from 7 as an amorphous powder in 90% yield by the procedure used to prepare 2. R_f: 0.24. $[\alpha]^{25}_{578}$ = +13.2° (c = 1, H_2O). Anal. ($C_{17}H_{28}N_4O_7F_3IS$): C, H, N.

N^2-[[N^α,N^ϵ-Bis(*tert*-butoxycarbonyl)-L-lysyl]-L-norvalyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (9). Compound 9 was obtained as described for 1, as an oil (1.22 g, 90%). Anal. ($C_{26}H_{46}N_5O_9I$): C, H, N.

N^2-(L-Lysyl-L-norvalyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid bis(trifluoroacetate salt) (10) was obtained from 9 as an amorphous powder in 94% yield by the procedure used to prepare 2. R_f: 0.12. $[\alpha]^{25}_{578}$ = –6.2° (c = 1, H_2O). Anal. ($C_{20}H_{32}N_5O_9F_6I$): C, H, N.

N^2-[N-(*tert*-Butoxycarbonyl)-L-methionyl]-N^3-(iodoacetyl)-L-2,3-diaminopropanoic Acid (11). According to the methodology described for 1, peptide 11 was obtained (3.13 g, 90% yield). Mp: 122–124 °C. ^{1}H NMR ($CDCl_3$): δ 1.1–1.3 (m, 2 H), 1.4 (s, 9 H), 2.1 (s, 3 H), 2.2–2.4 (m, 2 H), 2.8 (s, 2 H), 3.6–3.8 (m, 2 H), 4.0–4.3 (m, 1 H), 4.6–4.8 (m, 1 H), 5.6 (br s, 1 H), 7.1 (br s, 1 H). Anal. ($C_{15}H_{26}N_3O_6SI$): C, H, N.

N^2-(L-Methionyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoic acid trifluoroacetate salt (12) was obtained from 11 as an amorphous powder in 92% yield by the procedure used for preparation of 2. R_f: 0.30. $[\alpha]^{25}_{578}$ = +15.1° (c = 1, H_2O). Anal. ($C_{12}H_{19}N_3O_6F_3IS$): C, H, N.

N-Succinimidoyl N^2-(*tert*-butoxycarbonyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoate (13) was synthesized according to the procedure described earlier.[1] Crystallization from ethyl acetate–petroleum ether gave 1.8 g (77% yield) of **13**. Mp: 123–125 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9 H), 2.7 (s, 4 H), 2.8 (s, 2 H), 3.6–3.8 (m, 2 H), 4.1–4.3 (m, 1 H), 5.8 (br s, 1 H), 7.2 (br s, 1 H). Anal. ($C_{14}H_{20}N_3O_7I$): C, H, N.

[N^2-(*tert*-Butyloxycarbonyl)-N^3-(iodoacetyl)-L-2,3-diaminopropanoyl]-L-norvaline (14). Peptide 14 was obtained with use of the procedure described for 1 and crystallized from ethyl acetate–hexane to give 0.61 g (65%). Mp: 129–131 °C. ^{1}H NMR ($CDCl_3$): δ 0.8–1.2 (br m, 7 H), 1.45 (s, 9 H), 2.7 (s, 2 H), 3.6–3.8 (m, 2 H), 4.1–4.3 (m, 1 H), 4.5–4.7 (m, 1 H), 5.6 (br s, 1 H), 7.0 (br s, 1 H). Anal. ($C_{15}H_{26}N_3O_6I$): C, H, N.

[N^3-(Iodoacetyl)-L-2,3-diaminopropanoyl]-L-norvaline trifluoroacetate salt (15) was prepared from 14 in 90% yield as an amorphous powder by the procedure used for preparation of 2. R_f: 0.39. $[\alpha]^{25}_{578}$ = 2.4° (c = 1, H_2O). Anal. ($C_{12}H_{19}N_3O_6F_3I$): C, H, N.

Estimation of Peptidoglycan Synthesis. Bacteria were grown overnight with aeration at 37 °C in MBD LYC medium (Difco). Then, the cultures were transferred into similar medium and grown with aeration until an absorbance of approximately 0.6 at 660 nm was reached. An absorbance of 1.0 was an equivalent of 340 μg of dry weight per mL. Cultures were harvested by centrifugation and resuspended in the same medium supplemented with chloramphenicol (100 μg/mL) and prewarmed to 37 °C. Samples (10 mL) were then incubated for 30 min, [^{14}C]-DL-Ala (0.5 μCi/mL) was added to each of the 10-mL cultures, the appropriate amount of the peptide was added, and 0.5-mL aliquots were removed at intervals and diluted into 5 mL of an ice-cold trichloroacetic acid (10%, v/v). After storage on ice for at least 15 min, the samples were heated at 80 °C for 15 min to remove teichoic acid and then filtered through a glass-fiber filter (Whatman GF/C). The filters were washed with cold 5% trichloroacetic acid, ethanol, and ethyl ether and counted for radioactivity.

Reversal of the Growth Inhibition Effect of Tripeptide on *C. albicans* ATCC 26278 by *N*-Acetylglucosamine. *C. albicans* ATCC 26278 from logarithmic phase of growth on Sabouraud medium at 30 °C were harvested, washed with 0.9% saline, and suspended in YNB medium (Difco) at about 10^5 cells per mL. After 10 min of preincubation, a solution of peptide was added to obtain a final concentration of 10 μM, then GlcNAc (10 mM) was added at 90 and 180 min, and the optical density of the culture was measured at 660 nm in a Zeiss spectrocolorimeter.

Acknowledgment. We gratefully acknowledge the Technical University of Wrocław (Project No. CPBR 3.13.6.) for financial support of this work.

4-Heterocyclyloxy-2*H*-1-benzopyran Potassium Channel Activators

Rolf Bergmann,† Volker Eiermann,‡ and Rolf Gericke*,†

Preclinical Pharmaceutical Research and Central Analytical Laboratory, E. Merck, Frankfurter Strasse 250, D-6100 Darmstadt, Germany. *Received January 9, 1990*

The reaction of 2,4-dihydroxypyridine (2) with 3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (1) yielded the 4-[(1,2-dihydro-2-oxo-4-pyridyl)oxy] compound **3a**, accompanied by small amounts of the isomeric 4-(1,2-dihydro-4-hydroxy-2-oxo-1-pyridyl) compound **4**. This could also be prepared by hydrogenation of the benzyloxy derivative **5**. Reaction of 3,6-pyridazinediol (**10**) with 1 (R = CN) gave the 4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy] compound **11a**, which in turn rearranged on heating with NaH in DMSO into the 4-(1,6-dihydro-3-hydroxy-6-oxo-1-pyridazinyl) compound **12**. A series of 6-substituted analogues (R = CO_2Me, $CSNH_2$, NO_2, Br) of **3a** and **11a** were synthesized. N-Alkylation led to compounds **14a–c** (R = Me, Et, $CHMe_2$). The 4-heterocyclyloxychromenes **9** and **16a** were obtained by alkaline hydrolysis of their 3-camphorsulfonates. The racemic pyridazinyloxy compounds **11a** and **14a** could be resolved via their diastereomeric camphorsulfonates or camphanates. The differences between the 4-heterocyclyloxychromanols and the isomeric N-substituted compounds **4** and **12** were elucidated in the course of extensive NMR investigations. While in DMSO the former appeared to be conformationally flexible molecules the latter were rigid. All compounds were tested for oral antihypertensive activity in spontaneously hypertensive rats, using doses of 1 mg/kg. High and long lasting activities were found for the pyridyloxy compounds **3a** and **3d**, the pyridazinyloxy compound **11a**, and its N-alkylation products, as well as for the 3*S*,4*R*-enantiomers **20a** and **22a**. (–)-(3*S*,4*R*)-3,4-Dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**22a**) was selected for further development.

Angina pectoris, bronchial asthma, and essential hypertension are disorders in which intermittent or permanent narrowing of the vascular or bronchial lumina as a result of vaso- or bronchoconstriction is encountered. A growing number of people suffer from these disorders attributed to modern civilization, which cannot be treated adequately by current therapeutics. It would be desirable to have available agents acting more selectively on the coronary or bronchial systems combined with a smooth muscle relaxing effect. The recently characterized potassium channel activators may be helpful here.[1] At

† Preclinical Pharmaceutical Research.
‡ Central Analytical Laboratory.

(1) Weston, A. H. *Drug News Perspectives* **1988**, *1*, 205.

0022-2623/90/1833-2759$02.50/0

present, much interest is centered around the possible value in the therapy of obstructive airway diseases since animal studies[2,3] and administration to humans[4,5] have indicated useful bronchodilator activity. In addition, it has been described that compounds of this class have a direct coronary artery dilating effect.[6-8] In clinical studies in the therapy of angina pectoris[9-11] a longer duration of action than with a conventional nitro vasodilator was found. As the calcium antagonists, the potassium channel activators include substances of wide structural diversity: the cyanoguanidine pinacidil, the nitrate nicorandil, *N*-methyl-2-(3-pyridinyl)tetrahydrothiopyran-2-thiocarboxamide 1-oxide (RP 49 356), and the chroman derivatives. The latter class with cromakalim as its prototype appears to be of particular significance, which is emphasized by the high pharmacological potency of these substances.

