5-Acyl-6-aryl-4-nitro-3(2*H*)pyridazinones and Related 4-Amino Compounds: Synthesis and Pharmacological Evaluation

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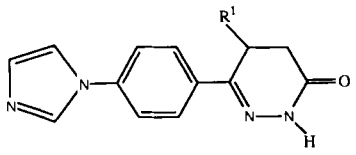
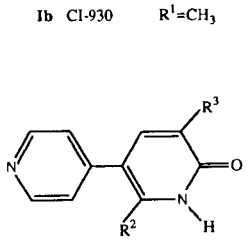
Abstract □ Several 4-nitro- and 4-amino-5-acyl-6-aryl-3(2*H*)pyridazinones were prepared and their in vitro and ex vivo antiaggregatory properties were evaluated. 4-Nitro derivatives **3** generally showed good activity in vitro towards arachidonic acid (AA)-induced human blood platelet aggregation. The 4-amino compound **4a**, which has weak in vitro activity, exhibited antiplatelet activity, particularly on adenosine dephosphate (ADP)-induced aggregation ex vivo in rabbit. Moreover, the same compound was shown to be active in platelet-activating factors (PAF)-induced rat paw hyperalgesia and to be endowed with low acute oral toxicity. The 4-amino derivatives **4a–m** and the other pyridazinones **5–9** administered orally to rats were also found to be more potent anti-inflammatory agents than acetyl salicylic acid (ASA). Compounds **3a** and **4a**, tested in vitro on lipopolysaccharide (LPS)-stimulated rat peritoneal macrophages, were seen to be active in the inhibition of prostaglandin E_2 (PGE_2) production and interleukin-1 activity. Structure–activity relationship studies in the series of antiaggregating pyridazinones **3** have shown the primary importance of the nitro and acetyl substituents at positions 4 and 5, respectively. Hydrophobic substituents at position 2 were also required for better activity.

6-Aryl-3(2*H*)pyridazinones, as well as their 4,5-dihydro derivatives, display a wide range of pharmacological activities, such as reduction of blood pressure,[1–3] anti-inflammatory activity,[4] inhibition of platelet aggregation,[3–5] and positive inotropic effect.[6–8] Several pyridazinones, like CI-914 (imazodan; **Ia**)[6] and CI-930 (**Ib**),[7] which are active as cardiotonic agents, are structurally related to amrinone (**IIa**; 5-amino-[3,4′-bipyridine]-6(1*H*)-one) and milrinone (**IIb**; 1,6-dihydro-2-methyl-6-oxo-[3,4′-bipyridine]-5-carbonitrile), the prototypes of a series of nonglycosidic, nonsympathomimetic, orally active compounds which are stimulating a remarkable interest as promising agents for congestive heart failure (CHF) therapy. The usefulness of this type of cardiotonic in the treatment of CHF patients is controversial. In fact, even though their use produces an initial hemodynamic improvement,[9–11] in long-term therapy the mortality is not reduced significantly.[12,13]



Ia imazodan R^1=H

Ib CI-930 R^1=CH_3



IIa amrinone R^2=H, R^3=NH_2

IIb milrinone R^2=CH_3, R^3=CN

The recent literature reports that milrinone and related compounds are also endowed with antiplatelet activity; the inhibition of cyclic-adenosine monophosphate (c-AMP) phosphodiesterase in cardiac muscle and platelets is assumed to be the primary mechanism for these activities.[14] Recently, Moos and co-workers[15] have elaborated in the field of pyridazinone derivatives a pharmacophore model for selective phosphodiesterase III (PDE III) inhibitors on the basis of solid-state, spectroscopic, and theoretical conformational analysis. The structural features of this model confirm the importance of a strong dipole (carbonyl) neighboring an NH group and of oxygenated substituents at the 5-position.

On these grounds, the 5-acyl-6-aryl-4-nitropyridazinones **3a–g**, some of which were described by us in a previous paper,[16] appear to be very attractive as potential platelet aggregation inhibitors and positive inotropic agents. In fact, taking into account the close resemblance between the pyridazinone and pyridone ring systems, the introduction of NO_2 instead of CN should not lead to remarkable differences in the geometric and electronic features between **3** and **IIb**. This hypothesis is supported by literature data on a series of positive inotropic pyrimidinones.[17]

We have also synthesized the amino derivatives **4 a–m** as amrinone analogues and the other pyridazinones **5–9**. Compounds **4**, in view of the well-known biological properties of several aminopyridazinones,[4] can also be considered interesting as anti-inflammatory agents.

Results and Discussion

Chemistry—The synthetic strategies employed in this work are shown in Schemes I and II. The difunctionalized isoxazoles **1a–c** were prepared as previously reported[16,18,19] from ethyl chloro(hydroximino)acetate[20] and the appropriate 1,3 diketones; the unknown **1d** was obtained starting from the same hydroximinic chloride and 1-(4-pyridyl)1,3-butandione.[21]

The key intermediates **2** (Table I) were obtained according to literature procedures[16,18,22,23] (**2a–g** and **2o–p**); **2h–l** were synthesized from **1d** or **1b** and the appropriate hydrazine; **2m** and **2n** were synthesized from **2d** by Michael-type addition with vinyl reagents of the type CH_2=CH-R. The target compounds **3** and **4** (Tables II and III, respectively) were synthesized by selective opening of the isoxazole ring of derivatives **2**. Compounds **4** were formed in very good yields

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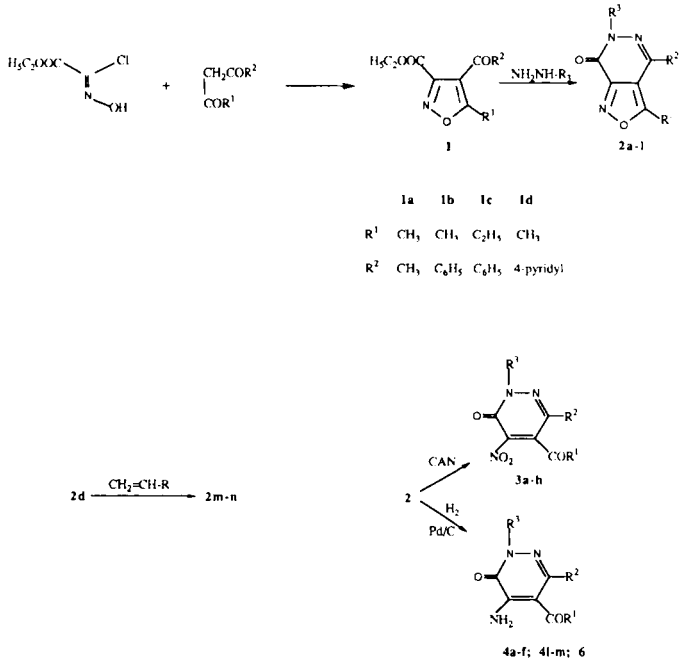


