anhydrous ethanol was hydrogenated with 1.88 g of 10% palladium on charcoal at atmospheric pressure for 20 min. The catalyst was removed by filtration through Celite. Evaporation provided a solid which was recrystallized from hexane to yield 8.64 g (94%) of ethyl-5-[1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-pentanoate: mp 84–86 °C; IR (CH_2Cl_2) ν 2940, 1728, 1490, 1445 cm^{-1}; NMR ($CDCl_3$) δ 1.2 (t, J = 7.0 Hz, 3 H), 1.45–1.95 (m, 4 H), 2.1–2.8 (m, 4 H), 4.1 (q, J = 7.0 Hz, 2 H), 6.50 (s, 1 H), 7.0–7.5 (m, 16 H). Anal. ($C_{29}H_{30}N_2O_2$) C, H, N.

5-[4-[(*tert*-Butylamino)carbonyl]phenyl]-6,7,8,9-tetrahydroimidazo[1,5-*a*]azepine (20b). A solution of 3.93 g (6.6 mmol) of 4-[5-[4-[(*tert*-butylamino)carbonyl]phenyl]-5-chloropent-1-yl]-1-(triphenylmethyl)-1*H*-imidazole in 180 mL of acetonitrile was refluxed for 15 h and cooled to room temperature, and 90 mL of methanol was added. The reaction mixture was refluxed an additional 15 h and evaporated to dryness. The residue was partitioned between ether and water. The ether layer was separated and washed with 1 N HCl (3 × 20 mL). The combined aqueous extracts were adjusted to pH 8 and extracted with methylene chloride which was dried over sodium sulfate, filtered, and evaporated to a white foam. The product was chromatographed on 90 g of silica gel (ethyl acetate/NH_4OH 98:2, R_f = 0.4) to yield 1.65 g (80%) of 5-[4-[(*tert*-butylamino)-carbonyl]phenyl]-6,7,8,9-tetrahydro-5*H*-imidazo[1,5-*a*]azepine, which crystallized from ether: mp 176–178 °C; IR (CH_2Cl_2) ν 3435, 2940, 1663, 1530, 1497 cm^{-1}; NMR ($CDCl_3$) δ 1.46 (s, 9 H), 1.5–3.2 (m, 8 H), 5.4–5.7 (m, 1 H), 6.83 (s, 1 H), 6.98 (d, J = 8.0 Hz, 2 H), 7.20 (s, 1 H), 7.72 (d, J = 8.0 Hz, 2 H). Anal. ($C_{19}H_{25}N_{30}$) C, H, N.

(+)- and (-)-5-(4-Cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine Hydrochloride. Racemic 5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine hydrochloride was applied, in 20-mg aliquots, to a 4.6 × 250 mm β-cyclodextrin-bonded silica gel column using 7:3 water/methanol as the eluant at a flow rate of 0.8 mL/min. The separate fractions were evaporated under vaccum to yield (-)-5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyridine hydrochloride (mp 82–83 °C (amorphous); $[\alpha]^{25}_D$ = -89.2°) and (+)-5-(4-cyanophenyl)-5,6,7,8-tetrahydroimidazol[1,5-*a*]pyridine hydrochloride (mp 218–220 °C; $[\alpha]^{25}_D$ = +85.02°).

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4′-Hydroxy-3-methoxyflavones with Potent Antipicornavirus Activity

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4′-Hydroxy-3-methoxyflavones are natural compounds with known antiviral activities against picornaviruses such as poliomyelitis and rhinoviruses. In order to establish a structure–activity relationship a series of analogues were synthesized, and their antiviral activities and cytotoxicities were compared with those of flavones from natural origin. The 4′-hydroxyl and 3-methoxyl groups, a substitution in the 5 position and a polysubstituted A ring appeared to be essential requirements for a high activity. The most interesting compound was 4′,7-dihydroxy-3-methoxy-5,6-dimethylflavone possessing in vitro TI_{99} values of >1000 and >200 against poliovirus type 1 and rhinovirus type 15, respectively. This compound was also active against other rhinovirus serotypes (2, 9, 14, 29, 39, 41, 59, 63, 70, 85, and 89) tested, having MIC_{50} values ranging from 0.016 to 0.5 μg/mL. Finally in contrast to quercetin it showed to be not mutagenic in concentrations up to 2.5 mg in the Ames test.

