Thromboxane Synthetase Inhibitors and Antihypertensive Agents. 3. *N*-[(1*H*-Imidazol-1-yl)alkyl]heteroaryl Amides as Potent Enzyme Inhibitors

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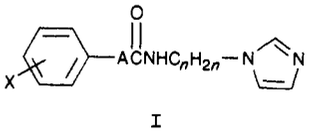
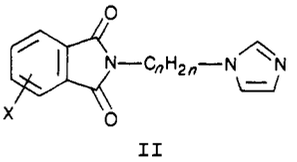
The title compounds were prepared as the heterocyclic analogues of thromboxane (TX) synthetase inhibitors and antihypertensive agents previously reported from our laboratories. These compounds were at least as active TX synthetase inhibitors as their benzene isosteres with the indole derivatives **50–55** having the most potent enzyme inhibiting activity measured to date in our laboratories. The best compound, **54**, is more than 200-fold more potent than the standard, dazoxiben. In contrast, the antihypertensive activity of these series of compounds was no better than their benzene counterparts and is far lower than the isoindoledione derivatives prepared in a related series. The structure–activity relationship results from this study were similar to our previous observations and include the fact that the amide moiety effectively replaces a carboxylic acid for potent TX synthetase inhibition and that a four to six methylene unit separation (approximately 8.5 Å) between amide and imidazole moieties achieves maximal activity.

For some time now, our laboratory has been investigating compounds that inhibit thromboxane (TX) synthetase as well as lower blood pressure.[2] In the first paper in this series,[3] we reported on a series of *N*-[(1*H*-imidazol-1-yl)alkyl]aryl amide derivatives I that were potent selective TX synthetase inhibitors with some antihypertensive activity. Subsequently, isoindole derivatives II, which had been initially prepared as intermediates for the synthesis of I, were found to be equipotent to I as TX synthetase inhibitors but were much more interesting as antihypertensive agents in our animal models.[2]

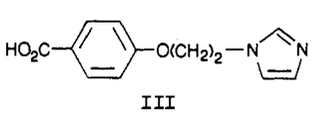


I



II

Other laboratories have also had active programs investigating TX synthetase inhibitors as potential therapeutic agents.[4-9] Compounds such as dazoxiben (UK 37248-01, III),[4] dazmegrel (IV),[5] CGS-13080 (V),[6] and furegrelate (VI)[7] have been intensively studied as such enzyme inhibitors. These agents may be useful for the treatment of cardiovascular disorders that might arise as a consequence of the proaggretory or vasoconstrictor



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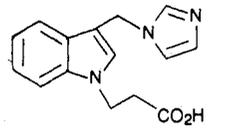

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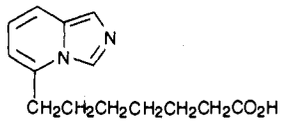

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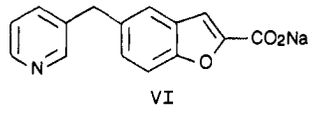

VI

properties of excess circulating TXA_2. Ischemia, arrhythmias, fibrillation, and sudden death as well as certain forms of hypertension are all disease states that might be treatable by selective inhibition of TX synthetase.[10] It is noteworthy to point out that selective TX synthetase inhibition increases the pool of the endoperoxide precursors PGG_2 and PGH_2, which in turn increase the synthesis of prostacyclin (PGI_2), which is a potent vasodilator and antiaggretory agent.[10]

As noted previously,[3] derivatives I differ from agents such as III–VI in that they lack the carboxylic acid moiety that has been found essential for potent selective TX synthetase in numerous laboratories.[4-9] While there appears to be some controversy concerning the ideal separation of the carboxylate moiety from the heterocyclic ring in these compounds,[11] there is general agreement that a pyridine or imidazole is essential for good TX synthetase inhibition.[4-9] Presumably the amide functionality of I[3] has either sufficient acidity or binding properties in our compounds that the amide adequately replaces the carboxylate moiety.

