nonradiolabeled drugs and nucleotides, and second containing triated EPA (100 μCi/mmol) and nonradiolabeled nucleotides, and the third containing ^{14}C-labeled nucleotides and [^{3}H]EPA; each reaction mixture had EPA (140 μM) and a nucleotide (140 mM) in buffer A. The reactions volumes were 50 mL in experiment 1, 5 mL in experiment 2, and 2 mL in experiment 3. The mixtures were incubated at 37 °C for 14 h. The samples were extracted with water-saturated EtOAc, and the aqueous fractions were chromatographed on a Sephadex LH-20 column as in the previous experiments. Unreacted nucleotides and nucleosides (occasional contaminants) were eluted 20 mM NH_4HCO_3 buffer. With use of a linear gradient of 20% MeOH–20 mM NH_4HCO_3 to 90% MeOH–20 mM NH_4HCO_3, the adducts were eluted with 50% MeOH–buffer. Treatment with alkaline phosphatase (bacterial) followed by chromatography of the digests on a Sephadex LH-20 column separated the nucleoside adducts from the enzymes and the free drug (DHPA). As before, successive elutions, first with water (to remove enzymes) and then with 20% MeOH–H_2O (v/v) and 50–80% MeOH–H_2O in a linear gradient, eluted all the drug–deoxyribonucleoside adducts. The solvents were removed under reduced pressure and the adducts were analyzed by HPLC (previous experiment in Figure 2). The specific activity ratios ($^{3}H/^{14}C$) and/or the UV absorbance ratios (A_{465}/A_{260}) were employed to calculate the molar ratios of the drug and nucleoside in the adducts. The molar extinction values of the adducts, which were determined from the specific activities, were found to be $\epsilon_{260} = (2.8 \pm 0.25) \times 10^4$ and $\epsilon_{465} = (1.6 \pm 0.2) \times 10^4$ M^{-1} cm^{-1}, respectively. These values affirm that one EPA is bound to one nucleoside molecule and that the chromophore of the drug is not stacked on the nucleoside base.

In order to examine the stability of EPA in the presence of nucleotides, solutions of EPA, with or without the nucleotides, were incubated for times as in the previous experiment. Actinomycin D was used as the standard in these reactions. In the above experiments the free drugs, EPA (when it is not completely hydrolyzed to DHPA) and DHPA, are eluted from the columns in 20% MeOH–H_2O.

Acknowledgment. This investigation was supported by Research Grants CH-259 from the American Cancer Society and CA 26281 from the National Cancer Institute, DHEW. We thank Dr. John B. Douros, Natural Products Branch, NCI, for a generous supply of actinomycin D, and Dr. Randall K. Johnson, previously of Arthur D. Little, Cambridge, MA, and at present at Smith Kline & French Laboratories, Philadelphia, for some antitumor test results. Albert Ross, an associate of Dr. Johnson at Cambridge, provided us with P388, P388/ADR, and L1210 leukemia in DBA_2 mice. Dr. Herbert Lazarus of Dana-Farber Cancer Institute gave us CCRF-CEM cells. Thanks are also due to Dr. Kenneth C. Edelin, Chairman of OB/GYN, and Dr. John I. Sandson, Dean of the Medical School of Boston University, for their support.

Trequinsin, a Potent New Antihypertensive Vasodilator in the Series of 2-(Arylimino)-3-alkyl-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinolin-4-ones

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Series of 3-substituted-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-diones and 2-substituted-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-ones were synthesized and tested for blood pressure lowering properties in anesthetized normotensive cats and conscious spontaneously hypertensive rats. Several compounds in the 2-(arylamino)-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one series display a high order of activity. The most active compounds are the alkyl derivatives of the 2-mesitylamino/2-mesitylimino tautomeric forms. The 2-(mesitylimino)-3-methyl analogue trequinsin is a potent antihypertensive agent and displays a hemodynamic profile characteristic of an arteriolar dilator. It is also a potent inhibitor of both cAMP phosphodiesterase and platelet aggregation.

Vasodilators in current use for the clinical management of hypertension are of two types: (1) receptor- or enzyme-dependent agents, such as the peripheral postsynaptic α_1-adrenoceptor blocking agents prazosin and indoramin, and the angiotensin converting enzyme inhibitor captopril and (2) the direct acting agents on arteriolar smooth muscle, such as hydralazine, diazoxide, nitroprusside, and minoxidil.[1] The attribute of these antihypertensive vasodilators that distinguishes them from other classes of antihypertensive agents, such as β-adrenoceptor blocking agents, centrally acting drugs and adrenergic neuron blocking drugs, is their ability to selectively lower peripheral resistance. They thereby reverse the major hemodynamic abnormality of a markedly elevated systemic vascular resistance that characterizes human essential hypertension. Both types of vasodilators, however, produce similar, although not absolutely identical, side effects that are related to their main hemodynamic action.

The major side effects of the directly acting vasodilators hydralazine, diazoxide, and minoxidil that attenuate their antihypertensive effectiveness are the baroreceptor reflex increases in sympathetic activity that raise cardiac output and produce tachycardia, the augmentation of plasma renin activity, and the retention of sodium with plasma volume expansion.[2] A need has thereby arisen for vasoactive antihypertensives that would lack completely or

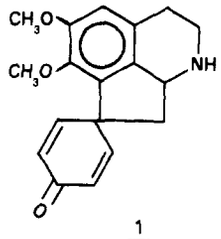
† Department of Chemistry.
‡ Department of Pharmacology.

(1) (a) Bannan, L. T.; Beevers, D. G.; Wright, N. *Br. Med. J.* **1980**, *281*, 1053, 1120, 1200. (b) Packer, M.; Le Jemtel, T. H. *Prog. Cardiovasc. Dis.* **1982**, *24*, 275.
(2) Koch-Weser, J. *Arch. Intern. Med.* **1974**, *133*, 1017.

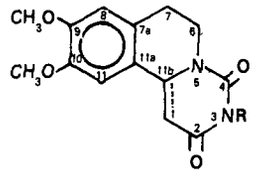
0022-2623/84/1827-1470$01.50/0

in part the above-mentioned side effects and that would act selectively on arteriolar smooth muscle with minimal interference with homeostatic reflexes and with the central and autonomous nervous system.[3]

Among efforts that have been concentrated on finding such substances is that of our strategically combined screening program of Indian plants and synthetic approaches. Subsequent to our finding of the peripheral vasodilatory properties of the antihypertensive alkaloid stepharine (1),[4] synthetic programs were undertaken that incorporated in their design the dimethoxyisoquinoline feature of the proaporphine alkaloid lead. One such program was based on hypotensive and tranquilizing 3-substituted pyrimido[6,1-*a*]isoquinoline-2,4-diones with a saturated pyrimido unit (**2**).[5] Our initial objective was to study the effect of the $\Delta^{1,11b}$ bond in the pyrimido unit on the biological properties of the pyrimido[6,1-*a*]isoquinoline-2,4-diones **3**. The evolution of this program leading to synthesis of the title compounds (**4**) and to trequinsin (HL 725, **56b**), one of the most potent antihypertensive peripheral vasodilators known and having platelet aggregation inhibitory properties, forms the subject of this paper.[6] Trequinsin is currently in clinical phase I trials.

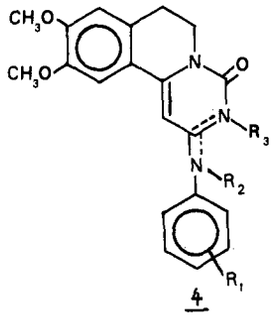


1



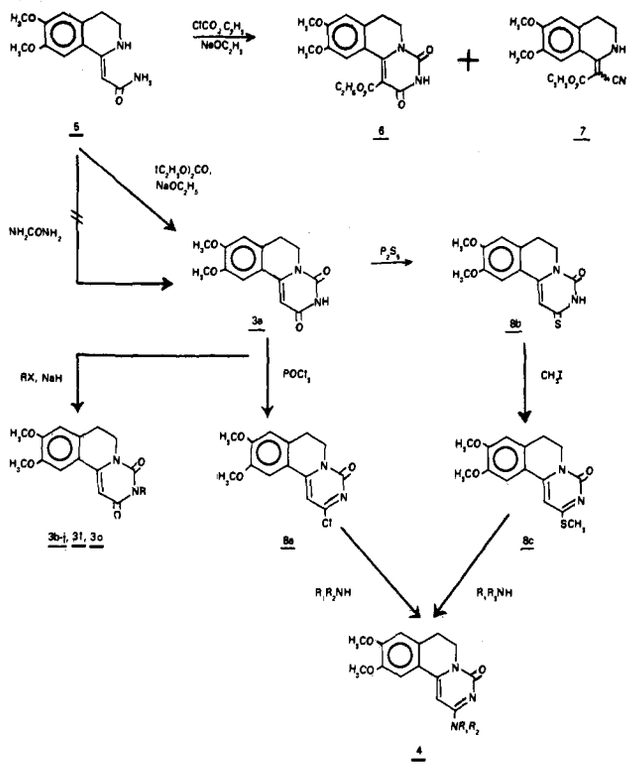
2 C_1, C_{11b}: single bond

3 C_1, C_{11b}: double bond

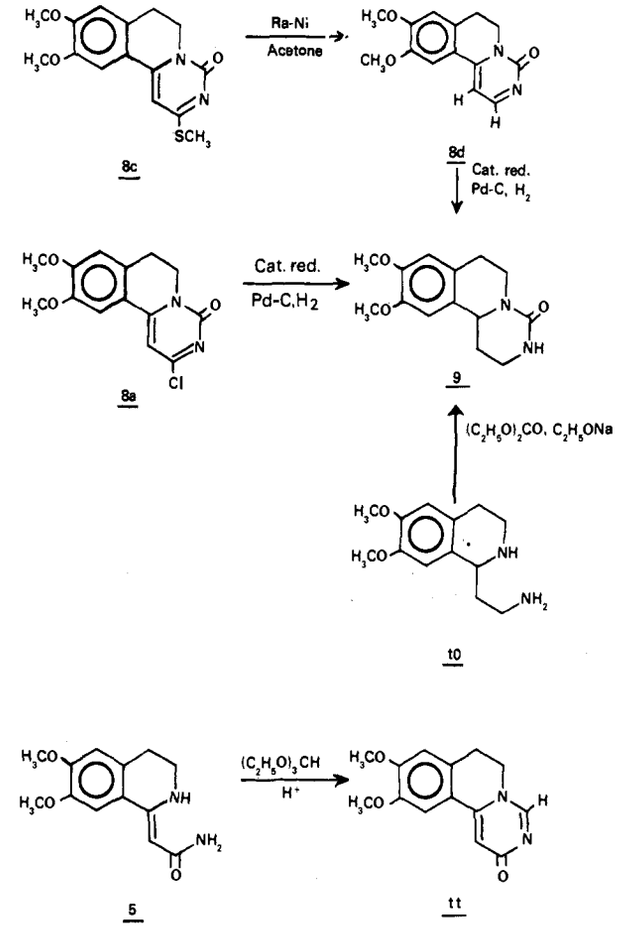


4

Scheme I



Scheme II



Chemistry. The synthesis of 9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (**3a**, R = H) was attempted by cyclization of 1-(carbamoylmethylene)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline

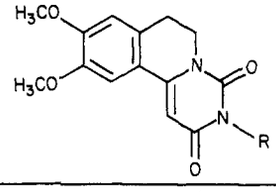
(3) (a) Gross, F. "Handbook of Experimental Pharmacology"; Gross, F., Ed.; Springer-Verlag: West Berlin, 1977; Vol. 39, pp 397–476. (b) Goldberg, L. I.; Rick, J. H.; Susan, O. "Hypertension, Physiopathology and Treatment"; Genest, J., Koiw, E., Kuchel, O., Eds.; McGraw-Hill: New York, 1977; pp 990–1924. (c) Prichard, B. M. C.; Tuckman, J. *Ibid.*, pp 1085–1117. (d) Opie, L. H. *Lancet* **1980**, *1*, 966.

