colorless foam. Anal. ($C_{24}H_{39}N_3O_7Si_2$) C, H, N.

5-(3-Furyl)-6-aza-2′-deoxyuridine (7). To a solution of **40** (0.54 g, 1.01 mmol) in THF (25 mL) was added a 1 N solution of tetra-*n*-butylammonium fluoride in THF (2.5 mL, 2.5 mmol) and the mixture stirred at room temperature for 3 h. The reaction mixture was concentrated to 5-mL volume and applied to a column of flash silica gel. Elution with dichloromethane–ethanol (5:1) gave 5-(3-furyl)-6-aza-2′-deoxyuridine (265 mg, 89%); mp 225–227 °C; UV (H_2O, pH 7) λ_{max} 308 nm (ϵ 7400), (H_2O, pH 13) 298 nm (ϵ 7100); ^1H NMR (Me_2SO-d_6) δ 2.15 (1 H, m, H-2′), 2.55 (1 H, m, H-2′), 3.5 (2 H, ABX, H-5′), 3.77 (1 H, m, H-4′), 4.45 (1 H, m, H-3′), 6.45 (1 H, dd, H-1′), 6.85 (1 H, d, furan H-4), 7.83 (1 H, t, furan H-5), 8.45 (1 H, d, furan H-2). Anal. ($C_{12}H_{13}N_3O_6$) C, H, N.

Antiviral Activity. Antiherpes activity was measured in a plaque reduction assay.[25] Confluent monolayers of Vero cells, in 24-mm diameter dishes, were infected with 30–40 plaque-forming units of either HSV-1 (strain HFEM) or HSV-2 (strain 196). The infected monolayers were incubated at 37 °C for 1 h and then overlayed with maintenance medium containing 0.75% carboxymethylcellulose and various concentrations of the test compound. The monolayers were incubated for a further 2 days at 37 °C, after which the cells were fixed and stained, the plaques counted, and the concentration of compound resulting in 50% inhibition of plaque formation was calculated.

Cytotoxicity Test. Cytotoxicity was measured by determining the incorporation of [*methyl*-^{3}H]thymidine into DNA of Vero cells. Subconfluent monolayers of Vero cells in 16-mm diameter dishes were overlayed with maintenance medium containing 1 μCi/mL [*methyl*-^{3}H]thymidine and various concentrations of the test nucleoside. After 18 h of incubation at 37 °C, DNA synthesis was determined by measuring the amount of [*methyl*-^{3}H]thymidine incorporated into acid-precipitable material.

Nucleoside Phosphorylation Studies. Thymidine kinases were isolated and purified from HSV1-infected Vero cells as previously described.[26] The relative phosphorylation rates of the nucleosides (50 μM) by these kinases were determined by an adenosine 5′-triphosphate transfer assay.[27]

Acknowledgment. We thank Drs. M. I. Brady, J. M. Cameron, and J. A. V. Coates for antiviral testing and Dr. J. Bradshaw for helpful discussions.

Registry No. **2**, 97776-64-2; (*E*)-**3**, 100348-16-1; (*Z*)-**3**, 100348-17-2; **4**, 100348-18-3; **5**, 100348-19-4; **6**, 97776-76-6; **7**, 100244-31-3; **8**, 4449-45-0; **9**, 24753-63-7; **10**, 97776-60-8; **11**, 100244-18-6; **12**, 97776-61-9; α-**13**, 97776-62-0; β-**13**, 97776-63-1; **14**, 97776-65-3; **15**, 20258-32-6; **16**, 97776-66-4; **17**, 100244-19-7; **18**, 97776-67-5; **19**, 97776-68-6; **20**, 100244-20-0; (*E*)-**21**, 97776-69-7; (*Z*)-**21**, 97776-70-0; (*E*)-**22**, 97776-71-1; (*Z*)-**22**, 97776-72-2; (*E*)-**23**, 97776-77-7; (*Z*)-**23**, 97776-78-8; (*E*)-**24**, 100244-21-1; (*Z*)-**24**, 100244-22-2; **25**, 100244-23-3; **27**, 100244-24-4; **28**, 100244-25-5; **29**, 100244-26-6; **30**, 100244-27-7; **31**, 54280-70-5; **32**, 100244-28-8; **33**, 100244-29-9; **34**, 100297-29-8; **35**, 100244-30-2; **36**, 100297-30-1; **37**, 100297-31-2; **38**, 100297-32-3; **39**, 100297-33-4; **40**, 100297-34-5; 2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentafuranosyl chloride, 52304-86-6; 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, 6974-32-9; 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride, 4330-21-6.