As a continuation of our studies, replacement of the benzene portion of I with other aryl moieties became a subject of interest. Thiophene, furan, benzothiophene, benzofuran, pyridine, and indole derivatives were all accessible synthetically and such molecular alteration of I might yield further insights into the structure–activity relationships within this system both as TX synthetase inhibitors as well as antihypertensive agents. It is clear from recent reports[5,7,8b] that such molecular modifications frequently enhance TX synthetase inhibiting properties.

(1) Present address: Ortho Pharmaceutical Corporation, Raritan, NJ 08869.
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(8) In addition to ref 4–7, see especially: (a) Tanouchi, T.; Kawamura, M.; Ohyama, I.; Kajiwara, I.; Iguchi, Y.; Okada, T.; Miyamoto, T.; Taniguchi, K.; Hayashi, M. *J. Med. Chem.* **1981**, *24*, 1149. (b) Tanouchi, T.; Kawamura, M.; Ohyama, I.; Kajiwara, I.; Iguchi, Y.; Okada, T.; Taniguchi, K.; Miyamoto, T.; Hayashi, M. *J. Med. Chem.* **1981**, *24*, 1139.
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(10) Chan, P. S.; Cervoni, P. *Drug Dev. Res.* **1986**, *7*, 341.
(11) Reference 4 reports that a separation of 8.1–8.8 Å was ideal for maximal activity while ref 8b and 9 report 8.5–9.0 Å to give optimal effect.

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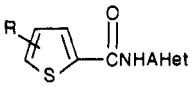
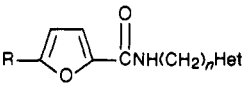
Table I. *N*-(Heteroalkyl)-thiophene-2-carboxamides



compd	R	A	Het[a]	yield, %	mp, °C	thromb formation inhibn[b]	antihypertensive act.[c]	formula
1	H	$(CH_2)_3$	Im	65[d]	135–137	93 (2)	I (2)	$C_{11}H_{13}N_3OS$
2	H	$(CH_2)_3$	Tr	53[d]	101–103	30 (2)	I (3)	$C_{10}H_{12}N_4OS$
3	H	$(CH_2)_4$	Im	59[d]	103–106	95 (2)	A (2)	$C_{12}H_{15}N_3OS$
4	5-CH_3	$(CH_2)_3$	Im	81[d]	120–122	96 (2)	I (2)	$C_{12}H_{15}N_3OS$
5	4,5-Br_2	$(CH_2)_3$	Im	85[d]	126–128	100 (2)	I (2)	$C_{11}H_{11}Br_2N_3OS$
6	5-Cl	$(CH_2)_3$	Im	82[e]	144–147	95 (2)	A (3)	$C_{11}H_{12}ClN_3OS$
7	5-Cl	$(CH_2)_3$	Tr	81[e]	103–105	64 (2)	I (3)	$C_{10}H_{11}ClN_4OS$
8	5-Cl	$(CH_2)_3$	2-MeIm	67[e]	139–141[f]	78 (2)	A (4)	$C_{12}H_{14}ClN_3OS$
9	5-Cl	$(CH_2)_3$	4-MeIm	17[e]	150–152[g]	95 (2)	I (3)	$C_{12}H_{14}ClN_3OS$
10	5-Cl	$(CH_2)_3$	4-PhIm	52[e]	122–124[h]	70 (2)	I (3)	$C_{17}H_{16}ClN_3OS$
11	5-Cl	$(CH_2)_4$	Im	46[e]	78–80	99 (2)	A (2)	$C_{12}H_{14}ClN_3OS$
12	5-Cl	$(CH_2)_4$	Tr	75[e]	97–99[f]	83 (2)	I (3)	$C_{11}H_{13}ClN_4OS$
13	5-Cl	$CH_2CH_2CH(CH_3)$	Im	67[e]	141–143	100 (4)	A (2)	$C_{12}H_{14}ClN_3OS$
14	5-Cl	$CH_2CH{=}CHCH_2$	Im	57[e]	115–117	100 (2)	I (2)	$C_{12}H_{12}ClN_3OS$
15	5-Cl	$(CH_2)_5$	Im	70[e]	123–125	100 (4)	I (4)	$C_{13}H_{16}ClN_3OS$
16	5-Cl	$(CH_2)_5$	Tr	94[e]	79–81[f]	100 (4)	I (2)	$C_{12}H_{15}ClN_4OS$
17	5-Cl	$(CH_2)_6$	Im	88[e]	96–98	100 (4)	I (3)	$C_{14}H_{18}ClN_3OS$
18	5-Cl	$(CH_2)_8$	Im	84[e]	95–97	96 (4)	I (2)	$C_{16}H_{22}ClN_3OS$