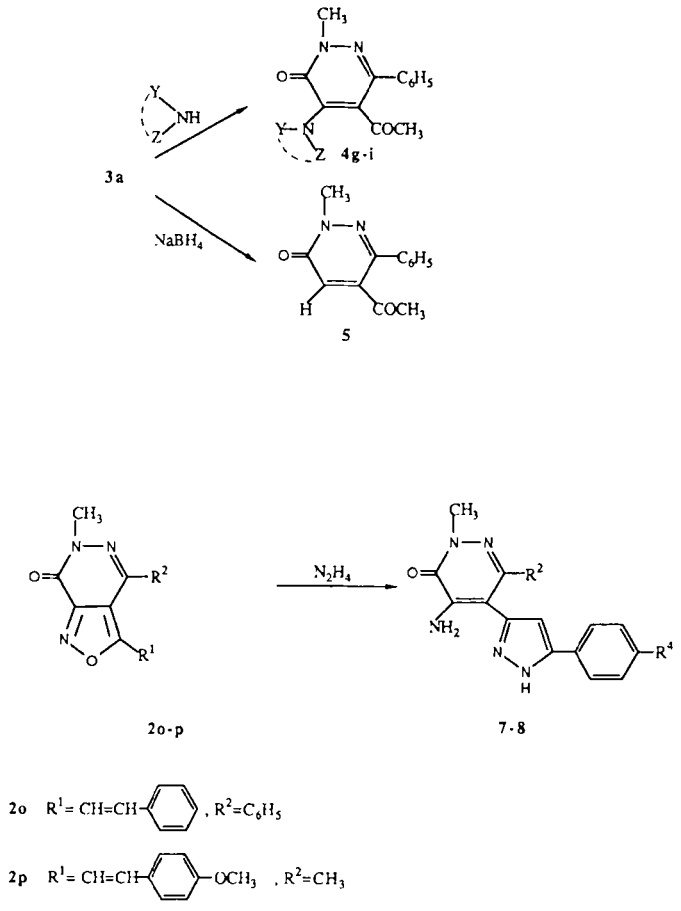
Scheme I

by treatment of 2 with H_2 on Pd/C.[24] This reaction, when performed on **2o**, yields **4l** through ring opening and hydrogenation of the exocyclic double bond. Following a procedure described by us in a previous work, treatment of 2 with ceric ammonium nitrate (CAN) in acetic acid and in the presence of nitric acid affords compounds 3 in moderate yields.[16]

Compound **3a** easily undergoes nucleophilic substitutions to give **4g–i**. When **3a** was treated with sodium borhydride, a loss of the nitro group took place and **5** was isolated in moderate yield by column chromatography. Compound **6** was obtained by catalytic hydrogenation on Pd/C of 3,4-dimethyl-6-phenylisoxazolo[3,4-*d*]pyridazinone **2b**.[18] Compound **7** was obtained according to literature procedures;[25] **8** was obtained by hydrazinolysis of **2p**. Compound **9** was prepared according to a literature procedure.[26] Synthetic and spectroscopic data of **5–9** are reported in Table IV.

Biology—The activity of 3 and 4, tested at a final concentration of 10 μM, towards arachidonic acid (AA)-, adenosine diphosphate (ADP)-, and platelet-activating factor (PAF)-acether-stimulated aggregation, in comparison with acetylsalicylic acid (ASA), indomethacin, and milrinone as reference drugs, is reported in Table V. The data show that **3a–c** and **3g** possess an interesting activity against AA-stimulated aggregation. In the series of 4-nitro derivatives 3, the AA-induced aggregation appears to be remarkably affected by the nature of the substituent at position 2. In fact the presence of hydrophobic substituents (alkyl, cycloalkyl, aryl, or arylalkyl groups) at N-2 produces the greatest inhibitory effect, while the introduction of a functionalized chain leads to compounds endowed with the lowest activity (i.e., **3e** and **3f**). Also, the extension of the alkyl chain on the ketone appears to be unfavorable towards this activity (see **3d**).

The in vitro antiaggregatory activity of these compounds is also closely connected to the presence of the NO_2 group. In fact, reduction of this substituent to NH_2 leads to a loss of activity (see **4a**); its substitution with a hydrogen has the same result (see **5**), as does the embodiment of the NO_2 and the $COCH_3$ in a cyclic system (see **2b**). The presence of the phenyl group at position 6 also plays an important role; in fact, replacement of this substituent with a methyl group gives a notable reduction of the activity (see **3h**).



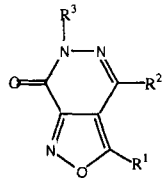
Scheme II

In Table V the IC_{50} of several compounds 3 on AA-induced aggregation are reported in comparison with that for ASA, indomethacin, and milrinone. It is remarkable that the IC_{50} for **3c** is very similar to that of indomethacin, lower than that of ASA, but greater than that of milrinone.

The 4-amino or substituted amino pyridazinones **4a–m** show weak activity or inactivity on AA- and ADP-stimulated aggregation. As regards the PAF-stimulated aggregation, the activity of 4-nitro derivatives 3 and related amino compounds 4 appears to not depend on the structure; on the other hand, it is moderate compared with that of other specific anti-PAF compounds.[27] In this type of inhibition test, in order to rule out platelet release-reaction and therefore prostaglandin involvement, we considered only reversible-type aggregometric curves, according to Kloprogge et al.[28]

Figure 1 shows the antiplatelet activity of **3a** (one of the most active antiaggregating agents in vitro) and of the corresponding amino **4a** in the ex vivo rabbit test in comparison with ASA. At a dose of 50 mg/kg, **3a** shows weak activity on the platelet aggregation stimulated by AA, ADP, and PAF-acether. Compound **4a**, at the same dose, shows weak activity towards AA- and PAF-stimulated aggregation, whereas it is very active in ADP-stimulated platelet aggregation. The apparent disagreement between the in vitro and in vivo pharmacological activities of the two compounds cannot be explained on the basis of previously reported data. For these compounds, metabolic activation (**4a**) and/or inactivation (**3a**) and/or different bioavailabilities might be envolved.