Several flavonoids have shown to inhibit the replication of picornaviruses. Two classes of compounds can be distinguished according to their antiviral spectrum and mechanism of action. Some chalcones and flavans inhibit selectively different serotypes of rhinoviruses.[1-3] Compounds such as 4′-ethoxy-2′-hydroxy-4,6′-dimethoxychalcone and 4′,6-dichloroflavan interact directly with specific sites on the viral capsid proteins, thereby uncoating and consequently liberation of viral RNA.[4-6] The sensitivity of the virus depends on the serotype and the compounds. Aza analogues such as 2*H*-pyrano[2,3-*b*]pyridines and *N*-benzylbenzamides show the same activity.[7]

A second class of compounds consists of flavones. Various substituted derivatives, which are active against a wide range of picornaviruses, except Mengo virus, and vesicular stomatitis virus was isolated from several plants.[8-11] They interfere with an early step in the viral RNA synthesis. Although the molecular mechanism is not completely understood yet, they probably inhibit the formation of minus-strand RNA of poliovirus by interacting with one of the proteins involved in the binding of the virus replication complex to vesicular membranes at which poliovirus replication takes place.[12-15]

† University of Antwerp.
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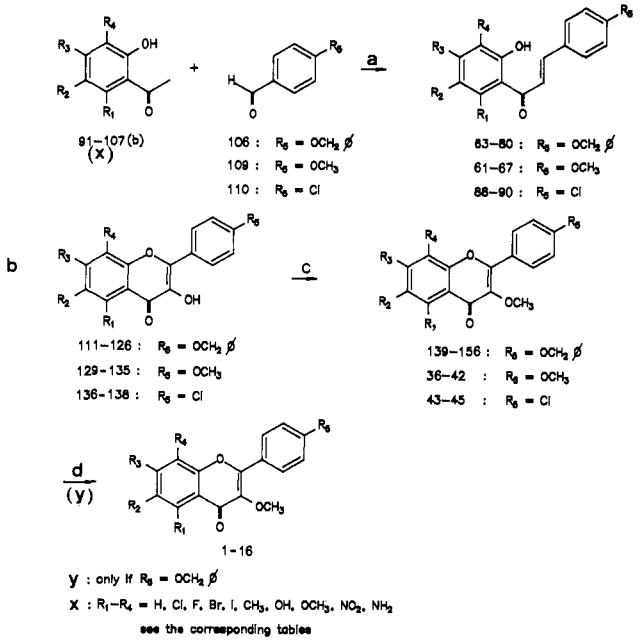
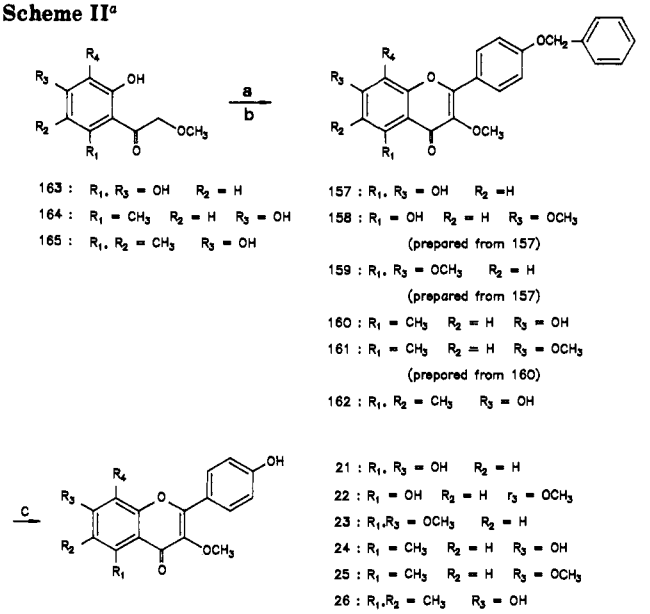
Scheme I[a]



[a] (a) NaOH, EtOH; (b) H_2O_2, NaOH, CH_3OH; (c) $(CH_3O)_2SO_2$, K_2CO_3, acetone; (d) AcOH, HCl.

Scheme II[a]



[a] (a) **166**, **167**; (b) NaOH, CH_3OH; (c) AcOH, HCl.

