Acknowledgment. We thank Dr. Nabil Seidah (Institut de Recherche Clinique de Montréal) for some of the amino acid analyses and Suzanne Forand for assistance in this project as a summer student. The manuscript was typed by Mrs. Cécile Pepin, whom we acknowledge. This work was supported by research grants from the Medical Research Council of Canada and from the Québec Heart Foundation.

Cyclic Guanidines. 14.[1] Imidazo[1,2-*a*]thienopyrimidin-2-one Derivatives as Blood Platelet Aggregation Inhibitors

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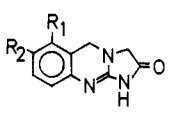
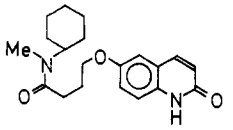
A series of novel 1,2,3,5-tetrahydroimidazo[1,2-*a*]thieno[2,3-*d*]-, -[3,2-*d*]-, and -[3,4-*d*]pyrimidin-2-one derivatives has been prepared and tested for the activity of inhibiting platelet aggregation in rats in vitro and ex vivo. These compounds were synthesized through the following reactions: sodium borohydride reduction of 2,4-dichlorothienopyrimidines, followed by ethoxycarbonylmethylation and successive amination. Most of the compounds were found to be potent inhibitors of blood platelet aggregation. Structure–activity relationships have indicated the essential contribution of the lactam structure and lipophilic substituents on the thiophene ring to the effective interaction of the compounds with a receptor site on the platelet. Among the compounds studied, 1,2,3,5,6,7,8,9-octahydro[1]benzothieno[2,3-*d*]imidazo[1,2-*a*]pyrimidin-2-one (**9m**) exhibited the most favorable activity.

In view of the important contribution of platelet functions to thrombus formation, considerable effort has been devoted in a search for inhibitors of platelet aggregation. Among the structures of reported inhibitors, we were interested in a lipophilic lactam structure as a common, essential prerequisite for the active compounds, e.g., 1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-ones[2] (I),



Ia R_1 = Me, R_2 = H
Ib R_1 = R_2 = Cl

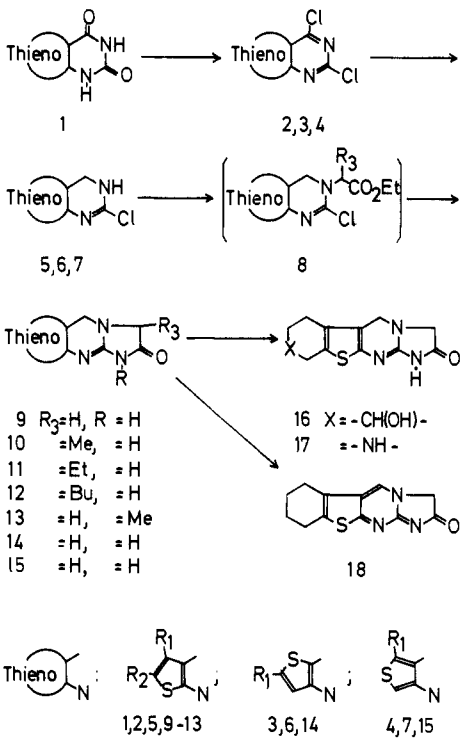


II

3,4-dihydroimidazo[1,2-*a*]benzimidazol-2(1*H*)-one derivatives,[3] 4-(3,4-disubstituted-benzyl)imidazolidin-2-one derivatives,[4] and 4-(6-carbostyryloxy)butyramide derivatives[5] (II). The amide structure may be essentially required for interaction of the compounds with an enzyme protein through hydrogen bondings, and the lipophilic moiety in these compounds may increase the effectiveness of the interaction. On the basis of this assumption, the present study was undertaken to prepare potent inhibitors of platelet aggregation and to confirm the structure–activity relationships along this line.

Our desired compounds were 1,2,3,5-tetrahydroimidazo[1,2-*a*]thienopyrimidin-2-ones (**9**–**17**) which have

Scheme I



the thieno moiety, in place of the benzo group of quinazoline compounds[1] (I), containing a 3,4-dihydrothienopyrimidine ring as a partial structure in the molecule. To our knowledge, the above 3,4-dihydro ring system has been found only in 4-alkyl-3,4-dihydrothieno[2,3-*d*]pyrimidines prepared by the reaction of thieno[2,3-*d*]pyrimidine with alkyllithiums.[6] The desired compounds having this ring system were easily obtained, however, by a novel synthetic route and were found to be highly potent inhibitors of platelet aggregation. This paper describes the synthesis and biological activities of a series of 1,2,3,5-tetrahydroimidazo[1,2-*a*]thieno[2,3-*d*]-, -[3,2-*d*]-, and -[3,4-*d*]pyrimidin-2-ones.

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Chemistry. Synthesis of 1,2,3,5-tetrahydroimidazo-[1,2-*a*]thienopyrimidin-2-ones was carried out by the sequence shown in Scheme I.

The ethyl 2-aminothiophene-3-carboxylates prepared according to the method of Gewald et al.[7] were treated with potassium cyanate or urea to give thieno[2,3-*d*]pyrimidine-2,4-diones (**1**), which were chlorinated with phosphoryl chloride to 2,4-dichlorothieno[2,3-*d*]pyrimidines (**2**). 2,4-Dichlorothieno[3,2-*d*]- (**3**)[8] and -[3,4-*d*]pyrimidines (**4**)[9] were obtained by known methods. Compounds **2h** and **2i** having a chlorine atom in the thiophene ring were prepared by heating **2b** and **2c** with *N*-chlorosuccinimide in acetic acid, respectively. The 2,4-dichloro derivatives (**2–4**) were treated with an excess amount of sodium borohydride in chloroform–ethanol to give 2-chloro-3,4-dihydrothienopyrimidines (**5–7**), whose NMR spectra showed the singlet signals due to the methylene group at position 4 at δ 4.5–4.8. The reaction rate of this reduction depended on the nature of the R_1 and R_2 substituents on the thiophene ring. In some cases, heating in aqueous tetrahydrofuran brought about a good result. Heating **5–7** with ethyl 2-bromoalkanoates in the presence of potassium carbonate predominantly gave 3-substituted intermediates (**8**), which were heated with ethanolic ammonia or methylamine in a sealed tube to yield the desired 1,2,3,5-tetrahydroimidazo[1,2-*a*]thienopyrimidin-2-ones (**9–15**).[10]

Compound **16**, having a hydroxy group in the ring fused to the thiophene ring, was prepared by catalytic hydrogenation of the corresponding benzyloxy derivative (**9r**). A basic compound, **17** or its water-soluble hydrochloride having a cyclic amino side chain attached to the thiophene ring, was obtained by heating the corresponding ethoxycarbonyl-substituted derivative (**9w**) with concentrated hydrochloric acid.