Thromboxane Synthetase Inhibitors and Antihypertensive Agents. 2. *N*-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones and *N*-[(1*H*-1,2,4-Triazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones as Unique Antihypertensive Agents

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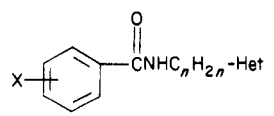
A series of *N*-[(1*H*-heteroaryl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones were prepared as part of a continuing investigation into the biological properties of compounds that were both thromboxane synthetase inhibitors and potential antihypertensive agents. The most active thromboxane synthetase inhibition was found for the title imidazole derivatives wherein a hexyl or octyl chain separated the heterocyclic ends of the molecule (**5**, **6**) or with substitution on the isoindole portion of the molecule (**18**, **19**, **21**, **22**, **25**, **26**). Compounds with shorter alkyl chain separations had good antihypertensive effects (**1–5**, **8–10**, **19–22**, **27–30**). Butyl derivative **3** was chosen for further evaluation as a potential antihypertensive agent with thromboxane synthetase inhibitory properties.

In a previous paper[2] we described a series of *N*-[(1*H*-imidazol-1-yl)alkyl]arylamides (I) and *N*-[(1*H*-1,2,4-triazol-1-yl)alkyl]arylamides (II) that were potent thromboxane (TX) synthetase inhibitors and also had interesting antihypertensive activity. A number of other laboratories have had active research programs investigating selective TX synthetase inhibitors as potential therapeutic agents,[3-5] and a number of such compounds are presently under clinical evaluation.



I, Het = 

II, Het = 

In addition to the possible treatment of ischemia, arrhythmias, fibrillation, and sudden death, we were interested in developing a class of TX synthetase inhibitors with antihypertensive activity as agents that might be useful in the treatment of certain forms of hypertension. Selective inhibition of the production of thromboxane A_2 (TXA_2) not only removes a source of potent vasoconstriction but also might lead to the enhancement of prostacyclin (a potent vasodilator) production through a

(1) Present address: Ortho Pharmaceutical Corp., Raritan, NJ 08869.

(2) Wright, W. B., Jr.; Press, J. B.; Chan, P. S.; Marsico, J. W.; Haug, M. F.; Lucas, J.; Tauber, J.; Tomcufcik, A. S. *J. Med. Chem.*, in press.

(3) (a) Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *J. Med. Chem.* **1985**, *28*, 1427. (b) See references 11 and 13–19 in ref 2.

(4) Lefer, A. M. *Drugs Future* **1984**, *9*, 437.

(5) Kato, K.; Ohkawa, S.; Terao, S.; Terashita, Z.-i.; Nishikawa, K. *J. Med. Chem.* **1985**, *28*, 287.

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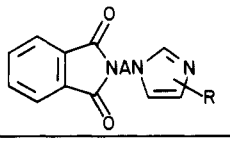
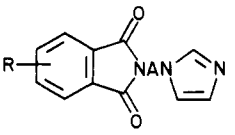
Table I. 2-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones



