NaOH solution to afford 3.2 g (99%) of 5-amino-2-(ethylamino)nicotinonitrile, which was used directly in the next step. An analytical sample, mp 94–96 °C, was obtained by recrystallization from heptane (Table I).

5-(Acetylamino)-2-(ethylamino)nicotinonitrile (13). To a solution of 1.6 g (0.01 mol) of 5-amino-2-(ethylamino)-nicotinonitrile in 75 mL of anhydrous EtOH was added 0.78 g (0.01 mol) of acetyl chloride. The mixture was stirred at room temperature for 30 min and filtered. The precipitate was triturated with 100 mL of 15% Na_2CO_3 solution. The product amounted to 1.0 g (49%) after recrystallization from EtOH, mp 196–198 °C (Table I).

Acknowledgment. We are indebted to Marie Politwoski and staff for the combustion analyses and Anna Sandor and George Palumbo for the biological assays. We also thank M. E. Fiala for preparing the manuscript.

Registry No. 1, 24517-64-4; 2, 52583-87-6; 3, 52583-89-8; 4, 74611-49-7; 5, 77276-32-5; 6, 77276-34-7; 7, 77276-35-8; 8, 77276-41-6; 9, 110457-38-0; 10, 5468-34-8; 11, 51560-67-9; 12, 110457-39-1; 13, 110457-40-4; 13A, 110457-41-5; 14, 31686-93-8; 15, 110457-42-6; 16, 76336-07-7; 17, 76335-99-4; 18, 76335-93-8; 19, 78997-37-2; 20, 76335-91-6; 21, 69407-71-2; 22, 76336-05-5; 23, 77276-17-6; 24, 69407-72-3; 25, 76336-15-7; 26, 76336-09-9; 27, 76336-11-3; 28, 77276-37-0; 29, 77276-63-2; 30, 77276-18-7; 31, 77276-20-1; 32, 110457-43-7; 33, 77276-16-5; 34, 77276-24-5; 35, 77276-25-6; 36, 77276-31-4; 37, 77276-36-9; 38, 77276-33-6; 39, 77289-97-5; 40, 77289-98-6; 41, 77276-28-9; 42, 77289-99-7; 43, 110457-45-9; 44, 110457-46-0; 45, 110457-47-1; 46, 110457-48-2; 47, 110457-49-3; 48, 77276-27-8; 49, 77276-42-7; 50, 110457-50-6; 51, 110457-51-7; 52, 110457-52-8; 53, 110457-53-9; 54, 110457-54-0; 55, 77276-45-0; 56, 77276-44-9; 57, 77276-40-5; 58, 110457-55-1; 59, 77276-29-0; 60, 110457-56-2; 61, 77276-39-2; 62, 110457-57-3; 63, 77276-53-0; 64, 82360-79-0; 65, 110457-58-4; 66, 82360-72-3; 67, 77276-19-8; 68, 82360-74-5; 69, 77276-61-0; 70, 110457-59-5; 71, 77276-47-2; 72, 110457-60-8; 73, 77276-21-2; 74, 82360-73-4; 75, 77276-46-1; 76, 77276-48-3; 77, 77276-49-4; 78, 77276-54-1; 79, 77276-56-3; 80, 110457-61-9; 81, 77276-38-1; 82, 76336-12-4; 83, 76336-08-8; di-*n*-butyl malonate, 1190-39-2; bis[2-(diethylamino)ethyl malonate, 92862-11-8; di-*sec*-butyl malonate, 32260-07-4; methyl 2-chloro-6-methylnicotinate, 53277-47-7; methyl 2-chloronicotinate, 40134-18-7; 2-chloro-6-methylnicotinonitrile, 28900-10-9; 2-chloronicotinonitrile, 6602-54-6; 2-chloro-4,6-dimethylnicotinonitrile, 14237-71-9; 2-(ethylamino)-5-nitronicotinonitrile, 31309-09-8; ethyl malonyl chloride, 36239-09-5; diethyl malonate, 105-53-3; *N*-methylpiperazine, 109-01-3; 1-ethyl-1,2-dihydro-4-hydroxy-7-melthyl-2-oxo-1,8-naphthyridine-3-carboxamide, 76335-95-0; 2-chloro-6-(ethylamine)-4-methylnicotinonitrile, 51561-60-5.

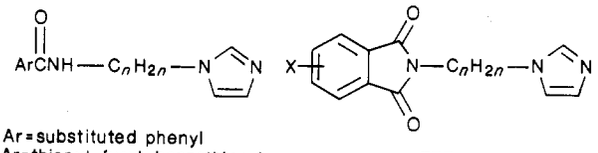
Thromboxane Synthetase Inhibitors and Antihypertensive Agents. 4. *N*-[(1*H*-Imidazol-1-yl)alkyl] Derivatives of Quinazoline-2,4(1*H*,3*H*)-diones, Quinazolin-4(3*H*)-ones, and 1,2,3-Benzotriazin-4(3*H*)-ones

William B. Wright, Jr.,* Andrew S. Tomcufcik, Peter S. Chan, Joseph W. Marsico, and Jeffery B. Press*[1]

CNS–Cardiovascular Disease Research Section, Medical Research Division of American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965. *Received May 6, 1987*

The quinazolinedione, quinazolinone, and 1,2,3-benzotriazinone title compounds were prepared as analogues of *N*-[(1*H*-imidazol-1-yl)alkyl]-1*H*-isoindole-1,3(2*H*)-diones which were the subject of a previous report from our laboratories. These compounds were evaluated as thromboxane (TX) synthetase inhibitors and as antihypertensive agents. While each series of compounds had activity both as TX synthetase inhibitors and as antihypertensives, the best compounds were *N*-[(1*H*-imidazol-1-yl)alkyl]quinazoline-2,4(1*H*,3*H*]-diones (V). In general these compounds were all selective enzyme inhibitors at least equipotent with the standard dazoxiben. These compounds were also very active antihypertensive agents as determined in SHR. The SAR is discussed for both types of activity. Compound **20a** was further evaluated for TX formation inhibiting properties in several other platelet types both in vitro and ex vivo and is between 100 and 1000 times more potent than dazoxiben.

Our laboratory has continued to pursue the goal of developing novel therapeutic agents that might be useful in the treatment of cardiovascular disorders. An ongoing series of *N*-[(1*H*-imidazol-1-yl)alkyl] and *N*-[(1*H*-1,2,4-triazol-1-yl)alkyl] derivatives of aryl amides I,[2] heteroaryl amides II,[3] and isoindole-1,3(2*H*)-diones III[4] was found to have interesting levels of thromboxane (TX) synthetase inhibiting activity as well as antihypertensive effects.



I: Ar = substituted phenyl
II: Ar = thienyl, furyl, benzothienyl benzofuryl, pyridyl, indolyl

III

Although TX synthetase inhibitors may well find clinical utility in the treatment of ischemia, arrhythmias, and sudden cardiac death,[5] we were particularly intrigued by the possibility that an agent that was a selective TX synthetase inhibitor might in fact produce antihypertensive effects by reducing levels of the potent vasoconstrictor TX and concomitantly raising the levels of the endogenous vasodilator prostacyclin (PGI_2) by the shunting of endoperoxides in the arachidonic acid cascade. While this attractive proposal seemed to have some merit initially for I,[2] it did not seem to generalize for the heterocyclic analogues II.[3] Isoindole-1,3(2*H*)-diones III,[4] on the other hand, did seem to have a unique biological profile with the most

(1) Current address: Ortho Pharmaceutical Corporation, 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.* **1986**, *29*, 523.

