6-Aryl-4,5-dihydro-3(2*H*)-pyridazinones. A New Class of Compounds with Platelet Aggregation Inhibiting and Hypotensive Activities†

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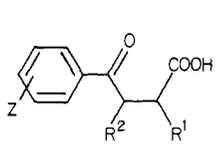
This paper reports on the synthesis and pharmacological activity of 6-aryl-4,5-dihydro-3(2*H*)-pyridazinone derivatives. The compounds exhibit an aggregation inhibiting action on human platelets in vitro and on rat platelets under ex vivo conditions, as well as a hypotensive action on rats. The strongest pharmacological effects were found with dihydropyridazinones, which have a 6-[*p*-[(chloroalkanoyl)amino]phenyl] substituent, together with a methyl group in the 5-position. The antiaggregation activity of compounds of this type is in vitro up to 16 000 times and ex vivo up to 370 times greater than that of acetylsalicylic acid; the hypotensive action is up to 40 times as great as that of the comparative substance dihydralazine.

The intravascular formation of platelet aggregates is an important pathogenetic factor in widespread cardiovascular diseases, such as myocardial and cerebral circulatory disorders, venous thromboses, cardiac and cerebral infarcts, and arteriosclerosis, and also in the risk of embolism in surgery on the heart and blood vessels. In these fields, the discovery of aggregation inhibiting drugs has revealed a new approach to therapy and prophylaxis.[1] The active compounds hitherto available for this indication, such as acetylsalicylic acid, sulfinpyrazone, and dipyridamole, however, have a relatively low activity, so that extended therapy proves unsatisfactory.

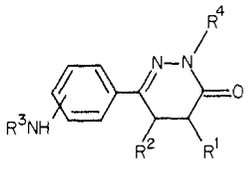
We present in this paper a new category of compounds, namely, 6-aryl-4,5-dihydro-3(2*H*)-pyridazinones II, that exhibit a strong platelet aggregation inhibiting action coupled with a hypotensive action. It is interesting that this pattern of action corresponds to that of prostacyclin, a prostaglandin that is present in the body and is regarded as a natural protective factor against undesirable thrombus formation but that, because of its short half-life, presents considerable difficulties in therapeutic use.[2]

The very first report of BASF on pharmacologically valuable dihydropyridazinones II appeared several years ago.[3] Among the substances cited were the compounds II ($R^1 = R^2 = R^4 = H$; R^3NH = *p*-H_2N, *p*-CH_3CONH, *p*-CH_3CH_2CONH, *m*-H_2N, or *m*-CH_3CONH). The three para-substituted dihydropyridazinones (compounds **1**, **3**, and **4**; Table I) were conspicuous in exerting a strong hypotensive action on anesthetized rats. The two meta compounds (dihydropyridazinones **11** and **12**; Table I) were less active. In more detailed pharmacological investigations, we have been able to demonstrate that the para-substituted substances **3** and **4** additionally exhibit, in vitro, a strong inhibiting effect on the collagen-induced and ADP-induced aggregation of human platelets. We report here on the synthesis and pharmacological action of new dihydropyridazinones II, as well as of those previously described.

Chemistry. Two methods were used to prepare the new dihydropyridazinones II and the five previously known



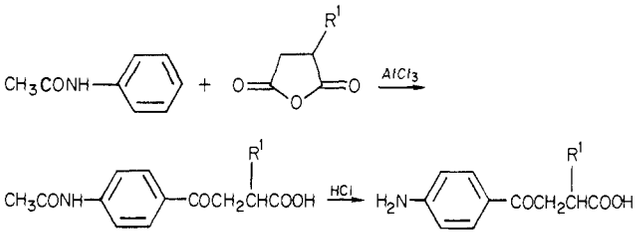
I



II

compounds. The first method entails the synthesis of a γ-keto acid I, where the radical Z is R^3NH, and its cyclization by means of hydrazine hydrate or methylhydrazine.[3,4] In the second method, a γ-keto acid I, where Z is a group that can be converted to R^3NH, is cyclized with hydrazine hydrate or methylhydrazine. Z is then converted to R^3NH.[3] Table I shows the known dihydropyridazinones **1**, **3**, **4**, **11**, and **12** and all the new compounds II and shows the method of synthesis followed for each substance.

Scheme I



40, R^1 = H; 44, R^1 = CH_3 41, R^1 = H; 45, R^1 = CH_3

In BASF, the work on the dihydropyridazinones II had been preceded by the development of a novel version of the Friedel–Crafts reaction. The Friedel–Crafts acylation of benzene or of suitable monosubstituted benzenes with succinic anhydride in the presence of aluminum chloride permits the preparation of γ-keto acids of type I, where R^1 and R^2 are hydrogen and Z is hydrogen or a para substituent.[5] With acetanilide as the benzene derivative, the reaction is lengthy under conventional reaction conditions, and only moderate yields are obtained.[6] The new version of the reaction, carried out in a dimethylformamide/aluminum chloride melt, on the other hand, allowed the γ-keto acid I ($R^1 = R^2 = H$; Z = p-CH_3CONH) (compound **40**) to be prepared rapidly and in good yield (see Experimental Section).[7] Hydrolysis of the acetylamino group of **40** with hydrochloric acid gave the amino acid **41**[6] (Scheme I) (the intermediate γ-keto acids are described in Table II). We obtained, via **40** and **41**, all those di-

† This paper has been compiled in honor of Professor Matthias Seefelder on the occasion of his 60th birthday.

(1) A. S. Gallus, *Drugs*, 18, 439 (1979).
(2) S. Moncada, R. Gryglewski, S. Bunting, and J. R. Vane, *Nature (London)*, **263**, 663 (1976).
(3) G. Bachmann and A. Amann, French Patent 1 507 475 (1967) to BASF; *Chem. Abstr.*, **70**, 37824*t* (1969).
(4) E. A. Steck, R. P. Brundage, and L. T. Fletcher, *J. Am. Chem. Soc.*, **75**, 1117 (1953); J. W. Mason and D. L. Aldous, *Chem. Heterocycl. Compd.*, **28**, 23 (1973).
(5) A. G. Peto in "Friedel-Crafts and Related Reactions", Vol. III, G. A. Olah, Ed., Interscience, New York, 1964, p 535; E. Berliner, *Org. React.*, **5**, 229 (1949).
(6) J. P. English, R. C. Clapp, Q. P. Cole, and J. Krapcho, *J. Am. Chem. Soc.*, **67**, 2263 (1945).
(7) G. Bachmann, unpublished work.

