, 1, NH).

Method D. (*R*)-*N*-Cyano-*N*′-4-pyridinyl-*N*″-(1,2,2-trimethylpropyl)guanidine (19a). A mixture of (*R*)-*N*-4-pyridinyl-*N*′-(1,2,2-trimethylpropyl)thiourea (free base of **18a**) (2.37 g, 10 mmol), cyanamide (0.84 g, 20 mmol), *N*,*N*′-dicyclohexylcarbodiimide (4.10 g, 20 mmol), and *N*-ethyldiisopropylamine (0.25 mL, 1.5 mmol) in dry Et_2O (15 mL) was stirred at room temperature for 72 h. The mixture was evaporated to dryness and the residue was purified by column chromatography (silica gel, 0.5% NH_4OH, 4.5% EtOH in CH_2Cl_2) and recrystallized from acetone–Et_2O to give **19a** (1.6 g, 65%) as a colorless crystalline solid: mp 164–165 °C; $[\alpha]^{20}_D$ −147.9° (*c* = 1.06, EtOH) [lit.[24] $[\alpha]^{20}_D$ −135° (*c* = 1, EtOH)]; NMR (10 mg/mL in $CDCl_3$, 300 K) δ 0.97 (s, 9, *t*-Bu), 1.15 (s, 3, CH*Me*), 3.86 (m, 1, C*H*Me), 5.15 (d, 1, N*H*CHMeBu), 7.18 (br d, 2, β-PyrH), 8.40 (br s, 1, N*H*Pyr) and 8.57 (br d, 2, α-PyrH); NMR (10 mg/mL in $CDCl_3$, 223 K) δ 0.96 (s, 9, *t*-Bu), 1.14 (s, 3, CH*Me*), 3.75 (br s, 0.11, C*H*Me), 3.97 (m, 0.89, C*H*Me), 5.4–5.7 (br m, 1, NH), 7.15 (br d, 1.78, β-PyrH), 7.40 (br d, 0.22, "β-PyrH"), 8.38 (br d, 0.22, "α-PyrH"), 8.53 (br d, 1.78, α-PyrH), 8.80 (br s, 0.11, NH), and 9.70 (br s, 0.89, NH).

Method E. (i) (*E*)-5-Methoxy-*N*-[1-(methylthio)-2-nitroethenyl]pyridin-3-amine (8). A mixture of 3-amino-5-methoxypyridine[56] (10.7 g, 86 mmol) and 1,1-bis(methylthio)-2-nitroethene (**5**)[57] (16.3 g, 90 mmol) in dry EtOH (500 mL) was heated under reflux for 72 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel, 0.2% NH_4OH, 1.8% EtOH in CH_2Cl_2) and recrystallized from EtOH–Et_2O to give **8** (6.4 g, 31%) as a yellow crystalline solid: mp 165–167 °C; NMR (DMSO-d_6) δ 2.46 (s, 3, SMe), 3.84 (s, 3, OMe), 6.80 (s, 1, =CH), 7.45 (dd, 1, 4-PyrH), 8.17 (d, 1, 2-PyrH), 8.27 (d, 1, 6-PyrH), and 11.4 (br s, 1, NH). Anal. ($C_9H_{11}N_3O_3S$) C, H, N, S.

(ii) (*S*)-*E*-*N*-(5-Methoxy-3-pyridinyl)-*N*′-(1,2,2-trimethylpropyl)-2-nitro-1,1-ethenediamine (38b). A solution of **2b** (3.95 g, 19.2 mmol) in EtOH (150 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (1.3 g). The calculated amount of H_2 was taken up in 45 min. The solution containing (*S*)-1,2,2-trimethylpropylamine (**3b**) was filtered, treated with **8** (4.6 g, 19.2 mmol), and heated under reflux for 22 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel, 0.3% NH_4OH, 2.7% EtOH in CH_2Cl_2) and recrystallized from CH_2Cl_2–Et_2O to give **38b** (1.5 g, 27%) as a colorless crystalline solid: mp 167–168 °C; $[\alpha]^{20}_D$ +169.4° (*c* = 1.062, EtOH); NMR (DMSO-d_6) δ 0.98 (s, 9, *t*-Bu), 1.19 (d, 3, CH*Me*), 3.83 (m, 1, C*H*Me), 3.85 (s, 3, OMe), 6.09 (s, 1, =CH), 7.31 (dd, 1, 4-PyrH), 8.07 (d, 1, 2-PyrH), 8.18 (d, 1, 6-PyrH), 9.25 (br s, 1, NH), and 10.6 (br s, 1, NH). Anal. ($C_{14}H_{22}N_4O_3$) C, H, N, O.