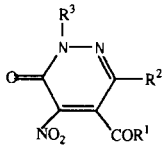
Table I—Some Synthetic and Spectroscopic Data of Isoxazolo[3,4-*d*]pyridazin-7(6*H*)-ones 2[a]



Compound	R^1	R^2	R^3	Formula[b]	mp, °C	Crystn. Solv.[c]	Yield, %	IR, (cm^{-1})	^1H NMR, (δ, $CDCl_3$), ppm
2h	CH_3	4-Pyridyl	H	$C_{11}H_8N_4O_2$	268–269	a	70	2900 (NH), 1690 (CO)	2.60(s, 3, CH_3), 7.50 (m, 2, Py H-3 and H-5), 8.85(m, 2, Py H-2 and H-6), 12.80(exch.br.s, 1, NH)
2i	CH_3	4-Pyridyl	CH_3	$C_{12}H_{10}N_4O_2$	190–192	a	86	1680 (CO)	2.61(s, 3, C-CH_3), 3.82(s, 3, N-CH_3), 7.50(m, 2, Py H-3 and H-5), 8.85(m, 2, Py H-2 and H-6)
2l	CH_3	C_6H_5	$CH_2C_6H_5$	$C_{19}H_{15}N_3O_2$	131–132	a	78	1685 (CO)	2.62(s, 3, C-CH_3), 5.43 (s, 2, CH_2), 7.25–7.80 (m, 10, 2xC_6H_5)
2m	CH_3	C_6H_5	$(CH_2)_2CN$	$C_{15}H_{12}N_4O_2$	165–167	a	94	2260 (CN), 1680 (CO)	2.55(s, 3, C-CH_3), 2.88 (t, 2, CN-CH_2, J = 7.0 Hz), 4.52(t, 2, N-CH_2, J = 7.0 Hz), 7.62(s, 5, C_6H_5)
2n	CH_3	C_6H_5	$(CH_2)_2COOCH_3$	$C_{16}H_{15}N_3O_4$	68–70	a	67	1740 ($COOCH_3$) 1675 (CO)	2.48(s, 3, C-CH_3), 2.85(t, 2, CO-CH_2, J = 7.2 Hz), 4.55(t, 2, N–CH_2, J = 7.2 Hz), 3.68 (s, 3, O-CH_3), 7.53(s, 5, C_6H_5)

[a] The following compounds were previously described: **2a** (R^1 = R^2 = R^3 = CH_3);[22] **2b** (R^1 = R^2 = CH_3; R^3 = C_6H_5);[18] **2c** (R^1 = R^3 = CH_3; R^2 = C_6H_5);[22] **2d** (R^1 = CH_3, R^2 = C_6H_5, R^3 = H);[24] **2e** (R^1 = CH_3; R^2 = R^3 = C_6H_5);[22] **2f** (R^1 = CH_3; R^2 = C_6H_5, R^3 = c-C_6H_{11});[16] **2g** (R^1 = C_2H_5; R^2 = C_6H_5; R^3 = CH_3);[16] **2o** (R^1 = CH = CH-C_6H_5; R^2 = C_6H_5; R^3 = CH_3);[23] **2p** (R^1 = CH = CH-C_6H_4-*p*-OCH_3; R^2 = R^3 = CH_3).[23] [b] All new compounds were analyzed for C, H, N. [c] a = EtOH.

Table II—Some Synthetic and Spectroscopic Data of 5-Acyl-6-aryl-4-nitropyridazin-3(2*H*)ones 3[a]

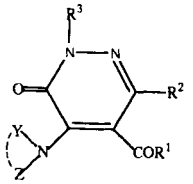


Compound	R^1	R^2	R^3	Formula[b]	mp, °C	Crystn. Solv.[c]	Yield, %	IR, cm^{-1}	^1H NMR (δ, $CDCl_3$), ppm
3e	CH_3	C_6H_5	$(CH_2)_2CN$	$C_{15}H_{12}N_4O_4$	141–142	a	38	2260 (CN), 1710 ($COCH_3$), 1690 (CO), 1540 and 1360 (NO_2)	2.13 (s, 3, CO—CH_3), 3.00 (t, 2, CN-CH_2, J = 7.0 Hz), 4.62 (t, 2, N-CH_2, J = 7.0 Hz), 7.53 (s, 5, C_6H_5)
3f	CH_3	C_6H_5	$(CH_2)_2COOCH_3$	$C_{16}H_{15}N_3O_6$	78–79	b	32	1730 ($COOCH_3$ and $COCH_3$), 1690(CO), 1550 and 1360 (NO_2)	2.13 (s, 3, CO—CH_3), 2.97 (t, 2, CO—CH_2, J = 7.2 Hz), 3.70 (s, 3, O-CH_3), 4.67 (t, 2, N-CH_2), 7.52 (s, 5, C_6H_5)
3g	CH_3	C_6H_5	$CH_2C_6H_5$	$C_{19}H_{15}N_3O_4$	140–142	a	51	1720 ($COCH_3$), 1685 (CO), 1560 and 1380 (NO_2)	2.12 (s, 3, CH_3), 5.48 (s, 2, CH_2), 7.22–7.65 (m, 10, 2xC_6H_5)

[a] The following compounds were previously described:[16] **3a** (R^1 = R^3 = CH_3; R^2 = C_6H_5); **3b** (R^1 = CH_3; R^2 = R^3 = C_6H_5); **3c** (R^1 = CH_3, R^2 = C_6H_5; R^3 = c-C_6H_{11}); **3d** (R^1 = C_2H_5; R^2 = C_6H_5; R^3 = CH_3); **3h** (R^1 = R^2 = R^3 = CH_3). [b] All new compound were analyzed for C, H, N. [c] a = EtOH; b = cyclohexane.

As a consequence of the above results, **3a** and **4a–m** were selected for further studies in vitro and in vivo. Table VI lists the anti-inflammatory activities in carrageenin-induced rat paw edema of **3a**, **4a–m**, and **5–9** in comparison with ASA as a reference substance. The pyridazinones **5–9**, in which the aryl group at position 6 and the functions at positions 4 and 5 are substituted or eliminated to various extents, were prepared and tested in order to investigate the importance of

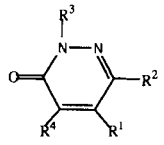
Table III—Some Synthetic and Spectroscopic Data of 5-Acyl-4-amino or Substituted Amino 6-Arylpyridazin-3(2*H*) ones 4