compd	R	A	yield,[a] %	mp, °c	thromboxane formn inhibn[b]	antihyper-tens act.[c]	formula
1	H	$(CH_2)_2$	d		20 (4)	A (2)	$C_{13}H_{11}N_3O_2$
2	H	$(CH_2)_3$	69	106–108[e]	94 (4)	A (2)	$C_{14}H_{13}N_3O_2$
3	H	$(CH_2)_4$	d		99 (4)	A (2)	$C_{15}H_{15}N_3O_2$
4	H	$(CH_2)_5$	d		92 (4)	A (2)	$C_{16}H_{17}N_3O_2$·HCl
5	H	$(CH_2)_6$	d		100 (2)	A (4)	$C_{17}H_{19}N_3O_2$
6	H	$(CH_2)_8$	d		100 (4)	I (4)	$C_{19}H_{23}N_3O_2$
7	H	$(CH_2)_{10}$	49	69–70[f,g]	92 (4)	I (3)	$C_{21}H_{27}N_3O_2$
8	H	$CH(CH_3)CH_2CH_2$	37	87–88[g,h]	94 (4)	A (3)	$C_{15}H_{15}N_3O_2$
9	H	$CH_2CH{=}CHCH_2$	d		85 (4)	A (2)	$C_{15}H_{13}N_3O_2$
10	H	$CH_2C{\equiv}CCH_2$	10	139–141[g,i]	100 (4)	A (2)	$C_{15}H_{11}N_3O_2$
11	2-CH_3	$(CH_2)_3$	78	122–125	36 (2)	I (2)	$C_{15}H_{15}N_3O_2$
12	2-CH_3	$(CH_2)_4$	71	120–122[f,j]	22 (2)	I (4)	$C_{16}H_{17}N_3O_2$
13	4-CH_3	$(CH_2)_3$	34	82–90	68 (2)	I (2)	$C_{15}H_{15}N_3O_2$
14	4-CH_3	$(CH_2)_4$	73	159–161[k]	86 (2)	I (3)	$C_{16}H_{17}N_3O_2$·$C_4H_4O_4$
15	2-C_2H_5	$(CH_2)_3$	73	104–106	0 (2)	I (2)	$C_{16}H_{17}N_3O_2$
16	2-C_2H_5	$(CH_2)_4$	74	191–201[f,l,m]	65 (2)	I (2)	$C_{17}H_{19}N_3O_2$·HCl
17	2-C_6H_5	$(CH_2)_4$	70	208–211[l,m]	57 (2)	I (3)	$C_{21}H_{19}N_3O_2$·HCl

[a] Prepared by procedure A unless otherwise noted. [b] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. UK37248 = 98%. [c] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A, active; I, inactive. Number of rats (one to four) as shown in parentheses. See ref 12. [d] Described in our previous publication.[2] [e] Literature reference reports mp 107–109 °C: Schwan, T. J. *J. Heterocycl. Chem.* **1967**, *4*, 633. [f] Procedure B. [g] Purified by HPLC (EtOAc/silica gel). [h] Procedure C. [i] C: calcd, 67.92; found, 67.37. [j] Recrystallized from EtOAc. [k] Fumarate salt. [l] HCl salt. [m] Recrystallized from EtOH.

Table II. 2-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones



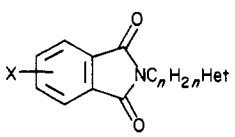
compd	R	A	yield,[a] %	mp, °C	thromboxane formn inhibn[b]	antihyper-tens act.[c]	formula
18	4-CH_3	$(CH_2)_3$	84	83–85[d,e]	100 (4)	I (3)	$C_{15}H_{15}N_3O_2$
19	4-CH_3	$(CH_2)_4$	68	196–199[f,g]	100 (6)	A (2)	$C_{16}H_{17}N_3O_2$·HCl
20	4-Cl	$(CH_2)_3$	48	92–94[d]	92 (4)	A (3)	$C_{14}H_{12}ClN_3O_2$
21	4-Cl	$(CH_2)_4$	82	80–83[d,e]	100 (4)	A (2)	$C_{15}H_{14}ClN_3O_2$
22	4-Br	$(CH_2)_4$	96	110–112[d,e]	100 (2)	A (2)	$C_{15}H_{14}BrN_3O_2$
23	4-Br	$(CH_2)_5$	25	102–104[h]	87 (4)	I (3)	$C_{16}H_{16}BrN_3O_2$
24	4-Br	$CH(CH_3)CH_2CH_2$	38	95–97[h]	89 (4)	I (3)	$C_{15}H_{14}BrN_3O_2$
25	4,5-Cl_2	$(CH_2)_3$	38	166–168[i]	100 (4)	I (2)	$C_{14}H_{11}Cl_2N_3O_2$
26	4,5-Cl_2	$(CH_2)_4$	30	108–110[d]	100 (2)	I (2)	$C_{15}H_{13}Cl_2N_3O_2$