The attractive mechanism of action, the pronounced and broad-spectrum antiviral activity, and the lack of resistance-induction[6] by these flavones prompted us to explore this class of flavonoids and to establish a link between structure and activity.

Preliminary studies with natural flavones have shown the 3-methoxyl and 4′-hydroxyl groups to be important for potent antiviral activity.[9-11] As further indications on structure–activity relationships are lacking, we synthesized a series of A-ring-substituted (methyl, hydroxy, methoxy, halo, amino, nitro) 4′-hydroxy-3-methoxyflavones **1–26** and compared their activities against polio- and rhinoviruses with those of some naturally occurring related compounds **27–35**. In order to confirm the importance of the 4′-hydroxyl group we also prepared some 4′-methoxy and 4′-chloroflavones (**36–42** and **43–45**) and compared their antiviral properties with those of natural 4′-methoxy- and 4′-acetoxyflavones (**46–56** and **57**). The importance of the 3-methoxyl group was confirmed by investigation of the antiviral properties of prepared 3-chloro-, 3-methyl-, 3-amino-, and lower 3-alkoxyflavones (**58**,**60** and **61**,**62**).

3-Hydroxy, 3-hydrogen, and 4′-hydrogen compounds were not considered in this work because they were proven to be inactive against picornaviruses.

Chemistry

3-Methoxyflavones were prepared by two well-known synthetic methods. As shown in Scheme I 2′-hydroxychalcones **63–90** were prepared from the corresponding 2′-hydroxyacetophenones **91–107** and para-substituted benzaldehydes **108–110** in alkaline medium.[16] These chalcones were cyclized with alkaline hydrogen peroxide to 3-hydroxyflavones **111–138** (Algar, Flynn, Oyamada-oxidation).[17,18] These 3-hydroxyflavones were then transformed into 3-methoxy derivatives **36–45** and **139–156** with dimethyl sulfate.[19] When the A ring or the 4′-position of the B ring was substituted with hydroxyl functions a benzyl ether protection was used. This group was eliminated by acid hydrolysis,[20] giving rise to compounds **1–18**.

The 7-amino-4′-hydroxy-3-methoxyflavone (**19**) was prepared by reduction of the 7-nitro analogue **18**.[21]

The AFO reaction gave rise to many side products in the preparation of 5-substituted flavones **20–26**. Therefore, compounds **21–26** were prepared by acid hydrolysis of the corresponding benzyl ethers **156–161**. These were synthesized by an Allan–Robinson reaction (Scheme II).[22] 2′-Hydroxy-2-methoxyacetophenones **163–165** were condensed with 4-(benzyloxy)benzoic acid anhydride (**166**) in the presence of the potassium salt of 4-(benzyloxy)benzoic acid (**167**) as base. Benzyl ether protecting was removed with acid. Selective methylation of the 7-hydroxyl group of **157** with dimethyl sulfate gave 4′-(benzyloxy)-5-hydroxy-3,7-dimethoxyflavone (**158**). **157** and **160** were also methylated to 4′-(benzyloxy)-3,5,7-trimethoxyflavone (**159**) and 4′-(benzyloxy)-3,7-trimethoxy-5-methylflavone (**161**), respectively. 4′,5-Dihydroxy-3-methoxyflavone (**20**) was prepared by nuclear reduction (Scheme III) of the 4′,5,7-trihydroxy-3-methoxyflavone derivative. Therefore the 7-hydroxyl group of **157** was selectively converted with 5-chloro-1-phenyltetrazole (**168**) into the phenolic ether **169**, which was then reduced with formic acid and palladium on charcoal.[23]

3-Ethoxy-4′-hydroxy-7-methylflavone (**58**) and 4′-hydroxy-3-isopropoxy-7-methylflavone (**59**) were prepared from the corresponding 3-hydroxy compound **113** by al-