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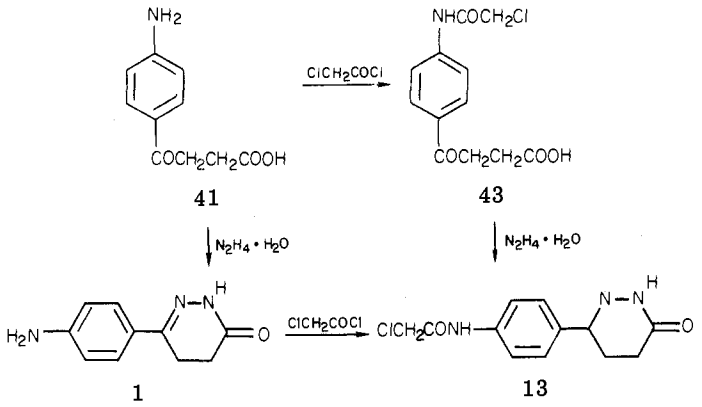
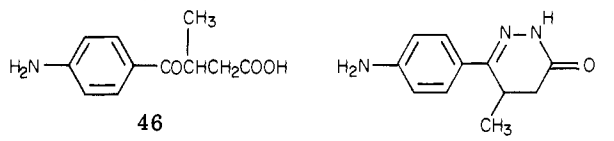
Scheme II



hydropyridazinones II in which R^1 and R^2 are hydrogen and R^3NH is a para substituent. Cyclization of **40** and **41** with hydrazine hydrate gave the amino- and (acetylamino)dihydropyridazinones **1** and **3**, respectively. Heating **1** in formic acid gave the formylamino compound **2**.[8] Treating the amino acid **41** with formic acid led to the γ-keto acid **42**, which with hydrazine hydrate again gave **2**. The (alkanoylamino)phenyl derivatives **4–6** were synthesized by acylating **1** with the corresponding acid chloride. Cyclizing the γ-keto acids **40** and **41** with methylhydrazine gave the 2-methyldihydropyridazinones **7** and **8**. Compound **8** was also prepared by treating the amino compound **7** with acetic anhydride. We obtained the [(chloroalkanoyl)amino]phenyl derivatives **13** to **18**[9,10] by acylating **1** with chloroalkanoyl chlorides. It is also possible to synthesize these compounds, as was shown for the example of **13**, by first acylating the amino acid **41** and then carrying out the cyclization (Scheme II). Treating the 2-methyldihydropyridazinone **7** with chloroacetyl chloride gave the (chloroacetyl)amino compound **19**. The amino acid derivatives **33** to **39**[9] were prepared by reacting the above [[(chloroacetyl)amino]phenyl]dihydropyridazinone **13** with amines.

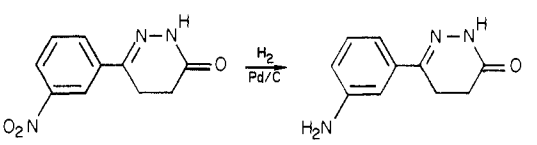
Friedel–Crafts acylation of acetanilide with methylsuccinic anhydride in a dimethylformamide/aluminum chloride melt gave the α-methyl keto acid **44** (Scheme I). This acetylamino derivative, like **40**, was convertible to the corresponding amino compound **45** by treating it with hydrochloric acid. We used the resulting γ-keto acids **44** and **45** as starting substances for the synthesis of the 4-methyldihydropyridazinones **9**, **10**, **20**, and **21**.[11] Compounds **9** and **10** are the products of cyclizing compounds **45** and **44** with hydrazine hydrate. We prepared the (chloroalkanoyl)amino compounds **20** and **21** by acylating the (aminophenyl)dihydropyridazinone **9** with chloroacetyl chloride and 2-chloropropionyl chloride. The α-methyl keto acid **44** and the 4-methyldihydropyridazinones **9** and **10** have also been described independently, at a slightly later date, by Curran and Ross.[12] These authors also synthesized **44** by a Friedel–Crafts acylation of acetanilide with methylsuccinic anhydride. They also presented proof that in the keto acid obtained, the methyl group is in the α-position and not in the β-position. Accordingly, a discussion of the structure of this Friedel–Crafts product is unnecessary.

We have also prepared some compounds of the category of the dihydropyridazinones II ($R^1 = R^4 = H$; $R^2 = CH_3$) (5-methyldihydropyridazinones). They are compounds **22** to **32**,[10] which all have a (haloalkanoyl)amino group in the para position of the phenyl ring. Two methods, which had already been used to prepare compound **13**, were used to synthesize **23** (see Scheme II). The one method involves acylating the known β-methyl keto acid **46**[12,13] with 2-



chloropropionyl chloride and cyclizing the resulting acylamino compound **47** with hydrazine hydrate. The second method comprises acylating the (aminophenyl)dihydropyridazinone **48**[12] with 2-chloropropionyl chloride. We synthesized the 5-methyldihydropyridazinones **22** and **24–32** by treating **48** with a chloroalkanoyl halide or bromoalkanoyl halide.

The (*m*-aminophenyl)dihydropyridazinone **11** was prepared by hydrogenating 6-(*m*-nitrophenyl)-4,5-dihydro-3-(2*H*)-pyridazinone (**49**)[12] in the presence of a Pd/C cata-



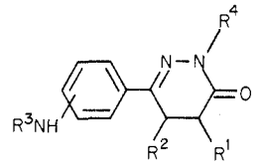
lyst. Treatment of **11** with acetyl chloride gave [*m*-(acetylamino)phenyl]dihydropyridazinone **12**.

Biology. Effect on Platelet Aggregation in Vitro (Table I). The collagen-induced and ADP-induced aggregation of human platelets in vitro is markedly inhibited by the (*p*-aminophenyl)dihydropyridazinone **1**, the *p*-(formylamino)phenyl compound **2**, and the [*p*-(alkanoylamino)phenyl]dihydropyridazinones **3–6**. In none of the investigations presented here is there a significant difference between the inhibition of collagen-induced aggregation and the inhibition of ADP-induced aggregation, so that there is no need for a differentiating assessment. The activity of compounds **1–6** surpasses that of acetylsalicylic acid, which is only effective in the case of collagen-induced aggregation, by factors of 33–668 (prostacyclin, the most effective inhibitor of platelet aggregation, and indomethacin, an inhibitor of platelet-cyclooxygenase, which, however, is not used in the therapy of thrombotic diseases, are about 300000 times and 2.5 times, respectively, more active than acetylsalicylic acid under our experimental conditions). The alkanoylamino derivatives **3–6** are more active than the amino compound **1**; the propionylamino compound **4** evidently represents an optimum, while further lengthening of the side chain (compounds **5** and **6**) diminishes the effect.

Moving the side chain of the (acetylamino)dihydropyridazinone **3** from the para position to the meta position

(8) R. Lebkücher, H. König, A. Amann, H. Giertz, and J. Schuster, German Laid-Open Patent Application DOS 2150685 (1973) to BASF; *Chem. Abstr.*, **79**, 32075*a* (1973).
(9) R. Lebkücher, H. König, A. Amann, H. Giertz, and J. Schuster, German Laid-Open Patent Application DOS 2123246 (1972) to BASF; *Chem. Abstr.*, **78**, 58445*a* (1973).
(10) R. Lebkücher, M. Thyes, H. König, H. D. Lehmann, J. Gries, D. Lenke, and J. Kunze, German Laid-Open Patent Application DOS 2727481 (1979) to BASF; *Chem. Abstr.*, **90**, 137849*m* (1979).
(11) R. Lebkücher, H. König, A. Amann, and H. Giertz, German Laid-Open Patent Application DOS 2304977 (1974), corresponding to Application P 2304977.6 (filed Feb. 1, 1973) to BASF; *Chem. Abstr.*, **81**, 136169*s* (1974).
(12) W. V. Curran and A. Ross, *J. Med. Chem.*, **17**, 273 (1974).
(13) F. J. McEvoy and G. R. Allen, Jr., *J. Org. Chem.*, **38**, 4044 (1973).

