hydroxyapatite, and gel filtration (details to be published elsewhere). The K_m values for ATP and PI for this PI kinase preparation were 47 and 105 μM, respectively, and the specific activity was 25 nmol of PIP formed min^{-1} mg^{-1}.

PI 4-kinase was assayed in a buffer containing 0.3% (w/v) Triton X-100, 100 μM [γ-^{32}P]ATP (50000 cpm $nmol^{-1}$), 200 μM PI, 1 mM dithiothreitol, 15 mM $MgCl_2$, 1 mM EGTA, 0.1 M NaCl, and 50 mM HEPES (pH 7.4, 30 °C) in a total volume of 0.2 mL. Reactions were linear over the time period used. The incubations were terminated by the addition of 0.75 mL of $CHCl_3$/MeOH/concentrated HCl (40:80:1). Two phases were obtained by the addition of 0.25 mL of $CHCl_3$ and 0.1 M HCl, and the organic phase was washed twice with 0.5 mL of $CHCl_3$/MeOH/0.1 M HCl (3:48:47). A 200-μL portion of the resulting organic phase was then counted for ^{32}P radioactivity. To confirm that the assay monitored specifically the formation of PI 4[^{32}P]P, the organic phase obtained as described above was dried under vacuum, the phospholipids were deacylated with $MeNH_2$, and the products were analyzed by ion-exchange HPLC on a partisphere wax column as described previously.[6] At least 95% of the ^{32}P was found to co-migrate with authentic 1-(*sn*-glycer-3-ylphospho)-D-*myo*-inositol 4-phosphate.

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Supplementary Material Available: X-ray crystal data on compound **2a** (Tables II–V) and NMR spectral comparison of compounds I and **10a** (Figure 2) (14 pages). Ordering information is given on any current masthead page.

Design of an Antithrombotic–Antihypertensive Agent (Wy 27569). Synthesis and Evaluation of a Series of 2-Heteroaryl-Substituted Dihydropyridines

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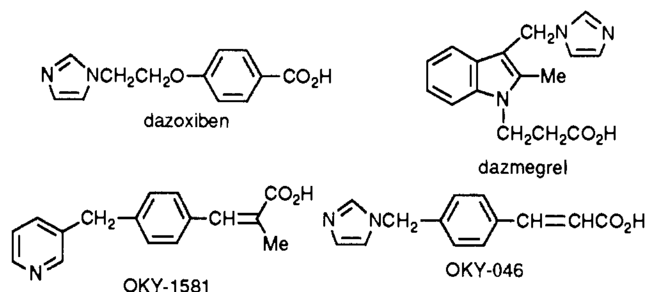
An approach to the design of potential combined antithrombotic–antihypertensive agents is described. A series of 1,4-dihydropyridines bearing a 1*H*-imidazol-1-yl or pyrid-3-yl substituted side chain in the 2-position were synthesized and tested for antihypertensive activity in spontaneously hypertensive rats and for inhibition of TXA_2 synthetase in rabbit platelets, in vitro. 1,4-Dihydro-2-(1*H*-imidazol-1-ylmethyl)-6-methyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid 3-ethyl 5-methyl diester (1) was shown to be similar in potency to nitrendipine as an antihypertensive agent. Compound 1 inhibited TXA_2 synthetase in rabbit and human platelets in vitro and reduced plasma TXB_2 levels in rats at antihypertensive dose levels. The reductions in thromboxane production observed in vivo and in vitro were accompanied by enhanced levels of 6-KPGF$_{1\alpha}$, reflecting diversion of the arachidonic acid cascade toward prostacyclin synthesis.

Hypertension is an important risk factor for a variety of cardiovascular disorders. With the availability of effective antihypertensive agents, therapy for marked and moderate hypertension is now routine. Although effective antihypertensive therapy reduces the incidence of cardiovascular related morbidity and mortality in previously hypertensive patients, the level of risk is not entirely reduced to that present in normotensive subjects. For example, extensive trials of antihypertensive drug therapy in hypertensive patients have shown a significant reduction in the incidence of stroke, congestive heart failure, and renal damage but little or no reduction in coronary artery disease (CAD) and associated myocardial infarction or sudden death.[1] Given the present availability of safe and effective antihypertensive agents with relatively few side-effects, further advance may require the development of agents having additional properties capable of reducing the incidence of CAD related events. One such approach would be to design a combined antithrombotic–antihypertensive agent.

CAD is characterized by the presence of atherosclerotic plaque on the coronary blood vessel wall. Acute myocardial infarction is believed to be initiated by plaque disruption and exposure of underlying collagen which stimulates platelet aggregation leading to thrombus formation and vasospasm due to release of vasoactive substances from the aggregating platelets.[2] Recent studies have highlighted the role of prostaglandins in CAD.[3,4] Thromboxane A_2 (TXA_2) is synthesized in platelets by the action of TXA_2 synthetase on prostaglandin endoperoxides and is one of the products released during platelet aggregation.[5] TXA_2 has been shown to be a potent vasoconstrictor and platelet-aggregating agent and these pro-

Chart I



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[§] Present address: Pfizer Central Research, Sandwich, Kent, England.

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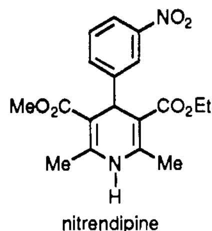
Chart II

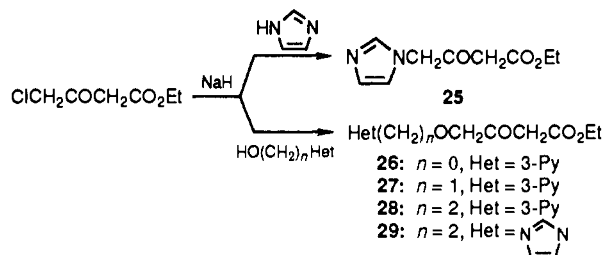


thrombotic actions may be implicated in CAD.[5,6] Inhibition of TXA_2 release by inhibition of TXA_2 synthetase is therefore of potential value for the prophylactic treatment of CAD. In addition, selective inhibition of TXA_2 synthetase redirects prostaglandin endoperoxides to the synthesis of prostacyclin, a powerful platelet-aggregation inhibitor and vasodilator.[6-8] Following an early observation that imidazole inhibited TXA_2 synthetase,[9] a number of laboratories have developed 1-substituted imidazole derivatives showing potent and selective TXA_2 synthetase inhibiting activity.[10-12] It has also been demonstrated that the 1-imidazolyl group may be replaced by 3-pyridyl.[11]