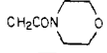
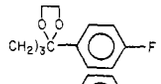
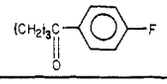
(4) (a) Dohadwalla, A. N.; Dadkar, N. K.; Bhattacharya, B. K., Proceedings of Indo-German Symposium on Medicinal Plants with Cardiovascular Activity, Central Drug Research Institute, Lucknow, India, Feb 22–26, 1977, A 20, p 24. (b) Bhat, S. V.; Bajwa, B. S.; Dohadwalla, A. N.; Kohl, H.; de Souza, N. J. *Ind. J. Pharm.* **1977**, *39*, 155.

(5) (a) Lombardino, J. G. *J. Med. Chem.* **1961**, *3*, 505. (b) Lombardino, J. G.; McLamore, W. M.; Laubach, G. D. U.S. Patent 3 081 306, 1963; *Chem. Abstr.* **1964**, *60*, 1769.

(6) (a) Lal, B.; Dornauer, H.; Bhattacharya, B. K.; Dohadwalla, A. N.; de Souza, N. J. Indian Patent 147 624, 1976; German Patent P 2720085.7, 1977. (b) Lal, B.; Bhattacharya, B. K.; Dadkar, N. K.; Dohadwalla, A. N.; Dornauer, H.; de Souza, N. J.; Schoelkens, B. A.; Ruppert, D.; Weithmann, U. *IRCS Med. Sci.* **1981**, *9*, 325. (c) Trequinsin is the INN assigned to HL 725.

Table I. 3-Substituted-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-diones



no.	R	mp, °C	recrystn solvent	yield, %	formula	anal.
3a	H	323–325	DMF	85	$C_{14}H_{14}N_2O_4$	C, H, N
3b	CH_3	260–262	EtOH–CH_2Cl_2	93	$C_{15}H_{16}N_2O_4$	C, H, N
3c	$CH(CH_3)_2$	190–192	EtOH	41	$C_{17}H_{20}N_2O_4$	C, H, N
3d	$CH(CH_3)C_2H_5$	164–165	EtOH	25	$C_{18}H_{22}N_2O_4$	C, H, N
3e	$CH_2CH(OH)CH_2OH \cdot 0.5H_2O$	184–185	C_6H_6–$CHCl_3$	83	$C_{17}H_{20}N_2O_5 \cdot 0.5H_2O$	C, H, N
3f	$CH_2C_6H_5$	220–221	EtOH	80	$C_{21}H_{20}N_2O_4$	C, H, N
3g	$CH_2CH_2C_6H_5$	198–200	MeOH	53	$C_{22}H_{22}N_2O_4$	C, H, N
3h	$CH_2CH_2CH_2N(CH_3)_2 \cdot HCl \cdot H_2O$	240–241		24	$C_{19}H_{25}N_3H_4 \cdot HCl \cdot H_2O$	C, H, N, Cl
3i	$CH_2P(O)(CH_3)_2 \cdot H_2O$	242–243	EtOH	65	$C_{17}H_{21}N_2O_5P \cdot H_2O$	C, H, N
3j	CH_2CN	217–218	MeOH–$CHCl_3$	68	$C_{16}H_{15}N_3O_4$	C, H, N
3k	CH_2COOH	287–288	DMF	91	$C_{16}H_{16}N_2O_6$	C, H, N
3l	$CH_2COOCH_2CH_3$	198–200	MeOH	76	$C_{18}H_{20}N_2O_6$	C, H, N
3m	$CH_2CONHCH_2CH_2N(C_2H_5)_2 \cdot HCl$	238–243	MeOH	57	$C_{22}H_{30}N_4O_5 \cdot HCl$	C, H, N
3n		247–248	MeOH	20	$C_{20}H_{23}N_3O_6$	C, H, N
3o		177–178	$CHCl_3$–Et_2O	31	$C_{26}H_{27}FN_2O_6$	C, H, N
3p		207–208	$CHCl_3$–Et_2O	68	$C_{24}H_{23}FN_2O_5$	C, H, N

(**5**) according to different procedures (Scheme I). Fusion of **5** with urea failed to yield any **3a**.[7] Treatment of **5** with ethyl chloroformate in pyridine or sodium ethoxide/ethanol gave instead of **3a** two compounds identified as 1-carbethoxy-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (**6**) and 1-(cyanocarbethoxy methylene)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**7**). The best results were obtained with diethyl carbonate and sodium ethoxide, which provided **3a** in 87% yield. Alkylation of **3a** with appropriate halides gave **3b–j**, **3l**, and **3o**. The amides **3m,n** and the carboxylic acid **3k** were prepared from **3l**. Compound **3p** was obtained from **3o** (Table I).

Treatment of **3a** with phosphorus oxychloride, or with phosphorus pentasulfide, provided the 2-chloro or the 2-thio derivatives **8a** and **8b**, respectively. Compound **8b** was readily converted to the methylthio derivative **8c** on methylation followed by liberation of the free base from the hydriodide. The location of the chlorine atom or the methylthio group at the 2-position was based on the following evidence. Hydrogenolysis of **8c** with Raney nickel in acetone gave the 2-demethylthio derivative **8d** (Scheme II). A singlet (δ 6.53) in the ^{1}H NMR spectrum of **8c** due to H-1 was replaced by two doublets (δ 6.66, J = 5 Hz and δ 8.5, $W_{1/2}$ = 5 Hz) in that of **8d**, due to H-1 and H-2, respectively. In spin decoupling experiments a collapse of the doublets to singlets confirmed the vicinal relationship of the newly generated hydrogen atom. Compound **8d**, on further hydrogenation at 50 psi using 10% Pd/C, was reduced to the derivative **9**, which was identical with that obtained through catalytic reduction of the chloro compound **8a** or through unambiguous synthesis by cyclization of 6,7-dimethoxy-1-(aminoethyl)-1,2,3,4-tetrahydroisoquinoline (**10**) with diethyl carbonate in the presence of sodium ethoxide. Synthesis of the 2-oxo derivative **11** was achieved through cyclization of **5** with triethyl orthoformate in acetic acid. The chemical shifts of its protons at positions 1 (δ 6.43) and 4 (s, δ 8.1) clearly distinguished **11** from its isomeric 4-oxo derivative, **8d**.

A more elegant synthesis of **8a** was devised through retrosynthetic considerations. A novel Bischler–Napieralski type condensation of 1-(3,4-dimethoxyphenethyl)-barbituric acid with phosphorus oxychloride gave **8a** in ≈100% yield.[8] The essential requirements and limitations of such a variation of the conventional reaction form the subject of a more detailed publication.[9]

The 2-*n*-butoxy and 2-phenoxy derivatives **8e** and **8f**, respectively (Table II), were prepared from the 2-chloro derivative **8a** by conventional methods. Treatment of the 2-chloro derivative **8a** or the 2-methylthio derivative **8c** with appropriate amines (Scheme I) readily provided the 2-(substituted amino) derivatives **12–49** (Tables II and III), which, because of the newly generated amidino moiety, permitted their existence in tautomeric equilibrium with their 2-imino isomers. Following alkylation studies, as described below, it was indeed possible to obtain separately the exocyclic and endocyclic *N*-alkyl tautomers.

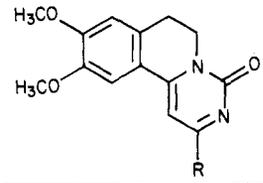
The alkyl derivatives **50–62** (Table IV) were prepared from their 2-(substituted anilino) precursors by treatment with alkyl halide in the presence of either potassium carbonate and acetone or sodium hydride and dimethylformamide. The ratios in which the exocyclic and endocyclic N-alkylated isomers were formed varied and appeared to depend on the size of the alkyl group and the reaction conditions (Table IV). Conditions of potassium carbonate and acetone and alkylation of the 2-mesitylamino analogue **45** with alkyl iodides of increasing chain length provided isomer ratios that altered progressively from nearly 1 exo:15 endo for the methyl derivatives **56a,b** through 1 exo:1 endo for the ethyl derivatives **57a,b** to exclusively the *exo*-alkyl isomer **59** for the *n*-butyl derivative. In the case of the isopropyl derivatives **58a,b**, a

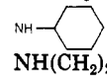
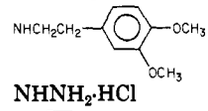
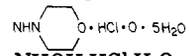
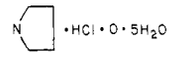
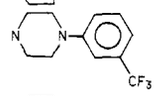
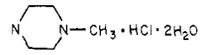
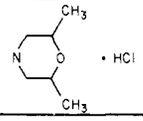
(7) Shaw, E.; Wooley, D. W. *J. Biol. Chem.* **1949**, *181*, 89.

(8) Lal, B.; D'Sa, A. S.; Dornauer, H.; de Souza, N. J. Indian Patent, 149432, 1978; German Patent P 28 47693, 1978.

(9) Lal, B.; D'Sa, A. S.; de Souza, N. J., unpublished results.