Compounds **9–17** were unstable under oxidative conditions. For example, air-oxidation of **9m** in methanol solution gave the corresponding dehydro derivative (**18**). The IR and NMR spectra of all compounds prepared were consistent with the assigned structures.

Structure–Activity Relationships. Some of the compounds obtained here were equally active in inhibiting platelet aggregation to the reference compounds, 6-methyl-(Ia, BL-3459)[2b] and 6,7-dichloro-1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazolin-2-one (Ib, BL-4162A)[2c] and 4-(6-carbostyryloxy)-*N*-cyclohexyl-*N*-methylbutyramide[5] (II, OPC-3689), in vitro and/or ex vivo as shown in Table I. It was found that the activity was considerably influenced by substitution on the thiophene ring. Unsubstituted compounds, **9a**, **14a**, and **15a**, were almost inactive. The activity was enhanced by most of the substituents which increased lipophilicity around the thiophene moiety (**9d–i** > **9b,c** ≫ **9a**; **15b** > **15a**). Cyclization of the alkyl substituents of R_1 and R_2 (**9m** > **91** > **9k** ≫ **9a**) resulted in a marked increase in the inhibitory activity. Replacement of the cyclohexane ring by an oxygen- or sulfur-containing ring also gave active compounds (**9s-u**), but replacement by a nitrogen-containing ring gave a less active compound (**17**). Substitution with a relatively small group on the cyclohexane ring or the related ring also gave potent, active compounds (**9n–p,w** and **16**). However, bulky substituents gave less active compounds, **9j,q,r,v,y,z**, suggesting some steric effect around this moiety.

Substitution with a methyl group at position 3 of the imidazopyrimidinone ring (**10**) gave a complexed effect: higher activity against collagen-induced platelet aggregation but much lower activity against ADP-induced aggregation, when compared with the corresponding unsubstituted parent compounds (**9**), except for **9m**. The methylation of **9m** at this position caused a marked decrease in the inhibitory activity against both collagen- and ADP-induced platelet aggregation. Although the reason for the complexed result is not clear, the inhibitory activity of these compounds against thrombin-induced aggregation and the antithrombic effect have been found to be parallel with the activity against ADP-induced aggregation in a preliminary study which will be reported elsewhere. The compounds with ethyl or a more bulky group on the same position (**11m** and **12m**) were also inactive and suggestive of steric hindrance around this position.

Substitution on the nitrogen atom at position 1 (**13m**) resulted in complete loss of the activity. The oxidation product (**18**) had also little activity in vitro, indicating essential contribution of the lactam structure to interaction with platelet sites probably through hydrogen bondings.

In addition, we previously reported that 6,7-dimethyl-5-phenyl-1,2,3,5-tetrahydroimidazo[1,2-*a*]thieno[2,3-*d*]pyrimidin-2-one[1] substituted with a bulky group at position 5 and 2,3,5,6-tetrahydro-1*H*-imidazo[2,1-*b*][1,3]benzodiazepin-2-one[11] deprived of the planeness of the three-ring system were almost inactive.

From these results, one can explain consistently the structure–activity relationships of this series of compounds by considering the important contribution of the lactam structure, steric hindrance around positions 3 and 5, lipophilicity around the thiophene moiety, and planeness of the multiring system.

Some of the active compounds were tested for the duration of their action ex vivo after a single oral administration of 50 mg/kg of body weight to rats. As shown in Table II, the compounds **9d,e,h** were relatively short-acting, while the compounds **9n,w** were rather long-acting inhibitors.

The reference compound Ia was also reported to possess a potent hypotensive activity.[2b] This may be rather a drawback of an antiaggregatory agent as an antithrombotic agent. Table III shows the effect of some of the present compounds on blood pressure and heart rate in normotensive rats after a single oral dose of 50 mg/kg of body weight, together with that of the reference compound I. Compounds **9m** and **17** little affected both blood pressure and heart rate of the rats, while compound **10m** caused some hypotensive response in the rats with little effect on heart rate. On the other hand, the reference compound I caused a considerable reduction of blood pressure, together with long-lasting tachycardia in the rats, and the other reference compound, II, increased heart rate without any effect on blood pressure.

Among the compounds obtained here, 1,2,3,5,6,7,8,9-octahydro[1]benzothieno[2,3-*d*]imidazo[1,2-*a*]pyrimidin-

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(10) Compound **9d** was allowed to react with lithium aluminum hydride to give 6,7-dimethyl-1,2,3,5-tetrahydroimidazo[1,2-*a*]-thieno[2,3-*d*]pyrimidine, which was identical with a sample obtained by a similar reduction of 6,7-dimethyl-1,2,3,5-tetrahydroimidazo[1,2-*a*]thieno[2,3-*d*]pyrimidin-5-one reported by Blaskiewicz. P. Blaskiewicz, H. Vorbrueggen, and H. Koch, German Offen. 2411273 (1975); *Chem. Abstr.*, **83**, 206324*f* (1975).