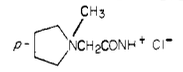
Table I. 6-Aryl-4,5-dihydro-3(2*H*)-pyridazinones: Chemical Data, Inhibition of Platelet Aggregation in Vitro, and Hypotensive Action



compd	R^3NH	R^2	R^1	R^4	method[a]	% yield[b]	mp, °C	recrystn solvent
A. 6-(Aminophenyl), 6-[(Formylamino)phenyl], and 6-[(Alkanoylamino)phenyl] Derivatives								
1	*p*-H_2N	H	H	H	A	65	237-238[h,i]	*j*
2	*p*-OHCNH	H	H	H	A	91	242.5-243.5[h]	
					B_1	77	237-243[h]	*l*
3	*p*-CH_3CONH	H	H	H	A	73	250-251[n]	*j*
4	*p*-CH_3CH_2CONH	H	H	H	B_2	79	238-239[o]	*j*
5	*p*-$CH_3(CH_2)_2CONH$	H	H	H	B_2	67	241-242	*j*
6	*p*-$CH_3(CH_2)_3CONH$	H	H	H	B_2	54	222-223	*j*
7	*p*-H_2N	H	H	CH_3	A	51	219-220	*l*
8	*p*-CH_3CONH	H	H	CH_3	A	68	207.5-208.5	*l*
					B_3	74	207-208	AcOEt
9	*p*-H_2N	H	CH_3	H	A	66	236-239[p]	*j*
10	*p*-CH_3CONH	H	CH_3	H	A	72	214-215[q]	EtOH
11	*m*-H_2N	H	H	H	B_4	77	167-168[r]	*l*
12	*m*-CH_3CONH	H	H	H	B_2	54	211-212[s]	*l*
B. 6-[[(Haloalkanoyl)amino]phenyl] Derivatives								
13	*p*-$ClCH_2CONH$	H	H	H	A	71	227-229[h]	*j*
					B_2	85	233[h]	*j*
14	*p*-$CH_3CH(Cl)CONH$	H	H	H	B_2	49	243-244[h]	(1) *j*, (2) *l*
15	*p*-$ClCH_2CH_2CONH$	H	H	H	B_2	74	235-236[h]	*j*
16	*p*-$CH_3CH_2CH(Cl)CONH$	H	H	H	B_2	42	184	(1) *j*, (2) AcOEt
17	*p*-$Cl(CH_2)_3CONH$	H	H	H	B_2	45	190-191	*j*
18	*p*-$ClCH_2C(CH_3)_2CONH$	H	H	H	B_2	27	200-202	*j*
19	*p*-$ClCH_2CONH$	H	H	CH_3	B_2	88	233-234	*l*
20	*p*-$ClCH_2CONH$	H	CH_3	H	B_2	92	from 247[h]	*l*
21	*p*-$CH_3CH(Cl)CONH$	H	CH_3	H	B_2	52	229-230	*j*
22	*p*-$ClCH_2CONH$	CH_3	H	H	B_2	43	235.5-236.5	*t*
23	*p*-$CH_3CH(Cl)CONH$	CH_3	H	H	A	87	222-223	
					B_2	54	229-230	(1) *l*, (2) *j*
24	*p*-$ClCH_2CH_2CONH$	CH_3	H	H	B_2	51	221-223[h]	MeOH
25	*p*-$CH_3CH_2CH(Cl)CONH$	CH_3	H	H	B_2	36	223-224	*j*
26	*p*-$CH_3CH(Cl)CH_2CONH$	CH_3	H	H	B_2	52	243-244	*l*
27	*p*-$Cl(CH_2)_3CONH$	CH_3	H	H	B_2	47	176-178	MeOH
28	*p*-$ClCH_2C(CH_3)_2CONH$	CH_3	H	H	B_2	42	203-204	*j*
29	*p*-$CH_3CH(Br)CONH$	CH_3	H	H	B_2	35	223-224[h]	*j*
30	*p*-$CH_3CH_2CH(Br)CONH$	CH_3	H	H	B_2	24	213-214[h]	(1) *v*, (2) *j*
31	*p*-$(CH_3)_2C(Br)CONH$	CH_3	H	H	B_2	34	205-206[h]	(1) *j*, (2) *w*
32	*p*-$BrCH_2C(CH_3)_2CONH$	CH_3	H	H	B_2	32	189-190	*j*
C. Amino Acid Derivatives								
33	*p*-$CH_3(CH_2)_2NHCH_2CONH\cdot HCl$	H	H	H	B_5	47	272-274[h]	
34	*p*-$(CH_3)_2CHNHCH_2CONH\cdot HCl$	H	H	H	B_5	44	260-261[h]	
35	*p*-$C_2H_5CH(CH_3)NHCH_2CONH\cdot HCl$	H	H	H	B_5	57	263[h]	
36	*p*-$CH_2{=}CHCH_2NHCH_2CONH\cdot HCl$	H	H	H	B_5	41	231[h]	