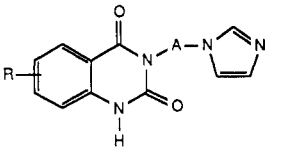
(3) Press, J. B.; Wright, W. B., Jr.; Chan, P. S.; Haug, M. F.; Marsico, J. W.; Tomcufcik, A. S. *J. Med. Chem.* **1987**, *30*, 1036.

(4) Press, J. B.; Wright, W. B., Jr.; Chan, P. S.; Marsico, J. W.; Haug, M. F.; Tauber, J.; Tomcufcik, A. S. *J. Med. Chem.* **1986**, *29*, 816.

(5) For a review of the biology of thromboxane synthetase inhibitors and their possible role in cardiovascular diseases, see: (a) Gorman, R. R. *Adv. Prostaglandin, Thromboxane, Leukotriene Res.* **1983**, *11*, 235; (b) Chan, P. S.; Cervoni, P. *Drug Dev. Res.* **1986**, *7*, 341.

0022-2623/87/1830-2277$01.50/0

Table I. *N*-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-quinazoline-2,4(1*H*,3*H*)-diones



compd	R	A	yield, %	mp, °C	antihypertens act.[a]	thromboxane formn inhibn:[b] IC_{50}, 10^{-7} M	formula
dazoxiben					I (2)	15	
1	H	$(CH_2)_3$	74[c]	197–200[e]	I (3)	6.0 (2)	$C_{14}H_{14}N_4O_2$
2	H	$(CH_2)_4$	66[c]	158–160[f]	A (2)		$C_{15}H_{16}N_4O_2$
3	H	$(CH_2)_5$	50[c]	160–162[e]	A (2)	3.5 (2)	$C_{16}H_{18}N_4O_2$
4	H	$(CH_2)_6$	62[d]	133–135[f]	A (2)	0.4 (2)	$C_{17}H_{20}N_4O_2$
5	H	$CH_2CH(CH_3)CH_2$	69[d]	206–208[g]	A (2)	0.3 (2)	$C_{15}H_{16}N_4O_2 \cdot H_2O$
6	H	$CH_2CH_2CH(CH_3)$	63[d]	130–133[g]	A (3)	7.0 (2)	$C_{15}H_{16}N_4O_2 \cdot H_2O$
7	6-Cl	$(CH_2)_3$	45[c]	239–242[e]	I (2)		$C_{14}H_{13}ClN_4O_2$
8[h]	6-Cl	$(CH_2)_4$	76[d]	214–216[e]	A (2)	0.02 (2)	$C_{15}H_{15}ClN_4O_2$
9	6-Cl	$(CH_2)_5$	23[d]	170–172[e]	A (2)	0.12 (2)	$C_{16}H_{17}ClN_4O_2$
10	6-Cl	$(CH_2)_6$	56[d]	200–203[e]	A (2)	0.04 (2)	$C_{17}H_{19}ClN_4O_2$
11	6-Cl	$(CH_2)_7$	69[d]	153–155	A (3)	0.02 (2)	$C_{18}H_{21}ClN_4O_2$
12	6-Cl	$(CH_2)_8$	67[d]	132–134[f,i]	I (2)		$C_{19}H_{23}ClN_4O_2$
13	6-Cl	$(CH_2)_{10}$	40[d]	109–113[f]	I (3)	0.07 (2)	$C_{21}H_{27}ClN_4O_2$
14[j]	6-Cl	$CH_2CH(CH_3)CH_2$	57[c]	214–216	I (3)	0.08 (2)	$C_{15}H_{15}ClN_4O_2$
15	6-Cl	$CH_2CH_2CH(CH_3)$	50[d]	193–195[e]	A (2)	1.5 (2)	$C_{15}H_{15}ClN_4O_2$
16	7-Cl	$(CH_2)_3$	69[d]	225–227[e]	I (2)	1.8 (2)	$C_{14}H_{13}ClN_4O_2$
17	7-Cl	$(CH_2)_4$	34[d]	196–198[e]	A (2)	0.09 (2)	$C_{15}H_{15}ClN_4O_2$
18	7-Cl	$(CH_2)_5$	68[c]	183–185[e]	A (2)	8.2 (2)	$C_{16}H_{17}ClN_4O_2$
19	7-Cl	$(CH_2)_6$	65[d]	186–188[e]	A (2)	2.0 (2)	$C_{17}H_{19}ClN_4O_2$
20[k]	7-Cl	$CH_2CH(CH_3)CH_2$	42[c]	200–203[e]	A (2)	0.03 (2)	$C_{15}H_{15}ClN_4O_2$
21	7-Cl	$CH_2CH_2CH(CH_3)$	43[d]	243–245[e]	I (2)		$C_{15}H_{15}ClN_4O_2$
22	8-Cl	$(CH_2)_4$	49[d]	155–157[e]	A (2)	0.3 (4)	$C_{15}H_{15}ClN_4O_2$
23	8-Cl	$(CH_2)_5$	38[d]	154–156[f]	A (2)	0.4 (2)	$C_{16}H_{17}ClN_4O_2$
24	8-Cl	$(CH_2)_6$	65[d]	155–157[e]	A (3)		$C_{17}H_{19}ClN_4O_2$
25	8-Cl	$CH_2CH(CH_3)CH_2$	35[d]	163–165[f]	I (3)	0.1 (2)	$C_{15}H_{15}ClN_4O_2$
26	8-Cl	$CH_2CH_2CH(CH_3)$	64[d]	219–221[e]	A (2)	10 (2)	$C_{15}H_{15}ClN_4O_2$
27	6-Br	$(CH_2)_3$	74[c]	257–260	I (3)	1.0 (2)	$C_{14}H_{13}BrN_4O_2$
28	6-Br	$(CH_2)_4$	87[c]	227–229[e]	A (2)	8.0 (2)	$C_{15}H_{15}BrN_4O_2$
29	6-CH_3	$(CH_2)_3$	32[d]	218–220[e]	I (2)	0.2 (2)	$C_{15}H_{16}N_4O_2$
30	6-CH_3	$(CH_2)_4$	70[c]	189–191[e]	A (3)	0.5 (2)	$C_{16}H_{18}N_4O_2$
31	6-CH_3	$(CH_2)_5$	77[d]	174–176[e]	A (2)	0.3 (2)	$C_{17}H_{20}N_4O_2$
32	6-CH_3	$CH_2CH(CH_3)CH_2$	58[d]	202–205[e]	I (2)	0.03 (4)	$C_{16}H_{18}N_4O_2 \cdot H_2O$
33	6-CH_3	$CH_2CH_2CH(CH_3)$	77[c]	195–196[e]	A (2)	0.7 (2)	$C_{16}H_{18}N_4O_2$
34	8-CF_3	$(CH_2)_4$	36[d]	145–147	A (2)	0.5 (2)[l]	$C_{16}H_{15}F_3N_4O_2$
35	8-CF_3	$(CH_2)_5$	68[d]	157–159	A (2)	0.1 (2)[m]	$C_{17}H_{17}F_3N_4O_2$
36	8-CF_3	$(CH_2)_6$	70[d]	149–151[f]	A (2)		$C_{18}H_{19}F_3N_4O_2$
37	8-CF_3	$CH_2CH(CH_3)CH_2$	60[d]	135–137	A (2)	4.0 (2)	$C_{16}H_{15}F_3N_4O_2$