***N*-3-Pyridinyl-3,3-dimethylbutanamide (11).** To a solution of 3-aminopyridine (9.40 g, 100 mmol) and triethylamine (20.9 mL, 150 mmol) in dry CH_2Cl_2 (200 mL) was added 3,3-dimethylbutyryl chloride (14.8 g, 110 mmol) at −5 °C. The reaction mixture was stirred at 0 °C for 1 h and allowed to warm to room

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temperature during 3 h. The solution was washed with aqueous $NaHCO_3$ (2 × 150 mL of 10%) and dried (Na_2SO_4) and the solvent was evaporated. The residue was recrystallized from Et_2O–pentane to give **11** (17.4 g, 91%) as a colorless crystalline solid: mp 100–101 °C; NMR ($CDCl_3$) δ 1.11 (s, 9, *t*-Bu), 2.27 (s, 2, CH_2), 7.27 (dd, 1, 5-PyrH), 8.20 (m, 1, 4-PyrH), 8.38 (dd, 1, 6-PyrH), 8.54 (dd, 1, 2-PyrH), and 9.04 (br s, 1, NH). Anal. ($C_{11}H_{16}N_2O$) C, H, N.

***N*-3-Pyridinyl-3,3-dimethylbutanethioamide (12).** A solution of **11** (9.4 g, 49 mmol) and Lawesson's reagent [2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide; 19.8 g, 49 mmol] in toluene (250 mL) was heated under reflux for 3 h. The solvent was evaporated and the residue was purified by column chromatography on basic alumina (500 g of activity grade 1, CH_2Cl_2) and recrystallized from Et_2O–pentane to give **12** (8.2 g, 80%) as a yellow crystalline solid: mp 118–119 °C; NMR ($CDCl_3$) δ 1.15 (s, 9, *t*-Bu), 2.84 (s, 2, CH_2), 7.36 (dd, 1, 5-PyrH), 8.38 (m, 1, 4-PyrH), 8.47 (dd, 1, 6-PyrH), 8.59 (d, 1, 2-PyrH), and 8.96 (br s, 1, NH). Anal. ($C_{11}H_{16}N_2S$) C, H, N, S.

***N*-(1,1-Dimethylethyl)-3-pyridineacetamide (13).** To a solution of 3-pyridineacetic acid (15.2 g, 100 mmol), triethylamine (34.8 mL, 250 mmol), and diphenyl phosphorazidate (21.6 mL, 100 mmol) in dry CH_2Cl_2 (350 mL) was added *tert*-butylamine (10.5 mL, 100 mmol) at 10 °C and the reaction mixture was stirred at room temperature for 15 h. The mixture was extracted with HCl (3 × 100 mL of 2 M), and the combined extracts were basified (Na_2CO_3) and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated to give the crude product which was purified by column chromatography (silica gel, 0.2% NH_4OH, 1.8% EtOH in CH_2Cl_2) and recrystallized from Et_2O–pentane to give **13** (16 g, 83%) as a colorless crystalline solid: mp 100–102 °C; NMR ($CDCl_3$) δ 1.32 (s, 9, *t*-Bu), 3.44 (s, 2, CH_2), 5.4 (br s, 1, NH), 7.27 (dd, 1, 5-Pyr), 7.65 (ddd, 1, 4-PyrH), 8.49 (d, 1, 2-PyrH), and 8.53 (dd, 1, 6-PyrH). Anal. ($C_{11}H_{16}N_2O$) C, H, N.

***N*-(1,1-Dimethylethyl)-3-pyridinethioacetamide (14),** prepared from **13** and Lawesson's reagent using the above procedure, was obtained as a colorless crystalline solid: mp 134–135 °C; NMR ($CDCl_3$) δ 1.51 (s, 9, *t*-Bu), 3.98 (s, 2, CH_2), 7.1 (br s, 1, NH), 7.30 (dd, 1, 5-PyrH), 7.71 (ddd, 1, 4-PyrH), 8.47 (d, 1, 2-PyrH), and 8.54 (dd, 1, 6-PyrH). Anal. ($C_{11}H_{16}N_2S$) C, H, N, S.