emp formula[c]	inhibn of platelet aggregation in vitro[d] collagen EC_{50}, mg/L (CL)[k]	rel act.[e]	ADP EC_{50}, mg/L (CL)[k]	hypotensive act.[f] ED_{20}, mg/kg, ip (CL)[k]	rel act.[g]
$C_{10}H_{11}N_3O$	14.8 (10.9/20.1)	33	46.4 (34.2/63.1)	0.18 (0.12/0.26)	1.88
$C_{11}H_{11}N_3O_2$[m]	5.27 (3.90/7.13)	94	3.74 (2.89/4.83)	0.32 (0.24/0.43)	1.06
$C_{12}H_{13}N_3O_2 \cdot 0.25H_2O$	1.09 (0.67/1.77)	453	1.00 (0.56/1.81)	0.20 (0.16/0.24)	1.69
$C_{13}H_{15}N_3O_2 \cdot 0.5H_2O$	0.74 (0.64/0.86)	668	0.64 (0.50/0.84)	0.060 (0.034/0.11)	5.63
$C_{14}H_{17}N_3O_2$	1.45 (1.19/1.76)	341	1.44 (1.22/1.69)	~0.46	~0.74
$C_{15}H_{19}N_3O_2$	3.34 (2.95/3.79)	148	2.64 (2.14/3.26)	~1.00	~0.34
$C_{11}H_{13}N_3O$	>46.4	<11	>46.4	>1.00	<0.34
$C_{13}H_{15}N_3O_2 \cdot 0.33H_2O$	>46.4	<11	>46.4	~1.00	~0.34
$C_{13}H_{15}N_3O_2$					
$C_{11}H_{13}N_3O$	46.9 (39.9/55.2)	11	43.8 (36.0/53.2)	>1.00	<0.34
$C_{13}H_{15}N_3O_2 \cdot H_2O$	~46.4	~11	~46.4	>1.00	<0.34
$C_{10}H_{11}N_3O$	>46.4	<11	>46.4	>1.00	<0.34
$C_{12}H_{13}N_3O_2$	>46.4	<11	>46.4	>1.00	<0.34
$C_{12}H_{12}ClN_3O_2$	0.25 (0.18/0.35)	1976	0.39 (0.30/0.53)	~0.37	~0.91
$C_{13}H_{14}ClN_3O_2$	0.28 (0.19/0.41)	1764	0.45 (0.33/0.62)	0.53 (0.44/0.64)	0.64
$C_{13}H_{14}ClN_3O_2$	0.90 (0.78/1.03)	549	0.98 (0.87/1.1)	>1.00	<0.34
$C_{14}H_{16}ClN_3O_2$	3.16 (2.94/3.41)	156	1.47 (1.25/1.73)	~0.37	~0.91
$C_{14}H_{16}ClN_3O_2$	>46.4	<11	>46.4	>1.00	<0.34
$C_{15}H_{18}ClN_3O_2$	9.24 (7.96/10.7)	54	11.4 (8.50/15.39)	>1.00	<0.34
$C_{13}H_{14}ClN_3O_2$	>46.4	<11	>46.4	>1.00	<0.34
$C_{13}H_{14}ClN_3O_2$	6.31 (5.09/7.83)	78	21.9 (16.3/29.5)	>1.00	<0.34
$C_{14}H_{16}ClN_3O_2$	33.8 (28.4/40.3)	15	32.9 (27.9/38.7)	>1.00	<0.34
$C_{13}H_{14}ClN_3O_2$	0.11 (0.042/0.28)	4490	0.15 (0.11/0.21)	0.15 (0.12/0.18)	2.25
$C_{14}H_{16}ClN_3O_2$[u]	0.031 (0.022/0.044)	15 936	0.099 (0.063/0.16)	0.0081 (0.0056/0.012)	41.7
$C_{14}H_{16}ClN_3O_2$	0.30 (0.22/0.41)	1647	0.16 (0.14/0.18)	0.044 (0.033/0.059)	7.68
$C_{15}H_{18}ClN_3O_2$	0.069 (0.061/0.077)	7159	0.035 (0.029/0.041)	0.65 (0.45/0.94)	0.52
$C_{15}H_{18}ClN_3O_2$	3.29 (2.87/3.78)	150	4.78 (4.26/5.36)	0.187 (0.14/0.25)	1.81
$C_{15}H_{18}ClN_3O_2$	1.66 (1.23/2.22)	298	1.63 (1.24/2.13)	~0.32	~1.06
$C_{16}H_{20}ClN_3O_2$	0.47 (0.38/0.57)	1051	0.28 (0.19/0.42)	~0.61	~0.55
$C_{14}H_{16}BrN_3O_2$	0.58 (0.42/0.81)	852	0.26 (0.21/0.34)	0.40 (0.28/0.57)	0.85
$C_{15}H_{18}BrN_3O_2$	0.27 (0.20/0.36)	1830	0.052 (0.036/0.076)	1.49 (1.20/1.85)	0.23
$C_{15}H_{18}BrN_3O_2$	0.89 (0.62/1.28)	555	0.33 (0.22/0.50)	2.93 (1.92/4.47)	0.12
$C_{16}H_{20}BrN_3O_2$	0.44 (0.36/0.53)	1123	0.50 (0.39/0.62)	0.34 (0.27/0.40)	0.99
$C_{15}H_{20}N_4O_2 \cdot HCl$[x]	~10	~49	~46.4	>1.00	<0.34
$C_{15}H_{20}N_4O_2 \cdot HCl \cdot 1.5H_2O$	~21.5	~23	~46.4	>1.00	<0.34
$C_{16}H_{22}N_4O_2 \cdot HCl \cdot 1.5H_2O$	~21.5	~23	~21.5	>1.00	<0.34
$C_{15}H_{18}N_4O_2 \cdot HCl \cdot H_2O$	~4.64	~107	~21.5	>1.00	<0.34

Table I (*Continued*)

compd	R^3NH	R^2	R^1	R^4	method[a]	% yield[b]	mp, °C	recrystn solvent
37	*p*-$(C_2H_5)_2NCH_2CONH\cdot HCl$	H	H	H	B_5	43	234–238[h]	MeOH
38	*p*-c-$O(CH_2CH_2)_2N$-CH_2CONH	H	H	H	B_5	82	198–199	MeOH
39		H	H	H	B_5	70	267–268[h]	EtOH
acetylsalicylic acid								
indomethacin								
prostacyclin								
dihydralazine								

[a] A = cyclization of the γ-keto acid with hydrazine hydrate or methylhydrazine. B_1–B_5 = modification of a dihydropyridazinone; B_1 = formylation of 1; B_2 = acylation of the corresponding (aminophenyl)dihydropyridazinone with an acid halide; B_3 = acylation of 7 with acetic anhydride; B_4 = catalytic hydrogenation of the corresponding nitro compound; B_5 = reaction of 13 with an amine. [b] Where a recrystallization solvent is stated, the yield relates to recrystallized product. [c] All the compounds shown here gave satisfactory elementary analyses (C, H, N, and, where present, halogen). [d] In vitro inhibition of collagen-induced or ADP-induced aggregation of human platelets. [e] Relative activity, based on acetylsalicylic acid.

gives a compound (**12**) having very greatly reduced antiaggregation properties.

An increase in antiaggregation activity (by a factor of 4.4) is found on replacing the acetylamino side chain of the dihydropyridazinone **3** by a (chloroacetyl)amino group (compound **13**). Replacing, however, the propionylamino side chain of the dihydropyridazinone **4** by an (α-chloropropionyl)amino group gives a compound (**14**) having about the same activity. The relative activities of **13** and **14** compared to acetylsalicylic acid are as high as 1976 and 1764, respectively. The (β-chloropropionyl)amino compound **15** is about as active as the corresponding substance with an (α-chloropropionyl)amino side chain (compound **14**). If the (α-chloropropionyl)amino side chain of **14** is replaced by longer (chloroalkanoyl)amino groups (compounds **16–18**), the activity compared to that of **13** and **14** is greatly reduced.

Replacing a hydrogen atom in the alkanoyl part of the side chain of the dihydropyridazinone **3** by an amine radical gives compounds (amino acid derivatives **33–39**) having a weak antiaggregation action. However, with the exception of the case of compound **39**, the relative antiaggregation activity is greater by a factor of about 23–107 than that of acetylsalicylic acid.