[a] Spontaneously hypertensive rats at 100 mg/kg, po, for 2 days. A = active; I = inactive. Number of rats is in parentheses. [b] Inhibition of thromboxane formation. IC_{50} determinations are carried out by plotting the 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. Numbers in parentheses represent the number of replications. [c] Procedure A. [d] Procedure B. [e] Recrystallized from EtOH. [f] Recrystallized from EtOAc. [g] Monohydrate. [h] HCl salt **8a** has mp 263–265 °C and IC_{50} = 0.07 (2). [i] N: calcd, 14.95; found, 14.47. [j] HCl salt **14a** has mp 305–308 °C and IC_{50} = 0.1 (6). [k] HCl salt **20a** has mp 287–290 °C and IC_{50} = 0.1 (6). [l] HCl salt **34a**, mp 260–263 °C. [m] HCl salt **35a**, mp 188–190 °C.

interesting compounds in the series (for example, III, n = 4) having TX synthetase inhibiting activity with potency similar to that of the standard dazoxiben[6] and also antihypertensive effects in spontaneously hypertensive rats (SHR) at 30 mg/kg, po. To expand upon this dual activity, we began a synthesis program to prepare analogues of III wherein other heterocycles replace the isoindole moiety.

Chemistry

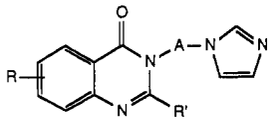
The imidazole derivatives that were the targets of this study were prepared as outlined in Scheme I. The *N*-(1*H*-imidazol-1-yl)alkanamines were obtained by imidazole condensation with the appropriate (bromoalkyl)phthalimide and subsequent hydrazinolysis as reported previously.[2-4] These amines were reacted either with an isatoic anhydride or with an appropriately substituted anthranilic acid that had been activated with carbonyldiimidazole to give IV. These intermediates formed in good yield, frequently crystallized, and could be used without further purification in the next steps. Conversion to the target compounds V–VII was accomplished by using standard chemical transformations. Thus, treatment of IV with ethyl chloroformate at 85–95 °C for 2 h and subsequent treatment of the reaction mixture in ethanolic potassium hydroxide with heating gave V in good yield. Heating IV in selected ortho esters produced the quinazolinones VI. Lastly, treating IV with sodium nitrite in hydrochloric acid solution gave the benzotriazinones VII, also in good yield. Tables I–III list the derivatives prepared in this study, and Table IV lists the intermediates.

Biology: Results and Discussion

Compounds V–VII were tested for TX synthetase inhibition by using platelets obtained from SHR and procedures described in previous papers.[2-4] As in these prior reports, all of the compounds were evaluated for selectivity by measuring effects on PGI_2 formation in aortic strips. None of the test compounds nor the standard dazoxiben

(6) Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *J. Med. Chem.* **1985**, *28*, 1427.

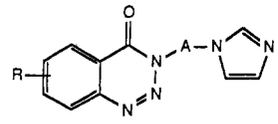
Table II. *N*-[(1*H*-Imidazol-1-yl)alkyl]quinazolin-4(3*H*)-ones



compd	R	A	yield, %	mp, °C	antihypertens act.[a]	thromboxane formn inhibn:[b] IC_{50}, 10^{-7} M	formula
dazoxiben					I (2)	15	
38	H	$(CH_2)_3$	35[c]	240–245[d,e,f]	I (2)		$C_{14}H_{14}N_4O{\cdot}2HCl{\cdot}^1/_2H_2O$
39	H	$(CH_2)_4$	65[c]	234–243[d,f]	A (3)		$C_{15}H_{16}N_4O{\cdot}2HCl{\cdot}^1/_2H_2O$
40	H	$(CH_2)_5$	52[g]	222–230[d,f]	A (2)		$C_{16}H_{18}N_4O{\cdot}2HCl$
41	H	$(CH_2)_6$	*g*	163–169[d,f,h]	A (2)		$C_{17}H_{20}N_4O{\cdot}2HCl{\cdot}H_2O$
42	H	$CH_2CH(CH_3)CH_2$	38[i]	245–248[d,f]	A (2)		$C_{15}H_{16}N_4O{\cdot}2HCl$
43	6-Cl	$(CH_2)_4$	61[c]	240–245[d,f]	A (2)	5.0 (2)	$C_{15}H_{15}ClN_4O{\cdot}2HCl$
44	6-Cl	$(CH_2)_5$	69[g]	111–113[g]	I (2)		$C_{16}H_{17}ClN_4O$
45	6-Cl	$(CH_2)_6$	35[g]	205–208[d,f]	A (5)		$C_{17}H_{19}ClN_4O{\cdot}2HCl$
46	6-Cl	$(CH_2)_8$	17[i]	93–96[j,k]	A (2)	0.4 (2)	$C_{19}H_{23}ClN_4O$
47	6-Cl	$CH_2CH(CH_3)CH_2$	21[i]	148–150[j]	I (2)	0.9 (2)	$C_{15}H_{15}ClN_4O$
48	6-Cl	$CH_2CH_2CH(CH_3)$	30[i]	133–135[j]	A (2)	80 (2)	$C_{15}H_{15}ClN_4O$
49	7-Cl	$(CH_2)_4$	10[i]	123–125[j]	I (2)		$C_{15}H_{15}ClN_4O$
50	6-CH_3	$(CH_2)_4$	40[c]	252–255[f,h]	A (3)	3.5 (2)	$C_{16}H_{18}N_4O{\cdot}2HCl{\cdot}H_2O$
51	6-Cl-2-CH_3	$(CH_2)_4$	85[c]	220–225[f,h]	A (2)		$C_{16}H_{17}ClN_4O{\cdot}2HCl{\cdot}H_2O$
52	6-Cl-2-C_2H_5	$(CH_2)_4$	95[c]	218–222[f]	A (2)		$C_{17}H_{19}ClN_4O{\cdot}2HCl$

[a] Spontaneously hypertensive rats at 100 mg/kg, po, for 2 days. A = active; I = inactive. Number of rats is in parentheses. [b] Inhibition of thromboxane formation. IC_{50} determinations are carried out by plotting the 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. Numbers in parentheses represent the number of replications. [c] Procedure C. [d] Recrystallized from EtOH. [e] Hemihydrate. [f] Dihydrochloride salt. [g] Procedure D. [h] Monohydrate. [i] Procedure E. [j] Recrystallized from EtOAc. [k] Cl: calcd, 9.89; found, 8.98.