Table II. 2-Substituted-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-ones



no.	R	mp, °C	recrystn solvent	yield, %	formula	anal.
8d	H	213–215	EtOH–Et_2O	18	$C_{14}H_{14}N_2O_3$	C, H, N
8e	$O(CH_2)_3CH_3$	158–159	EtOH	37	$C_{18}H_{22}N_2O_4$	C, H, N
8f	OC_6H_5	235–236	$CHCl_3$–petroleum ether	56	$C_{20}H_{18}N_2O_4$	C, H, N
8c	SCH_3·HI	220–225	$CHCl_3$–MeOH	71	$C_{15}H_{16}N_2O_3S$·HI	C, H, N, S
8a	Cl	235–236	EtOH	97	$C_{14}H_{13}ClN_2O_3$	C, H, N, Cl
12	$NHCH_3$·HCl·H_2O	179–181	EtOH	51	$C_{15}H_{17}N_3O_3$·HCl·H_2O	C, H, N, Cl
13		237–239	$CHCl_3$–petroleum ether	55	$C_{20}H_{25}N_3O_3$	C, H, N
14	$NH(CH_2)_2N(C_2H_5)_2$·2HCl·H_2O	147–150	EtOH–Et_2O	81	$C_{20}H_{28}N_4O_3$·2HCl·H_2O	C, H, N, Cl
15		179–180	MeOH–H_2O	67	$C_{24}H_{27}N_3O_5$	C, H, N
16	$NHNH_2$·HCl	236–238	EtOH	57	$C_{14}H_{16}N_4O_3$·HCl	C, H, N
17		251	EtOH–Et_2O	97	$C_{18}H_{22}O_4N_4$·HCl·0.5H_2O	C, H, N, Cl
18	NHOH·HCl·H_2O	264–266	MeOH–Et_2O	70	$C_{14}H_{15}N_3O_4$·HCl·H_2O	C, H, N, Cl
19	$N(CH_3)_2$·HCl·0.5H_2O	180–181	EtOH	81	$C_{16}H_{19}N_3O_3$·HCl·0.5H_2O	C, H, N, Cl
20		233–236	EtOH–Et_2O	82	$C_{18}H_{21}N_3O_3$·HCl·0.5H_2O	C, H, N, Cl
21		220	EtOH	73	$C_{25}H_{25}F_3N_4O_3$	C, H, N
22		260–263	EtOH–Et_2O	55	$C_{19}H_{24}N_4O_3$·HCl·2H_2O	C, H, N, Cl
23		227–228	EtOH–Et_2O	48	$C_{20}H_{25}N_3O_4$·HCl	C, H, N, Cl

fluctuation in the isomeric composition was observed in repeated experiments, for which there is no ascribable reason. Methylation of **45** under sodium hydride/dimethylformamide conditions provided an increased proportion of the *exo*-methyl isomer **56a**. Preferential formation of the *exo*-alkyl isomer under sodium hydride/DMF conditions was found to be generally true for alkylation of other 2-(substituted anilino) analogues.

Assignment of structures to the tautomers was based on characteristic UV, IR, and ^1H NMR spectral features as described below. These features were found to be diagnostic following identification of the isomers **56a** and **56b** through unambiguous synthesis of the compounds. The unambiguous synthesis of **56a** was readily carried out by treatment of **8a** with *N*-methylmesidine. For the unambiguous synthesis of **56b**, compound **3b** bearing the desired *N*-methyl group was converted through the 2-thione to the 2-methylthio quaternary iodide, which was then treated with mesidine. Confirmation of the structure of **56b** was also obtained by X-ray crystal analysis.[10]

Careful analysis of the spectral data for **56a** and **56b** (see Experimental Section) and comparison with that for the other alkylated isomers revealed that the following features were conclusively diagnostic: (a) in the UV spectra, bands at 275, 283.8, and 340 nm for the endo isomer and at λ 271.5 and 281 nm for the exo isomer; (b) in the IR spectra, three well-resolved bands at 1635 (C=N), 1625 (C=O), and 1615 (C=C) cm^{-1} for the endo isomer in contrast to broad bands at 1661, 1645, and 1637 cm^{-1} for the exo isomer consequent on conjugation between the C=C, C=N, and C=O bonds; (c) in the NMR spectra, downfield shifts for the NCH_3 protons and H-1 of the endo isomer (δ 3.46 and 5.26, respectively, for **56b**) relative to those for corresponding protons in the exo isomer (δ 3.25 and 5.13 for **56a**), in agreement with shielding/deshielding effects previously observed for cyclic amidines, guanidines, and related systems.[11]

Pharmacology. The pyrimido[6,1-*a*]isoquinolines (Tables I–IV) were screened for hypotensive activity by intravenous administration in anesthetized normotensive cats. Those compounds that met our criteria for activity in providing ED_{25} values at doses of 2 mg/kg or less, 10 min after administration, were evaluated further for antihypertensive activity on oral administration to spontaneously hypertensive (SH) rats. The procedures for compound administration and blood pressure measurement were essentially similar to those described in our earlier paper,[12] details of which are to be found in the Experimental Section.

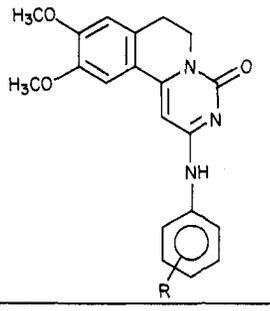
The detailed pharmacological studies carried out on trequinsin (**56b**) that established its peripheral vasodilatory

(10) Paulus, E., Hoechst AG., Frankfurt, West Germany, unpublished results. The X-ray analysis revealed that **56b** exists as the *Z* isomer with the mesidine moiety perpendicular to the plane of the ring system away from the *N*-methyl group. No attempt was made to study the geometrical isomerism of other alkyl derivatives.

(11) Jackman, L. M.; Jen, T. *J. Am. Chem. Soc.* **1975**, *97*, 2811.

(12) Bhat, S. V.; Dohadwalla, A. N.; Bajwa, B. S.; Dadkar, N. K.; Dornauer, H.; de Souza, N. J. *J. Med. Chem.* **1983**, *26*, 486.

Table III. Blood Pressure Lowering Activity of 2-(Substituted-anilino)-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-ones



no.	R	mp, °C (solvent)[a]	yield, %	formula[b]	hypotensive act.[c] ED_{25}: mg/kg	antihypertensive act.[d] mg/kg	antihypertensive act.[d] ΔBP, mmHg
24	2-C_2H_5	250–251 (A)	65	$C_{22}H_{23}N_3O_3$	10.0	NT	
25	2-COOH·0.5H_2O	324–326 (B)	61	$C_{21}H_{19}N_3O_5$·0.5H_2O	50.0	NT	
26	2-OC_2H_5·HCl	175–177 (C)	50	$C_{22}H_{23}N_3O_4$·HCl	1.2	25	−27 ± 5.1
27	2-Cl·HCl·H_2O	182–186 (E)	43	$C_{20}H_{18}ClN_3O_3$·HCl·H_2O	1.4	25	0
28	3-CF_3	301–302 (A)	63	$C_{21}H_{18}F_3N_3O_3$	2.0	NT	
29	4-OH	301–303 (A)	40	$C_{20}H_{19}N_3O_4$	10.0	NT	
30	4-OC_2H_5·HCl	247–250 (D)	50	$C_{22}H_{23}N_3O_4$·HCl	50.0	NT	
31	4-Cl	294–295 (A)	61	$C_{20}H_{18}ClN_3O_3$	50.0	NT	
32	2,4-$(CH_3)_2$	239–241 (A)	70	$C_{22}H_{23}N_3O_3$	1.0	25	−41 ± 4.9
33	2,4-Cl_2	274–276 (A)	42	$C_{20}H_{17}Cl_2N_3O_3$	1.0	25	0
34	2,5-$(OCH_3)_2$	268–269 (F)	48	$C_{22}H_{23}N_3O_5$	50.0	NT	
35	2,6-$(CH_3)_2$	285–287 (A)	58	$C_{22}H_{23}N_3O_3$	0.1	5	−27 ± 2.3
36	2-CH_3, 6-$CH(CH_3)_2$·HCl	225–228 (G)	67	$C_{24}H_{27}N_3O_3$·HCl	2.2	NT	
37	2-CH_3, 6-$C(CH_3)_3$·HCl	206–209 (H)	42	$C_{25}H_{29}N_3O_3$·HCl	1.8	25	0
38	2-C_2H_5, 6-$CH(CH_3)_2$·HCl·0.5H_2O	214–216 (I)	81	$C_{25}H_{29}N_3O_3$·HCl·0.5H_2O	3.0	NT	
39	2,6-F_2·CH_3SO_3H·0.5H_2O	283–285 (J)	73	$C_{20}H_{17}F_2N_3O_3$·CH_3SO_3H·0.5H_2O	3.5	25	0
40	2,6-Cl_2	228–230 (A)	10	$C_{20}H_{17}N_3Cl_2O_3$	2.0	NT	
41	3,4-$(CH_3)_2$	303–305 (F)	65	$C_{22}H_{23}N_3O_3$	10.0	NT	
42	3-NH_2, 4-CH_3	305–308 (A)	40	$C_{21}H_{22}N_4O_3$	2.4	NT	
43	3,5-$(OCH_3)_2$·HCl·H_2O	238–241 (K)	50	$C_{22}H_{23}N_3O_5$·HCl·H_2O	3.4	NT	
44	3,5-$(CH_3)_2$	285–287 (F)	60	$C_{22}H_{23}N_3O_3$	3.0	NT	
45	2,4,6-$(CH_3)_3$·HCl·2H_2O	167–169 (L)	85	$C_{23}H_{27}N_3O_3$·HCl·2H_2O	0.2	5	−37 ± 3.5
46	2,3-Cl_2, 4-CH_3·HCl	199–203 (C)	29	$C_{21}H_{19}Cl_2N_3O_3$·HCl	5.0	25	−19 ± 3.0
47	2,4,6-Cl_3·HCl·H_2O	288–290 (C)	12	$C_{20}H_{16}Cl_3N_3O_3$·HCl·H_2O	0.5	NT	
48	2,4,6-Br_3·HCl	285–287 (E)	10	$C_{20}H_{16}Br_3N_3O_3$·HCl			
49	3,4,5-$(OCH_3)_3$·HCl·H_2O	295–297 (J)	55	$C_{23}H_{25}N_3O_6$·HCl·H_2O	0.8	NT	
minoxidil					1.2	5	−51.2 ± 2.3
dihydralazine					1.0	5	−60.0 ± 8.9

[a] Crystallization solvents: A = MeOH, B = DMF, C = $CHCl_3$–Et_2O, D = MeOH–Et_2O, E = EtOH–CH_2Cl_2–petroleum ether (60–80 °C), F = $CHCl_3$–C_6H_6, G = Me_2CO–petroleum ether, H = Me_2CO–Et_2O, I = EtOAc–CH_2Cl_2–petroleum ether, J = EtOH, K = EtOH–CH_2Cl_2, L = EtOH–Et_2O. [b] Analytical results were obtained for C, H, and N, as well as for halogens and S when they were present, and are within ±0.4% of the theoretical values unless otherwise noted. [c] Dose estimated to produce 25 mmHg fall in mean systemic blood pressure of anesthetized cats at 10 min after the administration of compound; ED_{25} was calculated from the log dose–response curve. [d] ΔBP refers to the difference in systolic blood pressure of SH rats observed prior to the first application and 2 h after the fifth application. Values are the mean ± SEM of six animals. NT = not tested.

profile and inhibitory effect on cAMP phosphodiesterase and platelet aggregation are described below under the special pharmacology of **56b**.

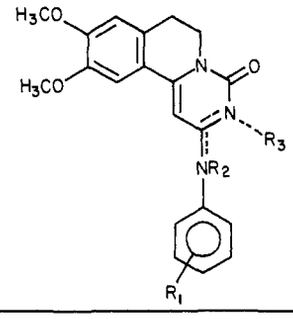
Results and Discussion

Of the sixteen 3-substituted pyrimido[6,1-*a*]isoquinoline-2,4-diones (Table I) investigated in the anesthetized cat, none provided ED_{25} values less than 2 mg/kg. Unlike the previously reported series of compounds **2** having a saturated pyrimido unit, compounds **3** bearing a $\Delta^{1,11b}$ bond in the pyrimido unit do not constitute a series of hypotensive compounds.