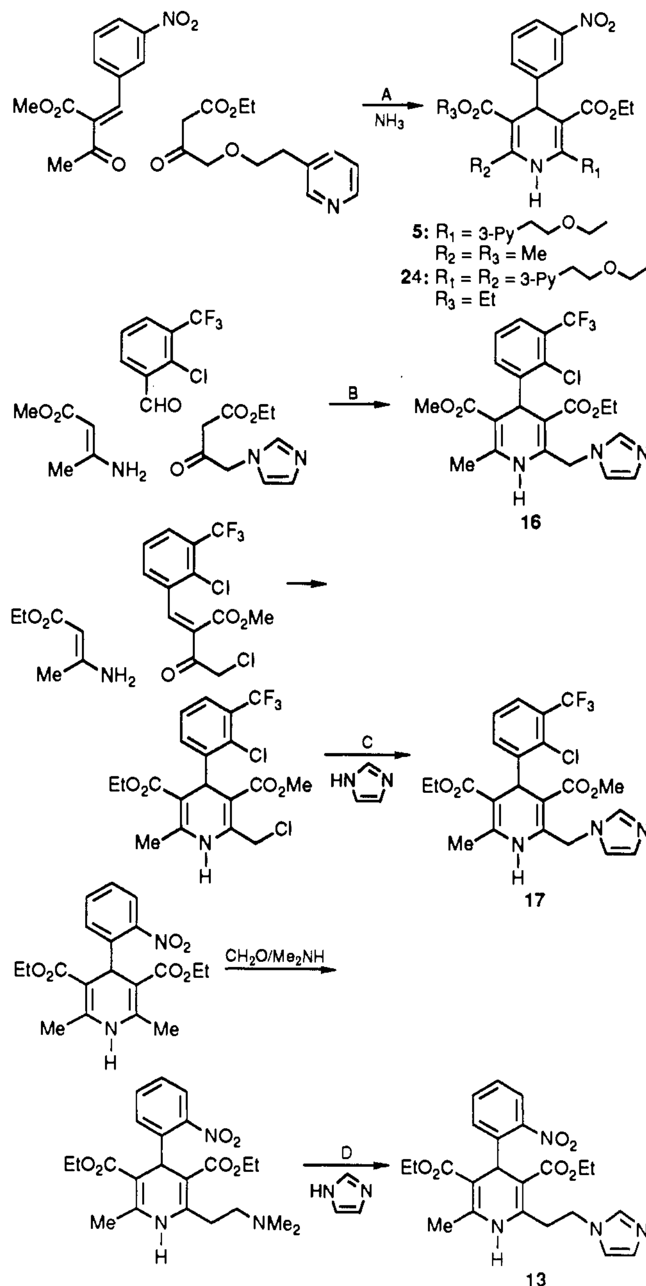
Clinical studies have been reported on dazoxiben,[13] dazmegrel,[14] OKY-1581,[15] and OKY-046[16] (Chart I). In general these compounds do not have antihypertensive activity although dazmegrel is reported to lower blood pressure in spontaneously hypertensive rats at high doses.[17] However, given the potent vasoconstricting action of TXA_2, the possibility of a synergistic effect of TXA_2 inhibition of blood pressure was envisaged when combined with antihypertensive activity in a single molecule.

The general structural requirements for TXA_2 synthetase inhibiting activity in this class of compounds consists of a basic heterocyclic ring and a polar, generally carboxyl, functionality separated by a wide variety of structural components such that the distance between the basic center and the polar function is about 8.5–10 Å.[11,12] We felt that these relatively nonspecific structural requirements might be incorporated into a known antihypertensive structure without loss of TXA_2 synthetase inhibiting activity. In particular we were attracted to the possibility of combining these structural components with a calcium antagonist of the dihydropyridine (DHP) type, typified by nitrendipine (Chart II).[18]

Scheme I



Scheme II



Because of their potency and selectivity for vascular smooth muscle, DHP calcium antagonists are important compounds for the treatment of hypertension and angina.[18-20] In the longer term, calcium antagonists have a

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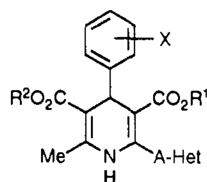
Table I. Dihydropyridines[a]