[a] Im = imidazolyl; Tr = triazolyl. [b] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. Differences of more than 2% are significant. UK 37248-01 (dazoxiben) = 98%. [c] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A = active; I = inactive; NT = not tested. Number of rats (two to four) as shown in parentheses. [d] Procedure B. [e] Procedure A. [f] Recrystallized from EtOAc. [g] Insoluble in boiling EtOAc. [h] Purified by HPLC (silica gel with 9:1 EtOAc/EtOH).

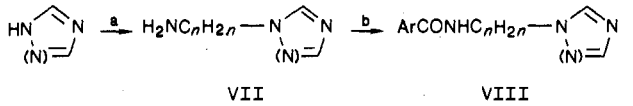
Table II. *N*-(Heteroalkyl)furan-2-carboxamides



compd	R	*n*	Het[a]	yield, %	mp, °C	thromb formation inhibn[b]	antihypertensive act.[c]	formula
19	H	2	Im	24[d]	131–133	65 (2)	NT	$C_{10}H_{11}N_3O_2$
20	H	3	Im	46[d]	111–113	97 (2)	I (3)	$C_{11}H_{13}N_3O_2$
21	5-Br	3	Im	57[e]	139–142	82 (2)	I (2)	$C_{11}H_{12}BrN_3O_2$
22	5-NO_2	3	Im	30[e]	151–153	64 (2)	A (3)	$C_{11}H_{12}N_4O_4$
23	H	4	Im	69[d]	88–90	87 (2)	I (4)	$C_{12}H_{15}N_3O_2$
24	H	4	Tr	61[d]	78–79[f]	22 (2)	I (2)	$C_{11}H_{14}N_4O_2$

[a] Im = imidazolyl; Tr = triazolyl. [b] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. Differences of more than 2% are significant. UK 37248-01 (dazoxiben) = 98%. [c] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A = active; I = inactive; NT = not tested. Number of rats (two to four) as shown in parentheses. [d] Procedure A. [e] Procedure B. [f] Recrystallized from EtOAc.

Scheme I[a]



VII VIII

[a] (a) Reference 3. (b) ArCOCl/NaOH or ArCO-imidazole/THF.

Chemistry

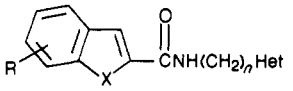
The imidazole and triazole derivatives for this study were prepared in a manner similar to that reported for the benzamide analogues I[3] and the synthesis is depicted in Scheme I. Imidazole or 1,2,4-triazole was condensed with the appropriate bromoalkylphthalimide to give the respective substituted heterocycle.[2] Hydrazinolysis gives the amines VII in good yield. Alternatively, the heterocycle was condensed with acrylonitrile and the resultant Michael-type product was reduced with Raney nickel catalyst to give VII (n = 3) in good yield. Condensation of VII with either an acid chloride or a carboxylic acid activated by the action of 1,1′-carbonyldiimidazole produces the target imidazole or triazole compounds VIII in good yields. Tables I–V list the derivatives prepared in this study.

Biology: Results and Discussion

The compounds prepared in this study (VIII) were tested for TX synthetase inhibition with platelets from spontaneously hypertensive rats (SHR) and procedures as described in previous papers.[2,3] Also similar to these prior reports, all of the compounds were evaluated for their selectivity by examining their effects on PGI_2 formation in aortic rings. None of the test compounds nor any of the standards such as dazoxiben[4] inhibited PGI_2; this result is consistent with a mechanism of selective TX synthetase inhibition. The biological results for TX synthetase inhibition are summarized in Tables I–VI.