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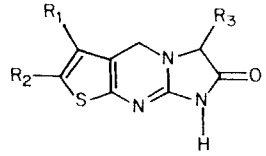
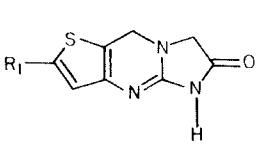
Table I. 1,2,3,5-Tetrahydroimidazo[1,2-*a*]thienopyrimidin-2-one Derivatives (9–18) and Their Inhibition of Blood Platelet Aggregation



9-12, 16, 17



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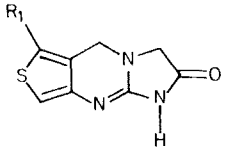

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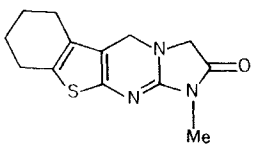
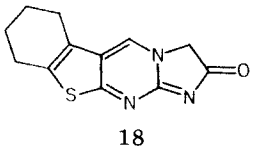
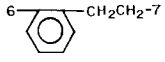
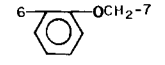


13m



18

								inhibn of blood platelet aggregation			
								ex vivo[d]			
								collagen		ADP	
						in vitro: EC_{50}[c]		control,			
compd	R_1	R_2	R_3	mp,[a] °C	yield, %	formula[b]	collagen, μM	ADP, μM	ΔA/min × 10^3	test, ΔA/min × 10^3	control, $\Delta A \times 10^3$	test, $\Delta A \times 10^3$
9a	H	H	H	unclear	67	$C_8H_7N_3OS \cdot HCl$	>250	>250				
9b	Me	H	H	253–257 dec	65	$C_9H_9N_3OS \cdot HCl \cdot 0.5H_2O$	15	19	210 ± 20	188 ± 57 (11)	152 ± 27	162 ± 74 (−6)
9c	H	Me	H	233–235 dec	50	$C_9H_9N_3OS \cdot HCl$	35	125	308 ± 23	162 ± 143 (47)	240 ± 33	204 ± 10 (15)
9d	Me	Me	H	249–253 dec	45	$C_{10}H_{11}N_3OS \cdot HCl$	1.0	5.0	170 ± 25	73 ± 52** (57)	170 ± 26	116 ± 26** (32)
9e	Me	Pr	H	209–212 dec	33	$C_{12}H_{15}N_3OS \cdot HCl$	0.08	0.58	226 ± 14	90 ± 90**[e] (60)	174 ± 27	128 ± 40[e] (26)
9f	Me	pentyl	H	206–208 dec	14	$C_{14}H_{19}N_3OS \cdot HCl$	0.19	2.0	117 ± 4	2 ± 4** (98)	175 ± 23	84 ± 45** (52)
9g	Pr	Et	H	214–216 dec	22	$C_{13}H_{17}N_3OS \cdot HCl$	2.2	3.0	186 ± 45	8 ± 4** (96)	94 ± 15	82 ± 27 (13)
9h	Me	Cl	H	>280	22	$C_9H_8ClN_3OS \cdot HCl$	1.9	4.2	290 ± 43	222 ± 36* (24)	146 ± 32	98 ± 24* (33)
9i	Cl	Me	H	241–242 dec	43	$C_9H_8ClN_3OS \cdot HCl \cdot H_2O$	17	36	290 ± 43	280 ± 14 (4)	146 ± 32	142 ± 27 (3)
9j	Ph	H	H	233–235 dec	68	$C_{14}H_{11}N_3OS \cdot HCl$	20	120	228 ± 42	198 ± 49 (13)	176 ± 18	178 ± 41 (−1)
9k	6-$CH_2CH_2CH_2$-7		H	249–255 dec	59	$C_{11}H_{11}N_3OS \cdot HCl$	0.25	40	156 ± 10	72 ± 61* (54)	242 ± 10	206 ± 40 (15)
9l	6-$CH_2(CH_2)_3CH_2$-7		H	230–232 dec	56	$C_{13}H_{15}N_3OS \cdot HCl$	0.17	18	210 ± 20	202 ± 26 (8)	152 ± 27	154 ± 24 (−1)
9m	6-$CH_2CH_2CH_2CH_2$-7		H	257–259 dec	71	$C_{12}H_{13}N_3OS \cdot HCl$	0.07	2.0	156 ± 10	0 ± 0** (100)	242 ± 10	141 ± 22** (42)
9n	6-$CH(CH_3)CH_2CH_2CH_2$-7		H	242–245 dec	10	$C_{13}H_{15}N_3OS \cdot HCl$	0.18	0.53	238 ± 32	0 ± 0**[e] (100)	166 ± 21	53 ± 16**[e] (68)
9o	6-$CH_2CH_2CH(CH_3)CH_2$-7		H	258–260 dec	43	$C_{13}H_{15}N_3OS \cdot HCl$	0.62	2.0	178 ± 23	0 ± 0** (100)	150 ± 21	111 ± 30 (26)
9p	6-$CH_2CH_2CH(t\text{-Bu})CH_2$-7		H	247–252 dec	31	$C_{16}H_{21}N_3OS \cdot HCl$	0.25	27	117 ± 4	4 ± 4** (97)	175 ± 23	125 ± 29* (29)
9q	6-$CH(CH_2Ph)CH_2CH_2CH_2$-7		H	266–269 dec	18	$C_{19}H_{19}N_3OS \cdot HCl$	4.0	160				
9r	ó-$CH_2CH_2CH(OCH_2Ph)CH_2$-7		H	247–250 dec	35	$C_{19}H_{19}N_3OS \cdot HCl$	2.2	4.2	117 ± 4	42 ± 74** (64)	175 ± 23	110 ± 29 (37)
9s	6-$CH_2CH_2OCH_2$-7		H	>280	78	$C_{11}H_{11}N_3O_2S \cdot HCl$	0.2	1.3	171 ± 18	113 ± 38* (34)	130 ± 24	100 ± 46 (23)
9t	6-$CH_2CH_2SCH_2$-7		H	>280	71	$C_{11}H_{11}N_3OS_2 \cdot HCl$	0.54	2.0	178 ± 23	0 ± 0** (100)	150 ± 21	100 ± 25** (33)