compd	R^1	R^2	A	Het	X	crystn solv	mp, °C	meth-od	% yield	formula[c]	dose, po, mmol/kg	% fall BP[b] (SEM) 2 h	6 h	TXA_2 inhibn:[d] IC_{50}, μM
1	Et	Me	CH_2	Im	3-NO_2	*i*-PrOH–MeOH	223–225	B	18	$C_{21}H_{22}N_4O_6$·HCl	0.03	51 (3)	38 (2)	5
								C	60		0.003	34 (4)	28 (9)	
2	Et	Et	CH_2CH_2	Im	3-NO_2	EtOH–Et_2O	174–175	D	41	$C_{23}H_{26}N_4O_6$·HCl[e]	0.03	40 (5)	NS	11
3	Et	Me	CH_2O	Py	3-NO_2	EtOAc	201–203	A	33	$C_{23}H_{23}N_3O_7$·HCl	0.03	31 (2)	17 (2)	12.8
4	Et	Me	CH_2OCH_2	Py	3-NO_2	EtOH–Et_2O	139–141	A	35	$C_{24}H_{25}N_3O_7$·HCl	0.03	18 (5)	NS	1.4
5	Et	Me	CH_2OCH_2-CH_2	Py	3-NO_2	EtOH–Et_2O	175–177	A	31	$C_{25}H_{27}N_3O_7$·HCl[f,f]	0.03	40 (6)	NS	72
6	Et	Me	CH_2OCH_2-CH_2	Im	3-NO_2	EtOAc–Et_2O	199–201	A	29	$C_{23}H_{26}N_4O_7$·HCl[f]	0.03	56 (4)	42 (7)	115
											0.003	NS	NS	
7	Me	Me	CH_2	Im	3-NO_2	MeOAc	223–224	C	25	$C_{20}H_{20}N_4O_6$·HCl	0.03	42 (3)	27 (2)	11.8
											0.003	NS	NS	
8	Et	Et	CH_2	Im	3-NO_2	EtOAc–EtOH	132–137	B	18	$C_{22}H_{24}N_4O_6$·HCl[h]	0.03	47 (7)	13 (5)	1.4
9	Me	Et	CH_2	Im	3-NO_2	*i*-PrOH–MeOH	204–207	B	14	$C_{21}H_{22}N_4O_6$·HCl	0.03	NS	NS	2.2
10	Et	*i*-Pr	CH_2	Im	3-NO_2	EtOH	115–117	B	8	$C_{23}H_{26}N_4O_6$·HCl	0.03	21 (9)	19 (5)	2
11	Et	*t*-Bu	CH_2	Im	3-NO_2	EtOAc–EtOH	150–156	B	14	$C_{24}H_{28}N_4O_6$·HCl[f]	0.03	NS	NS	23
12	Et	Me	CH_2	Im	2-NO_2	*i*-PrOH–MeOH	156–157	B	16	$C_{21}H_{22}N_4O_6$·$C_4H_6O_4$[i]	0.03	59 (3)	56 (2)	3.2
											0.003	44 (4)	14 (3)	
13	Et	Et	CH_2CH_2	Im	2-NO_2	EtOH	142–143	D	30	$C_{23}H_{26}N_4O_6$·$C_4H_4O_4$[j]	0.03	37 (3)	NS	2.3
14	Et	Me	CH_2	Im	2-$OCHF_2$	Et_2O	166–168	B	5	$C_{22}H_{23}F_2N_3O_5$	0.03	55 (3)	48 (2)	1.1
											0.003	NS	NS	
15	Me	Et	CH_2	Im	2-$OCHF_2$	EtOAc–MeOH	155–157	C	53	$C_{22}H_{23}F_2N_3O_5$·$C_4H_6O_4$	0.03	48 (4)	31 (5)	25
16	Et	Me	CH_2	Im	2-Cl,3-CF_3	EtOAc–MeOH	187–190	B	19	$C_{22}H_{21}ClF_3N_3O_4$·$C_4H_6O_4$	0.03	60 (3)	52 (5)	16
											0.003	16 (1)	NS	
17	Me	Et	CH_2	Im	2-Cl,3-CF_3	EtOAc–MeOH	190–193	C	60	$C_{22}H_{21}ClF_3N_3O_4$·$C_4H_6O_4$	0.03	31 (4)	15 (7)	125
18	Et	Me	CH_2	Im	2-Cl	EtOAc	127–130	B	11	$C_{21}H_{22}ClN_3O_4$·HCl[f]	0.03	56 (0)	48 (4)	0.5
											0.003	NS	NS	
19	Et	Me	CH_2	Im	2,3-Cl_2	EtOAc	186–187	C	12	$C_{21}H_{21}Cl_2N_3O_4$·HCl[h]	0.03	54 (3)	49 (3)	13
											0.003	40 (1)	20 (3)	
20	Et	Me	CH_2	Im	2,-Cl,6-F	*i*-PrOH–MeOH	227–229	B	12	$C_{21}H_{21}ClFN_3O_4$·HBr	0.03	48 (4)	35 (4)	19.5
											0.003	NS	NS	
nitrendipine											0.03	39 (9)	33 (7)	1000
dazoxiben											0.15	NS	NS	2.5

[a] All compounds exhibited IR and ^1H NMR spectra consistent with the assigned structure. [b] Mean percent fall in systolic blood pressure: standard errors of the mean (SEM) are indicated in parenthesis. All results were analyzed for statistically significant differences from control by analysis of variance, nonsignificant values ($p > 0.05$) are indicated by NS. [c] C, H, and N analyses were within ±0.4% of the theoretical values for the formulae given. [d] Molar concentration required to produce a 50% reduction in maximal stimulated release of thromboxane A_2 $(TXA)_2$ from rabbit platelet. [e] Quarter hydrate. [f] Half hydrate. [g] H: calcd, 5.55; found, 5.08. [h] Hydrate. [i] Succinate. [j] Maleate.

potentially protective role in atherosclerosis,[21] and incorporation of an antithrombotic component could be synergistic in further reducing morbidity and mortality among treated hypertensives and others with CAD. This approach received further support from a report of synergism between calcium antagonists and TXA_2 synthetase inhibitors, or prostacyclin, in terms of platelet antiaggregatory activity which appeared following initiation of this project in our laboratories.[22]

We also noted that this class of compounds contain ester groupings which could provide the desirable polar functionality present in previously reported TXA_2 synthetase inhibitors. In one approach to our goal of a combined antihypertensive–antithrombotic agent, we synthesized a series of analogues related to nitrendipine bearing a heteroarylalkyl substituent at the 2-position of the dihydropyridine ring. These studies led to the discovery of a number of compounds possessing both marked antihypertensive activity and TXA_2 synthetase inhibiting activity.

Chemistry

The compounds listed in Tables I and II were prepared by four general methods illustrated in Schemes I and II. β-Keto esters bearing a heterocyclic side chain were available by reaction of ethyl 4-chloroacetoacetate sodium enolate with sodium imidazolide or the sodium salt of a heterocyclic alcohol or phenol (Scheme I). Hantzsch type condensation[23] of these keto esters with a benzylidene keto ester and ammonia gave a mixture of DHPs bearing one or two heterocyclic side chains at the 2-position or 2,6-positions (route A, Scheme II). In a further variant of the Hantzsch condensation, ethyl 4-imidazolylacetoacetate was treated with a substituted benzaldehyde and methyl 3-aminocrotonate to give a 2-(imidazolylmethyl)-substituted DHP (route B). Routes A and B proved versatile but low yielding and accordingly other approaches to our target structures were examined; in particular, we sought improvements in routes to 2-(imidazolylmethyl) DHPs. This led to an alternative approach, route C, in which chlorobenzylidene derivatives, available by Knoevenagel con-

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Table II. Bis-Heterocyclic Substituted Dihydropyridines[a]

compd	R^1	R^2	A-Het	crystn solv	mp, °C	method	% yield	formula[c]	dose, po, mmol/kg	% bp[b] 2 h	% bp[b] 6h	TXA_2 inhibn:[d] IC_{50}, μM
21	Et	Me	CH_2N (imidazole)	EtOH	202–205	*i*	10	$C_{24}H_{24}N_6O_6 \cdot 2HCl$[e]	0.03	NS	NS	0.6
22	Et	Et	CH_2CH_2N (imidazole)	EtOH-EtOAc	210–211	D	35	$C_{27}H_{30}N_6O_6 \cdot 2HCl$[f]	0.03	NS	NS	5.3
23	Et	Et	CH_2OCH_2– (pyridine)	Me_2CO	169–171	A	25	$C_{31}H_{32}N_4O_8 \cdot 2HCl$[g]	0.03	NS	NS	NT[j]
24	Et	Et	$CH_2OCH_2CH_2$– (pyridine)	EtOAc	132–134	A	30	$C_{33}H_{36}N_4O_8 \cdot 2HCl$[h]	0.03	NS	NS	NT

[a–h] See footnotes to Table I. [i] See the Experimental Section. [j] Not tested.

densation of methyl or ethyl 4-chloroacetoacetates with a benzaldehyde, were condensed with an aminocrotonate and the intermediate chloromethyl DHP was reacted with imidazole. Route C was high yielding and could be applied to compounds in which the ester functions were unsymmetric. Route D utilized a Mannich base derived from a preformed DHP which was then subjected to base-catalyzed displacement with imidazole to give 2-(imidazolyl)-ethyl derivatives.