Recently, reactions between 3,4-epoxy-3,4-dihydro-2*H*-1-benzopyrans and 2-pyridones were reported to produce very potent 4-(1,2-dihydro-2-oxo-1-pyridyl)-2*H*-1-benzopyrans.[12] Pyrimidones, pyridazinones, and pyrazinones reacted analogously. Surprisingly a few heterocyclic compounds with two oxygen atoms as 2,4-dihydroxypyridine or 3,6-pyridazinediol behaved quite differently: Highly active benzopyran derivatives with a novel C-4 substitution were obtained.

Chemistry

With one exception, the (±)-epoxides **1** used as starting materials have been described before. (±)-6-Bromo-3,4-epoxy-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran (**1**, R = Br) was synthesized from 4-bromophenol, according to the method published by Evans et al.[13] All the 4-substituted 2*H*-1-benzopyran-3-ols obtained by opening of the epoxide precursors inevitably possessed the trans configuration. Unless stated otherwise, all products were racemic mixtures. Where tautomeric structures were possible, only one form was represented at random.

Reaction of 2,4-dihydroxypyridine (**2**) with the epoxide **1** (R = CN) in ethanol containing a catalytic amount of pyridine, according to the standard method for 2-pyridones,[12] gave **3a** almost exclusively (Scheme I). Several authors initially failed to recognize[14,15] that this was in fact the oxygen-linked compound **3a**, and not the isomeric nitrogen-bound **4**. The opening of the epoxide resulted as expected with N-substitution[12] (→ **5**), if one oxygen was protected, as for 4-benzyloxy-2-oxo-1,2-dihydropyridine. Catalytic hydrogenation of the benzyl

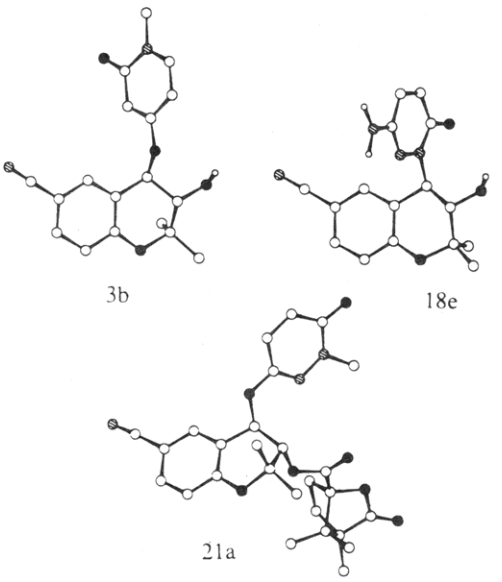
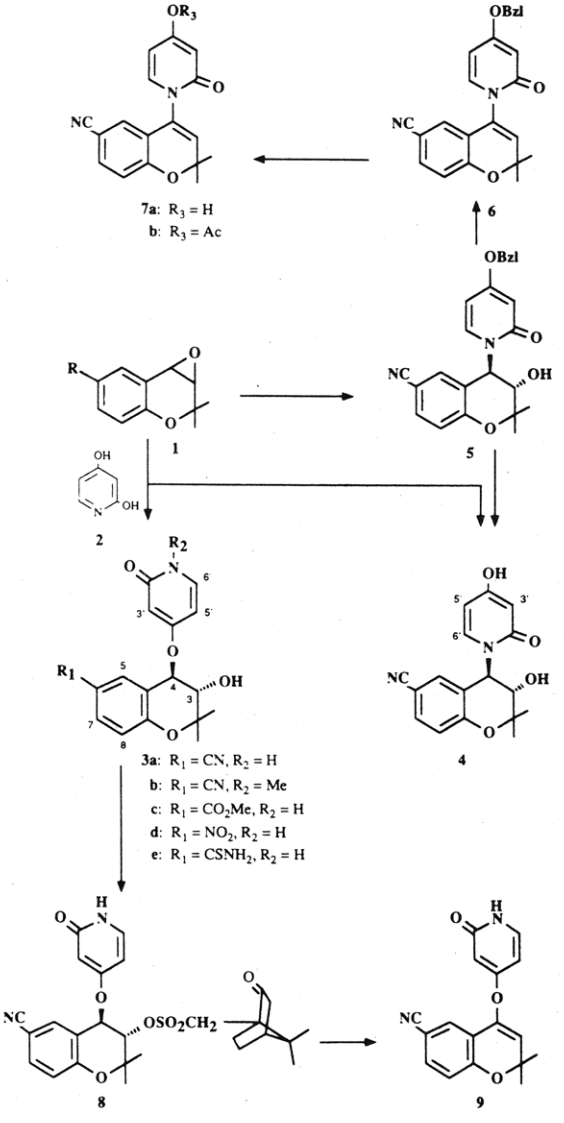


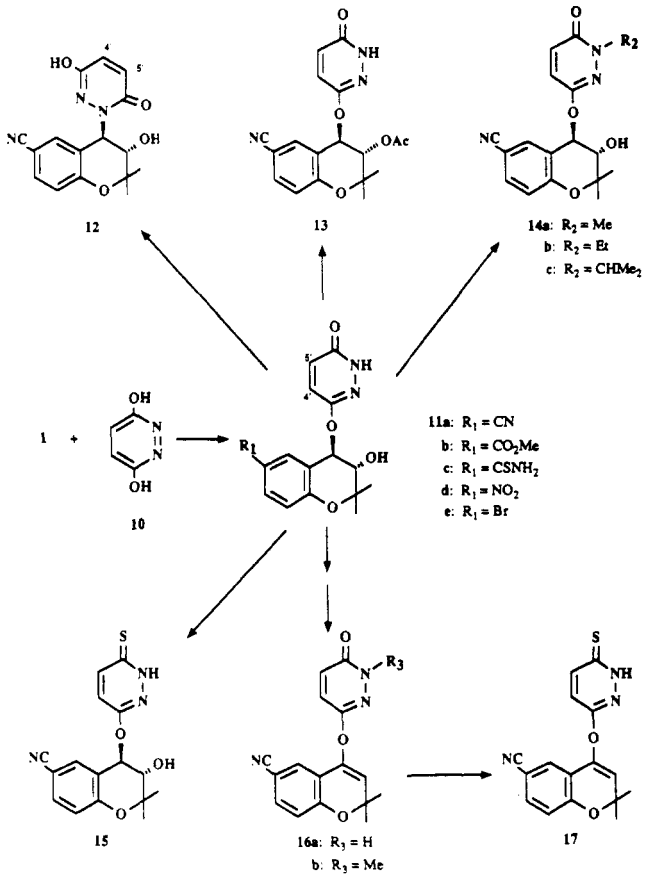
Figure 1. X-ray crystal structures of compounds **3b**, **18e**, and **21a**. Small circles represent hydrogen, large white circles carbon, black circles oxygen, and hatched circles nitrogen atoms. Hydrogen atoms attached to carbon are omitted for clarity.

Scheme I



(2) Arch, J. R. S.; Buckle, D. R.; Bumstead, J.; Clarke, G. D.; Taylor, J. F.; Taylor, S. G. *Br. J. Pharmacol.* **1988**, *95*, 763.
(3) McCann, J. D.; Welsh, M. J. *Annu. Rev. Physiol.* **1990**, *52*, 115.
(4) Baird, A.; Hamilton, T. C.; Richards, D. H.; Tasker, T.; Williams, A. J. *Br. J. Clin. Pharmacol.* **1988**, *25*, 114P.
(5) Williams, A. J.; Hopkirk, A.; Lavender, E.; Vyse, T.; Chiew, V. F.; Lee, T. H. *Am. Rev. Respir. Dis.* **1989**, *139*, A140.
(6) Maruyama, M.; Farber, N.; Gross, G. J. Presented at the FASEB, 73rd Annual Meeting, New Orleans, 1989; Abstr. 3894.
(7) Maruyama, M.; Gross, G. J. Presented at the FASEB, 74th Annual Meeting; Washington, DC, 1990; Abstr 315.
(8) Daut, J.; Maier-Rudolph, W.; von Beckerath, N.; Mehrke, G.; Günther, K.; Goedel-Meinen, L. *Science*, **1990**, *247*, 1341.
(9) Belz, G. G.; Matthews, J.; Heinrich, J.; Wagner, G. *Eur. J. Clin. Pharmacol.* **1984**, *26*, 681.
(10) Kishida, H.; Murao, S. *Clin. Pharmacol. Ther.* **1987**, *42*, 166.
(11) Thomas, P.; Dixon, M. S.; Winterton, S. J.; Sheridan, D. J. *Br. J. Clin. Pharmacol.* **1990**, *29*, 325.
(12) Bergmann, R.; Gericke, R. *J. Med. Chem.* **1990**, *33*, 492.
(13) Evans, J. M.; Fake, C. S.; Hamilton, T. C.; Poyser, R. H.; Watts, E. A. *J. Med. Chem.* **1983**, *26*, 1582.
(14) Häusler, G.; Gericke, R.; Wurziger, H.; Baumgarth, M.; Lues, I.; de Peyer, J.; Bergmann, R.; European Pat. Appl. 273262, 1988.
(15) Garcia, G.; Roux, R.; Nisato, D.; Gautier, P.; European Pat. Appl. 296975, 1988.

Scheme II



group yielded compound **4**, which was clearly not identical with **3a**. Careful investigation of the reaction with 2,4-dihydroxypyridine (**2**) showed that this also yielded traces of compound **4**. By changing the reaction conditions (DMSO/NaH; 130 °C) it was possible to shift the product ratio in favor of compound **4**. The final structural assignment was reached by X-ray structural analysis (Figure 1) of **3b**, obtained by methylation of **3a**.