Table III. *N*-[(1*H*-Imidazol-1-yl)alkyl]-1,2,3-benzotriazin-4(3*H*)-ones



compd	R	A	yield, %	mp, °C	antihypertens act.[a]	thromboxane formn inhibn:[b] IC_{50}, 10^{-7} M	formula
dazoxiben					I (2)	15	
53	H	$(CH_2)_3$	64[c]	82–84	A (2)	15 (2)	$C_{13}H_{13}N_5O$
54	H	$(CH_2)_4$	56[c]	96–98	A (2)		$C_{14}H_{15}N_5O$
55	H	$(CH_2)_5$	54[d]	188–190[e,f]	I (2)		$C_{15}H_{17}N_5O{\cdot}HCl$
56	H	$(CH_2)_6$	59[d]	154–156[e]	A (2)	0.5 (2)	$C_{16}H_{19}N_5O{\cdot}HCl$
57	6-Cl	$(CH_2)_3$	76[c]	65–70[g]	I (3)		$C_{13}H_{12}ClN_5O{\cdot}^1/_2H_2O$
58	6-Cl	$(CH_2)_4$	47[d]	121–123[h]	A (2)	2.4 (2)	$C_{14}H_{14}ClN_5O$
59	6-Cl	$(CH_2)_5$	37[d]	80–82[h]	A (2)		$C_{15}H_{16}ClN_5O$
60	6-Cl	$(CH_2)_6$	36[d]	99–101[h]	A (2)		$C_{16}H_{18}ClN_5O$
61	6-Cl	$(CH_2)_8$	66[d]	140–142[e]	A (2)		$C_{18}H_{22}ClN_5O{\cdot}HCl$
62	6-Cl	$CH_2CH(CH_3)CH_2$	61[c]	185–188[e]	I (2)	9.0 (2)	$C_{14}H_{14}ClN_5O{\cdot}HCl$
63	7-Cl	$(CH_2)_4$	41[c]	123–125	A (3)		$C_{14}H_{14}ClN_5O$
64	7-Cl	$(CH_2)_5$	85[c]	206–208[e]	A (2)		$C_{15}H_{16}ClN_5O{\cdot}HCl$
65	6-Br	$(CH_2)_4$	59[c]	203–205[e]	A (2)		$C_{14}H_{14}BrN_5O{\cdot}HCl$
66	6-Br	$CH_2CH(CH_3)CH_2$	71[c]	120–122[i]	I (4)	17 (2)	$C_{14}H_{14}BrN_5O$
67	6-CH_3	$(CH_2)_4$	80[c]	157–159[e,g]	A (2)	0.6 (2)	$C_{15}H_{17}N_5O{\cdot}HCl{\cdot}^1/_2H_2O$
68	6-CH_3	$(CH_2)_5$	75[d]	176–178[e]	A (2)		$C_{16}H_{18}N_5O{\cdot}HCl$

[a] Spontaneously hypertensive rats at 100 mg/kg, po, for 2 days. A = active; I = inactive. Number of rats is in parentheses. [b] Inhibition of thromboxane formation. IC_{50} determinations are carried out by plotting the 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. Numbers in parentheses represent the number of replications. [c] Procedure F. [d] Procedure G. [e] Hydrochloride. [f] Monohydrate. [g] Hemihydrate. [h] Purified by HPLC (EtOAc/silica gel). [i] Recrystallized from EtOAc.

inhibited PGI_2 formation. The antihypertensive effects of V–VII were measured in SHR.[7] The summary of the biological results is listed in Tables I–IV.

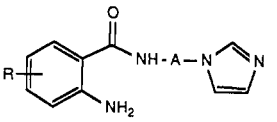
N-[(1*H*-Imidazol-1-yl)alkyl]-1*H*-quinazoline-2,4-(1*H*,3*H*)-diones (V, Table I) were examined most thoroughly for structural variations, and general SAR conclusions are derived from this series. In consideration of the TX synthetase activity, the SAR is consistent with that found in related series.[2-4] In general, these derivatives are all excellent selective enzyme inhibitors at least equipotent with the standard dazoxiben (UK 37248-01).[6]

Variation of chain length separation (A) between the imidazole and heterocycle had much less effect than in our previous series, but generally the shorter chain (A = *n*-propyl; **1**, **16**, **27**) derivatives were less potent than longer chain counterparts such as A = *n*-butyl (**8**, **17**, **22**, **34**), *n*-pentyl (**9**, **23**, **35**), and *n*-hexyl (**4**, **10**, **19**). Chain branching enhances potency for isobutyl separations (**5**, **14**, **20**, **25**, **32**) but slightly reduces potency for *sec*-butyl separations (**6**, **15**, **26**, **33**).

Substitution on the quinazolinedione portion of the molecule effected greater potency. As noted previously,[3,4] the electron-withdrawing substituents studied (6-Cl, **7–15**;

(7) Chan, P. S.; Poorvin, D. *Clin. Exp. Hypertens.* **1979**, *1*, 817.

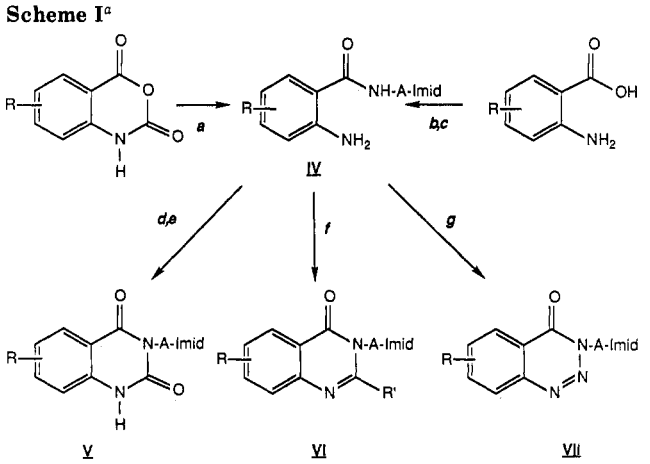
Table IV. 2-Aminobenzamides



compd	R	A	yield, %	mp, °C	antihypertens act.[a]	thromboxane formn inhibn:[b] IC_{50}, 10^{-7} M	formula
dazoxiben					I (2)	15	
69	H	$(CH_2)_3$	60[e]	118–120	I (2)		$C_{13}H_{16}N_4O$
70	H	$(CH_2)_4$	80[c]	91–93[d]	I (4)		$C_{14}H_{18}N_4O$
71	H	$CH_2CH(CH_3)CH_2$	57[e]	116–119	I (4)	25	$C_{14}H_{18}N_4O$
72	3-Cl	$(CH_2)_5$	81[e]	97–99[d]	I (3)		$C_{15}H_{19}ClN_4O$
73	4-Cl	$(CH_2)_4$	75[e]	103–105[g]	A (2)		$C_{14}H_{17}ClN_4O\cdot H_2O$
74	4-Cl	$(CH_2)_5$	75[e]	92–94[d]	I (3)		$C_{15}H_{19}ClN_4O$
75	4-Cl	$CH_2CH(CH_3)CH_2$	62[e]	124–126[d]	A (2)		$C_{14}H_{17}ClN_4O$
76	5-Cl	$(CH_2)_3$	54[c]	155–157[d]	A (1)		$C_{13}H_{15}ClN_4O$
77	5-Cl	$(CH_2)_4$	75[f]	98–101[d]	I (4)		$C_{14}H_{17}ClN_4O$
78	5-Cl	$(CH_2)_5$	55[e]	200–203[h]	A (5)		$C_{15}H_{19}ClN_4O\cdot 2HCl$
79	5-Cl	$(CH_2)_6$	91[e]	166–169[g,h]	A (3)		$C_{16}H_{21}ClN_4O\cdot 2HCl\cdot H_2O$
80	5-Cl	$(CH_2)_7$	50[e]	95[g,h,i]	I (4)		$C_{17}H_{23}ClN_4O\cdot 2HCl\cdot H_2O$
81	5-Cl	$(CH_2)_8$	34[e]	165–167[h]	A (2)		$C_{18}H_{25}ClN_4O\cdot 2HCl$
82	5-Cl	$CH_2CH(CH_3)CH_2$	70[f]	156–158	A (2)	0.9	$C_{14}H_{17}ClN_4O$
83	5-Cl	$CH_2CH_2CH(CH_3)$	60[e]	108–110[d]	A (2)	5	$C_{14}H_{17}ClN_4O$
84	5-Br	$(CH_2)_4$	66[c]	113–115[d]	A (2)		$C_{14}H_{17}BrN_4O$
85	5-Br	$CH_2CH(CH_3)CH_2$	67[f]	158–161	I (4)	2.9	$C_{14}H_{17}BrN_4O$
86	5-CH_3	$(CH_2)_4$	55[c]	82–84[d]	I (3)	3.4	$C_{15}H_{20}N_4O$
87	5-CH_3	$CH_2CH(CH_3)CH_2$	58[e]	120–122[j]	I (2)	13	$C_{15}H_{20}N_4O\cdot{}^1/_2H_2O$
88	5-CH_3	$CH_2CH_2CH(CH_3)$	50[c]	137–143[d]	A (1)	1.7	$C_{15}H_{20}N_4O$