Prior to consideration of the effects of the various heterocycles on TX synthetase activity, several conclusions concerning effects of varying the other portions of the molecule may be summarized. The effects of altering the length of the carbon chain are similar to those reported for the related benzamide derivatives 1.[3] Varying from C_3 to C_4 to C_5 to C_6 to C_8 in the thiophene derivatives (**6**, **11**, **15**, **17**, **18**), from C_3 to C_4 to C_5 to C_8 in the indole deriv-

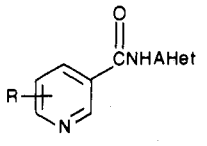
Table III. *N*-(Heteroalkyl)benzofuran- and -benzothiophene-2-carboxamides



compd	X	*n*	R	Het[a]	yield, %	mp, °C	thromb formation inhibn[b]	antihypertensive act.[c]	formula
25	O	3	H	Im	80[d]	105–107	97 (2)	I (2)	$C_{15}H_{15}N_3O_2$
26	O	3	H	Tr	47[d]	113–115	84 (2)	I (3)	$C_{14}H_{14}N_4O_2$
27	S	2	3-Cl	Im	31[e]	132–134[f]	70 (2)	A (3)	$C_{14}H_{12}ClN_3OS$
28	S	3	3-Cl	Im	50[e]	135–137	100 (4)	I (2)	$C_{15}H_{14}ClN_3OS$
29	S	3	5-Cl	Im	56[d]	155–157[g]	100 (4)	I (2)	$C_{15}H_{14}ClN_3OS$
30	S	3	3-Cl-6-F	Im	52[e]	127–129[h]	96 (2)	I (3)	$C_{15}H_{13}ClFN_3OS$
31	S	3	3-Cl-6-Me	Im	56[e]	122–125	96 (2)	I (2)	$C_{16}H_{16}ClN_3OS$
32	S	4	3-Cl	Im	60[e]	84–86	91 (2)	I (2)	$C_{16}H_{16}ClN_3OS$

[a] Im = imidazolyl; Tr = triazolyl. [b] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. Differences of more than 2% are significant. UK 37248-01 (dazoxiben) = 98%. [c] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A = active; I = inactive; NT = not tested. Number of rats (two to four) as shown in parentheses. [d] Procedure B. [e] Procedure A. [f] Recrystallized from EtOAc. [g] Recrystallized from EtOH. [h] Recrystallized from EtOH/Et_2O.

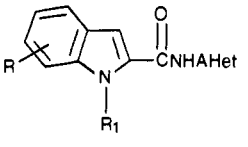
Table IV. *N*-(Heteroalkyl)pyridine-3-carboxamides



compd	R	A	Het[a]	yield,[b] %	mp, °C	thromb formation inhibn[c]	antihypertensive act.[d]	formula
33	H	$(CH_2)_3$	Im	24	112–114[e]	83 (2)	I (2)	$C_{12}H_{14}N_4O$
34	2-Cl	$(CH_2)_3$	Im	42	104–106	64 (2)	I (2)	$C_{12}H_{13}ClN_4O$
35	6-Cl	$(CH_2)_3$	Im	75	145–147	87 (4)	I (3)	$C_{12}H_{13}ClN_4O$
36	6-Cl	$(CH_2)_3$	2-MeIm	77	175–177	44 (4)	I (2)	$C_{13}H_{15}ClN_4O$
37	6-Cl	$(CH_2)_3$	4-MeIm	70	106–110[e]	86 (2)	I (2)	$C_{13}H_{15}ClN_4O$
38	6-Cl	$(CH_2)_4$	Im	62	95–97	94 (2)	I (2)	$C_{13}H_{15}ClN_4O$
39	6-Cl	$CH_2CH_2CH(CH_3)$	Im	79	137–139	98 (2)	A (2)	$C_{13}H_{15}ClN_4O$
40	6-Cl	$CH_2CH(CH_3)CH_2$	Im	52	102–104[e]	98 (2)	I (3)	$C_{13}H_{15}ClN_4O$
41	6-Cl	$(CH_2)_5$	Im	79	110–112	100 (2)	I (2)	$C_{14}H_{17}ClN_4O$

[a] Im = imidazolyl; Tr = triazolyl. [b] Procedure B. [c] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. Differences of more than 2% are significant. UK 37248-01 (dazoxiben) = 98%. [d] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A = active; I = inactive; NT = not tested. Number of rats (two to four) as shown in parentheses. [e] Recrystallized from EtOAc.