***N*-Cyano-*N'*-3-pyridinyl-3,3-dimethylbutanimidamide, Hydrochloride (41).** A solution of **12** (1.0 g, 5 mmol) and bis(triphenylstannyl)carbodiimide[58] (3.7 g, 5 mmol) in dry EtOH (25 mL) was heated under reflux for 7 h. The mixture was cooled to 5 °C and the precipitated bis(triphenyltin) sulfide was removed by filtration. The filtrate was evaporated to dryness and the residue was purified by column chromatography (silica gel, 2% EtOH in CH_2Cl_2) to give the free base as a colorless crystalline solid: mp 101–104 °C. The base was dissolved in CH_2Cl_2 (20 mL), treated with CH_2Cl_2/HCl (excess), evaporated to dryness under reduced pressure, and recrystallized from DMF–toluene to give hydrochloride salt **41** (0.19 g, 15%) as a colorless crystalline solid; mp 210–213 °C; NMR (DMSO-d_6) δ 1.11 (s, 9, *t*-Bu), 2.74 (s, 2, CH_2), 7.92 (m, 1, 5-PyrH), 8.63 (dd, 2, 4- and 6-PyrH), 9.21 (dd, 1, 2-PyrH), 11.56 (br s, 1, NH), and 12.3 (br s, 1, NH). Anal. ($C_{12}H_{16}N_4$·HCl) C, H, N, Cl.

***N*-Cyano-*N'*-(1,1-dimethylethyl)-3-pyridineacetimidamide (42),** prepared from **14** and bis(triphenylstannyl)carbodiimide using the above procedure, was obtained as a colorless crystalline solid: mp 137–139 °C; NMR ($CDCl_3$) δ 1.36 (s, 9, *t*-Bu), 3.86 (s, 2, CH_2), 7.0 (br s, 1, NH), 7.28 (m, 1, 5-PyrH), 7.67 (ddd, 1, 4-PyrH), and 8.52 (m, 2, 2- and 6-PyrH). Anal. ($C_{12}H_{16}N_4$) C, H, N.

***rac*-*N*-[4,5-Dihydro-4-(1,1-dimethylethyl)-2-thiazolyl]-3-pyridinamine (43).** To a solution of **26** (0.56 g, 2.2 mmol) and triethylamine (0.23 g, 2.3 mmol) in dry CH_2Cl_2 (25 mL) was added methanesulfonyl chloride (0.26 g, 2.3 mmol) at −10 °C. The reaction mixture was stirred for 2 h and then allowed to warm to room temperature during 17 h. The mixture was evaporated to dryness and the residue was purified by column chromatography (silica gel, 0.4% NH_4OH, 3.6% EtOH in CH_2Cl_2) and recrystallized from acetone–pentane to give **43** (0.18 g, 35%) as a colorless crystalline solid: mp 173–174 °C; NMR ($CDCl_3$) δ 0.99 (s, 9, *t*-Bu), 3.17 (dq, 2, NCH_2), 3.73 (dt, 1, *t*-BuC*H*), 5.9 (br s, 1, NH), 7.19 (dd, 1, 5-PyrH), 7.43 (dd, 1, 4-PyrH), 8.27 (dd, 1, 6-PyrH), and 8.35 (d, 1, 2-PyrH). Anal. ($C_{12}H_{17}N_3S$) C, H, N, S.

***N*-(4,5-Dihydro-4,4-dimethyl-2-thiazolyl)-3-pyridinamine (44),** prepared from **28** using the above procedure, was obtained as a colorless crystalline solid: mp 190–192 °C; NMR (DMSO-d_6) δ 1.36 (s, 6, CMe_2), 3.13 (s, 2, CH_2), 5.5 (br s, 1, NH), 7.16 (dd, 1, 5-PyrH), 7.56 (dd, 1, 4-PyrH), 8.11 (dd, 1, 6-PyrH), and 8.38 (d, 1, 2-PyrH). Anal. ($C_{10}H_{13}N_3S$·0.1H_2O) C, H, N.

***N*-2-[4-(1,1-Dimethylethyl)thiazolyl]-3-pyridinamine (45).** A solution containing 3-pyridylthiourea (1.53 g, 10 mmol) and 1-bromo-3,3-dimethyl-2-butanone (1.80 g, 10 mmol) in dry DMF (40 mL) was heated at 60 °C for 6 h. The solvent was evaporated and the residue was treated with aqueous $NaHCO_3$ (100 mL of 10%) and extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated to give the crude product which was purified by column chromatography (silica gel, 0.3% NH_4OH, 2.7% EtOH in CH_2Cl_2) and recrystallized from EtOAc to give **45** (1.3 g, 57%) as a colorless crystalline solid: mp 196–197 °C; NMR (DMSO-d_6) δ 1.28 (s, 9, *t*-Bu), 6.48 (s, 1, SCH), 7.33 (dd, 1, 5-PyrH), 8.13 (dd, 1, 4-PyrH), 8.17 (ddd, 1, 6-PyrH), 8.78 (d, 1, 2-PyrH), and 10.31 (s, 1, NH). Anal. ($C_{12}H_{15}N_3S$) C, H, N, S.