[a] Spontaneously hypertensive rats at 100 mg/kg, po, for 2 days. A = active; I = inactive. Number of rats is in parentheses. [b] Inhibition of thromboxane formation. IC_{50} determinations are carried out by plotting the 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. Numbers in parentheses represent the number of replications. [c] Procedure H. [d] Recrystallized from EtOAc. [e] Procedure I. [f] Procedure J. [g] Monohydrate. [h] Dihydrochloride. [i] Recrystallized from EtOH. [j] Hemihydrate.

Scheme I[a]



[a] (a) Imidazolylalkanamine/THF or DMSO, procedure H or procedure I; (b) CDI/THF, procedure J; (c) imidazolylalkanamine, procedure J; (d) EtOCOCl, 85–95°, 2 h, procedure A; (e) KOH/EtOH, heat, procedure A; (f) $(EtO)_3CR'$, procedure C; (g) $NaNO_2$, HCl, procedure F.

7-Cl, **16–21**; 8-Cl, **22–26**; 6-Br, **27**, **28**; 8-CF_3, **34–37**) improved potency, but, in contrast to the isoindole derivatives reported earlier,[4] methyl substitution (6-CH_3, **29–33**) also imparted improved potency. Overall, the 6-chloro derivatives were the most interesting derivatives of the study; for example, the C_4 derivative **8** had greater potency than the corresponding 7-Cl (**17**), 8-Cl (**22**), 6-Br (**28**), 8-CH_3 (**30**), and 8-CF_3 (**34**) compounds.

The best TX synthetase inhibitors in the quinazolinedione series were the 6-chloro derivatives with butyl, hexyl, heptyl, decyl, and isobutyl chain separation (**8**, **10**, **11**, **13**, and **14**), 7-chloro derivatives with butyl and isobutyl chain separation (**17** and **20**), and 6-methyl derivative with isobutyl chain separation (**32**).

Altering the quinazolinedione moiety to the quinazolinone series VI or the benzotriazinone series VII caused a reduction in potency of enzyme inhibition (Tables II, III). Of the compounds in each series in which we measured IC_{50} values, none matched the overall high potency of the quinazolinediones. For example, comparison of the 6-chloro derivatives with butyl chain separation (**8**, **43**, and **58**), the 6-chloro derivatives with isobutyl chain separation (**14**, **47**, and **62**), and the 6-methyl derivatives with butyl chain separation (**30**, **50**, and **67**) demonstrates this effect of heterocycle replacement. Another comparison showing this decreased potency between corresponding V and VI is **15** vs **48**.

The intermediates IV had some TX synthetase inhibiting properties as shown in Table IV. This activity is consistent with that reported earlier for other benzamide derivatives.[2]

The antihypertensive effects of these compounds, on the other hand, have little or no correlation with the heterocyclic lactam portion of the molecule. Very active compounds were found in each series: V, **3**, **11**, **17**, **22**, **23**, **31**, **34**; VI, **41**, **43**, **48**, **50**, **51**; VII, **56**, **58**, **64**, **67**. In all of the series, the *n*-propyl chain separated derivatives were only weakly active: **1**, **7**, **16**, **27**, **29**, **38**, **53**, **57**. This is similar to the SAR noted above for TX synthetase inhibition and is in contrast to the isoindoles described previously[4] wherein ethyl and propyl derivatives were among the derivatives with the best antihypertensive activity.

Peak antihypertensive potency was found for compounds containing butyl to hexyl chain separations as was also found in the enzyme inhibition SAR. Compounds in this group include **2–4**, **8–10**, **17–19**, **22–24**, **28**, **30**, **31**, **34–36**, **39–41**, **43–45**, **49–52**, **54–56**, **58–60**, **63–65**, **67**, and **68**. While branching of the alkyl chain reduced activity somewhat, the *sec*-butyl series was better than the isobutyl series (see especially **25**,**26**, **32**,**33**, and **47**,**48**). This result is a reversal of the trend noted above for TX enzyme inhibition.

Table V. In Vitro Inhibition of Thromboxane Formation in Human Platelets

compound	human platelet IC_{50},[a] M
20a	$2.12 \times 10^{-8} \pm 0.70$ ($n = 4$)
dazoxiben	$5.03 \times 10^{-6} \pm 1.28$ ($n = 4$)

[a] Inhibition of thromboxane formation in platelets drawn from human volunteers. IC_{50} values were determined as described in footnote *b* of Tables I–III. Numbers in parentheses represent the number of replications.

Substitution on the heterocyclic lactam rings generally maintained or enhanced antihypertensive activity regardless of substituent position. No overall trends may be determined. The best antihypertensive compounds in this study (**22**, **23**, **31**, **34**, and **56**) were incredibly active compounds with excellent blood pressure lowering properties in the SHR.

We examined the data to find compounds interesting with respect to our initial objective of discovering a TX synthetase inhibitor with potential as an antihypertensive agent, and several quinazolinediones appear to have sufficient activity in both screens for consideration for further studies. Compounds **8**, **10**, **11**, **17**, and **20** were all among the best enzyme inhibitors and have good activity in the SHR.

Compound **20a**[8] was further examined for additional TX synthetase inhibitory effects (Tables V, VI). We have routinely used platelets drawn from SHR for our initial TX measurements since we were interested in the best correlation between TX formation inhibition and antihypertensive effects in SHR.[2] To further study **20a**, the effects on human platelets were investigated. Blood samples were obtained from four healthy human donors, and the platelets were isolated by using a procedure modified slightly from that reported.[9] The IC_{50} of **20a** was almost 100 times greater than that of dazoxiben and again demonstrates the clear superiority of **20a** (Table V). The compound was also evaluated for effects ex vivo in rabbits (Table VI). Effects analogous to the in vitro results in SHR and human platelets generalized to the ex vivo rabbit platelet; **20a** once again exhibited significantly greater activity at 0.1 mg/kg as compared to dazoxiben at 0.3 mg/kg.