The methyl carboxylate **3c** and the nitro derivative **3d** were synthesized from the corresponding substituted epoxides **1**. The thiocarbamoyl compound **3e** was available by the addition of hydrogen sulfide to nitrile **3a**. As shown previously,[12] elimination of water to form chromenes is a simple process; it may be achieved, for example, by treatment with sodium hydroxide or sodium hydride in refluxing THF or dioxane. Chromene **6** was readily synthesized from **5** by using this method. Catalytic hydrogenolysis resulted in the formation of **7a**, leaving the chromene double bond intact. Treatment with acetic anhydride resulted in the fairly unstable acetate **7b**. In contrast to **5**, direct elimination of water with sodium hydroxide or sodium hydride was not possible with the pyridyloxy compound **3a**, but a small amount of chromene **9** could be obtained after activation to the camphorsulfonate **8** with sodium hydroxide in methanol.

3,6-Pyridazinediol (**10**) showed complex behavior during alkylations: N-alkylations, O-alkylations, or double alkylations could be observed,[16] depending on the reagents or conditions. On the treatment with epoxide **1** (R = CN) **10** showed a reaction pattern similar to that of 2,4-dihydroxypyridine (**2**) and a satisfactory yield of 3,4-dihydro-4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**11a**) was obtained (Scheme II). The N-bonded product **12** was not isolated. In contrast, warming **11a** with NaH in DMSO yielded the rearrangement product 3,4-dihydro-4-(1,6-dihydro-3-hydroxy-6-oxo-1-pyridazinyl)-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**12**), in addition to a few degradation products. Substances **11b**, **11d**, and **11e** were synthesized from the corresponding substituted epoxides **1**. The thiocarbamoyl compound **11c** was prepared by addition of hydrogen sulfide to **11a**.

Methylation of **11a** with dimethyl sulfate in acetone resulted in the *N*-methyl derivative **14a**. X-ray structural analysis of **14a** proved that epoxide **1** was preferentially attacked by an oxygen atom of 3,6-pyridazinediol. The ethyl group was later introduced into **11a** with diethyl sulfate (→ **14b**) and the isopropyl group correspondingly with 2-bromopropane (→ **14c**). Synthesis of **14a** using 3-hydroxy-1-methyl-6-oxo-1,6-dihydropyridazine[17] turned out to be even more favorable. Monoacetylation of the 3-hydroxy group of **11a** was brought about by treatment with excess acetic anhydride/pyridine (→ **13**). The chromene compound **16a** resulted from the treatment of the diasteromeric camphorsulfonates with alkali. This was used in the synthesis of enantiomers of **11a** (see below). Compound **16b** was synthesized from **16a** by using dimethyl sulfate, and thioxo compounds **15** and **17** were prepared from **11a** and **16a**, respectively, by using Lawesson's reagent in toluene.

Table I shows the results of the reactions of epoxide **1** (R = CN) with variously substituted 6-membered heterocyclic compounds. These were pyridines, pyrimidines, and pyridazines with either two oxygen atoms or one amino group and one oxygen atom. Compounds **18a–g** were obtained in the standard process of heating for several hours in ethanol/pyridine. As the only exception **18f** needed a 3-day heating period without any base. Only products occurring in significant quantities were isolated and trial conditions were not optimized. This led to moderate yields in some cases. With the scarcity of investigations on the reactivity of the bifunctional heterocyclic compounds in the literature it was not possible to make any predictions about the nature of the products. Structures were elucidated by NMR investigations with the exception of **18e**, for which an X-ray structure analysis was carried out (Figure 1).

2,5-Dihydroxypyridine,[18] 2,3-dihydroxypyridine, as well as, 4,6-dihydroxypyrimidine reacted analogously to 2,4-dihydroxypyridine (**2**) to give products **18a–c**. This showed their apparent preference for O-alkylation vs N-alkylation. Where there was a choice between an amino and a hydroxy group, as in 2-amino-3-hydroxypyridine, the reaction also took place at the oxygen group (→ **18d**). The cases of **18e** and **18f** were particularly interesting, since the reacting hydroxy groups were replaced by amino groups, compared to **10** and **2**. With both 3-amino-6-pyridazinol[19] and 4-amino-2-hydroxypyridine,[20] formation of an NH-link did not occur, and the reaction took place at the ring nitrogen of the pyridazinone and pyridone, respectively. The formation of the 4-(4-amino-1,2-dihydro-2-oxo-1-pyrimidinyl) compound **18g** from 4-amino-2-hydroxypyrimidine (cytosine) fits into this picture.

Cromakalim was resolved into its enantiomers via sep-

(16) Mason, J. W.; Aldous, D. L. *Pyridazines. The Chemistry of Heterocyclic Compounds*, Castle, R. N., Ed.; John Wiley & Sons: New York, 1973; Vol. 28, p 37.

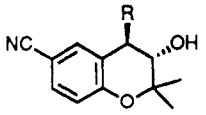
(17) Rubinstein, H.; Skarbek, J. E. *J. Org. Chem.* **1971**, *36*, 3372.

(18) Behrman, E. J.; Pitt, B. M. *J. Am. Chem. Soc.* **1958**, *80*, 3717.

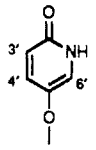
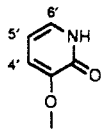
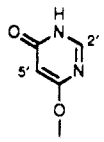
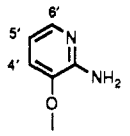
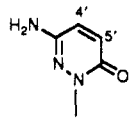
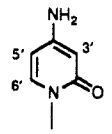
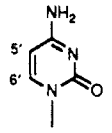
(19) Horie, T.; Kinjo, K.; Ueda, T. *Chem. Pharm. Bull.* **1962**, *10*, 580.

(20) Bäumler, J.; Sorkin, E.; Erlenmeyer, H. *Helv. Chim. Acta* **1951**, *34*, 499.

Table I. 4-Substituted *trans*-3,4-Dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitriles



18

no.	R	reaction time, h	yield, %	mp, °C	recryst solvent	formula	anal.[a]	max fall[b] in BP in mmHg ± SEM in SHR
18a		8	24	256.5–258	MeOH	$C_{17}H_{16}N_2O_4 \cdot 0.25H_2O$	C, H, N	23 ± 4
18b		5	31	262–265	EtOAc/MeOH	$C_{17}H_{16}N_2O_4$	C, H, N	NS[c]
18c		10	17	238–239.5	Et_2O	$C_{16}H_{15}N_3O_4 \cdot 0.25H_2O$	C, H, N	NS
18d		2.5	48	207–208.5	MeCN	$C_{17}H_{17}N_3O_3 \cdot 0.25H_2O$	C, H, N	NS
18e		13	23	276–278	EtOAc	$C_{16}H_{16}N_4O_3$	C, H, N	39 ± 8
18f		78	14	289	$MeOH/CH_2Cl_2$	$C_{17}H_{17}N_3O_3 \cdot 0.25H_2O$	C, H, N	NS
18g		7	32	>280	EtOH	$C_{16}H_{16}N_4O_3$	C, H, N	NS

[a] Analyses for the elements indicated were within ±0.4% of the theoretical values. [b] Mean arterial blood pressure ($N \geq 3$) was measured directly before and up to 210 min after oral administration of 1 mg/kg of the test substance. [c] Compounds that did not lower the blood pressure significantly (<18 mmHg).

aration of the diastereomeric 3-(α-methylbenzyl carbamates).[21] This method was disadvantageous in the case of **11a**. Chromatographic separation of the two carbamates proved to be difficult, and subsequent hydrolysis with trichlorosilane/triethylamine resulted in low yields. The camphorsulfonates **19a** and **19b** in contrast were easily synthesized by using the commercially available (1*S*)-(+)-camphor-10-sulfonic acid chloride. The diastereomers were readily separated by medium-pressure chromatography (Scheme III). However, the subsequent hydrolysis of the sulfonates required drastic alkaline conditions (NaOH in MeOH) and the enantiomers **20a** and **20b** were produced in only moderate yields. As expected, this process was accompanied by elimination of the 3-hydroxyl function resulting in the chromene **16a** (Scheme II). In the case of the *N*-methyl compound **14a**, the diastereomeric camphanates **21a** and **21b** were used in the synthesis of enantiomers **22a** and **22b**. The optically active camphanic acid could be recovered after hydrolysis. Both synthesis and mild alkaline hydrolysis of camphanates **21** gave high yields. The separation of **21a** and **21b** had to be carried out by preparative HPLC. The configuration of (−)-camphanic acid is known. With the X-ray structural analysis of (1*S*)-camphanate **21a** the absolute configuration of the active enantiomer **22a** could thus be determined to 3*S*,4*R* (Figure 1).

NMR Spectroscopic Studies

The synthetic pathway suggested the structures of most of the compounds. In combination with that, ^1H NMR was included to elucidate the structures. X-ray structural analysis of compounds **3b**, **14a**, **18e**, and **21a** proved the structures unambiguously. The ^1H NMR data of the synthesized compounds are listed in Table III. The signals of the C-2 methyl groups with chemical shifts of 1.21–1.56

(21) Ashwood, V. A.; Buckingham, R. E.; Cassidy, F.; Evans, J. M.; Faruk, E. A.; Hamilton, T. C.; Nash, D. J.; Stemp, G.; Willcocks, K. *J. Med. Chem.* **1986**, *29*, 2194.