Substitution of the 2-position (compounds **7**, **8**, and **19**) or of the 4-position (compounds **9**, **10**, **20**, and **21**) of the dihydropyridazinone ring by a CH_3 group greatly reduces the antiaggregation activity. This is also true of the (chloroalkanoyl)amino derivatives, among which compound **20**, having a relative activity of 78 compared to salicylic acid, is the most effective aggregation-inhibiting compound of this group.

The combination of a 6-[*p*-[(chloroalkanoyl)amino]-phenyl] group and a 5-methyl substituent has proved to be particularly effective. Compounds **23**, **25**, **27**, and **28** show a pronounced increase in antiaggregation activity compared to the corresponding CH_3-free substances. The relative activity increases to factors of up to 15 900 (compound **23**) [this corresponds to 5% (collagen-induced aggregation) or 18% (ADP-induced aggregation) of the activity of prostacyclin]. The position of the chlorine atom in the chloroalkanoyl group influences the antiaggregation activity: α-substitution gives more active compounds than β-substitution and γ-substitution (compounds **23**/**24**; **25**/**26**/**27**). The size of the side chain has also a substantial influence on the antiaggregation activity. In general, increasing the size of the chloroalkanoyl group to beyond three carbon atoms weakens the effect (compounds **23**/**26–28**). The (α-chlorobutyryl)amino compound **25** is, however, about as active as the (α-chloropropionyl)amino derivative **23**.

The bromine-substituted compounds (compounds **29–32**), in most cases, have a lower aggregation inhibiting activity than the corresponding chlorine derivatives (compounds **23**/**29** and **25**/**30**).

Effect on Platelet Aggregation ex Vivo. The dihydropyridazinones also show antiaggregation properties, if the substances are given orally to rats. Table III contains the results of ex vivo experiments (inhibition of the collagen-induced aggregation) for all the substances, which have EC_{50} values in vitro of less than 1.0 mg/L. The ED_{33} values range between 0.63 and 47.5 mg/kg po. This corresponds with a relative activity of 370 to 4.9 in a comparison with acetylsalicylic acid. There is no definite relation between the activity found after oral administration to rats and the effect on human platelets in vitro. The quotients of ED_{33}/EC_{50} vary between 3.9 and 158. It can be assumed that the differing quotients are due to differences in the substances' pharmacokinetics in rats.

Recently, Nakao et al.,[14] as well as Griffett et al.,[15] have reported on the aggregation inhibiting effect of dihydropyridazinones. The antiaggregation activity of these substances lies within the activity range of our own compounds. At the time being, it is not yet possible to assess to what extent there are pharmacological differences between the different dihydropyridazinones.

Effect on Blood Pressure (Table I). The (*p*-aminophenyl)dihydropyridazinone **1**, the *p*-(formylamino)phenyl compound **2**, and the [*p*-(alkanoylamino)phenyl]dihydropyridazinones **3–6** reduce the blood pressure of anesthetized rats (on intraperitoneal administration). The relative activity, taking that of dihydralazine as 1.00, is ~0.34 to 5.63. Only the propionylamino compound **4** is more active than the amino compound **1**; the dihydropyridazinones **2**, **3**, **5**, and **6** have either the same, or a lesser, activity than the amino compound **1**.

If the side chains of the amino- and (acetylamino)dihydropyridazinones, **1** and **3**, respectively, are moved from

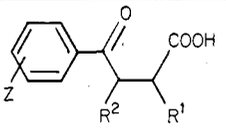
(14) T. Nakao, S. Setoguchi, and O. Yaoka, German Laid-Open Patent Application DOS 2 845 220 (1980) to Yoshitomi Pharmaceutical Industries, Ltd.

(15) E. M. Griffett, S. M. Konnon, A. Kumar, D. Lecker, G. M. Smith, and E. G. Tomich, *Br. J. Pharmacol.*, **72**, 697 (1981).

emp formula[c]	inhibn of platelet aggregation in vitro[d] collagen EC_{50}, mg/L (CL)[k]	collagen rel act.[e]	ADP EC_{50}, mg/L (CL)[k]	hypotensive act.[f] ED_{20}, mg/kg, ip (CL)[k]	hypotensive act.[f] rel act.[g]
$C_{16}H_{22}N_4O_2 \cdot HCl \cdot 0.5H_2O$	~10	~49	~10	>1.00	<0.34
$C_{16}H_{20}N_4O_3$	~21.5	~23	~46.4	>1.00	<0.34
$C_{17}H_{23}ClN_4O_2$	>46.4	<11	>46.4	>1.00	<0.34
	494 (381/639)	1.00			
	200 (169/237)	2.47			
	0.00165 (0.0014/0.0019)	299 394	0.0174 (0.0145/0.0208)	0.00067[y] (0.00052/0.00086)	
	149 (126/176)	3	310 (251/383)	0.34 (0.28/0.41)	1.00

[f] Anesthetized rat. [g] Relative activity, based on dihydralazine. [h] Decomposition. [i] Literature[3] mp 236 °C. [j] Dimethylformamide/water. [k] Confidence limits in parentheses for 95% of the measured values. [l] Propanol. [m] The product synthesized by method B_1 was subjected to elementary analysis. [n] Literature[3] mp 252 °C. [o] Literature[3] mp 237 °C. [p] Literature[12] mp 243-244 °C. [q] Literature[12] mp 219-220 °C. [r] Literature[3] mp 169 °C. [s] Literature[3] mp 208 °C. [t] Ethanol/water. [u] The product synthesized by method B_2 was subjected to elementary analysis. [v] Methanol/ether. [w] Propanol/water. [x] Only the element Cl was determined analytically. [y] Intravenous administration.

Table II. γ-Keto Acid Intermediates



compd	Z	R^2	R^1	method[a]	% yield[b]	mp, °C	recrystn solvent	emp formula[c]
40	p-CH_3CONH	H	H	A	62	202-203[d]	e	$C_{12}H_{13}NO_4$
41	p-H_2N	H	H	B	94	186-188[f,g]		$C_{10}H_{11}NO_3$
42	p-OHCNH	H	H	C	46	219-221[f]	PropOH	$C_{11}H_{11}NO_4$
43	p-$ClCH_2CONH$	H	H	D	53	184-185	acetone	$C_{12}H_{12}ClNO_4$
44	p-CH_3CONH	H	CH_3	A	40	205-206[h]	EtOH	$C_{13}H_{15}NO_4$
45	p-H_2N	H	CH_3	B	93	153-154		$C_{11}H_{13}NO_3$
47	p-$CH_3CH(Cl)CONH$	CH_3	H	D	36	139-140	i	$C_{14}H_{16}ClNO_4$

[a] A = Friedel-Crafts acylation. B = acid hydrolysis of the corresponding acetylamino compound. C = formylation of 41. D = acylation of the corresponding amino acid with an acid chloride. [b] Where a recrystallization solvent is stated, the yield relates to recrystallized product. [c] All the compounds shown here gave satisfactory elementary analyses (C, H, N, and, where present, Cl). [d] Literature[6] mp 202-205 °C. [e] Dimethylformamide/water. [f] Decomposition. [g] Literature[6] mp 189-190 °C. [h] Literature[12] mp 208-209 °C. [i] Ethyl acetate/petroleum ether (boiling range 40-60 °C).