Table V. *N*-(Heteroalkyl)-1*H*-indole-2-carboxamides



compd	R	R_1	A	Het[a]	yield,[b] %	mp, °C	thromb formation inhibn[c]	antihypertensive act.[d]	formula
42	H	H	$(CH_2)_3$	Im	47	197–199[e]	94 (2)	A (2)	$C_{15}H_{16}N_4O$
43	H	H	$(CH_2)_3$	4-MeIm	38	209–213[e]	100 (2)	I (4)	$C_{16}H_{18}N_4O$
44	H	CH_3	$(CH_2)_3$	Im	19	125–129	89 (6)	I (2)	$C_{16}H_{18}N_4O$
45	H	CH_3	$(CH_2)_3$	2-MeIm	70	190–192[e]	56 (2)	I (2)	$C_{17}H_{20}N_4O$
46	H	CH_3	$(CH_2)_3$	4-MeIm	68	137–142[f]	85 (2)	I (3)	$C_{17}H_{20}N_4O$
47	5-Cl	H	$(CH_2)_3$	Im	71	223–225[e]	95 (2)	I (2)	$C_{15}H_{15}ClN_4O$
48	5-Cl	H	$(CH_2)_3$	4-MeIm	13	220–225[e]	100 (2)	I (3)	$C_{16}H_{17}ClN_4O$
49	5-MeO	H	$(CH_2)_3$	Im	72	204–206[e]	96 (2)	I (2)	$C_{16}H_{18}N_4O_2$
50	H	H	$(CH_2)_4$	Im	74	137–139	100 (2)	I (3)	$C_{16}H_{18}N_4O$
51	5-Cl	H	$(CH_2)_4$	Im	54	183–185[e,g]	100 (2)	A (3)	$C_{16}H_{17}ClN_4O$
52	5-Cl	H	$CH_2CH_2CH(CH_3)$	Im	85	208–210	98 (4)	A (2)	$C_{16}H_{17}ClN_4O$
53	5-Cl	H	$CH_2CH(CH_3)CH_2$	Im	65	202–205	100 (4)	A (2)	$C_{16}H_{17}ClN_4O$
54	5-Cl	H	$(CH_2)_5$	Im	79	214–216	100 (2)	A (4)	$C_{17}H_{19}ClN_4O$
55	5-Cl	H	$(CH_2)_8$	Im	89	190–192[e]	93 (2)	I (2)	$C_{20}H_{25}ClN_4O$

[a] Im = imidazolyl; Tr = triazolyl. [b] Procedure B. [c] Inhibition of thromboxane formation at a concentration of 10^{-4} M. Numbers in parentheses represent the number of determinations. Differences of more than 2% are significant. UK 37248-01 (dazoxiben) = 98%. [d] Spontaneously hypertensive rats at 100 mg/kg po for 2 days. Key: A = active; I = inactive; NT = not tested. Number of rats (two to four) as shown in parentheses. [e] Recrystallized from EtOH. [f] Recrystallized from EtOAc. [g] C: calcd, 60.66; found, 60.04.

Table VI. Thromboxane Synthetase Inhibition IC_{50} Values for Selected Imidazolyl Compounds[a]

compd	IC_{50},[b] 10^{-6} M	compd	IC_{50},[b] 10^{-6} M
UK 37248-01[c]	1.5 (2)	**25**	0.3 (2)
3	3.0 (2)	**28**	0.3 ± 0.05 (4)
4	1.3 (2)	**29**	0.1 ± 0.025 (4)
5	0.4 (2)	**38**	8.0 (2)
6	0.6 (2)	**39**	3.0 (2)
11	0.2 (2)	**41**	3.0 (2)
13	2.0 ± 0.4 (4)	**50**	0.04 (2)
15	0.2 ± 0.06 (4)	**52**	0.05 (2)
17	0.2 ± 0.05 (4)	**54**	0.007 (2)
18	0.07 ± 0.0025 (4)	**55**	0.027 (2)

[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. [b] Numbers in parentheses represent the number of replications. [c] UK 37248-01 is the code number for dazoxiben.

atives (**47**, **51**, **54**, **55**), or from C_3 to C_4 to C_5 in the pyridine derivatives (**35**, **38**, **41**) shows that maximal activity of 100% TX synthetase inhibition is achieved for four to six methylene unit separation between the amide moiety and the imidazole or triazole. Examination of models shows this separation of the amide carbonyl with N-1 of the imidazole for maximal activity occurs near 8.5 Å. This distance is remarkably similar to the optimal distances found for the active carboxylic acid containing molecules[11] and suggests that the active site requires a carbonyl capable of binding rather than an acid moiety per se.