[a] Prepared by procedure C unless otherwise noted. [b] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. UK37248 = 98%. [c] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: active; I, inactive. Number of rats (one to four) as shown in parentheses. See ref 12. [d] Recrystallized from EtOAc. [e] Procedure D. [f] HCl salt. [g] Recrystallized from EtOAc/EtOH. [h] Purified by HPLC (EtOAc/silica gel). [i] Recrystallized from EtOH.

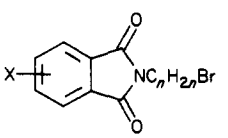
shunting of endoperoxides in the arachadonic acid cascade. The resultant elevated prostacyclin levels might lead to vasodilation with concomitant lowering of blood pressure, and this could present an attractive method of treating all forms of hypertension.

Chemistry

During the course of preparation of I and II, a routine Gabriel synthesis[6] was utilized with the preparation of III



III



IV

(6) Gibson, M. S.; Bradshaw, R. W. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 919.

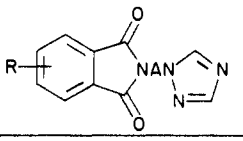
as an intermediate. In our previous report,[2] III (X = H) was subjected to hydrazinolysis and the resulting amine was converted to substituted benzamide derivatives. Interestingly, the intermediates III showed good levels of TX synthetase inhibitory activity. As a consequence of this activity and as part of our continuing development of this type of agent, a structure–activity relationship was undertaken.

The syntheses of these compounds (III) were straightforward and involved the preparation of a series of aryl-substituted *N*-(haloalkyl)phthalimides (IV) that were condensed with various substituted imidazoles and triazoles to produce the desired target compounds. A summary of these compounds is shown in Tables I–III.

Biology. Results and Discussion

For compounds unsubstituted in both the imidazole and aryl portions of the molecule (**1–10**, Table I), inhibition of TX formation in platelets was >90% at 10^{-4} M for alkyl

Table III. 2-[(1*H*-1,2,4-Triazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones



compd	R	A	yield,[a] %	mp, °C	thromboxane formn inhibn[b]	antihyper-tens act.[c]	formula
27	H	$(CH_2)_2$	*d*		0 (2)	A (2)	$C_{12}H_{10}N_4O_2$
28	H	$(CH_2)_3$		119–120[e]	64 (4)	A (2)	$C_{13}H_{12}N_4O_2$
29	H	$(CH_2)_4$	*d*		70 (2)	A (4)	$C_{14}H_{14}N_4O_2$
30	H	$(CH_2)_5$	*d*	*f*	79 (2)	A (2)	$C_{15}H_{16}N_4O_2$·HCl
31	H	$CH(CH_3)CH_2CH_2$	25	101–102[g,h]	47 (4)	I (4)	$C_{14}H_{14}N_4O_2$
32	4-Br	$(CH_2)_3$	51	113–115[i,j]	76 (4)	I (2)	$C_{13}H_{11}BrN_4O_2$
33	4,5-Cl_2	$(CH_2)_3$	48	163–165[i,j]	73 (2)	I (3)	$C_{13}H_{10}Cl_2N_4O_2$

[a] Prepared by procedure A unless otherwise noted. [b] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. UK37248 = 98%. [c] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A, active; I, inactive. Number of rats (one to four) as shown in parentheses. See ref 12. [d] Described in our previous publication.[2] [e] Literature reference reports mp 115–116 °C: Ainsworth, C.; Jones, R. G. *J. Am. Chem. Soc.* **1955**, *77*, 621. [f] Hydrochloride salt. [g] Recrystallized from EtOAc. [h] Procedure B. [i] Recrystallized from EtOH. [j] Procedure C.