Compound	R^1	R^2	R^3	$\overset{Y}{Z}>N$	Formula[a]	mp, °C	Crystn. solv.[b]	Yield, %	IR, cm^{-1}	^1H NMR (δ, $CDCl_3$), ppm
4a[c]	CH_3	C_6H_5	CH_3	NH_2		—	—	—	—	—
4b	CH_3	C_6H_5	$(CH_2)_2COOCH_3$	NH_2	$C_{16}H_{17}N_3O_4$	115	a	62	3420 and 3300 (NH_2), 1750 ($COOCH_3$), 1650 ($CO\text{-}CH_3$ and CO)	1.82 (s, 3, $CO\text{-}CH_3$), 2.90 (t, 2, $CO\text{-}CH_2$, J = 7.2 Hz), 3.71 (s, 3, $O\text{-}CH_3$), 4.50 (t, 2, $N\text{-}CH_2$, J = 7.2 Hz), 7.49 (s, 5, C_6H_5), 7.50 (exch.br.s.2, NH_2)
4c[d]	CH_3	C_6H_5	H	NH_2		—	—	—	—	—
4d	CH_3	C_6H_5	c-C_6H_{11}	NH_2	$C_{18}H_{21}N_3O_2$	214–215	a	71	3400 and 3290 (NH_2), 1650 ($CO\text{-}CH_3$ and CO)	1.50–2.20 (m, 13, 5xCH_2 cyclohexane and $CO\text{-}CH_3$), 5.10 (m, 1, cyclohexane H-1), 7.50 (s, 5, C_6H_5), 7.50 (exch. br.s.2, NH_2)
4e	CH_3	4-Py[e]	CH_3	NH_2	$C_{12}H_{12}N_4O_2$	200–203	a	68	3435 and 3335 (NH_2), 1640 ($CO\text{-}CH_3$ and CO)	1.80 (s, 3, $CO\text{-}CH_3$), 3.80 (s, 3, $N\text{-}CH_3$), 7.20–7.70 (exch.br.s.2, NH_2), 7.40 (m, 2, Py H-3 and H-5), 8.80 (m, 2, Py H-2 and H-6)
4f	CH_3	4-Py	H	NH_2	$C_{11}H_{10}N_4O_2$	264–266	a	73	3460 and 3150 (NH_2 and NH), 1670 ($CO\text{-}CH_3$ and CO)	1.80 (s, 3, $CO\text{-}CH_3$), 7.40 (m, 2, Py H-3 and H-5), 7.80 (exch.br.s. 2, NH_2), 8.80 (m, 2, Py H-2 and H-6), 13.20 (exch.br.s.1NH)
4g	CH_3	C_6H_5	CH_3	A[g]	$C_{19}H_{17}N_3O_2$	185–186	a	59	3260 (NH), 1720 (CO), 1630 ($CO\text{-}CH_3$)	1.68 (s, 3, $CO\text{-}CH_3$), 3.85 (s, 3, $N\text{-}CH_3$), 7.00–7.55 (m, 10, 2x C_6H_5), 8.25 (exch.br.s.1, NH)
4h	CH_3	C_6H_5	CH_3	D[h]	$C_{17}H_{21}N_3O_2$	Oil[i]		47	1720 (CO), 1650 ($CO\text{-}CH_3$)	1.10 (t, 6, 2xCH_3), 2.12 (s, 3, $CO\text{-}CH_3$), 3.43 (q, 4, 2xCH_2), 3.82 (s, 3, $N\text{-}CH_3$), 7.48 (s, 5, C_6H_5)
4i	CH_3	C_6H_5	CH_3	M[j]	$C_{17}H_{19}N_3O_3$	134–135	a	52	1715 (CO), 1635 ($CO\text{-}CH_3$)	2.03 (s, 3, $CO\text{-}CH_3$), 3.35 (m, 4, 2xCH_2 morpholine H-2 and H-6), 3.75 (m, 4, 2xCH_2, morpholine H-3 and H-5), 3.80 (s, 3, $N\text{-}CH_3$), 7.42 (s, 5, C_6H_5)
4l	$(CH_2)_2C_6H_5$	C_6H_5	CH_3	NH_2	$C_{20}H_{19}N_3O_2$	118	a	61	3415 and 3320 (NH_2), 1670 (CO), 1630 ($CO\text{-}CH_3$)	2.12–2.95 (m, 4, $CH_2\text{-}CH_2$), 3.89 (s, 3, $N\text{-}CH_3$), 6.90 (exch.br.s.2, NH_2), 7.10–7.70 (m, 10, 2x C_6H_5)
4m	CH_3	C_6H_5	$(CH_2)_2CN$	NH_2	$C_{15}H_{14}N_4O_2$	187–188	a	56	3435 and 3310 (NH_2), 2250 (CN), 1650 (2xCO)	1.78 (s, 3, $CO\text{-}CH_3$), 2.91 (t, 2, $CN\text{-}CH_2$, J = 7.0 Hz), 4.52 (t, 2, $N\text{-}CH_2$, J = 7.0 Hz), 7.30 (exch.br.s.2, NH_2) 7.52 (s, 5, C_6H_5)

[a] All new compounds were analyzed for C, H, N. [b] a = EtOH. [c] Reference 25. [d] Reference 24. [e] Py = pyridyl. [f] ^1H NMR in DMSO-d_6. [g] A = anilino. [h] D = D-diethlamino. [i] Purified by column chromatography. [j] M = morpholino.

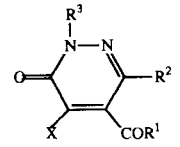
Table IV—Some Synthetic and Spectroscopic Data of Compounds 5–9



Compound	R^1	R^2	R^3	R^4	Formula[a]	mp, °C	Crystn. Solv.[b]	Yield, %	IR, cm^{-1}	^1H NMR (δ, $CDCl_3$), ppm
5	$COCH_3$	C_6H_5	CH_3	H	$C_{13}H_{12}N_2O_2$	108	b	34	1710 (CO-CH_3), 1670 (CO)	2.10 (s, 3, C-CH_3), 3.90 (s, 3, N-CH_3), 6.98 (s,1, CH), 7.49 (s, 5, C_6H_5)
6	$COCH_3$	CH_3	C_6H_5	NH_2	$C_{13}H_{13}N_3O_2$	142–144	a	73	3400 and 3280 (NH_2), 1670 (2xCO)	2.60 (s, 6, C-CH_3 and CO-CH_3), 7.30–7.82 (m, 7, C_6H_5 and NH_2)
7[c]	5-Phenylpyrazol-3-yl	C_6H_5	CH_3	NH_2	—	—	—	—	—	—
8	5-(4-Methoxphenyl) pyrazol-3-yl	CH_3	CH_3	NH_2	$C_{16}H_{17}N_5O_2$	191–192	a	65	3480 (NH), 3300 and 3200 (NH_2), 1640 (CO)	2.32 (s, 3, C-CH_3), 3.70 (s, 3, O-CH_3), 3.83 (s, 3, N-CH_3), 6.60 (exch.br.s, 2, NH_2), 6.64 (s, 1, pyrazole H-4), 7.42(m, AA'BB', 4, C_6H_4), 13.40 (exch.br. s, 1, NH)
9[d]	H	C_6H_5	CH_3	H	—	—	—	—	—	—

[a] All new compounds were analyzed for C, H, N. [b] a = EtOH; b = benzene:light petroleum (30:70). [c] Reference 25. [d] Reference 26.