The 2-substituted derivatives **8a,c–f**, and **12–23** (Table II) also did not provide a compound with ED_{25} values less than 2 mg/kg in the anesthetized cat. The compounds were, however, weakly hypotensive in general, with the (3,4-dimethoxyphenethyl)amino derivative **15**, the morpholinoamino derivative **17**, and the methylpiperazino derivative **22** displaying approximate ED_{25} values of 10 mg/kg.

In contrast, introduction of appropriately substituted anilino groups at the 2-position resulted in compounds with potent blood pressure lowering properties. Data for this series of compounds are shown in Table III along with that for minoxidil and dihydralazine for comparative purposes. The 2,4-dimethylanilino derivative **32** was one of the first compounds to show hypotensive activity in the anesthetized cat comparable to that of minoxidil. Its oral antihypertensive activity was, however, only about one-fifth that of minoxidil. The variety of substituted anilino derivatives shown in Table III was consequently investigated to assess the influence of substitution in various positions of the anilino ring on activity. Monosubstitution at the 2-, 3- and 4-positions (compounds **24–31**) with groups of varying electronic, steric, and lipophilic effects provided only the 2-ethoxy (**26**) and the 2-chloro (**27**) analogues as compounds with hypotensive activity of the order displayed by **32**. Both compounds were devoid of oral antihypertensive activity in the SH rat. Among the disubstituted analogues **32–44**, the 2,6-dimethylanilino derivative **35**

Table IV. Blood Pressure Lowering Activity of Alkyl/Acyl Derivatives of 2-(Substituted-anilino)-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-ones



no.	R_1	R_2	R_3	mp, °C (solvent)[a]	yield, %	formula[b]	hypotensive act.:[c] ED_{25}, mg/kg	antihypertensive act.[d] mg/kg	antihypertensive act.[d] ΔBP, mmHg
50	2,4-$(CH_3)_2$·HCl·H_2O		CH_3	203–206 (A)	80	$C_{23}H_{28}N_3O_4$·HCl·H_2O	10	NT	
51	2,6-$(CH_3)_2$		CH_3	168 (B)	44	$C_{23}H_{25}N_3O_3$	3.5	NT	
52	2-CH_3, 6-$CH(CH_3)_2$·HCl·0.5H_2O		CH_3	172–175 (B)	25	$C_{25}H_{29}N_3O_3$·HCl·0.5H_2O	1.0	5	0
53	2-C_2H_5, 6-$CH(CH_3)$-C_2H_5·HCl·0.5H_2O		CH_3	174–176 (C)	32	$C_{27}H_{33}N_3O_3$·HCl·0.5H_2O	3.5	25	−20 ± 4.7
54a	2,6-F_2·CH_3SO_3H·H_2O	CH_3		145–148 (D)	56	$C_{21}H_{19}F_2N_3O_3$·CH_3SO_3H·H_2O	6.0	25	0
							0.03	10	0
54b	2,6-F_2·CH_3SO_3H·H_2O		CH_3	237–239	23	$C_{21}H_{19}F_2N_3O_3$·CH_3SO_3H·H_2O	0.5	10	0
55a	2,6-F_2	$CH_2P(O)(CH_3)_2$		207–210 (E)	14	$C_{23}H_{24}F_2N_3O_4P$	2.4	25	0
55b	2,6-F_2·HCl·H_2O		$CH_2P(O)(CH_3)_2$	287–289 (D)	29	$C_{23}H_{24}F_2N_3O_4P$·HCl·H_2O	0.4	25	0
56a	2,4,6-$(CH_3)_3$·HCl·H_2O	CH_3		189–191 (D)	6	$C_{24}H_{27}N_3O_3$·HCl·H_2O	0.03	5	−45 ± 1.7
56b	2,4,6-$(CH_3)_3$·HCl		CH_3	210–211 (D)	87	$C_{24}H_{27}N_3O_3$·HCl	0.025	5	−41 ± 3.3
57a	2,4,6-$(CH_3)_3$·0.5H_2O	C_2H_5		116–117 (B)	48	$C_{25}H_{29}N_3O_3$·0.5H_2O	0.025	3	−13
57b	2,4,6-$(CH_3)_3$·HCl·0.5H_2O		C_2H_5	218–219 (D)	34	$C_{25}H_{29}N_3O_3$·HCl·0.5H_2O	0.1	5	−20 ± 4.0
58	2,4,6-$(CH_3)_3$·HCl·H_2O		$CH_2P(O)(CH_3)_2$	208–211 (F)	69	$C_{26}H_{32}N_3O_4P$·HCl·H_2O	0.1	5	−33 ± 8.3
59	2,4,6-$(CH_3)_3$	n-C_4H_9		177–178 (G)	65	$C_{27}H_{33}N_3O_3$	0.08	NT	
60a	2,4,6-$(CH_3)_3$	$CH(CH_3)_2$		182–183 (B)	27	$C_{26}H_{31}N_3O_3$	0.5	5	−37 ± 9.6
60b	2,4,6-$(CH_3)_3$		$CH(CH_3)_2$	178–179 (B)	5	$C_{26}H_{31}N_3O_3$	0.1	10	−34 ± 5.0
61	2,4,6-$(CH_3)_3$	$COCH_3$		210–212 (B)	95	$C_{25}H_{27}N_3O_4$	0.25	10	−24 ± 3.8
62	2,4,6-Cl_3		CH_3	187–189 (B)	29	$C_{21}H_{18}Cl_3N_3O_3$	3	25	−21 ± 9.0

[a] Crystallization solvents: A = MeOH–CH_2Cl_2, B = CH_2Cl_2–petroleum ether, C = Me_2CO–Et_2O, D = EtOH–Et_2O, E = EtOH, F = EtOAc–CH_2Cl_2, G = EtOAc–petroleum ether. [b–d] See footnotes *b–d* in legend to Table III.

stood out in displaying hypotensive activity 10 times more potent than the 2,4-dimethyl isomer **32** and antihypertensive activity nearly half as potent as that of minoxidil. Replacement of one or both of the methyl substituents of **35** with substituents with differing physical parameters, i.e., **36–40**, resulted in compounds much less potent than **35**. Decreased potency was also observed in the dimethylanilino analogues **41** and **44**, in which the methyl substituents were at the 3,4- and 3,5-positions, respectively. The 2,4-dichloroanilino derivative **33** was as potent as **32** in the cat but without activity in the SH rat. The 3,4-disubstituted-anilino derivatives **42** and **43** displayed less potent hypotensive activity than **32**. Following the trend indicated by the results of mono- and disubstitution, investigation of the influence of trisubstitution in the anilino ring on activity was limited to the methyl and halogen groups in varied positions (compounds **45–48**) and to the methoxy group in the 3,4,5-positions (**49**). Highly potent hypotensive activity in the cat was displayed by the mesidine analogue **45** and the 2,4,6-trihalo compounds **47** and **48**, whereas the 2,3-dichloro 4-methyl compound **46** and the 3,4,5-trimethoxy compound **49** were less active. In the SH rat, **45** was virtually as potent as minoxidil and thus far the most active compound in the series, whereas the 2,4,6-tribromoanilino derivative **48** was only weakly potent.

In summary, variations in hypotensive activity in the anesthetized cat of the substituted anilino derivatives are apparently a function not only of the electronic effects of the substituents but also of steric bulk and of the position of substitution. Furthermore, for oral antihypertensive activity in the SH rat, the contribution of the substituents to an increased lipophilicity of the compounds appears to play an additional significant role.

Data recorded in Table IV illustrate the effect of activity of alkylation of some of the more active substituted anilinopyrimido[6,1-*a*]isoquinolines. Among the disubstituted compounds, alkylation led to compounds (**50–55**) with lower potency in the hypotensive cat, except in the case of the 2,6-difluoro analogues **54a,b** and **55a,b**. None of the compounds had demonstrable antihypertensive activity in the rat. On the other hand, alkylation of the trisubstituted mesidine analogue **45** provided compounds **56–60** with considerably increased hypotensive potency. The effect of acetylation of **45** also resulted in the 2-(*N*-acetylmesidino) analogue **61** with potent activity. In the SH rat, all the alkylated mesidino analogues and the acetyl derivative produced significant falls in blood pressure, with the isomeric methylated analogues **56a** and **56b** being the most potent and almost comparable with the standard drug minoxidil. Alkylation of the 2,4,6-trichloroanilino

Table V. Comparison of Toxicity and Blood Pressure Lowering Activity of **56b** with Related Analogues[a]

no.	LD_{50}, mg/kg ip (mice)	blood pressure lowering activity: in anesthetized cats, ED_{25} mg/kg iv	blood pressure lowering activity: in conscious SH rats, units of AUC[b] (0–5 days)
45	82.5	0.2	117
56a	27	0.03	206
56b	150	0.025	217
57a	65	0.1	31[c]
57b	65	0.025	61[c]
58a	65	0.5	140
58b	300	0.1	112
60	47.5	0.1	90
61	825	0.25	49[c]

[a] For testing protocols, see the Experimental Section. [b] The test compounds were given in a dose of 5 mg/kg po once a day for 5 consecutive days. The antihypertensive activity was assayed by calculating the units of area under the curve (AUC by trapezoidal rule) for 0–5 days duration. Results were analyzed for statistical significant difference from control values using ANOVA. [c] Values are not significant ($p < 0.05$).

analogue provided only a weakly active derivative, **62**.

Pharmacology of 56b. In a comparison of activity and toxicity data for several nearly equipotent antihypertensive compounds in the series, compound **56b** was easily distinguishable as the most favored compound (Table V).

Compound **56b** was selected for special study of its antihypertensive and cardiovascular effects. It induced a dose-dependent fall in systemic blood pressure in different anesthetized animal species (Table VI). The minimum effective dose in the three species shown was 10–25 μg/kg iv. Prolonged hypotensive activity was obtained with 50–100 μg/kg iv, which lasted for 70–140 min. Similar results were obtained in anesthetized guinea pigs and rhesus monkeys.[6b]

In several models of experimental hypertension (spontaneous, renal, DOCA–saline) in conscious rats, oral applications of **56b** (1–5 mg/kg) daily for 5 days produced dose-related reductions in blood pressure in all the models (Figure 1). In conscious chronic renal hypertensive dogs, **56b** (0.01 mg/kg, tid) administered orally for 5 consecutive days caused significant decreases of arterial blood pressure with no signs of attenuation after repeated oral administration.[6b]

The maximum changes in cardiovascular parameters following intravenous infusion of **56b** in anesthetized dogs and rats were summarized in an earlier publication.[6b] Figure 2 illustrates some of the cardiovascular effects of administering **56b** intravenously (50 μg/kg) in anesthetized dogs. The profile displayed of a significant reduction in systemic blood pressure with concomitant increase in peripheral blood flow and increase in heart rate and rate of rise of left ventricular pressure (dp/dt max) is characteristic of a drug that is a generalized arteriolar dilator.