9u	6-$CH_2CH_2CH_2S$-7	H	262–265 dec	53	$C_{11}H_{11}N_3OS_2 \cdot HCl$	0.16	1.2	171 ± 18	125 ± 65 (27)	130 ± 24	102 ± 41 (22)
9v	6-$CH_2CH_2N(CH_2Ph)CH_2$-7	H	225–230 dec	63	$C_{18}H_{18}N_4OS \cdot 2HCl$	29	13.5	186 ± 45	121 ± 66 (35)	94 ± 15	45 ± 18** (52)
9w	6-$CH_2CH_2N(COOEt)CH_2$-7	H	245–247 dec	48	$C_{14}H_{16}N_4OS \cdot HCl \cdot H_2O$	0.12	1.2	171 ± 18	115 ± 70 (33)	130 ± 24	73 ± 53 (44)
9x	6-CH=CHCH=CH-7	H	252–254 dec	46	$C_{12}H_9N_3OS \cdot HCl$	0.15	5.3	183 ± 40	92 ± 106 (50)	145 ± 32	112 ± 31 (23)
9y		H	278–279 dec	39	$C_{16}H_{13}N_3OS \cdot HCl \cdot 0.5H_2O$	0.90	19	207 ± 28	191 ± 11 (8)	208 ± 43	149 ± 23 (28)
9z		H	>280	50	$C_{15}H_{11}N_3O_2S \cdot HCl$	1.6	34				
10c	H Me	Me	161–163	50	$C_{10}H_{11}N_3OS \cdot HCl \cdot H_2O$	10	230				
10d	Me Me	Me	264–268 dec	27	$C_{11}H_{13}N_3OS \cdot HCl \cdot 0.5H_2O$	0.13	20	183 ± 40	76 ± 86 (58)	145 ± 32	140 ± 49 (3)
10i	Cl Me	Me	260–262 dec	41	$C_{10}H_{10}ClN_3OS \cdot HCl \cdot H_2O$	0.9	150				
10m	6-$CH_2CH_2CH_2CH_2$-7	Me	232–235 dec	39	$C_{13}H_{15}N_3OS \cdot HCl \cdot 0.5H_2O$	0.60	48	180 ± 36	2 ± 4** (99)	207 ± 35	84 ± 20** (59)
10n	6-CH(Me)$CH_2CH_2CH_2$-7	Me	219–226 dec	11	$C_{14}H_{17}N_3OS \cdot HCl$	0.03	3.0	183 ± 40	47 ± 55** (74)	145 ± 32	92 ± 32** (37)
10o	6-$CH_2CH_2CH(Me)CH_2$-7	Me	270–276 dec	21	$C_{14}H_{17}N_3OS \cdot HCl \cdot 0.5H_2O$	0.2	32	207 ± 28	152 ± 80 (27)	208 ± 43	118 ± 75 (28)
11m	6-$CH_2CH_2CH_2CH_2$-7	Et	220–225 dec	36	$C_{14}H_{17}N_3OS \cdot HCl \cdot 0.5H_2O$	60	>250				
12m	6-$CH_2CH_2CH_2CH_2$-7	*n*-Bu	215–225 dec	24	$C_{16}H_{21}N_3OS \cdot HCl \cdot 0.5H_2O$	>250	>250				
13m			240–245 dec	40	$C_{13}H_{15}N_3OS \cdot HCl$	>250	>250				
14a	H		265–267 dec	60	$C_8H_7N_3OS \cdot HCl$	10	>250	308 ± 23	256 ± 38* (17)	240 ± 33	141 ± 18** (41)
14b	Me		257–260 dec	54	$C_9H_9N_3OS \cdot HCl$	12	250	228 ± 42	217 ± 26 (5)	170 ± 18	160 ± 15 (9)
15a	H		262–264 dec	52	$C_8H_7N_3OS \cdot HCl$	90	>250				
15b	Me		>280	58	$C_9H_9N_3OS \cdot HCl$	3.5	17	290 ± 43	198 ± 134 (32)	146 ± 32	106 ± 55 (27)
16	6-$CH_2CH_2CH(OH)CH_2$-7	H	255–256 dec	31	$C_{12}H_{13}N_3O_2S \cdot HCl \cdot 0.5H_2O$	0.1	2.0	117 ± 4	2 ± 4** (98)	175 ± 23	34 ± 25** (80)
17	6-$CH_2CH_2NHCH_2$-7	H	>280	46	$C_{11}H_{12}N_4OS \cdot 2HCl$	9.0	20	254 ± 19	228 ± 24[e] (10)	176 ± 5	108 ± 33**[e] (39)
18			263–265 dec	40	$C_{12}H_{11}N_3OS \cdot HCl$	84	250	183 ± 40	47 ± 55** (74)	145 ± 32	92 ± 32* (37)
Ia (BL-3459)[f]						0.8	5.0	244 ± 43	110 ± 70* (55)	182 ± 29	140 ± 37 (23)
Ib (BL-4162A)[f]						0.02	0.8	188 ± 16	126 ± 9 (33)	150 ± 32	150 ± 0 (0)
II (OPC-3689)[f]						1.2	12.0				

[a] Compounds were recrystallized from MeOH. [b] All compounds were analyzed for C, H, and N. Analytical results obtained for these elements were within ±0.4% of the calculated value. [c] Effective concentration required for 50% inhibition of platelet aggregation. [d] Data represent mean ± SD of five to seven animals given vehicle or compound at an oral dose of 50 mg/kg 2 h before the test. Figures in parentheses are the percent inhibition of platelet aggregation: * = $p < 0.05$; ** = $p < 0.01$ (vs. control). [e] Compound was given 3 h before the test. [f] BL-3459 (hydrochloride monohydrate, mp 260 °C dec), BL-4162A (hydrochloride hemihydrate, mp > 280 °C), and OPC-3689 (mp 180–181 °C) were synthesized in our Institute for experimental use.