Pharmacological Results and Discussion

Antihypertensive activity was determined in spontaneously hypertensive rats (SHR). Systolic blood pressure (BP) was measured by an indirect tail-cuff technique. All compounds were tested at an oral dose of 0.03 mmol/kg; BP was measured at 2- and 6-h time points. The more active compounds were retested at 0.003 mmol/kg. A measure of TXA_2 synthetase inhibition was obtained by determining the ability of compounds to inhibit release of the TXA_2 degradation product thromboxane B_2 (TXB_2) in rabbit platelets aggregated by addition of adenosine diphosphate and epinephrine.

Antihypertensive activity among the Hantzsch type DHPs is critically dependent on the nature of the 4-aryl substituent. Since the presence of a 3-nitrophenyl substituent in the 4-position confers good antihypertensive activity on a number of DHPs, we initially examined analogues in which this feature was retained while the nature of the ester and heterocyclic side chain was varied. As may be seen from Table I, introduction of an imidazolylmethyl side chain at the 2-position of a DHP gave a compound (**1**) which combined good antihypertensive activity and TXA_2 synthetase inhibiting activity similar in potency to dazoxiben. Antihypertensive activity of analogues related to **1** declined as the A chain increased from one to three atoms (**1**–**4**) but was partially restored for the four atom chain analogues (**5**, **6**). This observation may reflect increasing steric hindrance to binding at the calcium channel as the chain increases, up to the point where the chain becomes sufficiently long (four atoms) for the aromatic heterocyclic ring to fold away from the DHP ring. On the other hand, TXA_2 synthetase inhibiting activity was retained for A-chain lengths from one to three atoms but fell markedly when the chain reached four atoms. Introduction of a second heteroalkyl side chain at the 6-position (Table II) gave analogues devoid of significant antihypertensive activity, at the dose level tested, though retaining TXA_2 synthetase inhibition. Variation of the ester function within compounds **1** and **7**–**11** revealed that optimum antihypertensive activity was obtained in the unsymmetric ethyl methyl ester **1** in which the larger ester group is adjacent to the imidazole side chain. This observation agrees with previous studies showing unsymmetric esters to be more potent than their nearest symmetric analogues. However, we were surprised to observe that the reversed ester **9** showed greatly reduced antihypertensive activity relative to its isomer **1**. This marked difference was also observed for other corresponding pairs of unsymmetric esters within our series such as compouds **14**/**15** and **16**/**17**. TXA_2 synthetase inhibiting activity was relatively insensitive to changes in the ester functions.

Table III. Effect of Compounds on Plasma Levels of TXB_2 and 6-$KPGF_{1\alpha}$ in Rats 2 h after Oral Administration

compound	dose, mg/kg	*n*	TXB_2[a]	6-$KPGF_{1\alpha}$[a]
vehicle		13	572 ± 79	37 ± 13
1	0.3	10	484 ± 33	22 ± 5
	1	10	402 ± 88	NT
	3	10	330 ± 62[b]	68 ± 27
	10	10	280 ± 34[b]	94 ± 40
dazoxiben	3	10	384 ± 60[b]	86 ± 20
	10	10	270 ± 41[b]	202 ± 119[b]
nitrendipine	3	10	564 ± 57	62 ± 24
	10	10	562 ± 120	60 ± 25

[a] Plasma concentration expressed as pg/mL, values are mean ± SEM. [b] Statistically different from controls ($p < 0.05$).

Having established that the optimum values for the ester and side chain substituents were those present in compound **1**, these were retained while the 4-substituent was varied (compounds **12**–**20**). Variation of the 4-substituent gave a number of highly potent compounds in both of our test procedures although no general trends were observed.

A number of compounds were selected for further evaluation and results for **1** are shown in Tables III and IV. In normotensive rats, **1** produced a dose-related decrease in plasma TXB_2 levels over the range 0.3–10 mg/kg and at 3 and 10 mg/kg raised plasma levels of the prostacyclin degradation product 6-ketoprostaglandin $F_{1\alpha}$ (6-$KPGF_{1\alpha}$) (Table III). The effects of **1** on TXB_2 and 6-$KPGF_{1\alpha}$ release in human platelet-rich plasma (PRP) following aggregation by collagen was also determined (Table IV). Compound **1** (10^{-7}–10^{-3} M) produced a con-

Table IV. Effect of Compounds on TXB_2 and 6-$KPGF_{1\alpha}$ Production in Human Platelet-Rich Plasma

compound	molar conc	% TXB_2[a]	% 6-$KPGF_{1\alpha}$[a]
1	10^{-7}	92 ± 5	100
	10^{-6}	67 ± 14	112 ± 12
	10^{-5}	43 ± 11	119 ± 19
	10^{-4}	10 ± 4	431 ± 203
	10^{-3}	0 ± 1	460 ± 220
dazoxiben	10^{-7}	97.5 ± 2.5	115 ± 9
	10^{-6}	69 ± 18	463 ± 223
	10^{-5}	21 ± 7	603 ± 224
	10^{-4}	5 ± 3	460 ± 177
	10^{-3}	2 ± 1	345 ± 141
nitrendipine	10^{-7}	87 ± 13	112 ± 12
	10^{-6}	84 ± 16	113 ± 8
	10^{-5}	81 ± 19	136 ± 22
	10^{-4}	40 ± 12	121 ± 12
	10^{-3}	35 ± 17	96 ± 6

[a] Percentage release relative to controls, values are mean ± SEM calculated from four determinations at each concentration.

centration-related inhibition of collagen-stimulated TXB_2 production in PRP, with complete inhibition from 10^{-4} M, and a rise in 6-$KPGF_{1\alpha}$ levels over the same concentration range. The rise in 6-$KPGF_{1\alpha}$ production in PRP reflects diversion of endoperoxide intermediates to prostacyclin synthesis, by prostacyclin synthetase contained in leucocytes. Similar results were obtained with dazoxiben in human PRP whereas nitrendipine had no effect in concentrations up to 10^{-4} M.