Table IV. Thromboxane Synthetase IC_{50} Values for Selected Compounds[a]

compd	IC_{50} (10^{-6} M)	compd	IC_{50} (10^{-6} M)
UK 37,248-01	1.5	**7**	0.1
2	5	**18**	4
3	2	**19**	2
4	6	**22**	1
5	1.5	**23**	0.6
6	0.2	**25**	0.4

[a] IC_{50} determinations by plotting percent inhibition vs. log concentration of the test compound in concentration–response studies and measuring the concentration for 50% inhibition of TX formation from the graph. Each measurement was done in duplicate.

chain separations of n = 3–10. Peak activity of 100% inhibition occurred when n = 6–8. These results parallel those found for I.[2] If the imide moiety of III is considered to be a polar replacement for the carboxylic acid terminus of other reported TX synthetase inhibitors, the maximal chain separation of 6–8 in our compounds is in accord with the reported chain separation of 7 found best in other systems.[3] In this limited study, branching did not seem to lower activity. Substitution on the imidazole moiety (**11–17**, Table I) dramatically lowered TX inhibition, which is again consistent with previous reports of other imidazole-containing TX synthetase inhibitors.[2,3] Triazole derivatives (**27–33**, Table III) were less active in this study and were less active than compounds reported earlier.[2]

Substitution on the aryl moiety (**18–26**), Table II) maintained or enhanced the activity of the compounds such that 100% inhibition of TX formation was achieved for shorter alkyl chain (n = 3, 4) separated derivatives. Clearly 4-methyl, 4-chloro, 4-bromo, and 4,5-dichloro substitution was compatible with TX synthetase inhibitory activity. Concentration–response studies were run on the most interesting derivatives, and the results are listed in Table IV. There were small differences in potency among the best compounds with **3**, **5**, **6**, **7**, **19**, **22**, **23**, and **25** showing potencies equal to or greater than that of dazoxiben (UK 37,248).[7]

The compounds in this study did not inhibit PGI_2 formation in aortic rings. Such results are consistent with selective TX synthetase inhibition and are identical with those that we reported previously for related compounds[2] as well as those found for other imidazole derivatives such as dazoxiben,[3a] OKY-046, OKY-1581, and 1-benzylimidazole.[3b]

Compounds III also showed antihypertensive effects as measured in the spontaneously hypertensive rat (SHR) model. Generally in this assay, alkyl chain separations of n = 2–6 (**1–5**, **8–10**, **19–22**, **27–30**) gave consistent activity. Provided the chain separation was maintained within these limits, chain branching (**8**), chain unsaturation (**9**, **10**), and presence of either imidazolyl (**1–5**, **8–10**) or triazolyl (**27–30**) moieties had little effect on the antihypertensive activity at the screening level. Imidazole ring substitution (**11–17**) eliminated SHR activity reminiscent of the diminution of TX synthetase inhibitory activity described earlier for these same compounds.

Compounds derived in this program with optimal activity in both biological assays appear to be imidazolyl compounds with an alkyl chain separation n = 4 with or without aryl substitution (**3**, **19**, **21**, **22**). Since **3** was among the most interesting of these agents and the most readily available as a result of commercial availability of starting materials, it was chosen for further evaluation in various animal models as described by Chan et al.[8]

Compound **3** not only lowered mean arterial blood pressure (MABP) in SHR at the screening dose of 100 mg/kg po but also was effective at 30 mg/kg po. These two respective doses caused effects of 45 and 40 mmHg lowering of MABP 1 h after dosing. The clinical TX synthetase inhibitor Dazoxiben (UK 37,248) had no significant effects in this assay.