Table V—4-Nitro and 4-Amino-5-acylpyridazinones: Antiaggregatory Activity in Vitro



Compound[a]	R^1	R^2	R^3	X	Inhibition of Aggregation, %[b]			IC_{50}, μM[b,c]
					AA	ADP	PAF	
3a	CH_3	C_6H_5	CH_3	NO_2	86.7 ± 5.3	49.9 ± 9.8	60.6 ± 5.0	5.0 ± 2.4
3b	CH_3	C_6H_5	C_6H_5	NO_2	40.8 ± 8.2	35.7 ± 4.6	32.3 ± 5.1	12.5 ± 2.3
3c	CH_3	C_6H_5	c-C_6H_{11}	NO_2	63.0 ± 7.8	26.7 ± 6.9	48.5 ± 8.9	2.8 ± 1.1
3d	C_2H_5	C_6H_5	CH_3	NO_2	22.3 ± 9.8	24.3 ± 6.2	26.4 ± 9.5	25.2 ± 2.0
3e	CH_3	C_6H_5	$(CH_2)_2CN$	NO_2	8.6 ± 2.8	NT[d]	4.9 ± 3.5	—
3f	CH_3	C_6H_5	$(CH_2)_2COOCH_3$	NO_2	27.2 ± 4.0	38.9 ± 5.7	22.9 ± 3.1	—
3g	CH_3	C_6H_5	$CH_2C_6H_5$	NO_2	60.5 ± 5.6	35.5 ± 6.1	20.3 ± 5.7	—
3h	CH_3	CH_3	CH_3	NO_2	19.0 ± 3.1	45.0 ± 7.8	NT	10.1 ± 1.1
4a	CH_3	C_6H_5	CH_3	NH_2	0	0	28.8 ± 3.8	—
4b	CH_3	C_6H_5	$(CH_2)_2COOCH_3$	NH_2	10.5 ± 3.5	18.8 ± 11.0	39.5 ± 3.0	—
4c	CH_3	C_6H_5	H	NH_2	4.0 ± 2.5	10.3 ± 5.0	8.5 ± 3.3	—
4d	CH_3	C_6H_5	c-C_6H_{11}	NH_2	8.5 ± 2.2	19.2 ± 6.7	28.0 ± 3.0	—
4e	CH_3	4-Pyridyl	CH_3	NH_2	0	10.3 ± 9.0	38.0 ± 2.0	—
4f	CH_3	4-Pyridyl	H	NH_2	12.5 ± 3.5	26.5 ± 8.5	21.5 ± 5.0	—
4g	CH_3	C_6H_5	CH_3	Anilino	7.0 ± 2.1	0	25.0 ± 4.8	—
4h	CH_3	C_6H_5	CH_3	$N(C_2H_5)_2$	0	0	23.0 ± 7.1	—
4i	CH_3	C_6H_5	CH_3	Morpholino	0	0	23.0 ± 5.5	—
4l	$(CH_2)_2C_6H_5$	C_6H_5	CH_3	NH_2	8.2 ± 3.7	NT	NT	—
4m	CH_3	C_6H_5	$(CH_2)_2CN$	NH_2	5.3 ± 4.1	NT	NT	—
5	CH_3	C_6H_5	CH_3	H	0	0	33.7 ± 5.8	—
2b[c]	—	—	—	—	0	0	NT	—
ASA	—	—	—	—	25.0 ± 2.5	0	NT	30.0 ± 1.2
Indomethacin	—	—	—	—	100	30.0 ± 3.0	NT	2.0 ± 1.5
Milrinone	—	—	—	—	100	52.3 ± 3.4	91.5 ± 7.2	0.19 ± 0.1

[a] The compounds are tested at final concentration of 10 μM. [b] Average ± standard deviation of almost three experiments. [c] Values related to AA-induced aggregation. [d] NT: not tested. [e] See Table I.

these substituents on anti-inflammatory activity. Compound **3a** is inactive, whereas in this test, the other compounds show good activity which is generally greater than that of ASA. The presence of a nitro group on the pyridazinone skeleton seems to cause loss of the anti-inflammatory activity. The amino or substituted amino group, introduced on the basis of the well-documented importance of these functionalities for anti-inflammatory activity,[4] does in fact produce it to a considerable extent. Compound **9**, previously described in the literature[26] but not tested as an anti-inflammatory agent, is one of the most active in this test. Compound **4a**, which possesses a comparable anti-inflammatory activity to that of **9**, was also tested as an analgesic towards a PAF-induced hyperalgesia model in rat after oral administration (Figure 2). In this test,

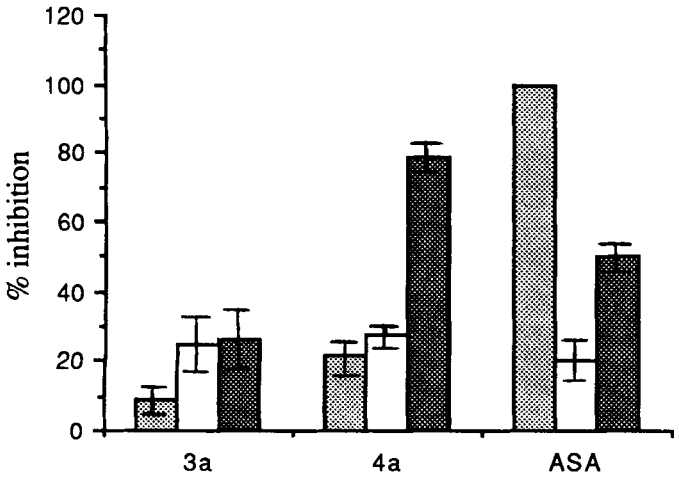


Figure 1—Percent inhibition of ex vivo rabbit platelet aggregation. Key: (▨) AA-induced; (□) PAF-induced; (■) ADP-induced. Compounds were given at a dose of 50 mg/kg (po).

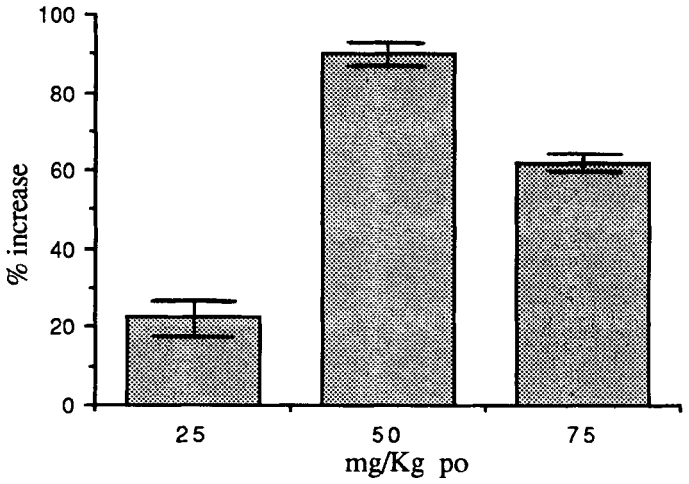


Figure 2—Percent increase of PAF-induced pain threshold by **4a** in rat (po).

Table VI—Anti-Inflammatory Activity of Several Pyridazinones

Compound	Percent Inhibition of Edema (mg/kg, po)[a]			
	100	50	25	10
3a	—	0	—	—
4a	74.3 ± 7.1	62.7 ± 6.1	32.1 ± 3.5	—
4b	—	30.3 ± 2.8	—	—
4c	—	52.1 ± 4.6	—	—
4d	—	44.9 ± 4.0	—	—
4e	—	63.3 ± 5.6	—	—
4f	—	NT[b]	—	—
4g	—	48.2 ± 4.2	—	—
4h	—	46.3 ± 3.9	—	—
4i	—	45.7 ± 3.8	—	—
4l	—	43.2 ± 3.9	—	—
4m	—	40.5 ± 4.1	—	—
5	—	42.3 ± 3.8	—	—
6	—	56.7 ± 5.0	—	—
7	—	35.2 ± 3.0	—	—
8	—	43.4 ± 3.9	—	—
9	—	66.5 ± 5.9	72.5 ± 6.4	35.4 ± 3.2
ASA	56.2 ± 5.0	29.1 ± 1.8	20.0 ± 6.0	—

[a] Values are average ± standard deviation of almost three experiments. [b] Not tested.