At the commencement of this work, published SAR[24] on dihydropyridines suggested that only methyl or amino groups were well tolerated at the 2/6-positions. Subsequently other workers showed that larger 2-substituents (2-[(aminoethoxy)methyl]) were tolerated.[25] Our results also show that a 2-(imidazolylmethyl) substituent can be incorporated with retention of antihypertensive activity and that this substituent confers marked TXA_2 synthetase inhibiting activity. Compound 1 was of particular interest as a potential combined antihypertensive–antithrombotic agent.

In vivo compound 1 lowered blood pressure and reduced plasma TXB_2 levels in rats over the same dose range. In vitro 1 reduced TXB_2 release from human and rabbit PRP. The reductions of TXB_2 release observed in vivo and in vitro were accompanied by enhanced release of 6-$KPGF_{1\alpha}$, reflecting diversion of the arachidonic acid cascade by selective inhibition of thromboxane synthetase. Compound 1 (Wy 27569) was selected for further studies in man.

Experimental Section

Melting points are uncorrected. IR spectra were obtained in Nujol with a Perkin-Elmer Model 521 spectrophotometer. NMR spectra were obtained on a Brucker WP200 instrument. C, H, and N analyses were within ±0.4% of theoretical values. 4-(2-Nitrophenyl)-1,4-dihydropyridines are sensitive to light; all chemical and biological procedures involving these were shielded from light wherever practical.

Ethyl 3-Oxo-4-(2-pyrid-3-ylethoxy)butanoate Oxalate (28). A solution of 3-(2-hydroxyethyl)pyridine (15 g, 0.116 mol) in 20 mL of THF was added dropwise over 1 h to a stirred suspension of sodium hydride (11.13 g, 0.231 mol as a 50% w/w dispersion in mineral oil) in 150 mL of THF, followed by addition of a solution of ethyl 4-chloroacetoacetate (18.16 g, 0.115 mol) in 50 mL of THF over a further 1 h. The mixture was allowed to stir overnight and the solvent was then evaporated. The residue was poured as a thin stream onto a mixture of concentrated hydrochloric acid (13.2 g) and ice–water (150 g). The mixture was extracted with $CHCl_3$; the extract was washed with brine, dried ($MgSO_4$), and evaporated. The residual oil was purified by chromatography on silica using $CHCl_3$–MeOH (96:4) as eluent to give an oil (17.4 g, 56%). A sample (2.6 g) was dissolved in EtOAc and treated with a solution of oxalic acid dihydrate (1.26 g) in EtOAc to precipitate the oxalate salt: 2.7 g; mp 66–68 °C; ^{1}H NMR (DMSO-d_6) δ 1.2 (3 H, t, OCH_2CH_3), 2.9 (2 H, t, CH_2CH_2O), 3.55 (2 H, s, $COCH_2CO$), 3.7 (2 H, t, CH_2CH_2O), 4.12 (2 H, q, OCH_2CH_3), 4.25 (2 H, s, OCH_2CO), 7.4 (1 H, m, 5-H), 7.78 (1 H, m, 4-H), 8.5 (2 H, m, 2,6-H), and 10.7 (2 H, br, H^+); IR 1747, 1631, 1554, 1322, and 805 cm^{-1}. Anal. ($C_{13}H_{17}NO_4$) C, H, N.

1,4-Dihydro-2-methyl-4-(3-nitrophenyl)-6-[(2-pyrid-3-ylethoxy)methyl]pyridine-3,5-dicarboxylic Acid 3-Methyl 5-Ethyl Diester Hydrochloride (5) and 1,4-Dihydro-4-(3-nitrophenyl)-2,6-bis[(2-pyrid-3-ylethoxy)methyl]pyridine-3,5-dicarboxylic Acid Diethyl Ester Dihydrochloride (24). Method A. A solution of the base of 28 (3 g, 0.01 mol), methyl (3-nitrobenzylidene)acetoacetate (3 g, 0.0075 mol), and concentrated aqueous ammonia (2 mL) in 50 mL of EtOH was heated at reflux for 6 h. The solvent was then evaporated and the residue was partitioned between Et_2O and 2 M hydrochloric acid. The aqueous phase was basified with aqueous ammonia, extracted with $CHCl_3$ and the extract was dried over Na_2SO_4. The extract was evaporated and the residue was subject to chromatography on silica using EtOAc as eluent to give 2.7 g of 5. The base was dissolved in EtOAc, acidified with ethanol-HCl, and diluted with Et_2O to precipitate the hydrochloride: 2 g (31%); mp 175–177 °C; ^{1}H NMR (DMSO-d_6) δ 1.16 (3 H, t, OCH_2CH_3), 2.34 (3 H, s, 2-CH_3), 3.1 (2 H, t, CH_2CH_2O), 3.6 (3 H, s, OCH_3), 3.8 (2 H, t, CH_2CH_2O), 4.05 (2 H, m, CH_2O), 4.65 (2 H, q, OCH_2CH_3), 5.03 (1 H, s, 4-H), 7.55 (2 H, m, ArH), 8.0 (3 H, m, ArH), 8.47 (1 H, m, ArH), and 8.75–9.05 (3 H, m, ArH + NH); IR 1710, 1680, 1528, 1205, 1100, and 780 cm^{-1}. Further elution using EtOAc/EtOH (4:1) gave 0.85 g of 24 (30%); treatment of the base with ethanol-HCl gave the dihydrochloride: mp 132–134 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (6 H, m, OCH_2CH_3), 3.1 (4 H, t, CH_2CH_2O), 3.85 (4 H, t, CH_2CH_2O), 4.0 (4 H, m, CH_2O), 4.7 (4 H, q, OCH_2CH_3), 5.0 (1 H, s, 4-H), 7.6 (2 H, m, ArH), 7.95–8.1 (4 H, m, ArH + NH), 8.35–8.55 (3 H, m, ArH), and 8.8–9.05 (4 H, m, pyridine 2,6-H); IR 1673, 1527, 1346, 1200, 1086, and 688 cm^{-1}.