There was no indication that the hypotensive action of **56b** was due to stimulation of central cardiovascular loci.

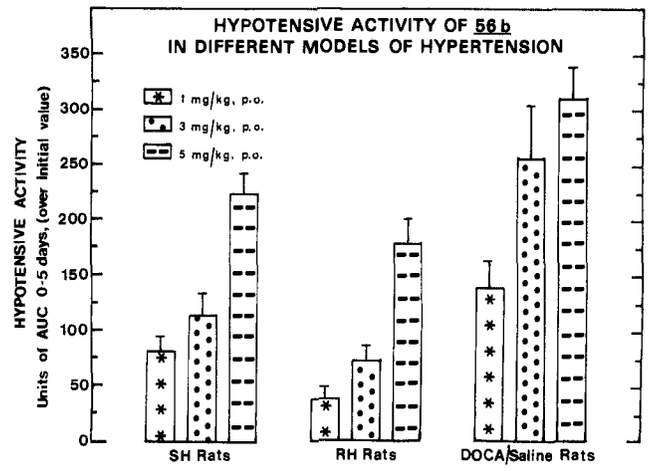


Figure 1. Effect of **56b** on systolic blood pressure in different models of hypertension. Values represent the mean ± SE of six animals.

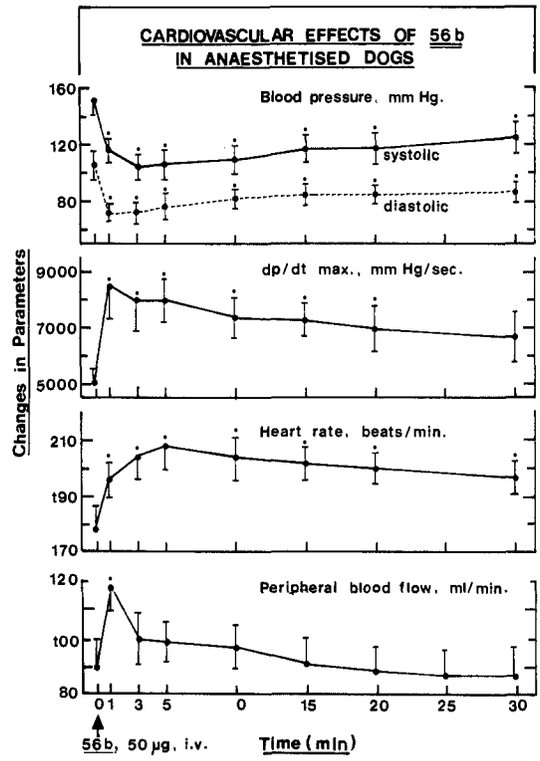


Figure 2. Effect of **56b** (50 μg iv) on cardiovascular parameters in the anesthetized dog. Values represent the mean of six animals. Asterisks indicate $p < 0.01$ significantly different from initial value.

Table VI. Hypotensive Activity of **56b** in Anesthetized Animals

animal species	dose, μg/kg iv	no. of expt	initial BP,[a] mmHg	fall in BP,[a,b] mmHg	% hypotension[a]	duration,[a] min
cats	10	5	110 ± 8.4	17 ± 3.7	16.8 ± 4.7	16 ± 7.5
	25	5	116 ± 10.5	25.8 ± 3.3	23.4 ± 4.5	45 ± 12.2
	50	5	125 ± 7.5	34.2 ± 4.7	28.6 ± 5.1	82.5 ± 17.2
dog	25	8	168.8 ± 11.9	37.5 ± 3.7	22.9 ± 3.7	50 ± 3.7
	50	6	163.3 ± 9.1	40.8 ± 5.2	25.4 ± 3.3	98.3 ± 18.7
	100	3	168.3 ± 6.7	56.1 ± 4.4	33.7 ± 2.6	140 ± 20
rabbits	10	7	93.8 ± 8.5	17.4 ± 3.9	18.6 ± 4.2	20.7 ± 5.5
	25	7	89.6 ± 5.2	26.4 ± 3.9	29.9 ± 4.2	57.8 ± 6.9
	50	6	84.2 ± 6.1	24.2 ± 3.5	28.5 ± 3.0	70 ± 12

[a] Values are the mean ± SEM. [b] The figures represent the maximum change in mean arterial blood pressure.

In the spinal transected cat preparations, the magnitude of the hypotension produced by administrations of **56b** (100 μg/kg iv) was the same as that observed in normal intact cats. Also, in anesthetized cats, intracerebroventricular administration of **56b** (5–50 μg) did not reduce the systemic blood pressure. Furthermore, the carotid occlusion pressor response in cats was not altered by intravenous administration of **56b**.[13]

The dose-dependent hypotensive activity of **56b** was still present following pretreatment with the α-adrenoceptor blocker phentolamine (1 mg/kg iv) and the β-adrenoceptor blocker propranolol (0.5 mg/kg iv), as well as after ganglionic blockade by pentolinium tartrate (5 mg/kg sc). The doses of the antagonists used in the anesthetized dog exhibited 70–80% inhibition of the response of the respective agonists, noradrenaline (2–4 μg/kg iv), isoprenaline (1–2 μg/kg iv), and 1,1-dimethyl-4-phenylpiperazinium iodide (20–30 μg/kg iv). Renin release was stimulated in anesthetized rats and conscious renal hypertensive dogs.[6b] Compound **56b** had a marked dose-dependent effect in hindquarter perfusion experiments. Following administration of 0.1 μg intraarterially to rats, the perfusion pressure was decreased by 34 ± 6 mmHg for 8 ± 1 min, after 0.2 μg by 40 ± 6 mmHg for 13 ± 1 min, and after 0.4 μg by 54 ± 8 mmHg for 25 ± 6 min. In isolated smooth muscle preparations, **56b** exerted concentration-related inhibition of acetylcholine-, histamine-, and barium chloride induced contractions with IC_{50} values of 2.4, 1.75, and 2.8 μg/mL, respectively.

In biochemical studies carried out by Ruppert and Weithmann, **56b** was shown to have a strong concentration-related inhibitory effect upon cAMP phosphodiesterase preparations from vascular tissues and platelets.[6b,14] Compound **56b** also inhibited in a concentration-related manner arachidonic acid induced platelet aggregation. The antiaggregatory activity of **56b** was shown to be due additionally to a concerted stimulation of vascular prostacyclin isomerase.[14]

These findings suggest that trequinsin (**56b**) reduces systemic blood pressure in normotensive as well as in hypertensive animals by a sequence of cardiovascular changes characteristic of a peripheral vasodilator agent and that an inhibition of cAMP phosphodiesterase might be involved in the mechanisms of action. Its profile indicated a potential for its use as an antihypertensive agent and for disease characterized by enhanced platelet aggregation. A further potential application of the compound is in therapy for chronic congestive heart failure in view of recent findings with antihypertensive vasodilators such as minoxidil.[15]

Experimental Section

Chemistry. All melting points are uncorrected and were obtained with a Kofler hot-stage apparatus. IR spectra were taken with a Perkin-Elmer Model 157 spectrophotometer using KBr disks and are reported in reciprocal centimeters. ^1H NMR (abbreviated as NMR throughout this section) spectra were obtained with a Varian T-60 spectrometer. Chemical shifts are reported in ppm relative to Me_4Si as the internal standard. UV spectra were recorded on a Carl Zeiss Specord spectrophotometer and λ_{max} values are shown in nanometers. Microanalytical data were determined for the elements whose symbols are shown and were within ±0.4% of the theoretical values.

1-Carbethoxy-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (6). To a solution of 1-(carbamoylmethylene)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline[16] (**5**; 2 g, 8.05 mmol) in anhydrous dichloromethane (50 mL) and pyridine (1 mL) at 0 °C was added slowly a solution of ethyl chloroformate (0.9 mL, 9.41 mmol) in anhydrous dichloromethane (10 mL) within 20 min. The reaction mixture was stirred at 0 °C for 5 h and then allowed to attain room temperature. It was washed with cold water, followed by dilute NaOH. The organic phase was worked up to give **7** as described below. The aqueous layer was acidified with cold 2 N HCl and extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to give a white solid, which on crystallization from chloroform–methanol (2:1) provided white crystals of **6** (0.5 g, 18%): mp 292–294 °C; IR 1725 (COOEt), 1700 (CO) cm^{-1}; NMR (Me_2SO-d_6) δ 1.17 (3 H, t, J = 7 Hz, OCH_2CH_3), 2.9 (2 H, t, J = 7 Hz, H-7), 3.7 (3 H, s, OCH_3), 3.9 (3 H, s, OCH_3), 3.9 (2 H, t, hidden at the base of OCH_3 signal), 4.2 (2 H, q, J = 7 Hz, OCH_2CH_3), 7.0 (2 H, s, Ar H), 11.3 (1 H, br, NH). Anal. ($C_{17}H_{18}N_2O_6$) C, H, N.

1-(Carbethoxycyanomethylene)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (7). The organic phase described for **6** above was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure to give a white solid of **7** (75 mg, 3%): IR 3200 (NH), 2200 (CN), 1670 (COOEt), 1600 (Ar) cm^{-1}, NMR ($CDCl_3$) δ 1.3 (3 H, t, J = 7 Hz, OCH_2CH_3), 2.8 (2 H, t, J = 5 Hz, H-4), 3.7 (2 H, m, H-3), 3.90 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 4.2 (2 H, q, J = 7 Hz, OCH_2CH_3), 6.73 (1 H, s, H-5), 8.0 (1 H, s, H-8), 10.6 (1 H, br, NH).

9,10-Dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (3a). A solution of **5** (5 g, 20.14 mmol) and an excess of sodium ethoxide (prepared from 12 g (0.52 mmol) of Na and 600 mL of anhydrous ethanol) in 100 mL of ethanol was heated to reflux. To the solution was added 150 mL of diethyl carbonate, and the reaction mixture was refluxed for an additional 8 h. The solvent was removed under reduced pressure and the residue was acidified to give a white precipitate of **3a** (4.8 g, 87%): mp 323–325 °C; IR 3200 (NH), 1700, 1690 (CONH), 1600 (Ar) cm^{-1}; NMR (CF_3COOD) δ 2.68 (2 H, t, J = 5 Hz, H-7), 3.6 (6 H, s, 9,10-OCH_3), 3.86 (2 H, t, J = 5 Hz, H-6), 6.21 (1 H, s, H-1), 6.50 (1 H, s, H-8), 6.96 (1 H, s, H-11). Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

9,10-Dimethoxy-3-methyl-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (3b). A mixture of **3a** (4.11 g, 14.98 mmol), benzene-washed NaH (0.75 g, 31.25 mmol), and anhydrous dimethylformamide (100 mL) was heated for 30 min. After the mixture cooled, methyl iodide (10 mL, 0.107 mol) was added, and the reaction mixture was maintained at 100 °C for 12 h. The reaction mixture was cooled and the excess of sodium hydride decomposed with methanol. The solvent was distilled under reduced pressure and the residue treated with water. The precipitate was filtered, washed with water, dried, and crystallized from ethyl acetate–dichloromethane (1:1) to give **3b** (4 g, 92%): mp 260–262 °C; IR 1700, 1665 (CONCO), 1640 (Ar) cm^{-1}; NMR ($CDCl_3$) δ 2.8 (2 H, t, J = 6 Hz, H-7), 3.2 (3 H, s, NCH_3), 3.75 (6 H, s, 9,10-OCH_3), 3.91 (2 H, t, J = 6 Hz, H-6), 5.83 (1 H, s, H-1), 6.46 (1 H, s, H-8), 6.83 (1 H, s, H-11). Anal. ($C_{15}H_{16}N_2O_4$) C, H, N.