***N*-[4,5-Dihydro-4-(1,1-dimethylethyl)-2-imidazolyl]-3-pyridinamine, Dihydrochloride (46).** A mixture of *N*-3-pyridinylcarbamimidothioic acid, methyl ester[59] (1.67 g, 10 mmol), 3,3-dimethyl-1,2-butanediamine, dihydrochloride[60] (1.89 g, 10 mmol), and triethylamine (3.1 mL, 30 mmol) in EtOH (100 mL) was heated under reflux for 16 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel, 0.4% NH_4OH, 3.6% EtOH in CH_2Cl_2) to give the base, which was dissolved in EtOH (10 mL), treated with ethanolic HCl (excess), evaporated to dryness, and recrystallized from EtOH–ether to give dihydrochloride **46** (1.18 g, 41%) as a colorless crystalline solid: mp 225–228 °C; NMR (DMSO-d_6) δ 0.89 (s, 9, *t*-Bu), 3.52 (dd, *J* = 7.0, 10.7, 1, *t*-BuCH), 3.72 (t, *J* = 10.7, 10.7, 1, *trans*-CHC*H*), 3.83 (dt, *J* = 7.0, 10.7, 1, *cis*-CHC*H*), 7.81 (dd, 1, 5-PyrH), 8.11 (dd, 1, 4-PyrH), 8.63 (d, 1, 6-PyrH), 8.75 (d, 1, 2-PyrH), 8.98 (br s, 1, NH), 9.15 (br s, 1, NH), and 11.35 (br s, 1, NH^+). Anal. ($C_{12}H_{18}N_4$·2HCl) C, H, N, Cl.

***N*-(4,5-Dihydro-4,4-dimethyl-2-imidazolyl)-3-pyridinamine, Dihydrochloride (47),** prepared from *N*-3-pyridinylcarbamimidothioic acid, methyl ester, and 1,2-diamino-2-methylpropane using the above procedure, was obtained as a colorless crystalline solid: mp 205–207 °C; NMR (DMSO-d_6) δ 1.36 (s, 6, CMe_2), 3.45 (s, 2, CH_2), 7.85 (dd, 1, 5-PyrH), 8.18 (dd, 1, 4-PyrH), 8.66 (dd, 1, 6-PyrH), 8.79 (d, 1, 2-PyrH), 8.85 (br s, 1, NH), 9.17 (br s, 1, NH), and 11.61 (br s, 1, NH^+). Anal. ($C_{10}H_{14}N_4$·2HCl) C, H, N, Cl.

***rac*-*N*-[4,5-Dihydro-3-(2-butyl)imidazolyl]-3-pyridinamine (48),** prepared from *N*-3-pyridinylcarbamimidothioic acid, methyl ester, and *N*-*sec*-butylethylenediamine using the above procedure, was obtained as a pale yellow oil: NMR ($CDCl_3$) δ 0.98 (t, 3, CH_3CH_2), 1.18 (d, 2, CH_3CH), 1.57 (m, 2, CH_2Me), 3.36 (m, 4, CH_2CH_2), 4.10 (br s, 1, NH), 4.12 (m, 1, CH), 7.13 (dd, 1, 5-PyrH), 7.24 (ddd, 1, 4-PyrH), 8.14 (dd, 1, 6-PyrH), and 8.25 (d, 1, 2-PyrH). Anal. ($C_{12}H_{18}N_4$·0.2H_2O) C, H, N, O.

Macroscopic Ionization Constants. These were calculated[61] from spectrophotometric data obtained at 25 °C, in ethylene glycol

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monomethyl ether/water (16 g/84 g), with a Hewlett-Packard UV/VIS 8450A spectrophotometer.