Ethyl 3-Oxo-4-(1*H*-imidazol-1-yl)butanoate Oxalate (25). A solution of imidazole (308.7 g, 4.54 mol) in 1800 mL of dry THF was added over 1 h to a stirred, ice-cooled, suspension of sodium hydride (218 g, 4.54 mol, as a 50% w/w dispersion in mineral oil) in 1150 mL of dry THF, maintained beneath argon and below 25 °C. After addition was completed, the solution was stirred for 20 h and a solution of ethyl 4-chloro-3-oxobutanoate (373.3 g, 2.27 mol) in 230 mL of dry THF was added below 25 °C over 1 h. The reaction was stirred for 24 h, and the suspended solid was collected and washed with THF. The solid was dissolved in a mixture of 1000 mL of H_2O, 270 mL of AcOH, and ice (260 g) and then extracted with 4 × 500 mL of $CHCl_3$. The combined extracts were washed with 500 mL of saturated NaCl solution, dried (Na_2SO_4), and evaporated. The residual oil was dissolved in 500 mL of EtOAc and filtered into a hot solution of oxalic acid dihydrate (245 g) in 1500 mL of 2-propanol to give the oxalate of 25: 418 g (64%); mp 112–114 °C dec; ^{1}H NMR (CD_3OD) δ 1.3 (3 H, t, OCH_2CH_3), 3.15 (2 H, s, CH_2), 4.1 (2 H, q, OCH_2CH_3), 5.25 (2 H, s, CH_2N), 7.5 (1 H, m, imidazole, 5-H), 7.6 (1 H, m, imidazole, 4-H), and 8.2 (1 H, m, imidazole, 2-H); IR 3151, 1724, 1692, 1213, 1050, 860, and 632 cm^{-1}. Anal. ($C_9H_{12}N_2O_3 \cdot C_2H_2O_4$) C, H, N.

4-[2-Chloro-3-(trifluoromethyl)phenyl]-1,4-dihydro-2-(1*H*-imidazol-1-ylmethyl)-6-methylpyridine-3,5-dicarboxylic Acid 3-Ethyl 5-Methyl Diester Succinate (16). Method B. The oxalate of compound 25 (2.72 g, 0.01 mol) was dissolved in a minimum volume of water, and the solution was brought to pH 6.5 by addition of aqueous potassium carbonate solution and extracted with $CHCl_3$. The extract was dried and evaporated to give the free base of 25 (1.24 g, 0.0068 mol), which was dissolved in 10 mL of EtOH and heated at reflux under nitrogen with 2-chloro-3-(trifluoromethyl)benzaldehyde (1.2 g, 0.004 mol) and

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methyl 3-aminocrotonate (0.46 g, 0.004 mol) for 20 h. The solvent was then evaporated and the residue was partitioned between Et_2O and 1 M hydrochloric acid to precipitate the product hydrochloride. The crude hydrochloride was collected by filtration and partitioned between $CHCl_3$ and aqueous potassium carbonate solution. The $CHCl_3$ phase was separated, dried (Na_2SO_4), and evaporated. The residue was dissolved in a mixture of 3 mL of EtOAc and 1 mL of MeOH and a solution of succinic acid (0.16 g) in the same volume of solvent added to precipitate the succinate salt (0.46 g, 19%): mp 187–190 °C; ^1H NMR (DMSO-d_6) δ 1.05 (3 H, t, OCH_2CH_3), 2.25 (3 H, s, 6-CH_3), 2.40 (4 H, s, succinic acid), 3.45 (3 H, s, OCH_3), 4.0 (2 H, m, OCH_2CH_3), 5.15 (2 H, d/d, CH_2N), 5.45 (1 H, s, 4-H), 6.9 (1 H, s, imidazole, 5-H), 7.18 (1 H, s, imidazole, 4-H), 7.25–7.0 (3 H, m, Ar-H), and 9.4 (1 H, s, imidazole, 2-H); IR 3151, 1724, 1692, 1213, 1050, 860, and 632 cm^{-1}.

1,4-Dihydro-2-methyl-6-[2-(dimethylamino)ethyl]-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic Acid Diethyl Ester. A solution of 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylic acid diethyl ester (7.5 g, 0.02 mol), dimethylamine hydrochloride (2.44 g, 0.03 mol), paraformaldehyde (0.9 g, 0.03 mol), and 0.4 mL of concentrated hydrochloric acid in 35 mL of EtOH was heated at reflux for 10 h. The solvent was then evaporated, and the residue was partitioned between 2 M hydrochloric acid and EtOAc. The aqueous phase was separated, basified with aqueous ammonia, and extracted into Et_2O. The extract was dried and evaporated, and the residue was chromatographed on neutral alumina (250 g, activity I) using $CHCl_3$ as eluent to give 4 g of the crude product (46.4%). Recrystallization from 10 mL of diisopropyl ether gave 1.8 g (21%): mp 111–113 °C; ^1H NMR ($CDCl_3$) δ 1.2 (6 H, m, OCH_2CH_3), 2.2 (3 H, s, 2-CH_3), 2.27 (6 H, s, NCH_3), 2.6 (2 H, m, CH_2CH_2N), 2.8 (1 H, m, CH_2CH_2N), 3.25 (1 H, m, CH_2CH_2N), 4.05 (4 H, m, OCH_2CH_3), 5.85 (1 H, s, 4-H), 7.2–7.75 (4 H, m, Ar-H), and 10.15 (1 H, s, NH); IR 1690, 1528, 1500, 1280, 1200, 1095, and 790 cm^{-1}.

4-[2-Chloro-3-(trifluoromethyl)phenyl]-1,4-dihydro-2-(1*H*-imidazol-1-ylmethyl)-6-methylpyridine-3,5-dicarboxylic Acid 5-Ethyl 3-Methyl Ester Succinate (17). Method C. A solution of 2-chloro-3-(trifluoromethyl)benzaldehyde (1.14 g, 0.00547 mol), methyl 4-chloroacetoacetate (0.82 g, 0.00545 mol), benzylamine (0.035 g), and acetic acid (0.02 g) in 5 mL of *i*-PrOH was stirred at room temperature for 20 h. Methyl 3-aminocrotonate (0.7 g, 0.00543 mol) was then added and the stirring was continued for a further 24 h. Concentrated hydrochloric acid (0.25 mL) was then added and after stirring for a further 2 h the precipitated chloromethyldihydropyridine was collected by filtration (1.01 g, 40.9%).