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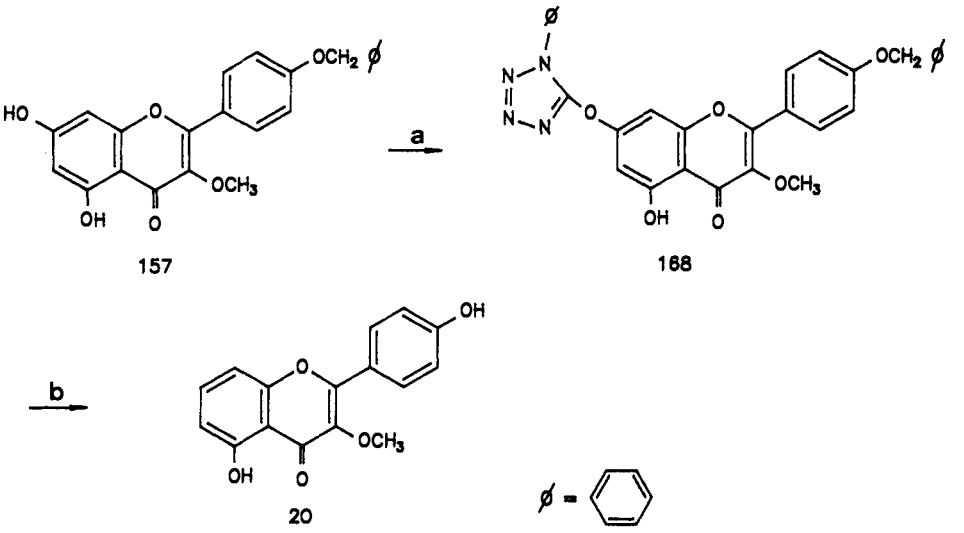
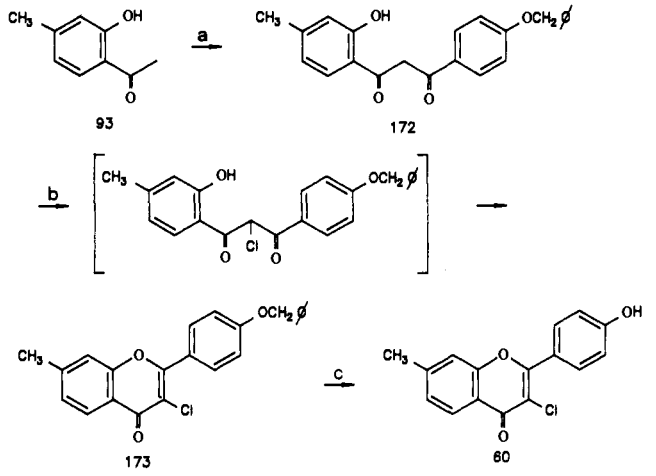
Scheme III[a]



[a] (a) 168, $(CH_3)_3CO^-K^+$, DMF; (b) HCOOH, Pd/C, C_6H_5, EtOH, H_2O.

Scheme IV[a]



[a] (a) 185, LDA, THF; (b) SO_2Cl_2, dioxane; (c) AcOH, HCl.

kylation with diethyl sulfate and isopropyl iodide/potassium carbonate and hydrolysis of the benzyl ethers **170** and **171**.

As indicated in Scheme IV, 3-chloro-4′-hydroxy-7-methylflavone (**60**) was synthesized by sulfuryl chloride chlorination of the dibenzoylmethane derivative **172** and simultaneous ring closure to **173** followed by hydrolysis.[24] **172** was prepared from *p*-(benzyloxy)benzoyl chloride (**185**) and 2′-hydroxy-4′-methylacetophenone (**93**).[25]

The same compound **172** was also used in the synthesis of 4′-hydroxy-3,7-dimethylflavone (**61**), which was obtained by methylation with methyl iodide to **174**, subsequent cyclization in acetate buffer to **175** and acid hydrolysis (Scheme V).

3-Amino-4′-hydroxy-7-methylflavone (**62**) was prepared by acid hydrolysis of the benzyl ether **176**. The latter was prepared by an alkaline transposition of α-azidochalcone **177** (Scheme VI). Starting material for these compounds was the acetophenone **93**. After tosylation of **93** to **178** and bromination with dioxane–bromine, 2-bromo-4′-methyl-2′-(*p*-tosyloxy)acetophenone (**179**) was obtained. Reaction with sodium azide gave rise to 2-azido-4′-methyl-2′-(*p*-tosyloxy)acetophenone (**180**), which was converted with aldehyde **108** (Scheme I) to α-azido-4-(benzyloxy)-4′-methyl-2′-(*p*-tosyloxy)chalcone (**177**).[26]