Further evaluation of the antihypertensive effects of compound **20a** as well as other highly active compounds in this series is beyond the scope of this paper. It is clear that these agents have useful antihypertensive properties from the activity reported herein. A complete report of antihypertensive effects and mechanistic studies is the subject of a future report from these laboratories and will be reported in due course.

In summary, compound **20a** represents one of the best TX synthetase inhibitors discovered in our studies to date. As a group, these compounds are superior TX synthetase inhibitors, with the best compounds having potencies nearly 1000-fold greater than the standard dazoxiben. The enzyme inhibiting properties of these compounds are similar across species such as rats, rabbits, dogs, and humans. Some of these compounds are also excellent antihypertensive agents, and much of the SAR found for the enzyme inhibitors in this study was parallel to that found for the antihypertensive effects. Control of blood pressure by altering the arachidonic acid metabolism pathway remains an intriguing possibility.

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, and halogen were within 0.4% of theoretical values, and ^1H NMR spectra were obtained for all compounds on a Varian Associates HA100A nuclear magnetic resonance spectrometer and were consistent with assigned structures. Melting points were determined on a Mel-Temp apparatus in open capillaries and are uncorrected. Compounds prepared by these general methods are listed in Tables I–IV. The designation **a** is used in the discussion to denote the hydrochloride salt of the numbered base.[8]

Preparation of the Quinazoline-2,4(1*H*,3*H*)-diones (Table I). Procedure A. A mixture of 0.01 mol of the *N*-[(1*H*-imidazol-1-yl)alkyl]-2-aminobenzamide and 10 mL of ethyl chloroformate was heated in an oil bath at 85–105 °C for 2 h and dissolved in EtOH. The reaction mixture was concentrated, 25 mL of EtOH and 2.0 g of KOH were added, and the mixture was heated at reflux temperature for 3 h and again concentrated. The residue was diluted with H_2O, HOAc was added to a pH of 6–7, CH_2Cl_2 was added, and the layers were separated. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated. The residue was triturated with Et_2O or EtOAc, and the crystalline product was isolated by filtration. In some reactions the product was not soluble in CH_2Cl_2 and was isolated by filtration from the mixed solvents. If the product did not crystallize, it was converted to an HCl salt.

Compound **20a** was prepared by using this procedure; the hydrochloride salt was prepared by dropwise addition of ethanolic HCl to an ethanol solution of **20**. The salt was isolated by dilution with EtOAc, collection of the crystalline product on filter, washing of the solid with EtOAc, and drying to give the analytical product: mp 287–290 °C; ^1H NMR (DMSO-d_6) δ 14.82 (v br s, 1 H, *H*Cl), 11.78 (br s, 1 H, N*H*), 9.25 (br s, 1 H, imidazolyl-*H*), 7.5 (br m, 5 H, aromatic, imidazolyl *H*), 4.20 (t, 2 H, $CH_2N(CO)_2$), 3.80 (t, 2 H, CH_2-imidazole), 2.5 (m, 1 H, C*H*), 0.80 (d, 3 H, CH_3CH). mass spectrum, m/z 318, 320 (M^+). Anal. Calcd for $C_{15}H_{15}ClN_4O_2$·HCl: C, 50.71; H, 4.54; N, 15.77; Cl, 19.96. Found: C, 50.76; H, 4.58; N, 15.78; Cl, 20.06.

Procedure B. The *N*-[(1*H*-imidazol-1-yl)alkyl]-2-aminobenzamide in the above reaction was isolated as a crude oil and was not triturated. The product was crystallized directly from a suitable solvent.

Preparation of the Quinazolin-4(3*H*)-ones (Table II). Procedure C. A mixture of 0.01 mol of the *N*-[(1*H*-imidazol-1-yl)alkyl]-2-aminobenzamide and 10–15 mL of the triethyl ortho ester was heated in an oil bath at 120–130 °C for 20 h and concentrated. The residue was triturated with Et_2O or EtOAc, and the product, if crystalline, was isolated by filtration. If the product did not crystallize, excess ethanolic HCl and Et_2O or EtOAc were added and the dihydrochloride salt was obtained.

Procedure D. The *N*-[(1*H*-imidazol-1-yl)alkyl]-2-aminobenzamide in the above reaction was isolated as a crude oil and and was not triturated. The product was crystallized directly from a suitable solvent.

Procedure E. The reaction was carried out as in procedure C, but for only 3 h. Under these conditions, reactions were often incomplete.

Preparation of the 1,2,3-Benzotriazin-4(3*H*)-ones (Table III). Procedure F. A mixture of 0.01 mol of the *N*-[(1*H*-imidazol-1-yl)alkyl]-2-aminobenzamide, 15 mL of H_2O, and 4.2 mL of concentrated HCl was cooled in an ice bath, and a solution of 0.72 g of $NaNO_2$ in 10 mL of H_2O was added over a 10-min period. The reaction mixture was stirred for 1 h, and 5 mL of 10 N NaOH was added. After 1 h, HOAc was added to a pH of 6–7 and the product was dissolved in CH_2Cl_2, washed with H_2O, dried over $MgSO_4$, and concentrated. The residue was triturated with Et_2O or EtOAc, and the product was recovered by filtration. If the product did not crystallize, it was converted to the hydrochloride salt.

Procedure G. The *N*-[(1*H*-imidazol-1-yl)alkyl]-2-aminobenzamide in the above reaction was isolated as a crude oil and

(8) The **a** designation refers to the use of the hydrochloride salt. The hydrochloride salt was easier to handle in our secondary biological assays.

(9) Vargas, J. R.; Radomski, M.; Moncada, S. *Prostaglandins* **1982**, *23*, 929.

Table VI. Ex Vivo Inhibition of Thromboxane Formation in Rabbits[a]

compound	dose, mg/kg	% TX formation inhibition at 0.5 h	1.0 h	2 h	3 h
20a	0.5/h (perfused) (n = 2)	95.9 ± 1.2	98.1 ± 1.9	–	–
20a	0.3 (iv, bolus) (n = 3)	88.9 ± 4.4	82.8 ± 4.6	60.8 ± 15.3	53.1 ± 13.6
20a	0.1 (iv, bolus) (n = 7)	73.6 ± 4.9	62.8 ± 8.0	39.5 ± 9.8	46.2 ± 11.2
dazoxiben	10 (iv, bolus) (n = 3)	–	94.7 ± 0.9	–	74.5 ± 1.5
dazoxiben	0.3 (iv, bolus)	45.9 ± 8.2	30.1 ± 10.6	13.6 ± 5.6[b]	22.8 ± 3.4 (n = 3)
saline	(n = 3)	−10.2 ± 9.3	3.7 ± 4.9	0.8 ± 4.9	10.6 ± 12.8

[a] Determinations were done on anesthetized rabbits by dosing in a 0.9% saline solution in the lateral ear vein. Blood samples were collected at 0.5-, 1.0-, 2-, and 3-h intervals, and TX levels were determined; n represents the number of replications. [b] n = 2.

was not triturated. The product was crystallized directly from a suitable solvent.