Table III. 6-Aryl-4,5-dihydro-3(2H)-pyridazinones: Inhibition of Rat Platelet Aggregation (Collagen-Induced) ex Vivo

compd	ED_{33}, mg/kg, po (CL)[a]	rel act.[b]	ED_{33}/EC_{50}[c]
4	41.6 (30.8/56.2)	5.6	56.3
13	15.7 (9.89/24.9)	14.8	62.8
14	22.6 (19.5/26.2)	10.3	82.7
15	41.7 (27/63)	5.6	46.3
22	12.2 (6.48/28.1)	19.1	111
23	0.63 (0.09/4.51)	370	20.3
24	47.5 (39.2/57.7)	4.9	158
25	0.66 (0.486/0.897)	353	9.6
28	5.00 (4.19/5.97)	46.6	10.6
29	8.18 (5.48/12.2)	28.5	14.1
30	2.58 (1.86/3.57)	90.3	9.6
31	3.49 (2.55/4.78)	66.8	3.9
32	3.50 (3.00/4.09)	66.6	8.0
acetylsalicylic acid	233 (124/438)	1.0	0.47

[a] Confidence limits in parentheses for 95% of the measured values. [b] Relative activity, based on acetylsalicylic acid. [c] In vitro inhibition of collagen-induced aggregation of human platelets; see Table I.

the para position to the meta position, the results are compounds (11 and 12) with clearly reduced hypotensive properties.

The introduction of a chlorine atom into the alkanoyl part of the side chain of the [(alkanoylamino)phenyl]dihydropyridazinones 3–5 yields compounds that have either a fairly similar (compounds 3/13; 5/16, 17) or a lesser (compounds 4/14, 15) hypotensive activity than the Cl-free analogues.

The replacement of a hydrogen atom in the alkanoyl part of the side chain of the dihydropyridazinone 3 by an amine radical entails a considerable decrease in hypotensive activity. The amino acid derivatives 33–39 are altogether less active than the comparative substance dihydralazine.

A substantial decrease in activity occurs, in general, as well, when the 2-position (compounds 7, 8, and 19) or the 4-position (compounds 9, 10, and 20) of the dihydropyridazinone ring is substituted by a CH_3 group.

On the other hand, substitution of the 5-position by a CH_3 group may entail an increase in activity. The most effective compound, actually, of the present series is the 6-[p-[(chloroalkanoyl)amino]phenyl] derivative 23, which has a relative activity of 41.7 compared to dihydralazine. In comparison to the corresponding compound without the 5-CH_3 group (14), the activity shows an increase by a factor of 65. A substantial increase in activity is found as well for compound 24 (by a factor of >23), while there are no apparent differences in activity between compounds 22, 25, 27, and 28 and the corresponding substances without the 5-methyl group. An increase in hypotensive activity

as a result of introducing a methyl group in the 5-position has been described previously by Curran and Ross.[12]

The position of the chlorine atom in the side chain of the 5-methyl (chloroalkanoyl)amino compounds has no systematic influence on the hypotensive effect: while in the case of the (chloropropionyl)amino group the α-chloro derivative is the more active (compounds **23** and **24**), the (β-chlorobutyryl)amino compound is about as active as the (α-chlorobutyryl)amino compound (compounds **26** and **25**).

Increasing the size of the chloroalkanoyl group to beyond three carbon atoms in the 5-methyl (chloroalkanoyl)amino derivatives weakens the hypotensive activity (compounds **23**/**25**–**28**).

The bromine-substituted compound **29** has a lower hypotensive activity than the corresponding chlorine derivative (compound **23**), but there is no difference in activity between the bromine-substituted compounds **30** and **32** and the corresponding chlorine-substituted derivatives (compounds **25** and **28**).

Experimental Section

Chemical Methods. Melting points were taken on a Büchi melting point apparatus and are uncorrected. IR and ^{1}H NMR spectra of all new compounds were consistent with their proposed structures.

Preparation of the γ-Keto Acids. Method A. 3-[*p*-(Acetylamino)benzoyl]propionic Acid (40). Dimethylformamide (220 mL, 2.8 mol) is added dropwise, in the course of about 30 min, with stirring, to 1335 g (10.0 mol) of anhydrous aluminum chloride; a vigorous exothermic reaction occurs. A mixture of 135 g (1.0 mol) of acetanilide and 100 g (1.0 mol) of succinic anhydride is then added, a little at a time, at 60–70 °C, while stirring, and after the addition, stirring is continued for 1 h at 70 °C. The melt, while still warm, is poured onto about 9 kg of ice, and 600 mL of concentrated hydrochloric acid is added to the resulting mixture. After about 15 min, the solid that precipitated is filtered off, washed thoroughly with water, and dried (191 g; mp 196–197 °C). Recrystallization from dimethylformamide/water gives 146 g (62%) of **40** as beige crystals, mp 202–203 °C (lit.[6] mp 202–205 °C). By a similar method, but with methylsuccinic anhydride instead of succinic anhydride, γ-keto acid **44** (Table II) is obtained. The method differs from that described for compound **40** in that first the acetanilide and then the anhydride are added to the mixture of aluminum chloride and dimethylformamide at 60–65 °C, after which the mixture is stirred for about 1.5 h at 70 °C and then worked up.

Method B. 3-(*p*-Aminobenzoyl)propionic Acid (41). 3-[*p*-(Acetylamino)benzoyl]propionic acid (**40**; 117.5 g, 0.50 mol) in 500 mL of concentrated hydrochloric acid is refluxed for 15 min. After dilution with 1 L of water, the mixture is brought to pH 4, at about 10 °C, by means of Na_2CO_3, and the product is filtered off. The solid thus isolated is washed with water and dried: yield 90.5 g (94%) of **41** as pale brown crystals; mp 186–188 °C (with decomposition) (lit.[6] mp 189–190 °C). The γ-keto acid **45** (Table II) is obtained in a similar manner.

Method C. 3-[*p*-(Formylamino)benzoyl]propionic Acid (42). 3-(*p*-Aminobenzoyl)propionic acid (**41**; 1.9 g, 9.8 mmol) in 10 mL of formic acid is stirred for 1 h at 60 °C, after which the mixture is diluted with water, and the product is filtered off, washed with water, and dried. The crude product obtained [2.1 g; mp 219–220 °C (with decomposition)] is recrystallized from propanol: yield 1.0 g (46%) of **42** as beige crystals; mp 219–221 °C (with decomposition).

Method D. 3-[*p*-[(Chloroacetyl)amino]benzoyl]propionic Acid (43). A mixture of 20.0 g (103 mmol) of 3-(*p*-aminobenzoyl)propionic acid (**41**) and 12.9 g (114 mmol) of chloroacetyl chloride in 200 mL of absolute toluene is stirred for 4 h at 80 °C and then cooled to 10 °C and filtered. The solid thus isolated is washed with water and dried (25.1 g; mp 176–177 °C). Recrystallization from acetone gives 14.8 g (53%) of compound **43** as pale brown crystals, mp 184–185 °C.