1,4-Dihydro-2-methyl-6-[2-(1*H*-imidazol-1-yl)ethyl]-4-(2-nitrophenyl)pyridinedicarboxylic Acid Diethyl Ester Maleate (13). Method D. A solution of 1,4-dihydro-2-methyl-6-[2-(dimethylamino)ethyl]-4-(2-nitrophenyl)pyridinedicarboxylic acid diethyl ester (2.15 g, 0.005 mol), 1,8-diazabicyclo[5.4.0]undec-7-ene (0.75 g), and imidazole (1.36 g, 0.02 mol) in 50 mL of chlorobenzene was heated at reflux for 22 h. The solution was diluted with 50 mL of $CHCl_3$, washed with 100 mL of H_2O, dried (Na_2SO_4), and evaporated. The residue was crystallized from 15 mL of EtOAc to give 1.5 g (66%) of yellow crystalline product. The base was suspended in 5 mL of EtOH, acidified with maleic acid (0.216 g), and diluted with EtOAc to precipitate the maleate: 0.85 g (30%): mp 142–143 °C; ^1H NMR (CD_3OD) δ 1.15 (6 H, m, OCH_2CH_3), 2.28 (3 H, s, 2-CH_3), 3.2 (2 H, m, CH_2CH_2N), 4.0 (4 H, m, OCH_2CH_3), 4.55 (2 H, t, CH_2CH_2N), 5.75 (1 H, s, 4-H), 6.2 (2 H, s, maleic acid) 7.3–7.75 (6 H, m, Ar-H), and 8.76 (1 H, t, imidazole 2-H); IR 1687, 1590, 1276, 1206, 1098, and 864 cm^{-1}.

The above product was added over 5 min to a stirred solution of imidazole (1.5 g) in 10 mL of MeCN to give a clear solution. After standing for 20 h, the solution was diluted with 15 mL of H_2O and the precipitated product was collected and washed with water to give 0.81 g (75.8%). The base was dissolved in 8 mL of hot EtOAc–MeOH (7:1), and succinic acid (0.2 g) was added in the same solvent (8 mL); on cooling the succinate crystallized and was collected by filtration and washed with EtOAc to give 0.8 g (61%): mp 190–193 °C; ^1H NMR (DMSO-d_6) δ 1.05 (3 H, t, OCH_2CH_3), 2.28 (3 H, s, 6-CH_3), 2.45 (4 H, s, succinic acid), 3.56 (3 H, s, OCH_3), 3.95 (2 H, m, OCH_2CH_3), 5.13 (2 H, d/d, CH_2N), 5.45 (1 H, s, 4-H), 6.85–7.7 (5 H, m, Ar-H), 7.8 (1 H, s, NH), and 9.33 (1 H, s, imidazole, 2-H); IR 1692, 1587, 1431, 1213, 1163, 1046, 806, 793, and 632 cm^{-1}.

1,4-Dihydro-2,6-bis(1*H*-imidazol-1-ylmethyl)-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic Acid Ethyl Methyl Diester Hydrochloride (21). Phenyltrimethylammonium perbromide (7.52 g, 0.02 mol) was added to a stirred, ice-cooled solution of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl diester (3.6 g, 0.01 mol) in 30 mL of MeCN maintained below 10 °C. The solution was allowed to stand for 0.75 h and a solution of imidazole (6.8 g, 0.1 mol) in 30 mL of MeCN was added with ice-cooling to maintain the mixture below 30 °C. After stirring for a further 2 h, the solvent was evaporated, and the residue was dissolved in water, basified with ammonia, and extracted with $CHCl_3$. The extract was dried and evaporated, and the residue was purified by chromatography on neutral alumina (100 g, activity I) using $CHCl_3$–MeOH (19:1) as eluent to give the base of 21 (1.5 g, 35.3 %). Treatment of the base with ethanol–HCl gave the hydrochloride: 0.6 g (10%); mp 202–205 °C; ^1H NMR (DMSO-d_6) δ 1.2 (3 H, t, OCH_2CH_3), 3.64 (3 H, s, OCH_3), 4.1 (2 H, m, OCH_2CH_3), 5.07 (1 H, s, 4-H), 5.55 (4 H, m, CH_2N), 7.5–8.1 (8 H, m, Ar-H), and 9.3 (2 H, m, imidazole 2-H); IR 1690, 1525, 1292, 1190, 1110, 750, and 625 cm^{-1}.

Antihypertensive Activity in SHR. The systolic blood pressure of female SHR (250–300 g) was measured in a 37 °C constant-temperature housing by a tail-cuff technique. Tail pulses were detected by a pneumatic pulse transducer (Narco Biosystems) and recorded on a Devices MX2 recorder. Rats with systolic pressures below 155 mmHg were discarded.

Groups of four hypertensive rats were dosed orally with the test substance in 0.5% (hydroxypropyl)methylcellulose–0.9% saline vehicle (10 mL/kg) or vehicle alone. Blood pressures were recorded before dosing and at 2, 6, and 24 h after dosing. Results were appraised statistically by an analysis of variance.

Inhibition of Thromboxane Release from Rabbit Platelets. Venous blood, approximately 75 mL, was obtained from an anesthetized rabbit and centrifuged at 200*g* for 10 min to obtain platelet-rich plasma (PRP). A 425-μL aliquot of PRP was incubated for 10 min at 37 °C in the presence of vehicle (25 μL, aqueous DMSO 2.5%) or test compound dissolved in the same volume. Platelet aggregation was induced by addition of adenosine diphosphate (10 μmol) and epinephrine (5 μmol) in 50 μL of water. After incubation for a further 5 min the platelets were removed by centrifugation at 10000g for 3 min and a 50-μL aliquot was taken for radioimmunoassay of TXB_2.[26] The difference between the amounts of TXB_2 in aggregated PRP and that in nonaggregated PRP was taken as the maximal stimulated release. The effect of compounds on TXB_2 release was expressed as a percentage relative to this value, and IC_{50} values were calculated from a computed linear-regression analysis of the log concentration effect curve.

Prostanoid Release from Human PRP. PRP was prepared from venous blood obtained from healthy volunteers and treated as above except that aggregation was induced by 50 μg of collagen in 50 μL of an isotonic glucose solution pH 2.7. The basal level of TXB_2 in human PRP was 0.98 ± 0.3 ng/mL, which increased to 6.4 ± 1.3 ng/mL in the presence of collagen. Basal levels of 6-$KPGF_{1\alpha}$ were normally less than 20 pg/mL but rose to a maximum of 580 ± 220 pg/mL in the presence of complete TXB_2 production.

Plasma Concentrations of TXB_2 and 6-$KPGF_{1\alpha}$ in the Rat. Female normotensive rats were killed by CO_2 asphyxiation 2 h after oral dosing with vehicle or 1. Blood was withdrawn from the posterior vena cava and 1% EDTA was added as anticoagulant. The blood samples were immediately centrifuged at 10000*g* for 10 min and the plasma was removed and stored at −30 °C prior to radioimmunoassay for TXB_2 and 6-$KPGF_{1\alpha}$.