Table VII—Prostaglandin E_2 Production from Rat Adherent Macrophages

Compound[a]	Concentration, μM	PGE_2, ng/mL[b]	Inhibition, %
—	—	9.37 ± 1.95[c]	—
3a	10	4.08 ± 0.63[d]	56.4
4a	10	5.52 ± 0.86	41.1
Indomethacin	5	0.18 ± 0.06[e]	98.1

[a] Drugs were added together with the stimulus and incubated for 24 h. [b] Values are mean ± SEM of one experiment performed in quadruplicate; similar results were obtained in two other experiments; PGE_2 production by unstimulated macrophages was 1.13 ± 0.22 and subtracted from reported values; unpaired t test was done versus LPS alone. [c] Base value obtained with 10 μg/mL of LPS in absence of inhibitor. [d] $p < 0.05$. [e] $p < 0.005$.

an ED_{50} was calculated for **4a** (40 mg/kg) which was comparable with that obtained for it in carrageenin-induced rat paw edema (41 mg/kg). On the other hand, it is well known that besides selective anti-PAF agents, several nonsteroidal anti-inflammatory (NSAI) compounds are also active in PAF-induced hyperalgesia.[29] The acute oral toxicity for **4a** was tested in mice: no toxic effect was seen until administration of a dose of 1 g/kg.

The in vitro and ex vivo antiplatelet, anti-inflammatory, and analgesic activities found for **3a** and **4a** can partially be explained by the inhibition of the AA metabolic pathways. In a first series of experiments, data obtained from in vitro studies on lipopolysaccharide (LPS)-stimulated rat peritoneal macrophages show that **3a** and **4a** are able to inhibit prostaglandin E_2 (PGE_2) production in this test (Table VII). The inhibition values obtained (56.4 and 41.1%, respectively) are in agreement with the results for the same compounds in the in vitro AA-induced platelet aggregation test. Similarly, in the interleukin-1 (IL-1) activity test using rat adherent macrophages, **3a** and **4a** show the same inhibition patterns (Table VIII). Since IL-1 plays an important role in chronic inflammatory diseases,[30] the results suggest that these compounds could also be active in this pathology type.

Conclusions

The pyridazinones studied here exhibit in vitro and in vivo biological activity. 4-Nitro derivatives **3**, which are endowed with in vitro activity towards AA-induced platelet aggregation (in some instances to the same degree as indomethacin), are only weakly active in vivo, perhaps because of unfavorable pharmacokinetics or metabolism.

Orally administered 4-amino derivatives **4** are found to be more active than ASA in carrageenin-induced rat paw edema. Compound **4a**, which is also endowed with activity in PAF-induced hyperalgesia, shows an attractive pharmacological profile. Bearing in mind its low toxicity, this

Table VIII—Interleukin-1 Activity from Rat Adherent Macrophages

Compound[a]	Concentration, μM	IL-1 Activity, units/mL[b]	Inhibition, %
—	—	296 ± 65[c]	—
3a	10	145 ± 20[d]	52.0
4a	10	174 ± 30	41.2
Indomethacin	5	245 ± 49	17.2

[a] Drugs were added together with the stimulus and incubated for 24 h. [b] Values are mean ± SEM of one experiment performed in triplicate; similar results were obtained in two other experiments, unstimulated production of IL-1 activity was 20 ± 2 and subtracted from reported values; unpaired t test was done versus LPS alone. [c] Base value obtained with 10 μg/mL of LPS in absence of inhibitor. [d] $p < 0.05$.

compound was selected for further pharmacological investigations.

Experimental Section

All melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. The IR spectra were measured for nujol mulls with a Perkin-Elmer 337 spectrometer. The ^1H NMR spectra were recorded with either Varian EM-360 or Perkin-Elmer R32 spectrometers. Microanalyses (C, H, N) are within ±0.4% of the theoretical values. Extracts were dried over sodium sulfate and solvents were removed under reduced pressure. Silica gel plates (Merck F_{254}) and silica gel 60 (Merck 70-230 mesh) were used for analytical TLC and for column chromatography, respectively.

Ethyl 5-methyl-4-(4-pyridyl-carbonyl)-isoxazole-3-carboxylate (1d)—To a cooled and stirred solution of sodium ethoxide (0.92 g, 13.5 mmol) in anhydrous ethanol (25 mL), a solution of 1-(4-pyridyl)-butan-1,3-dione[21] (2.2 g, 13.5 mmol) in anhydrous ethanol (25 mL) was slowly added. A solution of ethyl chloro(hydroxyimino)acetate[20] (2.05 g, 13.5 mmol) in anhydrous ethanol (8 mL) was added in a dropwise manner (over a 1-h period). The solution, neutralized with 6 M HCl, was evaporated to afford **1d**, after washing (with water) and drying (2.7 g, 10.3 mmol; mp 78–81 °C; 76.5%). Recrystallization from cyclohexane afforded white crystals (mp 85–87 °C); IR (cm^{-1}): 1750 ($COOC_2H_5$), 1670 (CO); ^1H NMR ($CDCl_3$): 1.12 (t, 3, J = 7.0 Hz, CH_3CH_2), 2.57 (s, 3, CH_3), 4.12 (q, 2, J = 7.0 Hz, CH_2CH_3), 7.50 (m, 2, Py H-3 and H-5), 8.82 (m, 2, Py H-2 and H-6).

Anal.—Calc. for $C_{13}H_{12}N_2O_4$: C, H, N.

Compounds 2h–i—To a cooled and stirred solution of **1d** (5 mmol) in ethanol (5 mL), the appropriate hydrazine (10 mmol) was added in a dropwise manner. After 2 h, the crude product was filtered and purified.

6-Benzyl-3-methyl-4-phenylisossazolo[3,4-*d*]pyridazin-7(6*H*)-one (2l)—A mixture of **1b** (3.1 mmol), benzylhydrazine dihydrochloride (12 mmol), and polyphosphoric acid (8 g; Merck-Schuchardt) in anhydrous ethanol (8 mL) was refluxed under stirring for 5 h and poured, after cooling, into ice-cold water (200 mL). The crude product was filtered, washed with water, and purified.

Compounds 2m–n—To a solution of **2d** (1.04 g, 4.6 mmol) and methyl acrylate or acrylonitrile (10 mmol) in pyridine (8 mL), a catalytic amount of 2 M NaOH (0.05 mL) was added and the mixture was warmed under stirring for 1 h at 85 °C. After dilution with water (80 mL), the suspension was extracted with chloroform (3 × 50 mL). After removal of the solvent, the crude product was recrystallized.