Table II. Compounds of Schemes I–III

no.	yield, %	mp, °C	recryst solvent	formula	anal.[a]	max fall[b] in BP in mmHg ± SEM in SHR	ED_{30},[c] μg/kg
3a	30	250–251	MeOH	$C_{17}H_{16}N_2O_4 \cdot 0.5H_2O$	C, H, N	104 ± 22	6
3b	34	202–203	Et_2O	$C_{18}H_{18}N_2O_4 \cdot 0.25H_2O$	C, H, N	83 ± 22	NT[d]
3c	12	251–252	Et_2O	$C_{18}H_{19}NO_6$	C, H, N	NS[e]	NT
3d	13	224–226	EtOAc/MeOH	$C_{16}H_{16}N_2O_6 \cdot 0.25H_2O$	C, H, N	117 ± 5	13
3e	22	242	MeOH	$C_{17}H_{18}N_2O_4S$	C, H, N, S	NS	NT
4	50	256–257	Me_2CO	$C_{17}H_{16}N_2O_4 \cdot 0.25H_2O$	C, H, N	28 ± 13	NT
7a	54	295	MeCN	$C_{17}H_{14}N_2O_3$	C, H, N	19 ± 5	NT
7b	70	170–172	Et_2O	$C_{19}H_{16}N_2O_4$	C, H, N	28 ± 6	NT
9	8	263–264	MeCN	$C_{17}H_{14}N_2O_3$	C, H, N	NS	NT
11a	47	255–256	$EtOH/H_2O$	$C_{16}H_{15}N_3O_4 \cdot 0.25H_2O$	C, H, N	117 ± 13	24
11b	23	242	Me_2CO/CH_2Cl_2	$C_{17}H_{18}N_2O_6 \cdot 0.25H_2O$	C, H, N	18 ± 7	NT
11c	63	142–144	MeOH	$C_{16}H_{17}N_3O_4S$	C, H, N, S	NS	NT
11d	46	288–290	EtOH	$C_{15}H_{15}N_3O_6$	C, H, N	78 ± 10	NT
11e	48	257–259	MeOH	$C_{15}H_{15}BrN_2O_4$	C, H, Br, N	NS	NT
12	57	294–296	Me_2CHOH	$C_{16}H_{15}N_3O_4 \cdot 0.25H_2O$	C, H, N	NS	NT
13	71	210–212	EtOAc	$C_{18}H_{17}N_3O_5$	C, H, N	97 ± 9	NT
14a	62	203–205	EtOH	$C_{17}H_{17}N_3O_4$	C, H, N	124 ± 26	18
14b	27	165–167	Et_2O	$C_{18}H_{19}N_3O_4$	C, H, N	127 ± 7	20
14c	42	201–203	CH_2Cl_2/Et_2O	$C_{19}H_{21}N_3O_4 \cdot 0.5H_2O$	C, H, N	32 ± 9	NT
15	14	192–193	CH_2Cl_2	$C_{16}H_{15}N_3O_3S \cdot 0.75H_2O$	C, H, N, S	NS	NT
16a	36	226–228	Et_2O	$C_{16}H_{13}N_3O_3$	C, H, N	NS	NT
16b	44	144–146	Et_2O	$C_{17}H_{15}N_3O_3$	C, H, N	NS	NT
17	30	191–192	Et_2O	$C_{16}H_{13}N_3O_2S$	C, H, N, S	NS	NT
20a	10	230–231	Et_2O	$C_{16}H_{15}N_3O_4$	C, H, N	129 ± 16[f]	14
20b	16	232–233	Et_2O	$C_{16}H_{15}N_3O_4$	C, H, N	NS	NT
22a	96	161–164	$EtOAc/Et_2O$	$C_{17}H_{17}N_3O_4$	C, H, N	151 ± 5	13
22b	98	161–162	$EtOAc/Et_2O$	$C_{17}H_{17}N_3O_4$	C, H, N	19 ± 9	NT
Cromakalim						78 ± 6	110
EMD 52 692						142 ± 9	10

[a,b] See footnotes in Table I. [c] Mean blood pressure; dose required to reduce blood pressure by 30 mmHg. [d] Not tested. [e] Compounds that did not lower the blood pressure significantly (<18 mmHg). [f] A dose of 0.3 mg/kg of the test substance was administered.

Table III. ^1H NMR Data[a] of Compounds of Schemes I–III and Table I

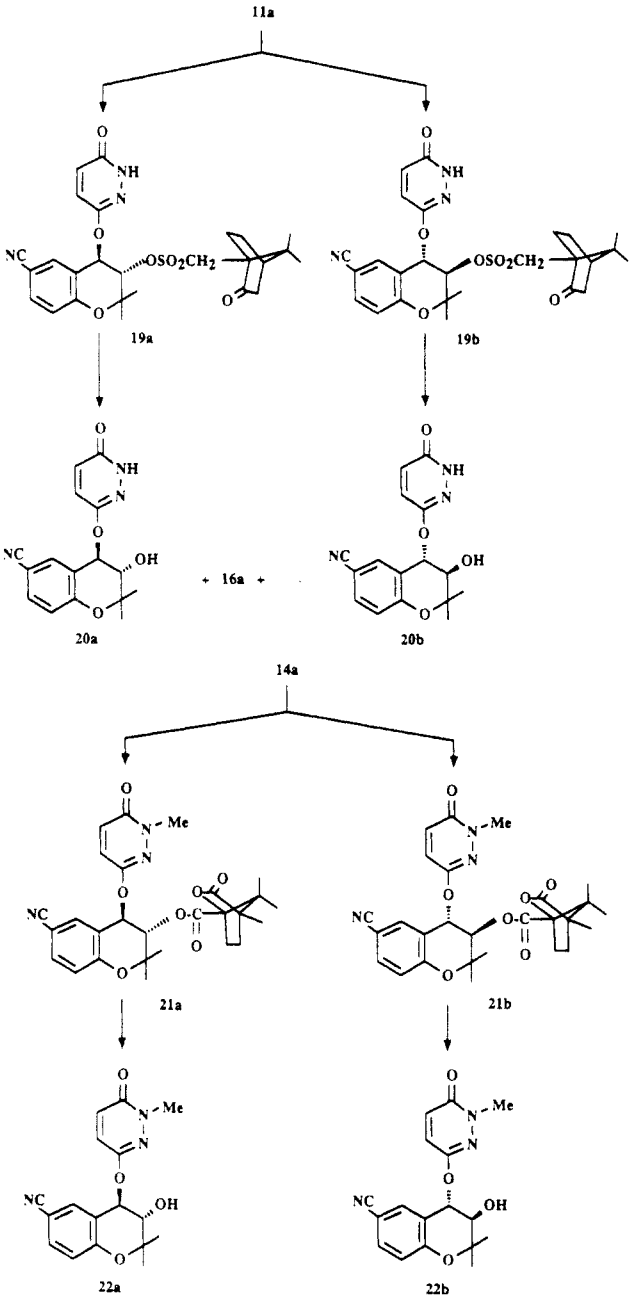
no.	H-3[b]	H-4	H-5	H-7	H-8	H-3′	H-4′	H-5′	H-6′
3a	3.79, t, 5.3	5.30, d, 5.3	7.66, m	7.66, m	6.97, d, 8.8	6.14, d, 2.1		5.96, m	7.29, d, 7.1
3b[c]	3.77, t, 5.3	5.94, d, 5.3[d]	7.65, m	7.65, m	6.96, d, 8.8	6.25, d, 2.8		6.02, dd, 7.4, 2.8	7.61, d, 7.4
3c[e]	3.78, m	5.89, d, 4.9[f]	7.83, s	7.82, dd, 9.5, 1.4	6.90, d, 9.5	6.14, d, 2.9		5.96, dd, 7.3, 3.1	7.29, d, 7.0
3d	3.82, t, 5.5	5.40, d, 5.5[g]	8.15, s	8.12, dd, 8.5, 2.8	7.03, d, 8.5	6.19, d, 2.8		5.96, dd, 7.1, 2.8	7.31, d, 7.0
3e	3.76, t, 5.1	5.83, d, 5.1[h]	7.92, d, 1.7	7.85, dd, 8.5, 1.7	6.82, d, 8.5	6.13, d, 2.5		5.95, dd, 7.4, 2.5	7.30, d, 7.4
4[i]	3.65, br	5.60, br	6.96, d, 2.0	7.50, dd, 8.5, 2.0	6.90, d, 8.5	5.68, d, 2.0		5.89, dd, 7.5, 2.0	7.21, d, 7.5
7a	6.05, s		6.92, d, 1.4	7.64, dd, 7.8, 1.4	7.00, d, 7.8	5.65, d, 2.8		6.01, dd, 7.0, 3.0	7.41, d, 7.2
7b[j,k]	5.80, s		6.98, d, 1.8	7.46, dd, 8.1, 1.8	6.91, d, 8.1	6.47, d, 2.5		6.22, dd, 7.4, 2.5	7.16, d, 7.4
9	5.74, s		7.42, d, 2.8	7.69, dd, 8.1, 2.8	7.03, d, 8.1	5.71, d, 2.8		6.12, dd, 7.1, 2.8	7.42, d, 7.1
11a[l]	3.81, dd, 6.8, 5.6	5.76, d, 6.8	7.74, dd, 2.0, 0.7	7.66, dd, 8.2, 2.3	6.97, d, 8.5		6.93, d, 9.8	7.20, d, 9.8	
11b[m]	3.80, d, 6.7	5.76, d, 6.7	7.85, d, 1.4	7.81, dd, 7.8, 1.4	6.90, d, 7.8		6.96, d, 9.9	7.24, d, 9.9	
11c	3.78, t, 5.8	5.78, d, 5.8[n]	7.91, d, 1.8	7.85, dd, 9.2, 1.8	6.81, d, 9.2		6.93, d, 10.2	7.20, d, 10.2	
11d	3.88, dd, 6.7, 5.6	5.82, d, 6.7	8.18, d, 3.1	8.12, dd, 9.5, 3.1	7.03, d, 9.5		6.97, d, 10.2	7.25, d, 10.2	
11e	3.76, dd, 7.0, 6.0	5.75, d, 7.0	7.36, m	7.36, m	6.94, d, 10.2		6.78, d, 9.5	7.22, d, 9.5	
12	4.08, dd, 9.8, 5.8	5.91, d br, 10.0	7.14, m, 2.6	7.59, dd, 8.5, 2.5	6.95, d, 8.5		7.00, d, 9.7[o]	7.07, d, 9.7[o]	
13[p]	5.31, d, 4.6[o]	5.84, d, 4.6[o]	7.90, d, 2.5	7.24, dd, 8.1, 2.5	7.05, d, 8.1		6.93, d, 10.2	7.18, d, 10.2	
14a[q]	3.87, dd, 6.9, 4.9	5.79, d, 6.9	7.80, d, 2.0	7.69, dd, 8.5, 2.0	7.00, d, 8.5		7.01, d, 10.2	7.23, d, 10.2	
14b[r]	3.92, d, 7.1	5.75, d, 7.3	7.53, d, 1.4	7.45, dd, 8.1, 1.4	6.86, d, 8.1		6.93, s	6.93, s	
14c[s]	3.95, dd, 6.7, 5.3	5.76, d, 6.7	7.80, d, 1.8	7.67, dd, 7.8, 1.8	6.99, d, 7.8		6.96, d, 10.2	7.20, d, 10.2	
15	3.84, d, 6.9	5.86, d, 6.9	7.82, d, 2.1	7.68, dd, 8.1, 2.1	6.99, d, 8.1		7.07, d, 9.9	7.59, dd, 9.9, 2.1[t]	
16a[j]	5.52, s		7.45, d, 2.1	7.47, dd, 8.8, 2.1	6.88, d, 8.8		7.07, d, 10.2	7.24, d, 10.2	
16b[u]	5.61, s		7.70, d, 2.1	7.69, dd, 8.1, 2.1	7.00, d, 8.1		7.08, d, 10.2	7.49, d, 10.2	
17	5.80, s		7.66, m	7.66, m	7.00, d, 8.5		7.31, d, 9.2[o]	7.66, m[o]	
18a	3.75, t, 5.6	4.87, d, 5.6[v]	7.84, d, 2.2	7.66, dd, 8.6, 2.1	6.95, d, 8.5	6.38, d, 9.6	7.55, dd, 9.6, 3.3		7.47, d, 3.0
18b	3.77, dd, 6.2, 5.1	5.13, d, 6.1	8.11, d, 2.2	7.66, dd, 8.6, 2.2	6.95, d, 8.6		7.16, dd, 6.5, 1.9[o]	6.20, t, 6.8	7.33, dd, 7.3, 1.9[o]
18c[w]	3.78, d br	5.98, d, 7.4	7.61, d, 1.0	7.66, dd, 7.6, 1.0	6.97, d, 7.6			5.69, s	
18d	3.81, t, 6.3	5.23, d, 6.4	7.66, m	7.66, m	6.97, d, 8.8		7.42, dd, 7.8, 1.0[o]	6.56, dd, 7.8, 4.6	7.59, dd, 4.8, 1.0[o]
18e	4.14, dd, 9.9, 6.3	5.92, d br, 9.9	7.06, d, 1.4	7.59, dd, 7.8, 1.4	6.94, d, 7.8		6.98, d, 9.5	6.86, d, 9.5	
18f[j,x]	4.11, br	5.70, br	7.20, s br	7.60, d br, 9.0	7.10, d, 9.0	br[y]		6.42, d, 9.0	7.60, br
18g[j]	4.11, d br, 9.0	5.30, d br, 9.0	7.10, d, 2.0	7.49, dd, 9.5, 2.0	6.89, d, 9.5			5.78, d, 7.0	7.38, d, 7.0
19a	5.15, d, 4.2[o]	5.99, d, 4.2[o]	7.95, d, 1.8	7.76, dd, 8.1, 1.8	7.07, d, 8.1		6.96, d, 9.9	7.19, d, 9.9	
19b	5.17, d, 4.2[o]	6.12, d, 4.2[o]	7.90, d, 1.5	7.75, dd, 8.1, 1.5	7.07, d, 8.1		6.95, d, 10.1	7.20, d, 10.1	
21a	5.56, d, 5.8[o]	5.92, d, 5.8[o]	7.58, d, 2.2	7.55, dd, 8.5, 2.2	6.98, d, 8.2		6.93, d, 9.8	7.00, d, 10.0	
21b	5.54, d, 5.5[o]	5.92, d, 5.5[o]	7.61, d, 2.3	7.55, dd, 8.5, 2.2	6.97, d, 8.5		6.93, d, 9.8	6.99, d, 9.8	