Table II. Duration of Inhibition of Platelet Aggregation in Rats after a Single Oral Dose of Imidazo[1,2-a]thienopyrimidin-2-one Derivatives at 50 mg/kg

	collagen-induced aggregation, $\Delta A/\text{min} \times 10^3$				ADP-induced aggregation, $\Delta A \times 10^3$			
		time after dose,[b] h				time after dose,[b] h		
compd	control[a]	3	6	24	control[a]	3	6	24
9d	170 ± 25	94 ± 74 (45)	153 ± 13 (10)	164 ± 25 (4)	170 ± 0	173 ± 14 (−1)	168 ± 18 (1)	168 ± 16 (1)
9e	226 ± 14	90 ± 90* (60)	213 ± 49 (6)	242 ± 21 (−7)	174 ± 27	128 ± 40 (26)	154 ± 44 (11)	176 ± 21 (−1)
9h	212 ± 29	77 ± 74* (64)	166 ± 47 (22)	166 ± 31 (22)	144 ± 10	106 ± 10** (26)	121 ± 13* (16)	122 ± 15 (15)
9m	194 ± 25	17 ± 42** (91)	41 ± 66** (79)	196 ± 12 (−2)	175 ± 32	92 ± 19** (48)	115 ± 28* (35)	160 ± 26 (9)
9n	238 ± 32	0 ± 0** (100)	36 ± 72** (85)	116 ± 96 (51)	166 ± 21	53 ± 16** (68)	79 ± 33 (52)	118 ± 29 (29)
9w	190 ± 14	120 ± 63 (37)	72 ± 58* (62)	30 ± 51** (84)	145 ± 19	68 ± 14** (53)	109 ± 15* (25)	58 ± 25** (80)
10m	120 ± 76	2 ± 5** (98)	1 ± 2** (99)	66 ± 77 (45)	170 ± 9	81 ± 9** (52)	80 ± 13** (53)	118 ± 27 (31)
17	254 ± 19	220 ± 24 (13)	66 ± 116* (74)	170 ± 88 (33)	176 ± 5	108 ± 33** (39)	126 ± 51 (28)	178 ± 35 (1)
18	256 ± 4	68 ± 93** (73)	195 ± 23** (24)	185 ± 112 (28)	171 ± 11	61 ± 20** (64)	121 ± 2** (29)	177 ± 8 (−4)

[a] Vehicle alone was administered 3 h before blood collection. [b] Data represent mean ± SD of five to seven animals. Figures in parentheses are percent inhibition of platelet aggregation. * = $p < 0.05$; ** = $p < 0.01$ (vs. control).

Table III. Effects of 9m, 10m, 17, and Reference Compounds on Systolic Blood Pressure and Heart Rate in Rats after an Oral Dose at 50 mg/kg

	initial value:[a] blood pressure, mmHg, and heart rate, bts/min	time after dose, h						
compd		1	2	3	4	5	6	24
9m	BP: 122 ± 3.9	4[b]		8*	8*	9	6	4
	HR: 467 ± 6.2	4[c]		8	12	9	7	−1
10m	BP: 128 ± 3.4	24**	20*	14*	11*	4		
	HR: 440 ± 4.5	5	5	2	4	4		
17	BP: 123 ± 2.0	8	13*	2	−2	−3		
	HR: 392 ± 14.6	−3	−4	2	6	−2		
Ia	BP: 129 ± 2.4	23**	28**	26**		34**	35**	12*
	HR: 464 ± 10.3	18**	20**	17**		18**	19**	−1
Ib	BP: 130 ± 3.2	11*	16*	17**	14**	14**	13*	
	HR: 377 ± 13.9	28**	37**	31**	37**	31**	33**	
II	BP: 125 ± 2.2	1	4	−1	−1	−1		
	HR: 391 ± 5.6	22*	20**	20**	21**	26**		

[a] Five animals were used in each group. Data represent mean ± SD. [b] Decrease in blood pressure. [c] Percent change in heart rate. * = $p < 0.05$; ** = $p < 0.01$ (vs. value).

Table IV. Inhibition of Blood Platelet Aggregation by 9m and Ia in Human, Rabbit, and Rat in Vitro (EC_{50},[a] μM)

	human		rabbit			rat			
compd	collagen	ADP	collagen	ADP	serotonin/ epinephrine	collagen	ADP	thrombin	arachidonic acid
9m	1.2	2.2	0.4	8	3	0.07	2	3	0.5
Ia	0.7	1.7	0.5	10	3	0.8	5	3	0.5

[a] Estimated concentration to produce 50% inhibition of aggregation, which was calculated from the inhibition rate at three to six concentrations of a test. Figures give the values as mean of two to three experiments.

2-one (**9m**, DH-6471) is the best compound in view of the biological evaluation, the ease of the preparation, and the chemical stability. As shown in Table IV, **9m** showed highly potent inhibitory activity against various aggregating agents, collagen, ADP, serotonin–epinephrine, thrombin, and arachidonic acid in rats, rabbits, and humans in vitro. The activities are almost equal to those of the reference compound Ia.[1a] Evaluation of the antithrombiotic effect of this compound is in progress.

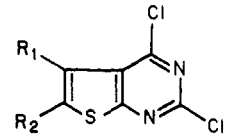
Experimental Section

Melting points were determined in open capillaries and are uncorrected. IR spectra were taken on a Hitachi 285 spectrometer. NMR spectra were recorded with Varian EM-360 (60 MHz) and Hitachi Perkin-Elmer R-20B (60 MHz) spectrometers (Me_4Si as an internal standard). UV spectra were taken on a Hitachi 323 spectrometer. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values.

2,4-Dichloro-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*]pyrimidine (2m).[12] A mixture of 23.0 g (103 mmol) of **1m** and 10 mL of *N,N*-dimethylaniline in 120 mL of $POCl_3$ was heated under reflux for 14 h and concentrated to dryness in vacuo. The residue was treated with ice–water and extracted with $CHCl_3$. The extract was washed with H_2O and dried over Na_2SO_4, and the solvent was evaporated to give 16.4 g (61%) of **2m**: mp 178–180 °C (recrystallization from $CHCl_3$–MeOH).

(12) M. Robba, P. Touzot, and R. M. Riquelme, *C. R. Hebd. Seances Acad. Sci., Ser. C*, **276**, 93 (1973).