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Registry No. 1, 103417-69-2; 1·HCl, 103433-96-1; **2**, 103417-86-3; 2·HCl, 103417-88-5; **3**, 103417-76-1; 3·HCl, 103417-78-3; 4, 123753-95-7; 4·HCl, 123753-75-3; **5**, 123753-96-8; 5·HCl, 123753-

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76-4; **6**, 123753-97-9; **6**·HCl, 123753-77-5; **7**, 123753-98-0; **7**·HCl, 103434-08-8; **8**, 103417-89-6; **8**·HCl, 103417-91-0; **9**, 123753-99-1; **9**·HCl, 123753-78-6; **10**, 123754-00-7; **10**·HCl, 103417-94-3; **11**, 123754-01-8; **11**·HCl, 103417-92-1; **12**, 123753-79-7; **12**·$C_4H_6O_4$, 123753-80-0; **13**, 103417-72-7; **13**·maleate, 103417-73-8; **14**, 123753-81-1; **15**, 123753-82-2; **15**·$C_4H_6O_4$, 123753-83-3; **16**, 123753-84-4; **16**·$C_4H_6O_4$, 123753-85-5; **17**, 123753-86-6; **17**·$C_4H_6O_4$, 123753-87-7; **18**, 103417-93-2; **18**·HCl, 123753-88-8; **19**, 123754-02-9; **19**·HCl, 123753-89-9; **20**, 123754-03-0; **20**·HBr, 123753-90-2; **21**, 123754-04-1; **21**·2HCl, 123753-91-3; **22**, 123775-19-9; **22**·2HCl, 123775-17-7; **23**, 123754-05-2; **23**·2HCl, 123775-18-8; **24**, 123754-06-3; **24**·2HCl, 123753-92-4; **25**, 103417-70-5; **25**·oxalate, 103417-90-9; **26**, 103417-77-2; **27**, 123753-93-5; **28**, 123753-94-6; **28**·oxalate, 123754-08-5; **29**, 90444-47-6; $ClCH_2COCH_2CO_2Et$, 638-07-3; *m*-$NO_2C_6H_4CH{=}CHCOCH_2CO_2Me$, 52604-00-9; $MeO_2CCH{=}C(NH_2)Me$, 14205-39-1; $ClCH_2COCH_2CO_2Me$, 32807-28-6; 3-(2-hydroxyethyl)pyridine, 6293-56-7; 3-hydroxypyridine, 109-00-2; 3-(hydroxymethyl)pyridine, 100-55-0; 1-(hydroxyethyl)imidazole, 1615-14-1; imidazole, 288-32-4; 2-chloro-3-(trifluoromethyl)benzaldehyde, 93118-03-7; 1,4-dihydro-2-methyl-3-[2-(dimethylamino)ethyl]-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid diethyl ester, 103417-71-6; 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid diethyl ester, 21829-26-5; 4-[2-chloro-3-(trifluoromethyl)phenyl]-6-(chloromethyl)-1,4-dihydro-2-methylpyridine-3,5-dicarboxylic acid 3-ethyl-5-methyl diester, 123754-07-4; 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl diester, 39562-70-4.

(8β)-6-Methylergoline Amide Derivatives as Serotonin Antagonists: N[1]-Substituent Effects on Vascular $5HT_2$ Receptor Activity

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A series of (8β)-6-methylergoline amide derivatives was synthesized with various alkyl substituents in the N[1]-position in order to evaluate their effectiveness in blocking vascular $5HT_2$ receptors. The influence of both the N[1] substituent and amide derivative proved to be of great importance on binding affinities to vascular $5HT_2$ receptors. Within each series of amides, however, maximum affinity was achieved with an *N*[1]-isopropyl substituent (**14**, **18**, **26**, **38**, and **41**; all with 2.7–50 times greater affinity than their N[1]–H analogues), with the exception of two cases (**22** and **37**) in the cyclohexylamide derivatives wherein *N*[1]-methyl equalled the isopropyl in potency. Other than these exceptions, affinities followed the pattern of H < Me < Et < iPr, with potencies falling off with larger alkyl substituents.

The suggestion has been made that the serotonin antagonist activity of ergot alkaloids may be selectively enhanced by methylation of N[1], the indole nitrogen,[1] but exceptions to this have been reported to exist.[2] Previous papers from these laboratories[2,3] expanded on the structure–activity relationships of the (8β)-ergoline-8-carboxylic acid esters as potent and selective serotonin antagonists as measured specifically by their vascular $5HT_2$ receptor binding affinities. The $5HT_2$ binding affinities of the ergoline esters were shown to be influenced by their ester substituents, alkyl substituents at N[6], but especially by their alkyl substituents at N[1], with isopropyl substitution resulting in maximum affinity. Because the ergoline esters are hydrolyzed in vivo to the less active carboxylic acid moiety,[4] we initiated studies of the structure–activity of a series of amide ergolines. We now report on the effect of N[1] substitution of a variety of ergoline amide derivatives on $5HT_2$ receptor affinity as measured by antagonism of serotonin-induced contractions in the rat jugular vein. This tissue is known to possess $5HT_2$ receptors that are responsible for contractions induced by serotonin.[5]

Chemistry

The N[1]-alkylated (8β)-6-methylergoline-8-carboxylic acids were prepared from dihydrolysergic acid as reported previously.[6] As depicted in Scheme I, ergoline acetic acid (**2**) was synthesized from dihydrolysergol (**1**) by the procedure of Semonsky and Kucharcyzk.[7] The N[1]-alkylation of **2** by the above procedure yielded the homologated ergoline carboxylic acids necessary to prepare the amides of Table III. The amides of both Tables I and III were synthesized by several methods, in part to evaluate the synthetic utility of these methods for the preparation of ergoline derivatives. Method A, using 1,1′-carbonyldiimidazole, and method E, using the acid chloride produced via $POCl_3$/DMF, proved to be the most convenient to use and gave excellent yields. Method B, acylation via the mixed anhydride with isobutyl chloroformate, also gave excellent yields, but required the use of less convenient (−45 °C) temperatures. Method C, condensation with EEDQ, and method D, oxidative amidation of the ergoline carboxylic acid hydrazide, proved to be least useful because of low and variable yields. Chromatographic procedures were not required to obtain pure products. Instead, crystallization of either the free base or maleate salt from various solvents proved sufficient.

The ergoline tetrazoles of Table II were produced from their respective parent amides by method F, which is derived from the standard method of making tetrazoles from N-substituted amides.[8,9] These were all isolated as maleate or mesylate salts.

The (8β)-aminoergolines (Scheme II) required as intermediates for the synthesis of the "reverse amides" in Table IV were made via the Curtius degradation of the parent acids.[10] The previously reported[11,12] syntheses of

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