Properties of **3c–i**, **3l**, and **3o**, prepared in a similar manner, are included in Table I.

3-(Carboxymethyl)-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (3k). A solution of **3l** (2.5 g, 6.9 mmol) and NaOH (0.6 g, 14.63 mmol) in ethanol (50 mL) was refluxed 12 h. Excess ethanol was evaporated under reduced pressure and the residue was treated with water and extracted with ethyl acetate. The aqueous layer was made acidic

(13) Dohadwalla, A. N.; Dadkar, N. K., unpublished results.
(14) (a) Ruppert, D.; Weithmann, K. U. *Life Sci.* **1982**, *31*, 2037. (b) Scholkens, B. A.; Dohadwalla, A. N.; Ruppert, D.; Weithmann, U. "Berichtsband 2, Kongress fur Thrombose und Hamostase, Munster"; Van de Loo, J., Asbeck, F., Schattauer, F. K., Ed.; Verlag: Stuttgart, 1982; pp 576–580. (c) Scholkens, B. A.; Dohadwalla, A. N. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *319*, Suppl. R 49. (d) Dohadwalla, A. N.; Dadkar, N. K.; Scholkens, B. A. *Indian J. Pharmacol.* **1982**, *14*, 43. (e) Weithmann, K. U. Proceedings of the 5th International Conference on Prostaglandins, Florence, May 1982.
(15) Mckay, C. R.; Chatterjee, K.; Porto, J. A.; Holly, A. N.; Parmley, W. W. *Am. Heart J.* **1982**, *104*, 575.
(16) (a) F. Hoffmann-La Roche & Co. A.G., Neth. Appl. 6, 401 827; *Chem. Abstr.* 1966, *64*, 6627. (b) Harsanyi, K.; Takacs, K.; Benedek, E.; Neszmelyi, A. *Liebigs Ann. Chem.* **1973**, 1606.

and the precipitated solid filtered, washed with water, and dried. The product was recrystallized from dimethylformamide to give **3k** (21 g, 87%): mp 287–288 °C. Anal. ($C_{16}H_{16}N_2O_6$) C, H, N.

3-[[[2-(Diethylamino)ethyl]carbamoyl]methyl]-9,10-dimethoxy-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinoline-2,4-dione (3m). A solution of **3l** (2.2 g, 6.1 mmol) and *N*,*N*-diethylethylenediamine (25 mL, 0.178 mol) was heated at 125–130 °C for 16–18 h. A solid separated out. The reaction mixture was cooled to allow further separation of the solid, filtered, and washed with ether. The product was crystallized from ethanol to give **3m** (2.3 g, 87.5%): NMR ($CDCl_3$) δ 1.0 (6 H, t, J = 7 Hz, $N(CH_2CH_3)_2$), 2.5 (4 H, q, J = 7 Hz, $N(CH_2CH_3)_2$), 2.53 (2 H, t, J = 7 Hz, CH_2NEt_2), 2.93 (2 H, J = 6 Hz, H-7), 3.33 (2 H, br t, $CONHCH_2$), 3.93 (3 H, s, OCH_3), 3.96 (3 H, s, OCH_3), 4.1 (2 H, t, J = 6 Hz, H-6), 4.67 (2 H, s, NCH_2CO), 6.1 (1 H, s, H-1), 6.48 (1 H, br t, $CONHCH_2$), 6.73 (1 H, s, H-8), 7.11 (1 H, s, H-11).

The above product was converted to its hydrochloride: mp 239–243 °C; IR 1700, 1640 (CONCO) cm^{-1}. Anal. ($C_{22}H_{30}N_4O_5$·HCl) C, H, N, Cl.

The properties of **3n**, prepared in a similar manner, are described in Table I.

2-Chloro-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (8a). (a) A mixture of **3a** (30 g, 0.109 mol) and $POCl_3$ (300 mL) was heated at 110 °C for 4 h. The excess of $POCl_3$ was distilled under reduced pressure. The residue was poured into ice-cold water and made basic with a solution of NaOH. A yellow solid separated, which was collected by filtration. This product was purified by passage through a silica gel column using chloroform as an eluent to give **8a** (28 g, 87%): mp 235–236 °C; IR 1665 (CO) cm^{-1}; NMR ($CDCl_3$) δ 2.96 (2 H, t, J = 6 Hz, H-7), 3.96 (6 H, s, 9,10-OCH_3), 4.2 (2 H, t, J = 6 Hz, H-6), 6.61 (1, s, H-1), 6.76 (1, s, H-8), 7.1 (1, s, H-11). Anal. ($C_{14}H_{13}ClN_2O_3$) C, H, N, Cl.

(b) A mixture of 1-(3,4-dimethoxyphenethyl)barbituric acid (20.2 g, 68.42 mmol) prepared as described hereunder) and $POCl_3$ (100 mL) was refluxed for 2.5 h at 100–110 °C. The excess of $POCl_3$ was distilled. The residue was poured onto crushed ice and made basic with a cold solution of 30% aqueous NaOH. A yellow gummy precipitate separated and was extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The residue was purified by passage over a silica gel column using chloroform as eluent to give **8a** (20.2 g, ca. 100%), with properties identical with those described above. Anal. ($C_{14}H_{13}ClN_2O_3$) C, H, Cl, N.

1-(3,4-Dimethoxyphenethyl)barbituric acid was prepared as follows. To an ethanolic solution of sodium ethoxide (7 g, 0.3 mol, of Na and 150 mL of ethanol) was added a solution of *N*-(3,4-dimethoxyphenethyl)urea (20.7 g, 0.092 mol) and diethyl malonate (15 g, 0.094 mol) in ethanol (250 mL), and the reaction mixture was refluxed for 16 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water (100 mL) and the solution made acidic with aqueous hydrochloric acid (1:1). A gummy mass separated, which solidified on chilling. This solid was filtered, washed with water, and dried to give the desired compound (20.2 g, 75%), which was crystallized from ethanol, mp 185–187 °C. Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

9,10-Dimethoxy-2-thioxo-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinolin-4-one (8b). A mixture of **3a** (10 g, 0.036 mol) and P_2S_5 (9 g, 0.02 mol) in dry dioxane (400 mL) was refluxed for 4 h. The solvent was evaporated under reduced pressure and the residue extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and filtered and the solvent removed under vacuum. The residue was passed through a silica gel column using dichloromethane as eluent. Evaporation of eluent gave a solid, which was crystallized from a chloroform–ether mixture to give **8b** (8 g, 76%): mp 272–273 °C; IR 1690 (CO), 1665 (Ar) cm^{-1}; NMR (Me_2SO-d_6) δ 2.93 (2 H, t, J = 6 Hz, H-7), 3.86 (6 H, s, 9,10-OCH_3), 3.93 (2 H, t, J = 6 Hz, H-6), 6.93 (1 H, s, H-1), 6.96 (1 H, s, H-8), 7.36 (1 H, s, H-11). Anal. ($C_{14}H_{14}N_2O_3S$) C, H, N, S.

9,10-Dimethoxy-2-(methylthio)-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (8c). To a suspension of **8b** (10 g, 0.034 mol) in tetrahydrofuran (200 mL) was added methyl iodide (20 mL, 0.211 mol) and the reaction mixture refluxed for 4 h. A white solid precipitated, which was collected by filtration and crystallized from a chloroform–methanol mixture. The crystalline material (mp 220–225 °C dec) was taken up in water, treated with cold dilute NaOH, and extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and filtered and the solvent evaporated under reduced pressure to give a white solid, which was crystallized from ethanol to give **8c** (10 g, 95%): mp 203–205 °C; IR 1665 (CO), 1640 (C=N) cm^{-1}; NMR ($CDCl_3$) δ 2.6 (3 H, s, SCH_3), 3.00 (2 H, t, J = 7 Hz, H-7), 3.96 (6 H, s, 9,10-OCH_3), 4.23 (2 H, t, J = 7 Hz, H-6), 6.53 (1 H, s, H-1), 6.76 (1 H, s, H-8), 7.11 (1 H, s, H-11). Anal. ($C_{15}H_{16}N_2O_3S$) C, H, N, S.

9,10-Dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (8d). To a solution of **8c** (450 mg, 1.48 mmol) in acetone (50 mL), was added Raney Ni (50 mg), and the reaction mixture was refluxed for 33 h. The reaction mixture was cooled and the Raney Ni filtered. The filtrate was concentrated under reduced pressure and the residue subjected to column chromatography on neutral alumina. Elution was started with petroleum ether. The pure compound was eluted with 90% chloroform–methanol. Evaporation of the eluent under vacuum gave **8d** as a colorless compound (200 mg, 52%): mp 213–214 °C; IR 1670 (CO), 1640 (CN) cm^{-1}; NMR ($CDCl_3$) δ 3.0 (2 H, t, J = 7 Hz, H-7), 4.0 (6 H, s, 9,10-OCH_3), 4.3 (2 H, t, J = 7 Hz, H-6), 6.66 (1 H, d, J = 5 Hz, H-1), 6.85 (1 H, s, H-8), 7.23 (1 H, s, H-11), 8.5 (1 H, d, J = 5 Hz, H-2). Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

9,10-Dimethoxy-1,2,3,4,6,7-hexahydro-11b*H*-pyrimido[6,1-*a*]isoquinolin-4-one (9). (i) **8d** (50 mg, 0.193 mol) was dissolved in methanol (50 mL), 10% palladium–carbon (50 mg) was added, and the reaction mixture was subjected to hydrogenation in a Parr hydrogenator at 40 psi for 4 h. The catalyst was removed by filtration and the filtrate evaporated under reduced pressure to give **9** as a white solid (48 mg, 95%): mp 190–192 °C; IR 3325 (NH), 1665 (CO) cm^{-1}; NMR ($CDCl_3$) δ 2–3.8 (7 H, m), 3.86 (6 H, s, 9,10-OCH_3), 4.6 (2 H, m, H-6, H-11b), 5.4 (1 H, m, NH), 6.63 (2 H, s, Ar H). Anal. ($C_{14}H_{18}N_2O_3$) C, H, N.

(ii) Compound **8a** (0.5 g, 1.708 mmol) was dissolved in methanol (50 mL), 10% palladium–carbon (100 mg) was added, and the reaction was subjected to hydrogenation at 50 psi in a Parr hydrogenator at room temperature for 6 h. The catalyst was removed by filtration and the solvent evaporated under pressure to give **9** (0.23 g, 51%), identical in all respects with the compound obtained by procedure (i) above.