Table V. 2,4-Dichlorothieno[2,3-*d*]pyrimidines (**2**)



2[a]

no.	mp, °C	yield, %	recrystn solvent	formula[b]
2c	144-145	65	Me_2CO	$C_7H_4Cl_2N_2S$
2e	60-61	58	petr. ether	$C_{10}H_{10}Cl_2N_2S$
2f	61-62	48	petr. ether	$C_{10}H_{10}Cl_2N_2S$
2g	51-52	38	petr. ether	$C_{11}H_{12}Cl_2N_2S$
2j	157-158	46	Me_2CO	$C_{12}H_6Cl_2N_2S$
2k	134-135	51	Me_2CO	$C_9H_6Cl_2N_2S$
2l	143-144	32	$CHCl_3$-MeOH	$C_{11}H_{10}Cl_2N_2S$
2n	75-77	22	Et_2O-petr. ether	$C_{11}H_{10}Cl_2N_2S$
2o	150-151	58	Me_2CO	$C_{11}H_{10}Cl_2N_2S$
2p	126-128	58	MeOH	$C_{14}H_{16}Cl_2N_2S$
2q	151-152	33	Me_2CO	$C_{17}H_{14}Cl_2N_2S$[c]
2r	105-106	40	Et_2O	$C_{17}H_{14}Cl_2N_2S$
2s	157-159	71	MeOH	$C_9H_6Cl_2N_2OS$
2t	152-154	85	$CHCl_3$-MeOH	$C_9H_6Cl_2N_2S_2$[d]
2u	184-186	87	$CHCl_3$-MeOH	$C_9H_6Cl_2N_2S_2$
2v	112-113	62	EtOH	$C_{16}H_{13}Cl_2N_3S$
2w	138-139	71	EtOH	$C_{12}H_{11}Cl_2N_3O_2S$
2x	179-181	68	$CHCl_3$-petr. ether	$C_{10}H_4Cl_2N_2S$
2y	157-160	50	Me_2CO	$C_{14}H_8Cl_2N_2S$
2z	171-173	82	$CHCl_3$-MeOH	$C_{13}H_6Cl_2N_2OS$

[a] Substituents R_1 and R_2 are alphabetized in Table I. [b] See footnote *b*, Table I. [c] C: calcd, 58.45; found, 58.02. [d] N: calcd, 10.11; found, 10.52.

By a similar method, other 2,4-dichloroderivatives (**2a-z**) were obtained, except for **2h,i**. The new compounds are listed in Table V.

2,4,5-Trichloro-6-methylthieno[2,3-*d*]pyrimidine (2i). A mixture of 21.9 g (100 mmol) of **2c** and 27.0 g (200 mmol) of *N*-chlorosuccinimide in 400 mL of glacial AcOH was heated at 80–90 °C for 2 h with stirring. After cooling, the mixture was concentrated to dryness in vacuo. The residue was mixed with 500 mL of 5% $NaHCO_3$ solution and a solid separated, which was collected, washed with H_2O, and dried to give 13.8 g (55%) of **2i**: mp 151–152 °C (Me_2CO); IR (KBr) 1535, 1480 cm^{-1}; NMR ($CDCl_3$) δ 2.62 (s, 3 H, CH_3). Anal. ($C_7H_3Cl_3N_2S$) C, H, N.

2,4,6-Trichloro-5-methylthieno[2,3-*d*]pyrimidine (**2h**) was obtained by a similar procedure in 57% yield: mp 134–135 °C (Me_2CO); IR (KBr) 1530, 1465 cm^{-1}; NMR ($CDCl_3$) δ 2.65 (s, 3 H, CH_3). Anal. ($C_7H_3Cl_3N_2S$) C, H, N.

2-Chloro-3,4,5,6,7,8-hexahydro[1]benzothieno[2,3-*d*]pyrimidine (5m). To a solution of 9.07 g (35 mmol) of **2m** in 100 mL of $CHCl_3$ and 40 mL of EtOH was added portionwise 7.95 g (210 mmol) of $NaBH_4$. The mixture was heated at 40–50 °C for 14 h with stirring. The solvent was evaporated and the residue was treated with H_2O. An insoluble solid was collected and washed with H_2O and EtOH to give 5.88 g (74%) of **5m**: mp 141–143 °C ($CHCl_3$–petroleum ether); IR (KBr) 3140, 1580 cm^{-1}; NMR ($CDCl_3$–Me_2SO-d_6) δ 1.5–2.0 (m, 4 H, C_6- and C_7-CH_2), 2.1–2.45 (m, 2 H, C_5-CH_2), 2.45–2.8 (m, 2 H, C_8-CH_2), 4.58 (s, 2 H, C_4-CH_2). Anal. ($C_{10}H_{11}ClN_2S$) C, H, N.

Compounds **5–7** except for **6p** and **6r**, were similarly prepared and are listed in Table VI.

7-(Benzyloxy)-2-chloro-3,4,5,6,7,8-hexahydro[1]benzothieno[2,3-*d*]pyrimidine (6r). To a mixture of 1.50 g (4.1 mmol) of **2r** in 20 mL of THF containing 0.4 mL of H_2O was added 0.78 g (20.5 mmol) of $NaBH_4$. The mixture was heated under reflux with stirring for 30 min and to the mixture was added another 0.4 mL of H_2O. The mixture continued to reflux for another 1.5 h. The solvent was evaporated, and the residue was treated as described above to give 0.87 g (64%) of **6r**.

Compound **6p** was similarly prepared. The results are shown in Table VI.