[a] Chemical shifts are referenced relative to TMS at 0 ppm; coupling constants are expressed in Hz. Spectra were run at 200 or 250 MHz in DMSO-d_6 at room temperature, except where noted. [b] The numbering of protons follows the indexing of compounds **3**, **4**, **11**, **12**, and **18** presented in Schemes I and II and Table I. [c] N-CH_3: 3.38, s. [d] Interchangeable with OH: 5.30, d, 5.3. [e] OCH_3: 3.78, s. [f] Interchangeable with OH: 5.34, d, 5.2. [g] Interchangeable with OH: 5.98, d, 5.5. [h] Interchangeable with OH: 5.25, d, 5.1. [i] Measured at 360 K. [j] $CDCl_3$ was used as solvent. [k] $COCH_3$: 2.32, s. [l] The data are identical with those of **20**. [m] OCH_3: 3.79, s. [n] Interchangeable with OH: 5.80, d, 5.8. [o] Interchangeable assignments. [p] $COCH_3$: 2.04, s. [q] NCH_3: 3.60, s. The data are identical with those of **22**. [r] C_2H_5: 4.05, m; 1.32, t, 7.1. [s] $CH(CH_3)_2$: 5.07, sept, 7.1; 1.30, d, 7.1; 1.21, d, 7.1. [t] $^4J_{H5'NH} = 2.1$. [u] NCH_3: 3.51, s. [v] Interchangeable with OH: 5.82, d, 5.6. [w] H-2′: 8.22, s. [x] TFA was added. [y] Due to severe broadening the proton cannot be identified.

ppm are omitted as well as those of the 3-hydroxy groups which appear at 4.10–5.99 ppm.

The protons at C-3 and C-4 have a trans configuration because of synthetic reasons. In the 4-heterocyclyloxy

Scheme III



compounds, coupling constants in the range of 4.6–7.4 Hz, in the N-substituted compounds **12**, **18e**, and **18g** values of 9.0–10.0 Hz (broadened doublets) can be measured. Because of rotamer mixtures in **4** and **18f** (see below), H-3 and H-4 protons appear as broad signals. Even at high temperatures (380 K) the determination of the coupling constants is impossible. Generally the signals to be assigned to various conformations show differences in chemical shifts of about 0.01–0.1 ppm. Coalescence temperature for the individual protons is between 320 and 370 K. The O-substituted compounds show greatly lowered rotation barriers across the oxygen bridge which give rise to sharp NMR signals. For the N- and O-substitution products, significant differences are seen in the chemical shift of the H-5 proton. Whereas δ values of 6.92–7.20 ppm are recorded for the N-substitution products **4**, **7**, **12**, and **18e–g**, the chemical shifts for all other compounds are in the range of 7.36–8.18 ppm.

The assignment of the protons to pyridazinone rings was done on the basis of chemical shifts. It appears that the protons next to the carbonyl group generally show lower δ values.[22] A striking feature in the NMR spectrum of **18e** is the marked broadening of the H-4 signal. Since N-substitution here is confirmed by X-ray analysis, the broadening must be attributed to hindered rotation about the C4–N bond in solution. Signal broadening is also seen for **12** (Scheme II). The chromenes **6** and **7** show uniformly sharp NMR lines, while **4**, **5**, **18f**, and **18g** show double signals. This phenomenon has been pointed out previously.[12] The four last-named compounds are probably present as a mixture of stable conformational isomers at room temperature (in a ratio generally of about 3:1), the hydroxy group at the 3-position being an essential condition for this. As in **12** and **18e**, interaction between the hydroxy group and the carbonyl group of the heterocyclic ring is conceivable, since **6** and **7**, which have no hydroxy function, are present in a single conformation. In **12** and **18e** only H-4 occurs with marked broadening. Evidently the rotation barrier is lower in pyridazinone compounds than in pyridone and pyrimidone compounds.

The solvent has an important influence on the equilibrium of conformational isomers and thus on spectrum. Provided that substances are soluble in $CDCl_3$ and NMR spectra are recorded herein, rather than the DMSO used here, then all types of compounds are present in single conformation. F. Cassidy et al.[23] recently also reported a single and rigid conformation for cromakalim in $CDCl_3$ at room temperature. ^1H NOE difference measurements and studies of ^{13}C relaxation times suggested the probable existence of a hydrogen bond between the hydroxyl group at C-3 and the carbonyl group of the pyrrolidone ring in solution.