Preparation of the *N*-[(1*H*-Imidazol-1-yl)alkyl]-2-aminobenzamides (Table IV). Procedure H. A mixture of 0.02 mol of an isatoic anhydride, 0.02 mol of a (1*H*-imidazol-1-yl)alkanamine, and 50 mL of THF was stirred overnight at room temperature and concentrated. The residue was shaken with CH_2Cl_2 and 10 mL of 1 N NaOH, and the layers were separated. The organic layer was washed twice with H_2O, dried over $MgSO_4$, and concentrated. The residue was triturated with Et_2O or EtOAc, and the product was recovered by filtration or converted to a dihydrochloride salt.

Procedure I. A mixture of 0.02 mol of an isatoic anhydride, 0.02 mol of a (1*H*-imidazol-1-yl)alkanamine, and 15 mL of DMSO was stirred overnight, diluted with 30 mL of H_2O, and shaken with 150 mL of CH_2Cl_2. The layers were separated, and the organic layer was washed twice with H_2O, dried over $MgSO_4$, and concentrated. The residue was triturated with Et_2O or EtOAc, and the product was recovered by filtration or converted to a dihydrochloride salt.

Procedure J. A mixture of 0.02 mol of an anthranilic acid derivative, 50 mL of tetrahydrofuran, and 0.02 mol of 1,1′-carbonyldiimidazole was stirred for 1 h while cooling in an ice bath and then for 2 h at room temperature. The (1*H*-imidazol-1-yl)alkanamine (0.02 mol) was added, and the reaction mixture was stirred overnight at room temperature. The mixture was warmed for 1 h, diluted with 20 mL of H_2O, and again warmed for 1 h. The mixture was concentrated, and the residue was shaken with 10 mL of 1 N NaOH and 150 mL of CH_2Cl_2. The layers were separated, and the organic layer was worked up as in the preceding reactions.

Thromboxane Synthetase Inhibition and Prostacyclin Synthetase Inhibition. Under urethane anesthesia, 9 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 platelets/μL. Platelets prepared by this procedure did not aggregate spontaneously. 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 released into the incubation fluid by the platelets. 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 containing the vehicle in lieu of a compound and background samples 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 picograms of TXB_2 formed per minute per sample. The percent inhibition of TXB_2 formation by the test compound was calculated by using the values from control samples containing the vehicle as 100%. Appropriate background and blank values have been subtracted accordingly from the test samples before this calculation. Incubations were carried out at different concentrations of test compounds to enable construction of dose–response curves. Experience with this IC_{50} determination in our laboratory indicates that the reproducibility of this assay is ±20–30%. The results of this test are summarized in Tables I–IV.

The inhibition of PGI_2 was similarly determined on guinea pig aortic ring preparations by using [^{3}H]-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 of PGI_2 from control values as reported by our laboratory.[10]

Ex Vivo Evaluation of Thromboxane Synthesis Inhibition in Rabbits. New Zealand white rabbits (Summit View Farms, Belvidere, NJ, 2.5–5 kg) were anesthetized with 32.4 mg/kg of sodium pentobarbital, iv. The carotid artery was isolated and cannulated for the collection of blood samples. The catheter was kept patent with sterile 0.9% saline solution. Blood samples (3.0 mL) were collected 5 min predose and at 0.5, 1, 2, and 3 h after dosing. The test compound was dissolved in 0.9% sterile saline solution and was administered intravenously at 0.1, 0.3, 0.5, or 10 mg/kg bolus or infused at 5 mg/kg per h by using a Razel syringe pump. The blood samples were placed in 12-mL glass centrifuge tubes and incubated in a metabolic shaker at 37 °C for 2 h. All blood samples were then stored on ice at 4 °C for 2 h, after which time they were centrifuged at 2000 rpm for 10 min at 4 °C. One milliliter of the serum was drawn off and added to 50 μL of indomethacin sodium solution (17.28 mg/100 mL) in a polypropylene tube. The serum samples were mixed well and stored at −20 °C until use. For determination of the TXB_2 content for each sample, the samples were thawed and diluted with sodium phosphate buffer (0.02 M Na_2HPO_4, pH 7.4). The TXB_2 was determined by a direct radioimmunoassay (RIA) as described above.

Inhibition of Thromboxane Formation in Human Platelets. Blood samples were collected from four healthy human volunteers, and platelets were isolated by using a small amount of prostaglandin E_1 to prevent tight aggregation of platelets.[9] The isolated platelets were treated with test substance, standard, or vehicle, and the TXB_2 content for each sample was determined by direct RIA measurement as described.

Antihypertensive Activity in Spontaneously Hypertensive Rats (SHR). The test compounds were tested for antihypertensive activity by the published methods.[7] 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.[7] The procedure

(10) Bielen, S. J.; Lucas, J.; Chan, P. S.; Cervoni, P. *IRCS Med. Sci.* **1985**, *13*, 334.

is repeated in a second and third rat, depending on the activity found in the first rat. 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.

Acknowledgment. We thank Dr. Ralph R. Ryall and associates for elemental analyses and Dr. John M. Baldoni, George Morton, and co-workers for spectral studies. We also express our appreciation to Arlene Hoffman and George Vice for determination of the biological properties of these compounds.