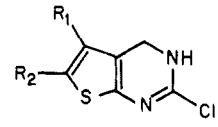
1,2,3,5,6,7,8,9-Octahydro[1]benzothieno[2,3-*d*]imidazo[1,2-*a*]pyrimidin-2-one Hydrochloride (9m). A mixture of 56.7 g (0.25 mol) of **5m**, 45.9 g (0.275 mol) of ethyl bromoacetate, and 104 g (0.75 mol) of finely powdered K_2CO_3 in 1.5 L of MeCOEt was heated under reflux with vigorous stirring for 14 h under an atmosphere of nitrogen. After the mixture cooled, an insoluble material was filtered off and washed with MeCOEt. The combined filtrate and washings were concentrated to dryness in vacuo to give an oily residue. The crude oil was dissolved in 400 mL of a 10% NH_3–EtOH solution, and the solution was heated at 120–130 °C for 5–8 h under an atmosphere of nitrogen in a sealed tube. After the solution cooled, a solid separated, which was collected, washed with H_2O, and dried to give 50.3 g (71%) of the free base of **9m**, which was converted to the hydrochloride by treatment with 5% HCl–EtOH solution: mp 257–259 °C dec (MeOH); IR (KBr) 1780, 1680, 1590, 1420 cm^{-1}; NMR (CF_3CO_2H) δ 1.6–2.3 (m, 4 H, C_7- and C_8-CH_2), 2.15–2.8 (m, 4 H, C_6- and C_9-CH_2), 4.55 (s, 2 H, C_3-CH_2), 4.80 (s, 2 H, C_5-CH_2). Anal. ($C_{12}H_{14}ClN_3OS$) C, H, N.

Other compounds (**9–15**) listed in Table I were similarly prepared.

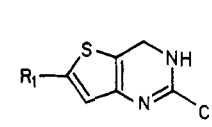
8-Hydroxy-1,2,3,5,6,7,8,9-octahydro[1]benzothieno[2,3-*d*]imidazo[1,2-*a*]pyrimidin-2-one Hydrochloride (16). The free base of 0.50 g (1.4 mmol) of **9r** was catalytically hydrogenated over 0.6 g of 10% Pd/C in 70 mL of MeOH containing 0.5 mL of concentrated HCl. After hydrogen absorption had been ceased, the catalyst was filtered off and washed with MeOH. The filtrate and washings were concentrated to give 0.13 g (31%) of the hydrochloride of **16**.

1,2,3,5,6,7,8,9-Octahydropyrido[4',3':4,5]thieno[2,3-*d*]imidazo[1,2-*a*]pyrimidin-2-one Hydrochloride (17). A mixture of 0.55 g (1.7 mmol) of **9w** in 45 mL of concentrated HCl was

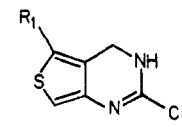
Table VI. 2-Chloro-3,4-dihydrothienopyrimidine Derivatives (**5-7**)



5[a]



6[a]



7[a]

no.	mp,[b] °C	yield, %	recrystn solvent	formula[c]
5a	146-148	84	$CHCl_3$-petr. ether	$C_6H_5ClN_2S$
5b	162-164	81	Me_2CO	$C_7H_7ClN_2S$
5c	unclear	49	Me_2CO	$C_7H_7ClN_2S$
5d	170-172	71	$CHCl_3$-EtOH	$C_8H_9ClN_2S$
5e	135-138	80	$CHCl_3$-petr. ether	$C_{10}H_{13}ClN_2S$
5f	102-105	72	$CHCl_3$-petr. ether	$C_{12}H_{17}ClN_2S$
5g	unclear	92	$CHCl_3$-petr. ether	$C_{11}H_{15}ClN_2S$
5h	unclear	71	Me_2CO	$C_7H_6Cl_2N_2S$[d]
5i	172-175	93	Me_2CO	$C_7H_6Cl_2N_2S$
5j	148-149	63	$CHCl_3$-petr. ether	$C_{12}H_9ClN_2S$
5k	151-153	68	Me_2CO	$C_9H_9ClN_2S$
5l	144-146	75	$CHCl_3$-petr. ether	$C_{11}H_{13}ClN_2S$
5n	unclear	76	$CHCl_3$-petr. ether	$C_{11}H_{13}ClN_2S$
5o	unclear	72	MeOH	$C_{11}H_{13}ClN_2S$
5p	unclear	71	$CHCl_3$-EtOH	$C_{14}H_{19}ClN_2S$
5q	unclear	68	MeOH	$C_{17}H_{17}ClN_2S$
5r	135-140	64	$CHCl_3$-petr. ether	$C_{17}H_{17}ClN_2OS$
5s	unclear	89	Me_2CO	$C_9H_9ClN_2OS$
5t	157-160	86	$CHCl_3$-petr. ether	$C_9H_9ClN_2S_2$
5u	unclear	50	$CHCl_3$-petr. ether	$C_9H_9ClN_2S_2$
5v	unclear	48	Me_2CO	$C_{16}H_{16}ClN_3S$
5w	unclear	28	Me_2CO	$C_{12}H_{14}ClN_3O_2S$
5x	173-177	83	MeOH	$C_{10}H_7ClN_2S$
5y	157-160	73	$CHCl_3$-petr. ether	$C_{14}H_{11}ClN_2S$
5z	207-210	93	$CHCl_3$-MeOH	$C_{13}H_9ClN_2OS$[e]
6a	138-140	84	$CHCl_3$-petr. ether	$C_6H_5ClN_2S$
6b	110-115	86	$CHCl_3$-petr. ether	$C_7H_7ClN_2S$
7a	130-131	87	Me_2CO	$C_6H_5ClN_2S$
7b	158-171	80	$CHCl_3$-Et_2O	$C_7H_7ClN_2S$

[a] Substituents R_1 and R_2 are alphabetized in Table I. [b] Some of the compounds did not show a clear melting point because of unstability under the heating. [c] See footnote *b*, Table I. [d] C: calcd, 38.02; found, 38.54. [e] C: calcd, 56.42; found, 56.00.

heated under reflux under an atmosphere of nitrogen for 15 h and concentrated to dryness in vacuo. The residue was triturated with Me_2CO to give 0.25 g (46%) of 17.