The chemical shift of H-5 and the mixtures of conformational isomers occurring in DMSO allow the 4-heterocyclyloxy compounds to be distinguished from their corresponding N-substituted isomers. Therefore the compounds **18a–d** shown in Table I are 4-heterocyclyloxy compounds. Their ^1H spectra at room temperature in DMSO are entirely uniform. The chemical shift of H-5 shows values of 7.61–8.11 ppm, while in **18e–g** values of 7.06–7.20 ppm are found. **18d** could additionally be confirmed by the signal of the amino group, which shows a broad singlet at 5.8 ppm. In contrast to **18a–d**, **18f**, and **18g** occur in the mixture of conformational isomers as described above; similarly H-5 shows a chemical shift of 7.10–7.20 ppm, suggesting the presence of the 2-oxo-1-pyridyl or the 2-oxo-1-pyrimidyl structures. As in the case of **18d**, signals for the amino group occur.

Results and Discussion

In an earlier study[12] it was reported that reaction of epoxides **1** with 2-pyridones resulted in the formation of hypotensive *trans*-3,4-dihydro-4-(1,2-dihydro-2-oxo-1-pyridyl)-2*H*-1-benzopyran-3-ols as the main products, as well as the inactive *trans*-3,4-dihydro-4-(2-pyridyloxy)-2*H*-1-benzopyran-3-ols. 2,4-Dihydroxypyridine (**2**) and 3,6-pyridazinediol (**10**) showed quite contrasting behavior, so that the 4-heterocyclyloxy structures **3a** and **11a** were not initially recognized as such. Both substances were highly active (Table II). These structures slightly resemble recently reported 4-[(3-oxy-1-cyclopent-1-enyl)oxy] compounds.[24] The actual 2-oxo-1-pyridyl and 3-oxo-2-

(22) Brügel, W. *Handbook of NMR spectral parameters*; Heyden: London, 1979; Vols. 2 and 3 and literature cited therein.

(23) Cassidy, F.; Evans, J. M.; Smith, D. M.; Stemp, G.; Edge, C.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1989**, 377.

pyridazinyl compounds **4** and **12**, were obtained as by-products in the standard reaction or by another method. They both lowered the blood pressure in spontaneously hypertensive rats (SHR) only weakly or were ineffective. It is assumed that 4-heterocyclyloxy compounds require a residual polar, amide-like pyridone or pyridazone structure for good efficacy; this structural feature was absent in previously described substances belonging to this structural class. As a rule of thumb, it can be stated that substituents on the heterocyclic ring weaken the substance's activity. This was obviously also the case with **4** and **12**.

The 4-pyridyloxy compounds **18a** and **18b**, isomeric to **3a**, and the pyrimidyloxy compound **18c** were only weakly active or inactive (Table I). From that fact it can be concluded that the structural variability for good antihypertensive efficacy in the 4-heterocyclyloxy series is very limited. The lack of effectivity in 3-pyridyloxy compound **18d** is due to the missing carbonyl dipole in the heterocyclic ring. The amino-substituted heterocyclic compounds **18e–18g** were all connected via their ring nitrogen atoms. In this subgroup only the pyridazine derivative **18e** showed some slight efficacy. Here, too, the introduction of substituents resulted in a reduction of activity and the corresponding compounds without amino groups in the heterocyclic ring were markedly superior to **18e–18g** as reported elsewhere.[12]

Powerful electron-withdrawing groups located at C-6 of the basic benzopyran system were essential for good blood pressure lowering activity.[12,21] In fact, high efficacy was shown by the nitro compounds **3d** and **11d**. The same applies for the cyano compounds **3a** and **11a**. Aside from the weakly active compound **11b**, introduction of other substituents led to total loss of activity (see methyl carboxylate **3c**, the thiocarbonic acid amides **3e** and **11c** and the bromo compound **11e**). The high efficacy was largely retained after N-alkylation of 4-heterocyclyloxy compounds **3a** and **11a**. This proves that the enforced pyridone and pyridazinone structures in **3b** and **14a–c**, with their carbonyl groups intact, are responsible for the activity. The *N*-methylpyridone **3b** was somewhat less potent than the unsubstituted compound **3a**. Among the alkylpyridazinones, the methyl and ethyl derivatives **14a** and **14b** were equally potent compared to the parent compound **11a**. Marked attenuation of activity was caused only by the bulkier 2-propyl residue in **14c**.

In the *N*-substituted compounds, the chromenes were frequently more potent than the corresponding chromanols. Here chromene **7a** was somewhat less potent than chromanol **4**, and acetate **7b** was equipotent. Surprisingly, the conversation of the highly efficacious chromanols in the 4-heterocyclyloxy series to chromenes produced completely ineffective compounds (**3a** → **9**; **11a** → **16a**; **14a** → **16b**). Transformation of chromanol **11a** to acetate **13** was accompanied only by a small reduction of the original activity. In contrast, the thioxo compounds **15** and **17** were ineffective.

To allow comparison of activities, the ED_{30} values of all highly active substances (blood pressure reduction > 100 mmHg after 1 mg/kg) were determined by using dose/response relationships (Table II). Assuming parallel dose/response relationships among all compounds tested, 3,4-dihydro-4-[(1,2-dihydro-2-oxo-4-pyridyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**3a**) was found to be the most potent compound in the group, with an ED_{30} of 6 μg/kg. The corresponding nitro compound **3d** and the pyridazone derivatives **11a**, **14a**, and **14b** were somewhat less potent, but the ED_{30} values of all these substances were in the range of 13–24 μg/kg. The racemic compound **11a** was resolved. This improved the antihypertensive efficacy: the activity resided in its levorotatory enantiomer **20a** (ED_{30} = 14 μg/kg); the dextrorotatory enantiomer **20b** was not significantly active. Similarily, racemate **14a** was resolved into a highly active **22a** (ED_{30} = 13 μg/kg) and a low-potent enantiomer **22b**. The relative differences in efficacy between the levo- and dextrorotatory enantiomers became even more marked when other pharmacological parameters such as smooth muscle relaxation and cell-membrane hyperpolarization were evaluated.[25] It became apparent that in accordance with cromakalim[26] the main activity resided in the respective levorotatory enatiomers **20a** and **22a**, while the dextrorotatory enantiomers **20b** and **22b** were virtually ineffective.

The antihypertensive efficacy of compounds **3a**, **3d**, **11a**, **14a**, **14b**, **20a**, and **22a** from the 4-heterocyclyloxychromanol series was of the same order of magnitude as that of 4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (EMD 52 692; ED_{30} = 10 μg/kg). They were thus considerably more potent than racemic cromakalim (ED_{30} = 110 μg/kg). Furthermore the mentioned compounds were found to possess a long duration of action. In a model to determine the duration of the antihypertensive effect in SHRs after intraveneous administration,[27] the effect of a compound, such as **22a**, lasted >13 times as long as that of cromakalim: intraveneous infusion of 14 μg/kg **22a** in 14 min led to a reduction in blood pressure by 69 mmHg. The time between the maximal blood pressure lowering effect and the achievement of 95% of the baseline blood pressure value was greater than 6.5 h. With equipotent dosages of cromakalim or EMD 52 692 this value was reached after approximately 30 min. These findings make this class of substances particularly attractive. (−)-(3*S*,4*R*)-3,4-Dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (**22a**) was selected for further development.

Experimental Section

Melting points were determined with a Büchi 535 melting point apparatus and are uncorrected. IR, NMR, and mass spectra are in agreement with the structures cited and were recorded on a Bruker IFS 48 IR spectrophotometer, a Bruker AC 200, WM 250 or AM 500 (TMS as internal standard), and a Vacuum Generators VG 70–70 or 70–250 at 70 eV, respectively. Crystal data were collected on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated Cu K_α radiation. Microanalyses were obtained with a Perkin-Elmer 240B CHN analyzer. Precoated silica gel 60 F_{254} plates with a layer thickness of 0.25 mm from E. Merck, Damstadt were used for thin-layer chromatography.

Syntheses. Compounds **3e**, **5**, **6**, **7b**, and **11c** were prepared according to procedures described in a previous paper.[12] The following procedures are representative of the general methods that are described in the text.

***trans*-3,4-Dihydro-4-[(1,2-dihydro-2-oxo-4-pyridyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (3a).** Epoxide **1** (R = CN; 4 g, 20 mmol), 2,4-dihydroxypyridine (**2**; 2.2 g, 19.8 mmol), and pyridine (1.6 mL, 19.8 mmol) were refluxed in EtOH (80 mL) for 7 h. The solution was evaporated and the residue chromatographed on silica gel (CH_2Cl_2 → EtOAc → 20% MeOH/EtOAc as a gradient elution). The homogeneous fractions

(24) Blarer, S.; UK Patent Appl. GB 2204868, 1988.

(25) Lues, I.; de Peyer, J.-E. Unpublished results.

(26) Hof, R. P.; Quast, U.; Cook, N. S.; Blarer, S. *Circ. Res.* **1988**, *62*, 679.