Typical Procedure for 3e–g—To a stirred suspension of the appropriate compound **2** (2.5 mmol) in acetic acid (50%, w/v; 20 mL) and nitric acid (65%, w/v; 2.5 mL), CAN (15 mmol) was added in a portionwise manner at 55–60 °C over a 60-min period. Ice-cold water (100 mL) was added and the mixture was extracted with ethyl acetate (3 × 50 mL). Removal of the solvent afforded a residue which was crystallized or purified by column chromatography on silica gel [**3e** eluant, toluene:ethyl acetate (7:3); **3f** eluant, toluene:ethyl acetate (9:1)], and then recrystallized.

General Procedure for 4b, 4d–f and 4l–m—Palladium on activated carbon (10% Pd/C, 0.5 g) was added to a suspension of suitable isoxazolopyridazinones (5 mmol) in ethanol (220 mL), and the mixture was hydrogenated in the Parr apparatus for 12 h. After filtration and several washings with ethanol, the reaction products were obtained from the solution by concentration under reduced pressure.

5-Acetyl-4-amino-6-methyl-2-phenylpyridazin-3(2*H*)-one (6)—Following the same procedure described above, catalytic hydrogenation of **2b** afforded **6** (63% yield).

General Procedure for 4g–i—To a stirred suspension of **3a** (1.5 mmol) in ethanol (15 mL) the appropriate amine was added. After 0.5 h, **4g** was obtained by filtration, while **4h–i** were isolated after dilution with water (100 mL), extraction with ethyl acetate (3 × 150 mL), and removal of the solvent. Compound **4h** (oil) was purified by column chromatography on silica gel (eluant, cyclohexane:ethyl acetate, 2:1).

5-Acetyl-2-methyl-6-phenylpyridazin-3(2*H*)-one (5)—To a stirred and cooled suspension of **3a** (1.5 mmol) in methanol (8 mL) a solution of sodium borhydride (1 mmol) in methanol (3 mL) was added. After 0.5 h, the mixture was diluted with water (80 mL) and extracted with ethyl acetate (3 × 50 mL). The solvent was removed and the residue was chromatographed on silica gel (eluant, cyclohexane:ethyl acetate, 1:1).

4-Amino-2,6-dimethyl-5-(4-methoxyphenyl)-pyrazol-3-yl-pyridazin-3(2*H*)-one (8)—A mixture of **2p**[23] (1.35 mmol), hydrazine hydrate (200 mmol), and ethanol (5 mL) was refluxed for 20 min. After cooling, the crude product was filtered and purified.

In Vitro Studies on Human Platelet Aggregation—Blood was obtained from healthy human volunteers who had not taken drugs for at least two weeks. Blood samples (9 mL) were collected in siliconated tubes containing 1 mL of 3.8% sodium citrate. The blood was centrifuged at 1000 rpm for 20 min to obtain platelet-rich plasma (PRP). Further centrifugation at 2500 rpm for 10 min was effected to obtain platelet-poor plasma (PPP). The platelet count was adjusted to ~3 × 10^8 cells/mL for aggregation studies. Measurement of platelet aggregation was performed according to the turbidimetric method of Born and Cross,[31] using a PA 3210 aggregometer (Daiichi, Kyoto, Japan).

All tested compounds were dissolved in DMSO and diluted until a maximum final concentration of 0.1% in residual solvent was obtained. This final concentration had no measurable effect on platelet aggregation in preliminary experiments. To induce platelet aggregation, a solution (0.050 mL) containing the aggregating agent (ADP, arachidonate, or PAF-acether) was added to the cuvette containing 0.450 mL of PRP. The percentage of aggregation was calculated from the amplitude of the trace in comparison with PRP from control donors (100% aggregation) and PPP (0% aggregation).

The ADP (Daiichi Kyoto, Japan) was used at a final concentration of 3.0 μM, arachidonic acid (A-6523 sodium salt, Sigma Chemical) at a final concentration of 1.0 mM, and PAF-acether (P-5029, Sigma Chemical) at a final concentration of 1.0 μM. The IC_{50} values were obtained from the dose–effect curves.

Rabbit Platelet Aggregation Ex Vivo Studies—Rabbits weighing ~2800 g were used. The animals were given the test compound by the oral route via a catheter placed in the stomach. Treated animals received 5 mL of 1% Carbowax containing the drug, while control animals were given only 5 mL of 1% Carbowax. Three hours after dosing, 14 mL of blood was collected by cardiac withdrawal, into a syringe containing 2 mL of 3.8% sodium citrate, from the anesthetized animals [anesthesia by injection of 0.5 mL sodium pentothal (1%, w/v) into the ear marginal vein].

Platelet-rich plasma (PRP) and PPP were prepared as previously described. The platelet count was adjusted to ~3 × 10^8 platelets/mL. The aggregation tests were performed as described for in vitro studies. Five animals were used for each experiment. The platelet aggregation of control rabbits showed that the anesthetic did not interfere with the basal values.

Anti-inflammatory Activity in Rats—The inhibition of carrageenin-induced rat paw edema by the tested substances was performed according to the method of Winter et al.[32] and studied as follows. Male rats (180–220 g) following an 18-h fast were used. The animals were given the compounds po in a 5 mL 1% Carbowax suspension 1 h before the injection of 0.05 mL of 1% carrageenin suspension into the right and left hind paws. The paw volume was measured 3 h after the dose by plethysmography (Ugo Basile Plethysmography, Milano, Italy). Five rats per compound or dose were tested. Carrageenin type I was purchased from Sigma Chemical (St. Louis, MO). Results are expressed as percent reduction of edema in comparison with controls.

Analgesic–Anti-inflammatory Activity—This activity was studied by a modification of the Randall and Selitto[33] method, using the same type and number of rats as in Winter's anti-inflammatory test and employing PAF-acether as the algogenic agent. The edema was induced in the right and left hind paws by intradermal injection (0.05 mL) of 2.5 μg PAF-acether. PAF-Acether (Sigma Chemical P-5029) was dissolved with a 0.9% (w/v) NaCl solution containing 0.01% serum albumin (final concentration 10^{-4} M). The drugs were administered orally 60 min before edema induction. The pain threshold of the inflamed paw was assessed 1 h after injection of PAF-acether using a Basile apparatus.