In two-kidney one-clip Goldblatt renal hypertensive dogs, **3** lowered MABP in a dose-dependent fashion by 12, 17, 20, and 31 mmHg at doses of 5, 10, 20, and 30 mg/kg, respectively, in groups of three to five dogs. Duration of action was between 2 and 4 h. No significant increases in heart rate were observed; some emesis occurred and there was some cutaneous vasodilation evident shortly after dosing.

In anesthetized normotensive dogs, **3** did not significantly alter vasopressor or vasodepressor responses to epinephrine, norepinephrine, isoproterenol, 1,1-dimethyl-4-phenylpiperazinium (DMPP, a ganglionic stimulant), tyramine, acetylcholine or angiotensin II.

Although it appeared that compound **3** might cause release of histamine as evidenced by the skin permeability test in mice using intravenous Evans Blue,[9] it was found not to cause histamine release when tested in human ba-

(7) (a) Tyler, H. M.; Saxton, C. A. P. D.; Parry, M. J. *Lancet* **1981**, 629. (b) *Drugs Future* **1982**, *7*, 849.

(8) Chan, P. S.; Cervoni, P.; Ronsberg, M. A.; Accomando, R. C.; Quirk, G. J.; Scully, P. A.; Lipchuck, L. M. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 726.

(9) Ovary, Z. *J. Immunol.* **1958**, *81*, 355.

sophils in vitro by using the method of Lichtenstein.[10] The antihypertensive effects in SHR were not altered by pretreatment with the H_1 and H_2 antagonists chlorpheniramine (10 mg/kg, po) plus cimetidine (20 mg/kg). In two-kidney one-clip Goldblatt renal hypertensive dogs, pretreatment with compound 48/80 (a known histamine-releasing agent)[11] caused only a slight attenuation of the hypotensive effects of **3** while a second dose of 48/80 did not cause hypotensive effects.

To summarize these findings, endogenous histamine release might contribute some small part of the antihypertensive effects of **3**. Although enhanced prostacyclin release, which might result from TX synthetase inhibition, may still play a role as a possible mechanism for lowering blood pressure, other mechanisms may be operating as well in order to achieve the biological profile described herein. Whether the inhibition of TX synthetase activity contributes a major role in the antihypertensive effects observed for **3** (as a representative of this class of compounds) requires further evaluation. In any event, it is certainly the case that the interesting hypotensive effects observed for this family of compounds makes them unique among the ever-increasing number of TX synthetase inhibitors.

Experimental Section

Although there was some variation in the procedures used in the preparation of these compounds, the general procedures described below are representative. Yields and melting points are recorded in the tables. Analyses for C, H, N, S, and halogen were within 0.4% of theoretical values unless otherwise noted in the footnotes, and NMR spectra were obtained on a Varian Associates CFT20 spectrometer for all compounds and were consistent with assigned structures. Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected.

Preparation of 2-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones and 2-[(1*H*-1,2,4-Triazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones. Procedure A. A mixture of 0.2 mol of the appropriate amine and 0.2 mol of 50% NaH (in oil) in 300 mL of DMF was stirred at 30–90 °C for 1–2 h, and 0.19 mol of the *N*-(bromoalkyl)phthalimide was added. The reaction mixture was heated on a steam bath for 8 h and concentrated to remove the DMF. The residue was extracted with hot toluene, and the toluene layer was washed with H_2O, dried over $MgSO_4$, and concentrated. The residue was triturated with Et_2O or EtOAc, and the desired produce was removed by filtration. If precipitation did not occur, the product was further purified by HPLC (EtOAc on silica gel column).

Procedure B. As above, but the reaction mixture after the removal of the DMF was treated with a mixture of CH_2Cl_2 and H_2O, the layers were separated, the organic layer was dried over $MgSO_4$ and concentrated, and the product was obtained by trituration with Et_2O or recrystallization from a suitable solvent.

Procedure C. A mixture of equal molar parts of the phthalic anhydride and the appropriate diamine was mixed in a small, round-bottom flask and immersed in an oil bath, which was held at 140–170 °C for 40–60 min. The reaction product was treated with toluene, and any insoluble material was removed by filtration. The toluene layer was concentrated, and the residue was washed onto a filter with Et_2O or recrystallized from a suitable solvent.