For this limited study, branching did not seem to lower activity (cf. **13** to **11**; **39** and **40** to **38**; **52** and **53** to **51**). Since we had already noted that incorporation of triazole in these systems did elicit TX synthetase activity albeit at less interesting levels of potency,[2,3] only a few triazole derivatives were prepared. As expected, imidazole derivatives are clearly superior to their triazole analogues (thiophenes, **1**:**2**; **6**:**7**; **11**:**12**; **15**:**16**; furans, **24**:**23**; benzofurans, **25**:**26**). Finally, the effects of substitution on the imidazole moiety were examined. Also similar to previous results, 2-methyl or 2-phenyl substitution significantly reduces while 4-methyl substitution maintains activity (cf. thiophenes, **8**, **10** < **9** ~ **6**; pyridines, **36** < **37** ~ **35**; indoles, **45** < **46** ~ **44**; **47** ~ **48**).

Alteration of the heterocyclic moiety in VIII produces some very interesting results (Table VI). First, it is important to note that, within the structure–activity relationship (SAR) limits discussed above, all of the heteroaromatic systems in this paper (with the exception of the furan systems, Table II) were at least as active as the substituted benzene analogues (e.g., I) previously reported.[3] The most potent thiophene derivative (**18**) was nearly 10-fold more potent than the best substituted benzene analogue,[3] while the benzofuran and benzothiophene derivatives were as potent as the previously reported compounds. There was a 10-fold reduction in potency in the pyridyl derivatives (**38**–**41**) while the indoles (**50**–**55**) were by and large the most potent TX synthetase inhibitors as measured to date in our laboratories.

The most potent inhibitor (**54**, IC_{50} = 0.007 μM) is 15 times more potent than the best compounds that we previously reported[2,3] and more than 200 times more potent than the standard, dazoxiben.

The reasons that the varying heterocycles affect TX synthetase inhibiting activity are not clear. Certainly very electron rich aromatic systems (such as the furan derivatives listed in Table II) are inconsistent with good activity. On the other hand, while electron-poor heteroaromatics such as 5-chlorothiophenes (Table I) or pyridines (Table IV) produce good activity, other more subtle effects must play a major role in both the activity and potency of these systems since the benzofuran, benzothiophene, and especially indole derivatives have the highest overall TX synthetase inhibiting activity.

In contrast to the thromboxane synthetase inhibition, the antihypertensive activity of the compounds in this study is far lower (Tables I–V) than that reported previously for the most interesting isoindoledione derivatives in related studies.[2] This lower antihypertensive activity is similar to that found for the majority of arylamide derivatives reported in our first paper[3] and emphasizes the unique role that the isoindoledione moiety plays in this activity. At this juncture in our studies, it is difficult to discuss SAR for antihypertensive effects other than to point out that electron-deficient aryl systems such as 5-chlorothiophene (**6**, **8**, **11**, **13**) and 5-nitrofuran (**22**) among others are consistent with but neither sufficient nor predictive of such activity (see, for example, **5**, **7**, **9**, **14**, **15**, **21**). This generalization also holds for the indole derivatives (**51**–**54**, Table V) but weakens when examining the antihypertensive effects of benzothiophenes (Table III), 6-chloropyridines (Table IV).

While our laboratory continues to seek novel TX synthetase inhibitors as agents that might control blood pressure, the possible correlation between TX synthetase inhibition and antihypertensive effects that was strongly implicated in our previous paper[2] does not follow for derivatives in this current study. Further papers in this area of research will be forthcoming from our laboratories.

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 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–V.