In Vitro Studies on Lipopolysaccharide (LPS)-Stimulated Rat Peritoneal Macrophages—Cells in the peritoneal washing from male Wistar rats (220–280 g) were plated at 2 × 10^6 cells/well in multiwell plates (24 × 10 mm; Costar, Cambridge, MA) and incubated in RPMI 1640 (Gibco, Paisley, U.K.) containing 2 mM l-glutamine, 25 mM HEPES buffer, 50 μg/mL of gentamycin sulfate (Sigma, St. Louis, MO), and supplemented with 20% fetal calf serum (FCS; Seromed, Berlin, FRG). After 2 h of incubation at 37 °C in a 5% CO_2 atmosphere, nonadherent cells were removed by washing thor-

oughly and the adherent population was further incubated in a solution of RPMI 1640 and 5% FCS with 10 μg/ml of LPS from *E. coli* (055:B5, Difco) with or without test compounds. After 24 h, supernatant aliquots were collected for the PGE_2 assay, the remaining supernatants being dialyzed against phosphate buffer solution (1:200, v/v) twice and sterile filtered before the IL-1 assay. The PGE_2 concentration was determined by specific radioimmunoassay (N.E.N., Dreieich, FRG). The IL-1 activity in dialyzed supernatants was assessed by a murine thymocyte costimulation assay.[34] Thymocytes (6×10^5) from C3H/HeJ mice were incubated in multiwell plates (Costar) in 0.2 mL of RPMI 1640 and 5% FCS, with 1.5 μg/mL of phytohemoagglutinin (PHA; The Wellcome Foundation, Beckenham, U.K.) and serial twofold dilutions of supernatants. After 72 h, cells were pulsed for a further 15 h with 1 μCi/well of [^{3}H]thymidine (185 GB_9/mmol, Amersham International, U.K.). Data are presented as units/mL, as calculated for a sample dilution, giving 50% of the maximal thymidine incorporation as compared with standard curves obtained with endotoxin-free human recombinant IL-1 beta (1×10^7 units/mg; Sclavo, Siena, Italy).

Acute Oral Toxicity of Compound 4a—Compound **4a** was administered po to mice at doses of 300, 500, and 1000 mg/kg in a 1% Carbowax suspension. Ten mice per dose were tested.

References and Notes

1. Curran, W. V.; Ross, A. *J. Med. Chem.* **1974**, *17*, 273–281.
2. McEvoy, F. J.; Allen, G. R. *J. Med. Chem.* **1974**, *17*, 281–286.
3. Thyes, M.; Lehmann, H. D.; Gries, J.; Koning, H.; Kretzschmar, R.; Knuze, J.; Lebkucher, R.; Lenke, D. *J. Med. Chem.* **1983**, *26*, 800–807.
4. Takaja, M.; Sato, M.; Terashima, K.; Tanizawa, H. *J. Med. Chem.* **1979**, *22*, 53–58 and references therein cited.
5. Griffed, E. M.; Kinnon, S. M.; Kuman, A.; Lecker, D.; Smith, G. M.; Tonnich, E. G. *Br. J. Pharmacol.* **1981**, *72*, 697–705.
6. Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaar, R. E. *J. Med. Chem.* **1984**, *27*, 1099–1101.
7. Sircar, I.; Duell, B. L.; Bobowski, G.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1985**, *28*, 1405–1413.
8. Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Wyss, V.; Pollosk, G. D.; Wilson, H.; Kanffman, R. F.; Hayes, J. S. *J. Med. Chem.* **1986**, *29*, 1832–1840.
9. Ludmer, P. L.; Wright, R. F.; Arnold, J. M.; Ganz, P.; Braunwald, E.; Colucci, W. S. *Circulation* **1986**, *73*, *I*, 130–137.
10. Jafri, S. M.; Burlew, B. S.; Goldberg, A. D.; Rogers, A.; Goldstein, S.; *Am. J. Cardiol.* **1986**, *57*, 254-259.
11. Terris, S.; Bourdillon, P. D. V.; Cheng, D.; Latts, J.; Olsen, S.; Nicklas, J.; Pitt, B. *Am. J. Cardiol.* **1986**, *58*, 596–600.
12. Lejemthel, T. H.; Gumbardo, D.; Chadwick, B.; Rutman, H. I.; Sonnenblick, E. H. *Circulation* **1986**, *73*, *III*, 213–218.
13. Baim, D. S.; Colucci, W. S.; Momrad, E. S.; Smith, H. S.; Wright, R. F.; Lanoue, A.; Gauthier, D. F.; Ransil, B. J.; Grossman, W.; Braunwald, E.; *J. Am. Coll. Cardiol.* **1986**, *7*, *III*, 661–670.
14. Sircar, I.; Steffen, R. P.; Bobowski, G.; Burke, S. E.; Newton, R. S.; Weishaar, R. E.; Bristol, J. A.; Evans, D. B. *J. Med. Chem.* **1989**, *32*, 342–350 and references cited therein.
15. Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishar, R. E.; Bristol, J. A.; McPhail, A. J. *J. Med. Chem.* **1987**, *30*, 1963–1972.
16. Dal Piaz, V.; Ciciani, G.; Turco, G. *Synthesis* **1989**, 213–214.
17. Bagli, S.; Bogri, T.; Palameta, B.; Rahkit, S.; McQuillant, J.; Lee, D. K. H. *J. Med. Chem.* **1988**, *31*, 814–823.
18. Renzi, G.; Dal Piaz, V. *Gazz. Chim. Ital.* **1965**, *95*, 1478–1491.
19. Renzi, G.; Dal Piaz, V.; Musante, C. *Gazz. Chim. Ital.* **1968**, *98*, 656–666.
20. Skinner, G. S. *J. Am. Chem. Soc.* **1924**, *46*, 731–741.
21. Bauer, V. J.; Lanzilotti, A. E. *J. Labelled Compd.* **1969**, *5*, 87–89.
22. Renzi, G.; Pinzauti, S. *Il Farmaco ed. sc.* **1969**, *24*, 885–892.
23. Dal Piaz, V.; Pinzauti, S.; Lacrimini, P. *J. Heterocycl. Chem.* **1976**, *13*, 409–410.
24. Sprio, V.; Aiello, E.; Mazza, A. *Annali di Chimica* **1967**, *57*, 836–845.
25. Dal Piaz, V.; Ciciani, G.; Chimichi, S. *Heterocycles* **1986**, *24*, 3143–3148.
26. Duffin, G. F.; Kendall, J. D. *J. Chem. Soc.* **1959**, 3789–3799.
27. Braquet, P.; Touqui, L.; Shen, T. Y.; Vargeftig, B. B. *Pharmacol. Rev.* **1987**, *39*, 97–145.
28. Kloprogge, E.; De Haas, G. H.; Gorter, G.; Akkerman, J. W. N. *Thromb. Res.* **1983**, *30*, 107–112.
29. Bonnet, J.; Loiseau, A. M.; Orvoen, M.; Bessin, P. *Agents Actions* **1981**, *11*, 559–563.
30. Dinarello, C. A. *FASEB J.* **1988**, *2*, 108–115.
31. Born, G. V. R.; Cross, M. J. *J. Physiol.* **1963**, *168*, 178–195.
32. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544–547.
33. Randall, L. O.; Selitto, J. *J. Arch. Int. Pharmacodyn.* **1957**, *111*, 409–419.
34. Becherucci, C.; Donati, D.; Perretti, M.; Parente, L. *Agents Actions* **1989**, *28*, 243–247.

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