3-[*p*-[(2-Chloropropionyl)amino]benzoyl]butyric Acid (47). A mixture of 6.0 g (29.0 mmol) of 3-(*p*-aminobenzoyl)butyric acid (**46**)[12,13] and 4.4 g (34.7 mmol) of 2-chloropropionyl chloride in 100 mL of absolute tetrahydrofuran is stirred for 6 h under reflux and then concentrated. Water (200 mL) is added to the oily residue, and the mixture is left to stand for 16 h at room temperature. The water is then decanted, and the oil that remains is stirred with petroleum ether (boiling range 40–60 °C). The solid that forms is filtered off and recrystallized twice from ethyl acetate/petroleum ether (boiling range 40–60 °C): yield 3.1 g (36%) of compound **47** as colorless crystals; mp 139–140 °C.

Preparation of the 6-Aryl-4,5-dihydro-3(2*H*)-pyridazinones. Method A. 6-(*p*-Aminophenyl)-4,5-dihydro-3(2*H*)-pyridazinone (1). 3-(*p*-Aminobenzoyl)propionic acid (**41**; 38.6 g, 0.20 mol) and hydrazine hydrate (12.0 g, 0.24 mol) in 500 mL of ethanol are refluxed for 6 h. After the mixture has been cooled, the product is filtered off. The solid thus obtained is washed with ethanol and dried [34.1 g; mp 237–238 °C (with decomposition)]. Recrystallization from dimethylformamide/water gives 24.7 g (65%) of compound **1** in the form of pale beige crystals, mp 237–238 °C (with decomposition) (lit.[3] mp 236 °C). The dihydropyridazinones **3** and **7** (Table I) are prepared in a similar manner.

4,5-Dihydro-6-[*p*-(formylamino)phenyl]-3(2*H*)-pyridazinone (2). A mixture of 2.30 g (10.4 mmol) of 3-[*p*-(formylamino)benzoyl]propionic acid (**42**) and 0.65 g (13.0 mmol) of hydrazine hydrate in 14 mL of water is heated at 100 °C for 3 h, cooled, and filtered. The product isolated is washed with water and dried: yield 2.05 g (91%) of compound **2** in the form of beige crystals; mp 242.5–243.5 °C (with decomposition); the ^{1}H NMR spectrum is identical with that of compound **2** synthesized by method B_1. Compounds **9** and **10** (Table I) are synthesized by a similar method.

6-[*p*-(Acetylamino)phenyl]-4,5-dihydro-2-methyl-3-(2*H*)-pyridazinone (8). 3-[*p*-(Acetylamino)benzoyl]propionic acid (**40**; 6.0 g, 25.5 mmol) and methylhydrazine (1.4 g, 30.4 mmol) in 150 mL of ethanol are refluxed for 6 h. The mixture is concentrated, and the residue obtained is recrystallized from propanol: yield 4.4 g (68%) of compound **8** (containing 0.33 mol of water per mole) in the form of off-white crystals; mp 207.5–208.5 °C.

6-[*p*-[(Chloroacetyl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (13). A mixture of 4.0 g (14.8 mmol) of 3-[*p*-[(chloroacetyl)amino]benzoyl]propionic acid (**43**) and 0.74 g (14.8 mmol) of hydrazine hydrate in 70 mL of ethanol is boiled for 3 h and then cooled, and the product is filtered off. It is recrystallized from dimethylformamide/water, giving 2.8 g (71%) of compound **13** as pale yellow crystals, mp 227–229 °C (with decomposition). Compound **23** (Table I) is synthesized by a similar method.

Method B_1. 4,5-Dihydro-6-[*p*-(formylamino)phenyl]-3(2*H*)-pyridazinone (2). 6-(*p*-Aminophenyl)-4,5-dihydro-3(2*H*)-pyridazinone (**1**; 9.5 g, 50.2 mmol) in 50 mL of formic acid is refluxed for 1 h and then poured onto 500 mL of ice–water. After 2 h, the product is filtered off, and the crystals thus isolated are washed thoroughly with water, dried, and recrystallized from propanol: yield 8.4 g (77%) of **2** as colorless crystals; mp 237–243 °C (with decomposition).

Method B_2. 4,5-Dihydro-6-[*p*-(propionylamino)-phenyl]-3(2*H*)-pyridazinone (4). Propionyl chloride (3.5 g, 37.8 mmol) is added dropwise to 6-(*p*-aminophenyl)-4,5-dihydro-3-(2*H*)-pyridazinone (**1**; 6.0 g, 31.7 mmol) in 100 mL of absolute tetrahydrofuran. The mixture is then stirred for 6 h under reflux, cooled, and filtered. The product thus isolated is washed with tetrahydrofuran and then with water and then recrystallized from dimethylformamide/water: yield 6.4 g (79%) of compound **4** (containing 0.5 mol of water per mole) in the form of colorless crystals; mp 238–239 °C (lit.[3] mp 237 °C). Compounds **5**, **6**, **12**, **16**, **21**, **23**, **25**, and **26** (Table I) are prepared similarly. However, for the synthesis of compound **16**, the reaction mixture is worked up differently; after stirring under reflux, the mixture is concentrated, the residue is suspended in water, and the product is filtered off and recrystallized.

6-[*p*-[(Chloroacetyl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (13). Chloroacetyl chloride (7.5 g, 66.4 mmol) is added dropwise to 6-(*p*-aminophenyl)-4,5-dihydro-3(2*H*)-pyridazinone (**1**; 10.0 g, 52.8 mmol) in 150 mL of absolute toluene. The mixture is then stirred under reflux for 6 h, cooled, and filtered, and the solid thus isolated is washed first with toluene and then with water. Recrystallization from dimethylform-

amide/water gives 11.9 g (85%) of compound **13** in the form of off-white crystals, mp 233 °C (with decomposition). Compounds **14**, **15**, **17–20**, **22**, **24**, and **27–32** (Table I) are synthesized by a similar method (with a 10–50 mol % excess of acid halide). For the preparation of **19**, the workup of the reaction mixture is modified; after stirring under reflux, the mixture is concentrated, the residue is suspended in water, and the product is filtered off and recrystallized.

Method B_3. 6-[*p*-(Acetylamino)phenyl]-4,5-dihydro-2-methyl-3(2*H*)-pyridazinone (8). Acetic anhydride (4.5 g, 44.1 mmol) is added to 8.1 g (39.9 mmol) of 6-(*p*-aminophenyl)-4,5-dihydro-2-methyl-3(2*H*)-pyridazinone (**7**) in 80 mL of absolute toluene, and the mixture is stirred for 2 h at 80 °C. After cooling, the product is filtered off. The solid obtained is washed with toluene and recrystallized from ethyl acetate: yield 7.2 g (74%) of **8** as beige crystals; mp 207–208 °C.