Procedure A. Reaction of a (1*H*-Imidazol-1-yl)alkanamine or a (1*H*-1,2,4-Triazol-1-yl)alkanamine with an Acid Chloride. A mixture of 0.01 mol of the amine, 0.01 mol of 1 N NaOH (10 mL), and 50 mL of CH_2Cl_2 was stirred and 0.01 mol of the acid chloride was added. The reaction mixture was stirred overnight and treated with more CH_2Cl_2 and 5 mL of 1 N NaOH. The layers were separated, and the organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated. The residue generally crystallized upon trituration with Et_2O and was isolated by filtration. Satisfactory microanalyses were generally obtained without recrystallization. When necessary, recrystallization from EtOH or EtOAc was usually satisfactory.

In some of the early reactions, the dihydrochloride of the amine and 0.03 mol of 1 N NaOH were used.

Procedure B. Reaction of a (1*H*-Imidazol-1-yl)alkanamine or a (1*H*-1,2,4-Triazol-1-yl)alkanamine with an Acid and 1,1′-Carbonyldiimidazole. A mixture of 0.01 mol of the acid and 0.01 mol of 1,1′-carbonyldiimidazole in 50 mL of THF was stored at room temperature for 2 h and 0.01 mol of the amine (or the dihydrochloride) was added. The reaction mixture was then stirred at room temperature for 18–24 h, refluxed for 2–5 h, treated with 5 mL of H_2O, and again heated for 1 h. The solvent was removed in vacuo and the residue was treated with CH_2Cl_2 and 0.01–0.03 mol of 1 N NaOH. The layers were separated, and the organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated to give the desired product, which was washed onto a filter with Et_2O or recrystallized from a suitable solvent.

Thromboxane Synthetase Inhibition and Prostacyclin Synthetase Inhibition. Under urethane 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 minutes 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 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, 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 subtracted from the test samples before this calculation. The results of this test are summarized in Tables I–VI.

The inhibition of PGI_2 was similarly determined on guinea pig aortic ring preparations with $^3H_6$-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 of PGI_2 from control values.

Antihypertensive Activity in Spontaneously Hypertensive Rats. The test compounds were tested for antihypertensive activity by the published methods.[12] Male, 16 weeks 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 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.

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Nonequilibrium Opioid Antagonist Activity of 6,14-Dideoxynaltrexone Derivatives

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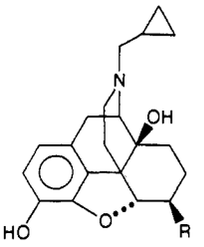
A series of 6,14-dideoxynaltrexones that contain different electrophiles in the 6-position were synthesized and evaluated for nonequilibrium opioid antagonist activity in the guinea pig ileum and mouse vas deferens preparations. Members **3–5** of the series possessed irreversible antagonist activity profiles similar to those previously reported for the 14-hydroxy analogues. In contrast, the 14-deoxy-β-funaltrexamine (14-deoxy-β-FNA) analogue (**6**) exhibited a profile of irreversible antagonist activity that differed from that of β-FNA. It was concluded that the 14-hydroxy group is not essential for irreversible blockage when the electrophile is capable of reacting with a broad spectrum of nucleophiles. However, with a highly selective electrophile such as the fumarate group, the 14-hydroxy function appears to play a role in aligning the molecule to optimize attack by a receptor-based nucleophile.

β-Chlornaltrexamine[1] (**1**, β-CNA) and β-funaltrexamine[2] (**2**, β-FNA) are potent nonequilibrium opioid antagonists and are employed widely as tools in opioid research.[3]



1 (β-CNA), R = $N(CH_2CH_2Cl)_2$

2 (β-FNA), R = $NCOC{=}CCO_2Me$ (H, H)

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β-CNA blocks the three major opioid receptor types (μ, κ, δ), while β-FNA is highly selective for μ opioid receptors. Because these ligands become attached covalently to opioid receptors, they produce ultralong-lasting antagonism in vivo and irreversible blockage in vitro.

A variety of other electrophilic moieties have been attached to the naltrexamine pharmacophore in order to study the relationship between intrinsic reactivity and selectivity at opioid receptors.[4-6] In this paper we examine

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