2,3,6,7,8,9-Hexahydro[1]benzothieno[2,3-*d*]imidazo[1,2-*a*]pyrimidin-2-one Hydrochloride (18). To a solution of 5.0 g (17.5 mmol) of **9m** in 1.5 L of MeOH was introduced air at 50–55 °C for 24 h. The solution was treated with charcoal and concentrated in vacuo to give 2.4 g (49%) of 18: mp 263–265 °C dec (MeOH); IR (KBr) 1785, 1650, 1545, 1360 cm^{-1}; NMR (CF_3CO_2H) δ 1.85–2.4 (m, 4 H, C_7- and C_8-CH_2), 2.4–3.2 (m, 4 H, C_6- and C_9-CH_2), 5.64 (s, 2 H, C_3-CH_2), 9.15 (s. 1 H, C_5-CH). Anal. ($C_{12}H_{12}ClN_3OS$) C, H, N.

Biological Method. Test Animals. Male Wistar-Imamichi rats weighing 250 g (8 weeks old) were used as routine test animals in our laboratory.

Preparation of Platelet-Rich Plasma. Blood was taken from the carotid artery of rats into a plastic syringe containing 0.1 volume of 3.13% sodium citrate dihydrate solution under pentobarbital anesthesia (Nembutal, Abbott Laboratories, 40 mg/kg, ip). The citrated blood was centrifuged at 230*g* for 7 min at room temperature to obtain platelet-rich plasma (PRP). The sediment was further centrifuged at 1500*g* for 10 min to obtain platelet-poor plasma (PPP). The platelet count of PRP was adjusted to approximately $5 \times 10^5/\mu L$ by adding PPP.

Platelet Aggregation Test in Vitro. Platelet aggregation was measured by a Bryston Aggregometer at 30 °C under stirring at 1100 rpm. Aggregation was initiated by adding 50 μL of an aggregating agent to 0.45 mL of PRP. The agents used were 10 μM ADP (Sigma Chemical Co.) in 25 mM Tris-HCl, 0.13 M NaCl buffer solution, pH 7.4, containing 10 mM $CaCl_2$ and a collagen suspension, which was prepared by homogenizing collagen (bovine achilles tendon, Sigma Chemical Co.) in the Tris buffer, centrifuging the homogenate at 200*g* for 5 min, and diluting supernatant with the Tris buffer to give 0.270 of absorbancy at 420 nm.[13] The fine collagen suspension of this strength induced aggregation after a lag period of 2–3 min. The compound to be tested for antiaggregatory action was added as a saline or MeOH solution in a volume of 5 μL to PRP before addition of the aggregating agent.

Platelet aggregation was expressed by the maximum decrease in absorbancy of PRP (extent, ΔA) for ADP-induced aggregation and by the maximum rate of decrease in absorbancy at the initial aggregation phase (rate, ΔA/min) for collagen-induced aggregation.[13]

Platelet Aggregation Test ex Vivo. The compounds to be tested were dissolved or suspended in 0.5% Tween 80 solution and given to rats fasted overnight at a dose of (50 mg/10 mL)/kg of body weight. Control rats received vehicle alone (0.5% Tween 80, 10 mL/kg). At the time indicated, blood samples were taken to prepare PRP under pentobarbital anesthesia as described above. Platelet aggregation was tested as in the in vitro test, and the antiaggregatory action of the test compounds was expressed as inhibition percent, calculated from comparison of the platelet aggregation between the test and control rats.[13]

Measurement of Blood Pressure and Heart Rate. The compounds to be tested were suspended in 0.5% CMC solution, except for **9m** which was suspended in 0.5% Tween 80 solution. They were orally given to rats at a dose of (50 mg/7.5 mL)/kg of body weight. Systolic blood pressure and heart rate were measured by the tail-cuff method[14] with a blood pressure recorder 8002 (W+W Electronic Inc.) after prewarming the rats at 55–60 °C for 3 min prior to and at specified time intervals after the administration of a test compound.

(13) S. Ashida and Y. Abiko, *Thromb. Haemostasis*, **40**, 542 (1978).
(14) M. Gerold and H. Tschirky, *Arzneim.-Forsch.*, **18**, 1285 (1968).

Antihypertensive Activity of 6-Arylpyrido[2,3-*d*]pyrimidin-7-amine Derivatives

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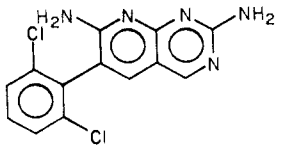
Received October 6, 1980

A series of 51 6-arylpyrido[2,3-*d*]pyrimidin-7-amine derivatives was prepared and evaluated for antihypertensive activity in the conscious spontaneously hypertensive rat. A number of these compounds, notably 6-(2,6-dichlorophenyl)-2-methylpyrido[2,3-*d*]pyrimidin-7-amine (**36**), lowered blood pressure in these rats in a gradual and sustained manner to normotensive levels at oral doses of 10–50 mg/kg. Normalized blood pressure levels could then be maintained by single daily oral doses. The effect of structural variation in the 6-aryl group and in the 2 and 4 positions of the pyridopyrimidine ring on activity is reported and discussed.

In the course of an ongoing program to develop novel agents for the treatment of hypertension, we had occasion to reexamine certain compounds in a series of pyrido[2,3-*d*]pyrimidine-2,7-diamines, disclosed previously from these laboratories as potent potassium-sparing diuretics.[1] One compound in particular, 6-(2,6-dichlorophenyl)-pyrido[2,3-*d*]pyrimidine-2,7-diamine (**1**), showed promising



1

antihypertensive effects when tested in the spontaneously hypertensive rat (SHR). The magnitude of this effect was clearly greater than that previously observed with known diuretic substances, suggesting that **1** might be working through a mechanism other than diuresis and the associated blood volume reduction. Examination of other potent diuretics of this series supported this possibility, since none possessed comparable antihypertensive effects in this model.[2]

The present study was undertaken to prepare analogues of **1** to explore further the relationship between the diuretic and antihypertensive effects in this series of compounds. The combination of effects might well be desirable, considering the effectiveness and popularity of antihypertensive regimens which combine a diuretic agent with another hypotensive agent operating by a different mechanism of action.[3] However, it was recognized that

(1) J. Davoll, U.S. Patent 3 534 039 (1970).
(2) Unpublished results.

0022-2623/81/1824-0382$01.25/0