(iii) 1-(2-Aminoethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline[17] (0.6 g, 2.54 mmol) in absolute ethanol (20 mL) was treated with sodium ethoxide (0.7 g of Na, 0.03 mol and 10 mL of ethanol) and diethyl carbonate (3 mL, 24.76 mmol) added. The reaction mixture was refluxed for 4 h. Excess ethanol was removed under reduced pressure and the residue treated with water and extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and filtered and the solvent evaporated under reduced pressure to give **9** (0.425 g, 64%), which was crystallized from dichloromethane–petroleum ether (bp 60–80 °C): mp 190–192 °C. Spectral data were identical with those recorded for the compound obtained by experiment (i) above.

9,10-Dimethoxy-6,7-dihydro-2*H*-pyrimido[6,1-*a*]isoquinolin-4-one (11). To a solution of **5**[16] (15 g, 0.06 mol) in 1-butanol (100 mL) were added triethyl orthoformate (88.8 g, 0.534 mol) and 2 drops of concentrated hydrochloric acid. The reaction mixture was refluxed for 18 h. Excess solvent and triethyl orthoformate were removed under reduced pressure. The residue was diluted with water and extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and filtered and the solvent evaporated under reduced pressure to give a residue, which was passed through a silica gel column using chloroform as eluent. Evaporation of the eluent under reduced pressure gave **11** as a white solid (10 g, 64%): mp 268–270 °C; IR 1650 (CO), 1620 (CN) cm^{-1}; NMR ($CDCl_3$) δ 3.05 (2 H, t, J = 6.5 Hz, H-7), 3.91 and 3.95 (6 H, s, 9,10-OCH_3), 4.06 (2 H, t, J = 6.5 Hz, H-6), 6.43 (1 H, s, H-1), 6.68 (1 H, s, H-8), 7.08 (1 H, s, H-11), 8.1 (1 H, s, H-4). Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

2-Butoxy-9,10-dimethoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (8e). To a mixture of NaOH (1 g, 0.025 mol) and 1-butanol (50 mL) was added **8a** (1.469 g, 5.01 mmol) and the mixture refluxed for 6 h. The solvent was removed under

(17) Yamazaki, T. *Yakugaku Zasshi* **1959**, *79*, 1008.

reduced pressure and the residue treated with water and extracted with chloroform. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give a white solid, which on crystallization from a chloroform–ether mixture yielded **8e** (0.7 g, 37%): mp 158–159 °C; IR 1665 (CO), 1640 (CN) cm^{-1}; NMR ($CDCl_3$) δ 0.95 (3 H, t, J = 7 Hz, CH_2CH_3), 1.16–1.93 (4 H, m, 4, $OCH_2CH_2CH_2CH_3$), 2.93 (2 H, t, J = 7 Hz, H-7), 3.93 (6 H, s, 9,10-OCH_3), 4.16 (2 H, t, J = 7 Hz, H-6), 4.4 (2 H, t, J = 7 Hz, OCH_2), 6.1 (1 H, s, H-1), 6.71 (1 H, s, H-8), 7.1 (1 H, s, H-11). Anal. ($C_{18}H_{22}N_2O_4$) C, H, N.

9,10-Dimethoxy-2-phenoxy-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (8f). Sodium hydride (1 g, 41.6 mmol) was covered by benzene (50 mL). A solution of phenol (2 g, 21.25 mmol) in benzene (20 mL) was added slowly and the reaction mixture refluxed until evolution of hydrogen stopped. To the above slurry was added (3 g, 10.2 mmol) and the mixture refluxed overnight. Excess sodium hydride was decomposed by methanol. The solvent was removed under reduced pressure, the residue was treated with water, and the product was extracted with chloroform. The organic layer was washed with water and dried over anhydrous sodium sulfate and the solvent distilled under vacuum to give a pale yellow solid. The product was crystallized from chloroform–petroleum ether (bp 60–80 °C) to give **8f** (2 g, 56%): mp 235–236 °C. Anal. ($C_{20}H_{18}N_2O_4$) C, H, N.

General Synthetic Procedure for Compounds 12–49. Compound **8a** and the appropriate amino compound in a 1:2 molar ratio were dissolved in a suitable solvent, more often in chloroform, and the reaction mixture was refluxed for 12–24 h. The end of the reaction was determined by TLC. The solvent was evaporated, the residue was treated with aqueous NaOH, and the product was extracted with an organic solvent. The organic solvent was evaporated, and the residue was purified first by column chromatography and finally by crystallization from a suitable solvent. The preparation of compound **45** as a typical example is described below. The properties of the other compounds **12–49** are included in Tables II and III.

9,10-Dimethoxy-2-(mesitylamino)-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (45). A solution of **8a** (76.68 g, 0.26 mol) and mesitylamine (107 g, 0.79 mol) in chloroform (1.5 L) was refluxed with stirring for 20 h. The solution was cooled and washed first with 10% aqueous NaOH and then with water. The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was crystallized from dichloromethane–petroleum ether (bp 60–80 °C) to give **45** (75.5 g, 74%): mp 287–288 °C; IR 3226 (NH), 1639 (CN) cm^{-1}; NMR ($CDCl_3$) δ 2.23 (9 H, s br, 2′,4′,6′-CH_3), 2.9 (2 H, t, J = 6 Hz, H-7), 3.80 and 3.93 (6 H, s, 9,10-OCH_3), 4.6 (2 H, t, J = 6 Hz, H-6), 5.46 (1 H, s, H-1), 6.70–6.93 (4 H, m, Ar H). Anal. ($C_{23}H_{25}N_3O_3$) C, H, N.

General Procedure for Synthesis of Compounds 50–62. To a suspension of the 9,10-dimethoxy-2-(substituted-anilino)-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one and anhydrous K_2CO_3 or NaH in acetone or DMF was added the appropriate alkyl halide and the reaction mixture heated overnight. The reaction mixture was cooled, ice water was added, and the product was extracted with an organic solvent. The organic layer was washed with water and dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The residue, which contained a mixture of isomers, was subjected to column chromatography on a silica gel column, and the isomers were separated and identified by spectral methods (vide infra). The preparation of **56a** and **56b** is described below as a typical example. The properties of the other compounds obtained are included in Table IV.

9,10-Dimethoxy-2-(mesitylimino)-3-methyl-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinolin-4-one (56b) and 9,10-Dimethoxy-2-(methylmesitylamino)-6,7-dihydro-4*H*-pyrimido[6,1-*a*]isoquinolin-4-one (56a). A suspension of **45** (50 g, 0.127 mol), anhydrous K_2CO_3 (160 g, 1.23 mol), and methyl iodide (228 g, 1.6 mol) in acetone (1.5 L) was heated with stirring at 60 °C for 6 h. The mixture was cooled and filtered. The filtrate was evaporated under reduced pressure to give a residue. Chromatography of the residue over silica gel using as eluents first benzene–chloroform (60:40) and later benzene–chloroform (50:50) gave two compounds. The first compound eluted was **56b** (43 g, 83%): mp 151–152 °C; mp of **56b**·HCl 198–200 °C; UV (MeOH) (free base) 207.3, 230, 275, 283.8, 340.0; IR (free base) 1653, 1625, 1615 cm^{-1}; NMR ($CDCl_3$) δ 2.0 (6 H, s, 2′,6′-CH_3), 2.18 (3 H, s, 4′-CH_3), 2.76 (2 H, t, J = 6 Hz, H-7), 3.46 (3 H, s, NCH_3), 3.6 (3 H, s, 9-OCH_3), 3.71 (3 H, s, 10-OCH_3), 3.9 (2 H, t, J = 6 Hz, H-6), 5.26 (1 H, s, H-1), 6.36 (1 H, s, H-8), 6.44 (1 H, s, H-11), 6.55 (2 H, s, H-3′,5′). Anal. ($C_{24}H_{27}N_3O_3$) C, H, N.

The second compound isolated was **56a** (2.86 g, 5.53%): mp 118–120 °C (**56b**·HCl mp 189–191 °C dec); UV (MeOH) (free base) 271.5, 281; IR (free base) 1661, 1645 (humps), 1637 cm^{-1}; NMR ($CDCl_3$) δ 2.06 (6 H, s, 2′,6′-CH_3), 2.23 (3 H, s, 4′-CH_3), 2.76 (2 H, t, J = 6 Hz, H-7), 3.25 (3 H, s, NCH_3), 3.56 (3 H, s, 9-OCH_3), 3.73 (3 H, s, 10-OCH_3), 3.98 (2 H, t, J = 6 Hz, H-6), 5.13 (1 H, s, H-1), 6.36 (1 H, s, H-8), 6.4 (1 H, s, H-11), 6.66 (2 H, s, H-3′,5′). Anal. ($C_{24}H_{27}N_3O_3$) C, H, N.

A small yield of a mixture of the methiodides of **56a** and **56b** was also isolated, mp 221–222 °C. Anal. ($C_{25}H_{30}IN_3O_3$) C, H, N, I.

Unambiguous Synthesis of 56a. Compound **8a** was treated with *N*-methylmesidine[18] according to the general procedure described above for compounds **12–49**. The product obtained was identical in all respects with **56a** as described above.

Unambiguous Synthesis of 56b. Compound **3b** (1.0 g, 3.46 mmol) was dissolved in pyridine (15 mL) on warming. To this solution P_2S_5 (2 g, 8 mmol) was added and the reaction mixture refluxed for 15 h. Excess pyridine was distilled under reduced pressure. The residue was taken up in methylene chloride and washed with water, dilute HCl, and then with water. The organic layer was dried (Na_2SO_4), and the solvent was evaporated under vacuum to give a residue, which on passage through a neutral alumina column with benzene as eluent gave the corresponding 2-thio **9a** as a yellow compound (0.9 g, 85%). Anal. ($C_{15}H_{16}N_2O_3S$) C, H, N, S.

Compound **9a** (0.9 g, 2.95 mmol) was dissolved in hot tetrahydrofuran (75 mL), methyl iodide (4 mL, 42.37 mmol) was added, and the reaction mixture was refluxed for 3 h. A solid separated, which was filtered and crystallized from methylene chloride–petroleum ether to give the methiodide **10** (1.3 g, 100%): IR 1709 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 3.1 (3 H, s, SCH_3), 3.73 (3 H, s, NCH_3), 7.63 (1 H, s, H-1). Anal. ($C_{16}H_{19}IN_2O_3S$) C, H, N, S.

Compound **10** (0.5 g, 1.12 mmol) and mesidine (2 mL, 14.25 mmol) were heated at 100 °C for 3 h. TLC showed no starting material. The reaction mixture was washed with petroleum ether. The petroleum ether washings provided **56b**·HI (0.04 g). The petroleum ether insoluble sticky material was treated with dichloromethane–petroleum ether, when orange crystals of compound **9a** (80 mg) were isolated. The mother liquor was concentrated, treated with cold dilute HCl, and extracted with ethyl acetate. The aqueous layer was made basic by addition of dilute NaOH and extracted with chloroform. The 40 mg of **56b**·HI isolated above were also converted to the free base and combined with the chloroform layer. Evaporation of the solvent gave a solid, which on passage through a silica column, using chloroform as eluent, gave a solid (100 mg, 22%) identical in its properties with an authentic sample of **56b**.