Procedure D. A mixture of equal molar portions of the anhydride and the diamine in CH_2Cl_2 was stirred for 3–20 h, and the acid intermediate was recovered by filtration or by concentration. The carboxylic acid was then placed in a small round-bottom flask, immersed in an oil bath at 140–170 °C for 40–60 min, and worked up as in the preceding procedure.

Thromboxane Synthetase Inhibition and Prostacyclin Synthetase Inhibition. Under urethan anesthesia, 10 mL of arterial blood was collected in 1 mL of 3.2% sodium citrate in a polystyrene tube from Okamoto-Aoki spontaneously hypertensive rats (SHR) (Taconic Farms, Germantown, NY) between 19 and 24 weeks of age. The blood was diluted with 3 mL of cold saline and centrifuged at room temperature for 15 min at 460*g*. The platelet-rich plasma (PRP) was separated. The platelets were isolated by centrifuging the PRP at 4 °C for 10 min at 1060*g* and were washed in 4 mL of cold oxygenated Krebs phosphate buffer, pH 7.4. The chilled platelets recovered from centrifuging at 800*g* for 10 min were resuspended in oxygenated Krebs phosphate buffer and diluted to contain (4.5–6.0) × 10^4 platelts/μL. Platelets prepared by this procedure did not aggregate.

The inhibition of thromboxane (TX) formation was studied by determining the concentration of thromboxane B_2(TXB_2), the stable hydrolysis product of TXA_2. Assay samples, prepared on ice, contained 200 μL of platelet suspension, 50 μL of saline, and 50 μL of vehicle or drug under study. The samples were incubated for 10 min at 37 °C in a metabolic shaker. The reaction was terminated by immersing the tubes in an ice bath and adding 50 μL of 0.5 M citric acid. The samples were centrifuged for 10 min in a refrigerated centrifuge, and the supernatants thus obtained were decanted and stored at −20 °C. Controls wherein platelets, vehicle, and incubation buffer were inactivated in boiling water for 3 min prior to 37 °C incubation were run in parallel. The TXB_2 content for each sample was determined by a direct radioimmunoassay (RIA) utilizing a TXB_2 specific RIA kit purchased from New England Nuclear, Boston, MA, and instructions contained therein and expressed as pg of TXB_2 formed min^{-1} $sample^{-1}$, from which the percent inhibition of TXB_2 formation was calculated. The small amount of TXB_2 measured in the controls was considered released before incubation and was substracted from the test samples before this calculation. The results of this test are summarized in Tables I–IV.

The inhibition of PGI_2 was similar determined on guinea pig aortic ring preparations using [$^3H_6$]-keto-$PGF_{1\alpha}$ (the stable hydrolysis product of PGI_2) levels as measured with a RIA method obtained from New England Nuclear. None of the test compounds altered levels from control values.

Antihypertensive Activity in Spontaneously Hypertensive Rats (SHR). The test compounds were tested for antihypertensive activity by the published methods.[12] Male, 16-week-old, spontaneously hypertensive rats of the Okamoto strain, from Taconic Farms, Germantown, NY, having an average mean arterial blood pressure of 170 ± 1.5 mmHg are used in the test. One to three rats are used per test compound. A rat is dosed by gavage with a test compound, suspended in 2% preboiled starch at a concentration of 50 mg/mL, at a dose of 100 mg/kg of body weight or less, with 0.9% sodium chloride loading at a dose of 25 mL/kg of body weight. A second identical dose of the test compound, without sodium chloride loading, is given 24 h later. At 28 h after the initial dose, the mean arterial blood pressure is measured by the method of Chan and Poorvin vide supra.[12] The procedure is repeated in a second and third rat when activity is determined. Compounds are considered active when blood pressure in one test SHR has been reduced to ≤116 mmHg or when the average of two test SHR has been reduced to ≤122 mmHg.

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