Method B_4. 6-(*m*-Aminophenyl)-4,5-dihydro-3(2*H*)-pyridazinone (11). 4,5-Dihydro-6-(*m*-nitrophenyl)-3(2*H*)-pyridazinone (**49**;[12] 28.5 g, 0.13 mol) dissolved in 350 mL of dimethylformamide is hydrogenated at room temperature in the presence of 2.5 g of 10% palladium on charcoal. When the absorption of hydrogen has ceased, the catalyst is filtered off, the filtrate is concentrated, and the residue is recrystallized from propanol, in the presence of active charcoal: yield 19.0 g (77%) of **11** in the form of off-white crystals; mp 167–168 °C (lit.[3] mp 169 °C).

Method B_5. 4,5-Dihydro-6-[*p*-[[(propylamino)acetyl]-amino]phenyl]-3(2*H*)-pyridazinone Hydrochloride (33). Propylamine (29.5 g, 0.50 mol) is added dropwise, with stirring, to 26.5 g (0.10 mol) of 6-[*p*-[(chloroacetyl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (**13**) in 150 mL of ethanol. The mixture is then stirred for 2 h under reflux. After the mixture has been cooled, the product is filtered off, and the solid substance thus obtained is washed with cold ethanol and then recrystallized from ethanol. The 4,5-dihydro-6-[*p*-[[(propylamino)acetyl]-amino]phenyl]-3(2*H*)-pyridazinone thus obtained (13.6 g; mp 153–155 °C) is dissolved by heating with 50 mL of 1 N HCl and 25 mL of water. The solution is concentrated and the product dried, giving 15.3 g (47%) of compound **33** as a colorless solid, mp 272–274 °C (with decomposition). The compounds **34–37** (hydrochlorides) and **38** (free base) (Table I) are obtained by a similar method. However, for the preparation of **34–36**, the hot solution of the free base (which has not been recrystallized) in dilute hydrochloric acid is filtered, the filtrate is cooled, and the crystals that precipitate are filtered off, washed with cold water, and dried. In the case of compound **37**, the free base (which has not been recrystallized) dissolves in dilute hydrochloric acid at room temperature; the solution is concentrated and the residue is recrystallized from methanol.

1-[[[[*p*-[4,5-Dihydro-3-oxo-6(2*H*)-pyridazinyl]phenyl]-amino]carbonyl]methyl]-1-methylpyrrolidinium Chloride (39). 6-[*p*-[(Chloroacetyl)amino]phenyl]-4,5-dihydro-3(2*H*)-pyridazinone (**13**; 7.9 g, 29.7 mmol) and 1-methylpyrrolidine (12.7 g, 149 mmol) in 100 mL of ethanol are stirred for 3 h under reflux. After the mixture has been cooled, the product is filtered off, and the solid obtained is washed with cold ethanol and then recrystallized from ethanol: yield 7.3 g (70%) of compound **39** in the form of off-white crystals; mp 267–268 °C (with decomposition).

Biological Methods. Determination of the Inhibition of Platelet Aggregation in Vitro. The determination is, in principle, carried out by the method described by Born and Cross.[16] Human blood obtained by vein puncture is rendered nonclotting by adding 10% v/v sodium citrate solution (3.8% strength), and a plasma rich in platelets is then obtained from the blood by centrifuging (300*g* for 10 min at 4 °C). The platelet-rich plasma is used within 2 h. Aggregation is triggered by adding collagen (from Stago) to give a final concentration of 0.02 mg/mL or by adding ADP (from Stago) to give a final concentration of 0.85 μg/mL. The maximum rate of aggregation is determined in the Born aggregometer (Mark 3). The incubation of the platelets with the test compounds (dissolved in Me_2SO; final concentration of Me_2SO in the platelet-rich plasma, 1%) or the solvent (Me_2SO) is carried out for 10 min at room temperature. To characterize the inhibiting action, the regression of log dose and effect is used to calculate the EC_{50}, i.e., the concentration that produces 50% inhibition, as well as the 95% confidence limits of the measurements. Acetylsalicylic acid (dissolved in 0.1 M sodium acetate) is used as the reference compound. In addition, data on the antiaggregation activity of indomethacin (dissolved in Me_2SO) and prostacyclin (dissolved in Tris buffer, pH 8.3) are given (prostacyclin was generously provided by Schering AG).

Determination of the Inhibition of Platelet Aggregation ex Vivo. The substances are administered orally to male Sprague–Dawley rats weighing 200–250 g. Three rats are used for each dose. One hour after administration, blood is taken under ether narcosis by heart puncture. Platelet-rich plasma is obtained by centrifuging, and aggregation is induced by collagen as indicated above. The ED_{33} is determined as the dose that inhibits the collagen-induced platelet aggregation by 33%.

Hypotensive Action on Anesthetized Rats. Carotid arterial pressure of male Sprague–Dawley rats, weighing 230–280 g, under urethane anesthesia (1.78 mg/kg, ip), is measured by means of a Statham transducer, and the signal is recorded continuously. The test compounds are administered by intraperitoneal injection, in a volume of 2 mL/kg. The regression of log dose and hypotensive effect is used to determine the ED_{20}, i.e., the dose that lowers the mean carotid pressure by 20%. Dihydralazine is used as the reference compound. In addition, data on the hypotensive activity of prostacyclin (dissolved in Tris buffer, pH 8.3; intravenous injection, in a volume of 1.0 mL/kg) are given.

Registry No. 1, 21282-90-6; 2, 41896-97-3; 3, 21394-91-2; 4, 21282-94-0; 5, 85234-89-5; 6, 85221-79-0; 7, 52241-06-2; 8, 85221-80-3; 9, 52239-87-9; 10, 52239-86-8; 11, 21282-91-7; 12, 14714-33-1; 13, 39754-32-0; 14, 39754-33-1; 15, 69635-64-9; 16, 85221-81-4; 17, 69635-72-9; 18, 69635-71-8; 19, 85221-82-5; 20, 53885-82-8; 21, 85221-83-6; 22, 69635-62-7; 23, 69635-63-8; 24, 69635-65-0; 25, 85221-84-7; 26, 85221-85-8; 27, 69635-66-1; 28, 69635-69-4; 29, 69635-73-0; 30, 69635-67-2; 31, 69635-74-1; 32, 69635-70-7; 33, 85221-86-9; 34, 85221-87-0; 35, 85221-88-1; 36, 85221-89-2; 37, 85221-90-5; 38, 39754-24-0; 39, 39754-30-8; 40, 5473-15-4; 41, 6945-94-4; 42, 41897-00-1; 43, 69635-61-6; 44, 52240-23-0; 45, 53885-72-6; 46, 42075-29-6; 47, 85221-91-6; 48, 36725-28-7; 49, 52239-76-6; 2-chloropropionyl chloride, 7623-09-8; acetanilide, 103-84-4; succinic anhydride, 108-30-5; hydrazine hydrate, 7803-57-8; methylhydrazine, 60-34-4; propionyl chloride, 79-03-8.

(16) G. V. R. Born and M. J. Cross, *J. Physiol.*, 168, 178 (1963).