Registry No. 1, 110551-96-7; 2, 110551-97-8; 3, 110551-98-9; 4, 110551-99-0; 5, 110552-00-6; 6, 110552-01-7; 7, 110552-02-8; 8, 110552-03-9; 8·HCl, 110552-96-0; 9, 110552-04-0; 10, 110552-05-1; 11, 110552-06-2; 12, 110552-07-3; 13, 110552-08-4; 14, 110552-09-5; 14·HCl, 110552-97-1; 15, 110552-10-8; 16, 110552-11-9; 17, 110552-12-0; 18, 110552-13-1; 19, 110552-14-2; 20, 110552-15-3; 20·HCl, 110552-98-2; 21, 110552-16-4; 22, 110552-17-5; 23, 110552-18-6; 24, 110552-19-7; 25, 110552-20-0; 26, 110552-21-1; 28, 110567-72-1; 29, 110552-23-3; 30, 110552-24-4; 31, 110552-25-5; 32, 110552-26-6; 33, 110552-27-7; 34, 110552-28-8; 34·HCl, 110552-99-3; 35, 110552-29-9; 32·HCl, 110553-00-9; 36, 110552-30-2; 37, 110552-31-3; 38, 110552-32-4; 38·2HCl, 110553-01-0; 39, 110552-33-5; 39·2HCl, 110553-02-1; 40, 110552-34-6; 40·2HCl, 110553-03-2; 41, 110552-35-7; 41·2HCl, 110553-04-3; 42, 110552-36-8; 42·2HCl, 110553-054; 43, 110552-37-9; 43·2HCl, 110553-06-5; 44, 110552-38-0; 45, 110552-39-1; 45·2HCl, 110553-07-6; 46, 110552-40-4; 47, 110552-41-5; 48, 110552-42-6; 49, 110552-43-7; 50, 110552-44-8; 50·2HCl, 110553-08-7; 51, 110552-45-9; 51·2HCl, 110553-09-8; 52, 110552-46-0; 52·2HCl, 110553-10-1; 53, 110552-47-1; 54, 110552-48-2; 55, 110552-49-3; 55·HCl, 110553-11-2; 56, 110552-50-6; 56·HCl, 110553-12-3; 57, 110552-51-7; 58, 110552-52-8; 59, 110552-53-9; 60, 110552-54-0; 61, 110552-55-1; 61·HCl, 110553-13-4; 62, 110552-56-2; 62·HCl, 110553-14-5; 63, 110552-57-3; 64, 110552-58-4; 64·HCl, 110553-15-6; 65, 110552-59-5; 65·HCl, 110553-16-7; 66, 110552-60-8; 67, 110552-61-9; 67·HCl, 110567-73-2; 68, 110552-62-0; 68·HCl, 110553-17-8; 69, 90259-60-2; 70, 90260-14-3; 71, 110552-63-1; 72, 110552-64-2; 73, 110552-65-3; 74, 110552-66-4; 75, 110552-67-5; 76, 90259-73-7; 77, 110552-68-6; 78, 110552-69-7; 78·2HCl, 110553-18-9; 79, 110552-70-0; 79·2HCl, 110553-19-0; 80, 110552-71-1; 81, 110552-72-2; 81·2HCl, 110553-20-3; 82, 110552-73-3; 83, 110552-74-4; 85, 110552-76-6; 86, 110552-77-7; 87, 110552-78-8; 88, 110552-79-9; $ClCO_2CH_2CH_3$, 541-41-3; $CH(OC_2H_5)_3$, 122-51-0; $CCH_3(OC_2H_5)_3$, 78-39-7; $CC_2H_5(OC_2H_5)_3$, 115-80-0; 2-$H_2NC_6H_4CONH(CH_2)_5$Imid, 110552-80-2; 2-$H_2NC_6H_4CONH(CH_2)_6$Imid, 110552-81-3; 2-$H_2NC_6H_4CONH(CH_2)_2CH(CH_3)$Imid, 110552-82-4; 2-$H_2N$-5-$ClC_6H_3CONH(CH_2)_{10}$Imid, 110552-83-5; 2-$H_2N$-4-$ClC_6H_3CONH(CH_2)_3$Imid, 110552-84-6; 2-$H_2N$-4-$ClC_6H_3CONH(CH_2)_6$Imid, 110552-85-7; 2-$H_2N$-3-$ClC_6H_3CONH(CH_2)_4$Imid, 110552-86-8; 2-$H_2N$-3-$ClC_6H_3CONH(CH_2)_6$Imid, 110552-87-9; 2-$H_2N$-3-$ClC_6H_3CONHCH_2CH(CH_3)CH_2$Imid, 110552-88-0; 2-$H_2N$-3-$ClC_6H_3CONH(CH_2)_2CH(CH_3)$Imid, 110552-89-1; 2-$H_2N$-5-$BrC_6H_3CONH(CH_2)_3$Imid, 110552-90-4; 2-$H_2N$-5-$BrC_6H_3CONH(CH_2)_4$Imid, 110637-22-4; 2-$H_2N$-5-$CH_3C_6H_3CONH(CH_2)_3$Imid, 110552-91-5; 2-$H_2N$-3-$CF_3C_6H_3CONH(CH_2)_4$Imid, 110552-92-6; 2-$H_2N$-3-$CF_3C_6H_3CONH(CH_2)_5$Imid, 110552-93-7; 2-$H_2N$-3-$CF_3C_6H_3CONH(CH_2)_6$Imid, 110552-94-8; 2-$H_2N$-3-$CF_3C_6H_3CONHCH_2CH(CH_3)CH_2$Imid, 110552-95-9; $H_2N(CH_2)_3$Imid, 5036-48-6; $H_2N(CH_2)_4$Imid, 67319-76-0; $H_2NCH_2CH(CH_3)CH_2$Imid, 93668-15-6; $H_2N(CH_2)_5$Imid, 78415-62-0; $H_2N(CH_2)_6$Imid, 78415-63-1; $H_2N(CH_2)_7$Imid, 68887-60-5; $H_2N(CH_2)_8$Imid, 78415-64-2; $H_2N(CH_2)_2CH(CH_3)$Imid, 93668-14-5; 2-H_2N-5-Cl-$C_6H_3CO_2H$, 635-21-2; 2-H_2N-5-Br-$C_6H_3CO_2H$, 5794-88-7; 2-H_2N-5-Cl-$C_6H_3CONH(CH_2)_4$Imid, 78-39-7; 2-H_2N-5-$ClC_6H_3CONH(CH_2)_4$Imid, 115-80-0; 2-H_2N-5-$BrC_6H_3CONHCH_2CH(CH_3)CH_2$Imid, 110613-11-1; isotoic anhydride, 118-48-9; 8-chloroisatoic anhydride, 63497-60-9; 7-chloroisatoic anhydride, 40928-13-0; 6-chloroisatoic anhydride, 4743-17-3; 6-bromoisatoic anhydride, 4692-98-2; 6-methylisatoic anhydride, 4692-99-3; thromboxane synthetase, 61276-89-9.

Chiral DNA Gyrase Inhibitors. 2. Asymmetric Synthesis and Biological Activity of the Enantiomers of 9-Fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic Acid (Ofloxacin)†

Lester A. Mitscher,*‡ Padam N. Sharma,‡ Daniel T. W. Chu,§ Linus L. Shen,§ and Andre G. Pernet§

Department of Medicinal Chemistry, Kansas University, Lawrence, Kansas 66045, and Antiinfective Research Division, Abbott Laboratories, North Chicago, Illinois 60064. *Received April 17, 1987*

A short and efficient synthesis, starting with (*R*)- and (*S*)-alaninol, of the two optical antipodes of the quinolone antimicrobial agent ofloxacin has been devised. Testing in vitro of the products against a range of bacteria and in an assay system incorporating purified DNA gyrase from different bacterial species demonstrates that the *S*-(−) enantiomer is substantially the more active.

Recently there has been great interest in preparing and testing the enantiomers of drugs that exert their pharmacological action via specific receptors or enzymes. In favorable cases this has been shown to result in enhanced selectivity, greater potency, and fewer side effects.[1]

Among the quinolone antimicrobial agents, relatively few fused tricyclic analogues have been found to possess outstanding antibacterial activity.[2] Some of the more prominent exceptions are flumequine (**1**),[3] methylflumequine (S-25930) (**2**),[3] and ofloxacin (**3**)[4-6] (Chart I). Recently their optically active enantiomers have been separated and isolated through high-performance column chromatogra-

† This paper is based upon work presented at the American Institute of Chemists National Meeting, Chicago, IL, Sept 24–26, 1986.

‡ Kansas University.

§ Abbott Laboratories.

(1) Ariens, E. J. *Med. Res. Rev.* **1986**, *6*, 451.
(2) Wentland, M. P.; Cornett, J. B. *Annu. Rep. Med. Chem.* **1985**, *20*, 145.
(3) Gerster, J. F.; Rohlfing, S. R.; Pecore, S. E.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. *Abstracts 25th Interscience Conference on Antimicrobial Agents and Chemotherapy*, Minneapolis, MN, 29 Sept–2 Oct, 1985; Vol. 114, Abstract 134.
(4) Daiichi Seiyaku, *Drugs Future* **1983**, *8*, 395.
(5) Hayakawa, I.; Hiramitsu, T.; Tanaka, Y. *Chem. Pharm. Bull.* **1984**, *32*, 4907.
(6) Tanaka, Y.; Suzuki, N.; Hayakawa, I.; Suzuki, K. *Chem. Pharm. Bull.* **1984**, *32*, 4923.

0022-2623/87/1830-2283$01.50/0