(27) Giardino, E. C.; Katz, L. B.; Casey, T. M.; Osifchin, E. M.; Shriver, D. A.; Falotico, R.; Tobia, A. J. *Pharmacologist* **1988**, *30*, A152.

were combined to give 1.9 g (30%) of **3a**, mp 250–251 °C (MeOH). Anal. ($C_{17}H_{16}N_2O_4 \cdot 0.5H_2O$) C, H, N. A small amount (<1%) of compound **4** was detected by HPLC in the nonpolar fractions. Compounds **3c**, **3d**, **11a**, **11b**, **11d**, and **11e** were prepared in an analogous manner from the appropriate epoxides **1** and 2,4-dihydroxypyridine (**2**) or 3,6-pyridazinediol (**10**). Similar treatment of **1** (R = CN) with various 6-membered-ring heterocycles yielded compounds **18a–e** and **18g** (see Tables I and II).

***trans*-3,4-Dihydro-4-[(1,2-dihydro-1-methyl-2-oxo-4-pyridyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (3b).** Compound **3a** (10 g, 31.1 mmol), dimethyl sulfate (6 mL, 63.3 mmol), and anhydrous K_2CO_3 (12 g, 86.8 mmol) in Me_2CO (300 mL) were stirred and heated under reflux for 1.5 h. The reaction mixture was cooled, filtered, and evaporated, and the crude residue was purified by chromatography (silica gel, $CH_2Cl_2 \rightarrow$ EtOAc $\rightarrow$ 20% MeOH/EtOAc as a gradient elution). After crystallization from Et_2O compound **3b** (3.6 g, 34%) was collected, mp 202–203 °C. Anal. ($C_{18}H_{18}N_2O_4 \cdot 0.25H_2O$) C, H, N.

***trans*-3,4-Dihydro-4-(1,2-dihydro-4-hydroxy-2-oxo-1-pyridyl)-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (4).** Compound **5**[12] (14.6 g, 36.3 mmol) dissolved in MeOH (150 mL) was hydrogenated over 5% Pd/C (4 g). After 4 h the catalyst was removed by filtration. Evaporation of the solvent gave a gum which was chromatographed on silica gel (gradient elution EtOAc $\rightarrow$ 20% MeOH/EtOAc). Recrystallization from Me_2CO afforded 5.8 g (50%) crystalline **4**, mp 256–257 °C. Anal. ($C_{17}H_{16}N_2O_4 \cdot 0.25H_2O$) C, H, N. Similar treatment of benzyloxy compound **6** furnished compound **7a**.

***trans*-3,4-Dihydro-4-(1,6-dihydro-3-hydroxy-6-oxo-1-pyridazinyl)-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (12).** Compound **11a** (4.6 g, 14.5 mmol) was stirred at 90 °C with 80% NaH (450 mg, 23.4 mmol) in DMSO (60 mL) under N_2. After 4 h the mixture was cooled and poured on ice. The resulting alkaline solution was washed with EtOAc and after acidification with 1 N HCl extracted with EtOAc. The combined EtOAc extracts were dried and evaporated to give compound **12** (2.6 g, 57%), mp 294–296 °C (Me_2CHOH). Anal. ($C_{16}H_{15}N_3O_4 \cdot 0.25H_2O$) C, H, N.

***trans*-3-Acetoxy-3,4-dihydro-4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy]-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (13).** Compound **11a** (1 g, 3.1 mmol) was dissolved in Ac_2O (6.5 mL) and pyridine (35 mL). The resulting mixture was stirred at room temperature for 12 h. Evaporation gave an oil that was purified by chromatography on silica gel ($CH_2Cl_2 \rightarrow$ EtOAc) to give compound **13** (800 mg, 71%), mp 210–212 °C. Anal. ($C_{18}H_{17}N_3O_5$) C, H, N.

***trans*-3,4-Dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (14a).** Epoxide **1** (R = CN; 300 g, 1.49 mol), 3-hydroxy-1-methyl-1,6-dihydropyridazin-6-one[17] (195 g, 1.55 mol), and pyridine (150 mL, 1.86 mol) were boiled in EtOH (3 L) for 6 h. Epoxide **1** (30 g, 0.15 mol) was added and the mixture refluxed for an additional 2 h. The solution was concentrated to half volume, and the crystals obtained on cooling were isolated and washed with Me_2CHOH and Et_2O to give compound **14a** (335 g, 62%), mp 203–205 °C (EtOH). Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

***trans*-3,4-Dihydro-4-[(1,6-dihydro-6-oxo-1-isopropyl-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (14c).** A mixture of compound **11a** (1 g, 3.15 mmol), 2-bromopropane (5 mL, 53.3 mmol), and K_2CO_3 in Me_2CO (50 mL) was stirred and heated under reflux for 18 h. After cooling, the reaction mixture was filtered and evaporated. The residue was taken up in EtOAc (100 mL) and washed with H_2O. After the organic phase was dried and evaporated, the residue was recrystallized from CH_2Cl_2/Et_2O to give **14c** (480 mg, 42%), mp 201–203 °C. Anal. ($C_{19}H_{21}N_3O_4 \cdot 0.5H_2O$) C, H, N. The corresponding ethyl and methyl derivatives **14b** and **16b** were prepared similarly from compounds **11a** and **16a** (see Table II).

4-[(1,6-Dihydro-6-thioxo-3-pyridazinyl)oxy]-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (17). A solution of compound **16a** (1 g, 3.4 mmol) and Lawesson's reagent (800 mg, 2 mmol) in PhMe (160 mL) was stirred at 65 °C for 30 min. Evaporation of solvent, followed by chromatography (silica gel, CH_2Cl_2) and recrystallization from Et_2O furnished compound **17** (320 mg, 30%), mp 191–192 °C. Anal. ($C_{16}H_{13}N_3O_2S$) C, H, N, S. Similar treatment of compound **11a** yielded **15**.

***trans*-3,4-Dihydro-4-(4-amino-1,2-dihydro-2-oxo-1-pyridyl)-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (18f).** 4-Amino-2-hydroxypyridine[20] (900 mg, 8.2 mmol) and epoxide **1** (R = CN; 2 g, 9.9 mmol) in EtOH (40 mL) were heated under reflux. Additional **1** (1.5 g, 7.5 mmol) was added in portions over a 78 h period. The resulting precipitate of compound **18f** (350 mg, 14%) was collected after cooling, mp 289 °C (MeOH/5% CH_2Cl_2). Anal. ($C_{17}H_{17}N_3O_3 \cdot 0.25H_2O$) C, H, N.

(3*S*,4*R*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy]-2,2-dimethyl-3-chromanyl (1*S*)-Camphor-10-sulfonate (19a) and (3*R*,4*S*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy]-2,2-dimethyl-3-chromanyl (1*S*)-Camphor-10-sulfonate (19b). Compound **11a** (4 g, 12.6 mmol) and (1*S*)-(+)-camphorsulfonyl chloride (4 g, 16 mmol) in pyridine (40 mL) were stirred and heated at 90 °C for 8 h. The mixture was poured into 1 N HCl (200 mL) and extracted with EtOAc. After drying and evaporation of the combined organic phases the residue was chromatographed (Labomatic column, volume 615 mL; silica gel 60, particle size 0.040–0.063 mm, 230–400 mesh ASTM; pressure 8–10 bar; gradient elution $CH_2Cl_2 \rightarrow$ EtOAc). Crystals obtained from the nonpolar fractions were recrystallized from Me_2CHOH to give **19a** (1.3 g, 20%): mp 224–226 °C; $[\alpha]^{20}_D$ (MeOH, *c* 1) -93.2°. Anal. ($C_{26}H_{29}N_3O_7S$) C, H, N, S.

19b was obtained similarly after recrystallization from Et_2O from the polar fractions (1.8 g, 26%): mp 202–203 °C; $[\alpha]^{20}_D$ (MeOH, *c* 1) +116.7°. Anal. ($C_{26}H_{29}N_3O_7S \cdot 0.25H_2O$) C, H, N, S. The mixture of the diastereomeric camphorsulfonates **8** was prepared accordingly from **3a** and (1*R*)-(−)-camphorsulfonyl chloride and used directly in the next reaction.

4-[(1,6-Dihydro-6-oxo-3-pyridazinyl)oxy]-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (16a) and (−)-(3*S*,4*R*)-3,4-Dihydro-4-[(1,6-dihydro-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (20a). Compound **19a** (500 mg, 0.95 mmol) was stirred in MeOH (20 mL) containing NaOH on a carrier (0.8–1.6 mm, ~14–25 mesh ASTM, Cat. No. 1567, E. Merck; 2 g) at room temperature for 20 h. By adding an additional amount of NaOH on carrier (2 g) the reaction was finished within 4 h (TLC monitoring). Filtration and evaporation of the reaction mixture gave a residue, which was dissolved in H_2O and extracted with EtOAc after acidification. The organic phase was dried and evaporated. The residue was chromatographed on a silica gel column ($CH_2Cl_2 \rightarrow$ EtOAc as a gradient elution). The chromatographically homogeneous nonpolar fractions (100 mg, 36%) were combined and recrystallized from Et_2O to give **16a**, mp 226–228 °C. Anal. ($C_{16}H_{13}N_3O_3$) C, H, N. The polar material was recrystallized from Et_2O to yield **20a** (30 mg, 10%): mp 230–231 °C; $[\alpha]^{20}_D$ (MeOH, *c* 1) -173.6°. Anal. ($C_{16}H_{15}N_3O_4$) C, H, N. Similar treatment of the camphorsulfonates **8** (diastereomeric mixture) yielded compound **9**. See Table II.