Pharmacology. Acute Toxicity. Acute toxicity (LD_{50}) was evaluated in mice (18–22 g) of either sex. Test compounds were dissolved in distilled water or suspended in 0.5% (carboxymethyl)cellulose and injected intraperitoneally at dose levels of 10, 30, 100, 300, and 1000 mg/kg in a volume of 1 mL/100 g of body weight. The animals were observed for 48 h and approximate LD_{50} values were calculated according to the method described by Campbell and Richter.[19]

Hypotensive Activity in Anesthetized Animals. Experiments were performed on anesthetized cats, dogs, and rabbits. Cats (2.5–4.0 kg) of either sex were anesthetized with ether and maintained on chloralose (70 mg/kg iv). Mongrel dogs (10–16 kg) of either sex were anesthetized with pentobarbitone sodium (35 mg/kg iv). Rabbits (2.5–3.5 kg) were anesthetized with pentobarbitone (50 mg/kg iv). The mean arterial blood pressure was measured through the femoral artery by using a Statham P23Db pressure transducer. Arterial blood pressure was amplified

(18) Emerson, S. Wm. *J. Am. Chem. Soc.* **1941**, *63*, 2023.
(19) Campbell, D. G. S.; Richter, W. *Acta. Pharmacol. Toxicol.* **1967**, *25*, 345.

and registered on a Hellige two-channel recorder. All injections were made via a catheter placed in the femoral vein.

For primary screening, only cats were used. Test compounds were dissolved in distilled water or propylene glycol and administered through the femoral vein at dose levels of 0.01, 0.025, 0.05, 0.1, 0.3, 1.0, 3.0, and 10 mg/kg. Ten minutes after administration of the test compounds, the fall in mean arterial blood pressure was recorded in cats. ED_{25} values were calculated from the log dose–response curves. Minoxidil and dihydralazine were used as standard drugs. Their ED_{25} values were 1.2 and 1.6 mg/kg iv, respectively.

Antihypertensive Assay in Conscious Hypertensive Rats. Blood pressure lowering activity was evaluated in conscious spontaneously hypertensive (SH), renal hypertensive (RH), and metacorticoid hypertensive (DOCA/saline) rats. Male SH rats (250–300 g, 12–16 weeks old), derived from Wistar–Okamoto strain and obtained from in-house breeding facilities, were used. For renal hypertension, male Wistar rats (90–100 g, 4–6 weeks old) were made hypertensive by clamping the left renal artery with a silver clip (0.2-mm gap), leaving the contralateral kidney intact.[20] Metacorticoid hypertension was induced in male Wistar rats (90–100 g, 4–6 weeks old) by implanting four pellets (25 mg each) of deoxycorticosterone acetate (DOCA) subcutaneously after removing the left kidney and substituting 1.0% sodium chloride solution for drinking water.[21] Rats were warmed at 37–38 °C in a heating chamber for 10 min prior to blood pressure determination. Systolic blood pressure was measured in conscious rats by the tail-cuff method, utilizing a piezo electric crystal for the detection of pressure pulse, an aneroid manometer for measuring pressure, and a cardioscope (BPL-India) for visualizing the disappearance and/or appearance of the pressure pulse. The test compound was administered in SH rats in doses ranging from 3 to 25 mg/kg po daily once for 5 days, as a solution in 0.9% sodium chloride or as a suspension in 0.5% (carboxymethyl)-cellulose in a volume of 10 mL/kg. One group served as control and received vehicle. Systolic blood pressure was recorded before the first application and 2 h after each drug administration. A fall in systolic blood pressure was determined as the difference between the posttreatment blood pressure and the initial reading and evaluated statistically by using the paired "t" test. Compounds exhibiting more than a 15 mmHg fall in systolic blood pressure and having a level of significance of $p < 0.05$ are included in Table II.

Cardiovascular Studies on 56b. The experiments were conducted with Beagle dogs of either sex, weighing between 11 and 15 kg. The dogs were anesthetized with pentobarbitone sodium (35 mg/kg iv), and the femoral vein was cannulated to allow intravenous administration of drugs. The arterial blood pressure was recorded from the femoral artery through a cannula connected to a statham P23Db pressure transducer. The pressure in the left ventricle of the heart was measured with a Milar-tip catheter. By use of an electronic differentiator, dp/dt was determined. Heart rate was obtained from an ECG Lead II recording, using fine needle electrodes inserted through the skin. Peripheral blood flow through the femoral artery was measured by using an electromagnetic flow probe (Statham). All responses were recorded on a six-channel Hellige recorder. Compound **56b** was studied at a dose of 50 μg/kg iv in six animals. All the parameters were measured at 1, 3, 5, 15, 20, 25, and 30 min after drug administration (Figure 2).

Peripheral Vasodilatory Activity of 56b. The activity was investigated by using autoperfused hindquarters of rats, at a constant flow, as described by Bhattacharya et al.[22] Blood from the proximal part of the abdominal aorta was forced by a peristaltic pump (DESAGA) into the distal part of the aorta. The pump speed was so adjusted that the perfusion pressure was almost equal to the systemic blood pressure. The systemic blood pressure and perfusion pressure were measured with Statham P23Db pressure transducers and recorded on a Hellige two-channel recorder. Intraarterial injections of **56b** (0.1, 0.2, and 0.4 μg) were made into the rubber tubing leading toward the periphery. The fall in perfusion pressure and the duration of action were recorded in six animals. Mean values ± SE were calculated.

Smooth Muscle Relaxant Activity of 56b. Isolated guinea pig ileum preparations were set up according to Burn.[23] Contraction of the ileum was recorded on a two-channel potentiometric recorder (Omniscribe) through TF 3V3 strain gauge. Compound **56b** was added to the bath at different concentrations (0.5–8.0 μg/mL) and a 3-min contact period permitted. The effect of the compound on acetylcholine- (5–10 ng/mL), histamine- (5–10 ng/mL), and barium chloride (15–20 μg/mL) induced contractions was studied. The percent inhibition was calculated from the mean of four experiments. IC_{50} values were determined from the log dose–response curves.

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Registry No. **3a**, 68619-18-1; **3b**, 68619-19-2; **3c**, 68619-20-5; **3d**, 91209-60-8; **3e**, 91209-61-9; **3f**, 91209-62-0; **3g**, 91209-63-1; **3h**, 91209-65-3; **3h**·HCl, 91209-64-2; **3i**, 91209-66-4; **3j**, 91209-67-5; **3k**, 91209-68-6; **3l**, 91209-69-7; **3m**, 91209-71-1; **3m**·HCl, 91209-70-0; **3n**, 91209-72-2; **3o**, 91209-73-3; **3p**, 91228-83-0; **5**, 91209-74-4; **6**, 91209-75-5; **7**, 91209-76-6; **8a**, 75535-96-5; **8b**, 80023-72-9; **8c**, 91209-78-8; **8c**·HI, 91209-77-7; **8d**, 91209-79-9; **8e**, 91209-80-2; **8f**, 91209-81-3; **9**, 91209-82-4; **10**, 91209-83-5; **11**, 78915-35-2; **12**, 91209-85-7; **12**·HCl, 91209-84-6; **13**, 76536-29-3; **14**, 91209-86-8; **14**·2HCl, 76536-23-7; **15**, 91209-87-9; **16**, 91209-88-0; **16**·HCl, 76536-03-3; **17**, 91209-89-1; **17**·HCl, 76536-04-4; **18**, 91209-90-4; **18**·HCl, 76536-02-2; **19**, 91209-91-5; **19**·HCl, 76536-11-3; **20**, 91209-92-6; **20**·HCl, 76536-35-1; **21**, 76536-40-8; **22**, 91209-93-7; **22**·HCl, 76536-38-4; **23**, 91209-95-9; **23**·HCl, 91209-94-8; **24**, 76536-53-3; **25**, 91209-96-0; **26**, 91209-97-1; **26**·HCl, 76536-52-2; **27**, 91209-98-2; **27**·HCl, 76536-56-6; **28**, 91209-99-3; **29**, 91210-00-3; **30**, 76536-46-4; **30**·HCl, 91210-01-4; **31**, 76536-44-2; **32**, 76536-49-7; **33**, 76536-51-1; **34**, 91210-02-5; **35**, 76536-45-3; **36**, 91210-03-6; **36**·HCl, 91228-84-1; **37**, 91210-05-8; **37**·HCl, 91210-04-7; **38**, 91210-07-0; **38**·HCl, 91210-06-9; **39**, 91210-08-1; **39**·CH_3SO_3H, 91210-09-2; **40**, 76536-61-3; **41**, 76536-43-1; **42**, 91210-10-5; **43**, 91210-12-7; **43**·HCl, 91210-11-6; **44**, 76536-47-5; **45**, 76536-66-8; **45**·HCl, 76536-65-7; **46**, 91210-14-9; **46**·HCl, 91210-13-8; **47**, 91210-15-0; **47**·HCl, 76536-70-4; **48**, 91210-17-2; **48**·HCl, 91210-16-1; **49**, 91210-18-3; **49**·HCl, 76536-64-6; **50**, 91210-19-4; **50**·HCl, 83070-43-3; **51**, 91210-20-7; **52**, 91210-22-9; **52**·HCl, 91210-21-8; **53**, 91210-24-1; **53**·HCl, 91210-23-0; **54a**, 91210-25-2; **54a**·CH_3SO_3H, 91210-26-3; **54b**, 91210-27-4; **54b**·CH_3SO_3H, 91210-28-5; **55a**, 91210-29-6; **55b**, 91210-31-0; **55b**·HCl, 91210-30-9; **56a**, 83070-45-5; **56a**·HCl, 76536-67-9; **56a**·CH_3I, 91210-32-1; **56b**, 79855-88-2; **56b**·HCl, 78416-81-6; **56b**·CH_3I, 91210-33-2; **57a**, 76536-69-1; **57b**, 80023-91-2; **57b**·HCl, 80023-97-8; **58**, 91210-35-4; **58**·HCl, 91210-34-3; **59**, 76536-68-0; **60a**, 83070-39-7; **60b**, 80023-89-8; **61**, 83070-40-0; **62**, 91210-36-5.

(20) Goldblatt, H.; Lynch, J.; Hanzel, R. F.; Summervilles, W. W. *J. Exp. Med.* **1934**, *59*, 347.
(21) Peterfalvi, M.; Jequier, R. *Arch. Int. Pharmacodyn. Ther.* **1960**, *124*, 237.
(22) Bhattacharya, B. K.; Dadkar, N. K.; *Dohadwalla*, A. N. *Br. J. Pharmacol.* **1977**, *59*, 243.
(23) Burn, J. H. "Practical Pharmacology"; Blackwell Scientific Publication: Oxford, 1952; pp 17–18.