Biology. Measurement of $^{86}Rb^+$ Efflux and Spontaneous Activity of Rat Portal Veins. Male Wistar rats were anesthetized with CO_2 and exsanguinated. The portal vein was exposed and attached at either end to a cotton thread. After removal of the surrounding connective tissue it was cut along the length axis. The lumen was washed free of blood, the vessel excised, and a tension of 500 mg was applied. The vein was then incubated for 30 min in a HEPES-buffered physiological salt solution (PSS) gassed with 95% O_2/5% CO_2 at 32 °C, pH 7.4. The PSS contained NaCl (120 mM), KCl (5 mM), $NaHCO_3$ (15 mM), NaH_2PO_4 (1.2 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.5 mM), glucose (11 mM), and HEPES (20 mM) and had pH 7.4 at 37 °C. For loading with $^{86}Rb^+$, the vein was incubated for an additional 80 min in PSS to which 5 μCi/mL $^{86}RbCl$ had been added.

After loading with $^{86}Rb^+$, the vein was shortly dipped into PSS to remove excess radioactivity and mounted in a thermostated perfusion chamber. A preload of 500 mg was applied and the chamber was perfused with PSS at 37 °C at a rate of 2.5 mL/min. The upper cotton thread was attached to an isometric force transducer (Gould cell, Statham) that was connected to a custom-built amplifier from which the signal was given to a recorder and to an integrator for quantitation of myogenic activity.

For measurement of $^{86}Rb^+$ efflux, the perfusate was collected at a sampling rate of 2 min and counted for radioactivity in the Cerenkov mode at 50% efficiency. The radioactivity remaining in the portal vein at the end of the assay was determined by dissolving the vessel in 0.5 mL of Lumasolve (Lumac) at 50 °C overnight. The sample was then supplemented with 0.5 mL of 1 M HCl and 10 mL of Optifluor (Packard) and counted in the ^{32}P channel at 100% efficiency.

The rate constant, k, of $^{86}Rb^+$ efflux, defined as the radioactivity released from the vessel per minute divided by the concurrent radioactivity of the vessel,[62] was calculated as described.[36] Drug effects on the efflux rate constant were calculated as the peak value of k obtained during drug application compared to the basal value of k averaged over 6–10 min before drug application and are expressed as a percentage scale (Δk%). Concentration–effect curves were fitted to the law of mass action or to the Hill equation by nonlinear least-squares analysis.

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Communications to the Editor

7-Oxabicycloheptylprostanoic Acids: Potent, Time-Dependent Cyclooxygenase Inhibitors That Induce a Conformational Change in the Prostaglandin Endoperoxide Synthase Protein

Inhibition of the cyclooxygenase activity of prostaglandin endoperoxide (PGH) synthase is believed to be the basis for the pharmacological action of nonsteroidal antiinflammatory agents.[1,2] Understanding the structural basis of cyclooxygenase inhibition by diverse classes of inhibitors has taken on added significance with the recent discovery of a second cyclooxygenase gene that is expressed in response to mitogenic stimulation of cells.[3-5] The protein coded by this gene is 60% similar to the constitutively expressed cyclooxygenase protein but its substrate specificity, sensitivity to inhibitors, etc., is not known.

The Squibb group recently described a novel series of 7-oxabicycloheptylprostanoic acid derivatives that inhibit arachidonic acid-induced platelet aggregation and arachidonic acid oxygenation by platelet and bovine seminal vesicle microsomes.[6-8] The most potent compound among a series of structural analogs is **1**, which possesses bis-exo

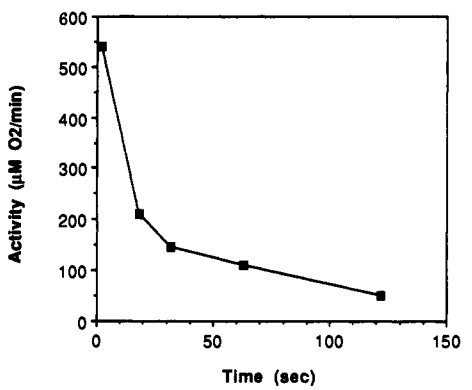

Figure 1. Time course of loss of cyclooxygenase activity following addition of **2**. The assay mixture contained 0.20 μM protein (specific activity 17 μmol O_2 min^{-1} mg^{-1} protein), 1 μM hematin, 1 mM phenol, and 0.075 μM **2** in 1.25 mL Tris-HCl (100 mM, pH 7.4). At varying times after addition of **2**, 97 μM arachidonic acid was added and the rate of O_2 uptake determined. The O_2 uptake rate in the absence of **2** was 629 μM O_2/min.

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(8) The Squibb numbers for compounds **1** and **2** are SQ 28,852 and SQ 29,535, respectively.[6]

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