(3*S*,4*R*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-2,2-dimethyl-3-chromanyl (1*S*)-Camphanate (21a) and (3*R*,4*S*)-6-Cyano-3,4-dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-2,2-dimethyl-3-chromanyl (1*S*)-Camphanate (21b). Under stirring at 85 °C in pyridine (1.8 L) compound **14a** (275.6 g, 842 mmol) was treated with (−)-camphanic acid chloride (206 g, 951 mmol) and 4-(dimethylamino)pyridine (6 g, 49 mmol) for 4 h. The solution was evaporated and the residue was mixed with EtOH (1 L). Evaporation gave a crystalline residue which was recrystallized from Me_2CHOH (1 L) to yield a mixture of diastereomeric camphanates **21a** and **21b** (368 g, 86%). A portion (20 g) dissolved in CH_2Cl_2 (50 mL) was separated by using HPLC (steel column 400–100, LiChrosorb Si 60, 10 μm; E. Merck; Me_3COMe). The nonpolar material was recrystallized from Et_2O/CH_2Cl_2 to give **21a** (8.9 g, 45%): mp 230–232 °C; $[\alpha]^{20}_D$ (MeOH, *c* 1) -96.0°. Anal. ($C_{27}H_{29}N_3O_7$) C, H, N. The homogeneous polar fractions were combined and recrystallized from Et_2O/CH_2Cl_2 to give **21b** (6 g, 30%): mp 224–226 °C; $[\alpha]^{20}_D$ (MeOH, *c* 1) +91.1°. Anal. ($C_{27}H_{29}N_3O_7$) C, H, N.

(−)-(3*S*,4*R*)-3,4-Dihydro-4-[(1,6-dihydro-1-methyl-6-oxo-3-pyridazinyl)oxy]-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carbonitrile (22a). NaOH (1 N; 500 mL) was added in one portion to a solution of the camphanate **21a** (80 g, 158

mmol) in THF (500 mL). After stirring at room temperature for 15 min the reaction mixture was diluted with H_2O (800 mL) followed by extraction with EtOAc. The organic layer was dried and evaporated to a small volume. On addition of Et_2O, crystals separated, which were dried in a vacuum oven at 120 °C for 10 h to yield 49.6 g (96%) **22a**: mp 161–164 °C; $[\alpha]^{20}_D$ (MeOH, c 1) −173.7°. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

Crystal Data. 3b: $C_{18}H_{18}N_2O_4$; M = 326.4; monoclinic; $P2_1/c$; a = 1503.1 (4) pm; b = 803.1 (3) pm; c = 1397.5 (4) pm; α = 90°; β = 96.99° (4); γ = 90°; V = 1674.4 × 10^6 pm^3; Z = 4; ρ_x = 1.295 g cm^{-3}; μ(Cu K$_\alpha$) = 7.240 cm^{-1}; F(000) = 688; no. of reflections with $I \geq 3\sigma(I)$ = 1931; no. of refinement parameters = 272; final R values, R = 0.050; R_w = 0.048.

18e: $C_{16}H_{16}N_4O_3$; M = 312.4; orthorhombic; $Pca2_1$; a = 1075.8 (2) pm; b = 966.8 (2) pm; c = 1489.7 (4) pm; $\alpha = \beta = \gamma$ = 90°; V = 1549.4 × 10^6 pm^3; Z = 4; ρ_x = 1.339 g cm^{-3}; μ(Cu K$_\alpha$) = 7.487 cm^{-1}; F(000) = 656; no. of reflections with $I \geq 3\sigma(I)$ = 2568; no. of refinement parameters = 208; final R values, R = 0.047; R_w = 0.049.

21a: $C_{27}H_{29}N_3O_7$; M = 507.6; orthorhombic; $P2_12_12_1$; a = 1097.5 (2) pm; b = 1853.9 (3) pm; c = 1301.1 (2) pm; $\alpha = \beta = \gamma$ = 90°; V = 2647.1 × 10^6 pm^3; Z = 4; ρ_x = 1.273 g cm^{-3}; μ(Cu K$_\alpha$) = 7.314 cm^{-1}; F(000) = 1072; no. of reflections with $I \geq 3\sigma(I)$ = 4454; no. of refinement parameters = 335; final R values, R = 0.057; R_w = 0.053.

Antihypertensive Studies in Conscious Spontaneously Hypertensive Rats. Compounds were tested for antihypertensive activity in conscious spontaneously hypertensive male rats (280–330 g; blood pressure >180 mmHg; origin: Okamoto strain). Mean arterial pressure was recorded directly via an aortic catheter in unrestrained animals. A HSE setup (Statham pressure transducer, Watanabe recorder, HSE oscilloscope) was used for the recording of arterial blood pressure. Blood pressure was recorded continuously over a period from 1 h before to 3.5 h after administration of the substance; to assess the effects of the substance, the mean of the maximum individual changes in the 3.5-h period after administration was used. For each compound 1 mg/kg was administered orally as a screening dose; 2–4 additional doses of selected compounds which proved active at 1 mg/kg in reducing blood pressure were tested, and an ED_{30} (= dose in μg/kg which reduces blood pressure by 30 mmHg) was calculated from a linear regression of effect vs log dose. The substances were suspended in 5% gum arabic and administered orally by gavage.

Acknowledgment. We wish to extend our thanks to Dr. W. Hiller, University of Tübingen, for providing us with X-ray data and Dr. Gerhard Barnickel and Christine Schittenhelm for representations of the crystal structures. For their skillful experimental work we would like to thank Günther Kritzinger, Heinrich Kübler, Gabriele Pöschl, Horst Schiefer, and Sylvia Wild. We also thank Marion Gerbig for preparing the manuscript.

Supplementary Material Available: X-ray crystallographic data, including positional parameters, bond distances, bond angles, and anisotropic displacement parameter expressions, for **3b**, **18e**, and **21a** (29 pages). Ordering information is given on any current masthead page.

Anti-HIV-1 Activity, Toxicity, and Stability Studies of Representative Structural Families of Polyoxometalates

Craig L. Hill,*[‡] Mark S. Weeks,[‡] and Raymond F. Schinazi*[†]

Department of Chemistry, Emory University, Atlanta, Georgia 30322, and Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30033.
Received February 26, 1990

The anti-HIV-1 activity and toxicity of representative structural families of polyoxotungstates in human lymphocytes was determined. The 21 compounds examined include those derived from the following structural families: $[NaSb_9W_{21}O_{86}]^{18-}$ (HPA-23), $X^{n+}W_{12}O_{40}^{(8-n)-}$ (Keggin), $P_2W_{18}O_{62}^{6-}$ (Wells–Dawson), $W_6O_{19}^{2-}$ (Lindqvist), $[NaP_5W_{30}O_{110}]^{14-}$ (Preyssler), and $W_{10}O_{32}^{4-}$ (decatungstate). The molecular architecture of each of these structural families is constituted principally by a network of bonds between d^0 W^{VI} and oxide ions. Of these, 10 show median effective concentration (EC_{50}) values of approximately 1 μM and six have marked toxicity with a median inhibitory concentration (IC_{50}) of less than 50 μM. Only compounds containing more than six metal atoms showed appreciable antiviral activity. Beyond this, however, no marked correlation existed between the molecular size, charge, or charge density of the polyoxometalates and their anti-HIV-1 activity. Examination of an exemplary class of polyoxotungstates, the phosphotungstates of formula A- and B-$PW_9O_{34}^{9-}$ under physiological conditions (buffered neutral aqueous media), illustrates that both isomers equilibrate rapidly to generate the same distribution of products and that this distribution depends principally on the buffer. These heretofore unappreciated complexities in the chemistry of these compounds under neutral aqueous conditions indicates interpretation or evaluation of these compounds in cell culture and other biological screens must be done with care.

Introduction

Various nucleoside analogues show high activity against human immunodeficiency virus type 1 (HIV-1), the causative agent in acquired immunodeficiency syndrome (AIDS), and substantial promise for the treatment of this disorder.[1] Despite this early promise, the one drug to garner full approval by the FDA for treatment of AIDS, 3′-azido-3′-deoxythymidine (AZT), exhibits several manifestations of toxicity.[1,2] Furthermore, recent research has also indicated that a high percentage of patients maintained on AZT for a period of 6 months or longer develop resistance to the drug.[3] Although a number of other

* Address correspondence to Professor Craig L. Hill, Department of Chemistry, Emory University, Atlanta, GA 30322.

† Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine.

‡ Department of Chemistry, Emory University.

(1) (a) Richman, D. D.; Fischl, M. A.; Grieco, M. H.; Gottlieb, M. S.; Volberding, P. A.; Laskin, O. L.; Leedom, J. L.; Groopman, J. E.; Mildvan, D.; Hirsch, M. S.; Jackson, G. G.; Durack, D. T.; and Nusinoff-Lehrman, S. *N. Engl. J. Med.* **1987**, *317*, 192. (b) Yarchoan, R.; Klecker, R. W.; Weinhold, K. J.; Markham, P. D.; Lyerly, H. K.; Durack, D. T.; Gelmann, E.; Lehrmann, S. N.; Blum, R. M.; Barry, D.; Shearer, G.; Fischl, M. A.; Mitsuya, H.; Gallo, R. C.; Collins, J. M.; Bolognesi, D. P.; Myers, C. E.; Broder, S. *Lancet* **1985**, *i*, 575. (c) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Nusinoff-Lehrman, S.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096. (d) Schinazi, R. F. *AIDS in Children, Adolescents and Heterosexual Adults: An Interdisciplinary Approach to Prevention*; Schinazi, R. F., Nahmias, A. J., Eds.; Elsevier North-Holland: New York, 1988; pp 126–143.

0022-2623/90/1833-2767$02.50/0