Biology

The antiviral potency of the flavones against poliovirus type 1 Brunhilde strain 1A/S3 and human rhinovirus type 15 was determined by the end-point titration technique (EPTT) as described earlier.[27] The antiviral activity has been expressed as the viral strength reduction factor (RF) after three days (polio) or seven days (rhino) of incubation in the presence of the maximal non-toxic dose of the compound (MNTD). The RF_{MNTD} was calculated as the ratio of the viral titer of the virus control to the viral titer in the presence of the MNTD of the test compound. The viral titer was expressed in the EPTT as the 50% tissue culture dose end point (TCD 50)/mL i.e. the highest dilution of the viral suspension which produced a cytopathogenic effect (CPE) in 50% of the cell cultures inoculated. A RF_{MNTD} value of 10^3 could be considered as a pronounced antiviral activity. Therefore, the minimal dose of the test compound, which produced such a titer reduction (mD_{RF10^3}; 10 μg/mL) was used as a criterion to compare the antiviral activities of the flavones (Tables I, IV, and V). Active compounds were selected and their therapeutic index 99 (TI_{99}) was determined. The TI_{99} was calculated as the ratio of the maximum drug concentration at which 50% of the growth of normal cells is inhibited (CyD_{50}) to the minimum drug concentration at which 99% of the virus is inhibited (ED_{99}) (Table II). The culture cells used for antipolio- and antirhinovirus testing were Vero cells and human skin fibroblasts, respectively. The most interesting compound was then tested against 17 different rhinovirus serotypes by the EPTT using HeLa culture cells, and its activity was compared with that of known antiviral products such as guanidine and 2-α-(hydroxybenzyl)benzimidazole (HBB). The results were expressed as the 50% inhibitory concentration (MIC_{50}; μg/mL) i.e. the lowest concentration of compound that protected 50%

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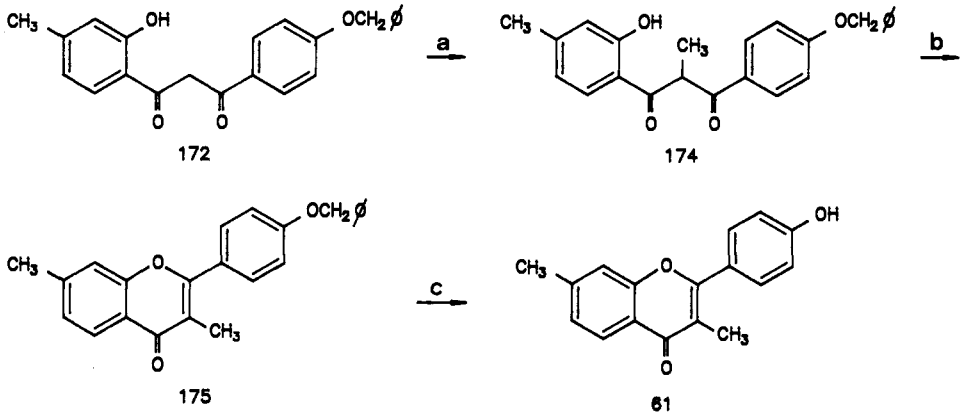
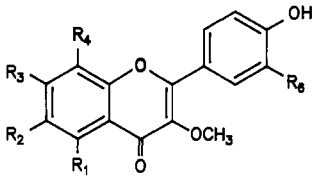
Scheme V[a]



[a] (a) CH_3I, K_2CO_3, acetone; (b) sodium acetate, AcOH; (c) AcOH, HCl.

Table I. Antipoliovirus Activity of 4'-Hydroxy-3-methoxyflavones 1–35



no.[a]	R_1	R_2	R_3	R_4	R_6	mp, °C	crystn solvent	yield, %	MNTD,[b] μg/mL	RF_{MNTD}[c]	mD_{RF10^3},[d] μg/mL	anal.	
1	H	H	H	H	H	232–233	ethanol	72	15	10^4	6	$C_{16}H_{12}O_4$	C,H
2	H	CH_3	H	H	H	228–230	methanol	70	12	$10^{4.5}$	$C_{17}H_{14}O_4$	$C_{17}H_{14}O$	C,H
3	H	H	CH_3	H	H	219–220	methanol	70	25	$10^{4.5}$	4	$C_{17}H_{14}O_4$	C,H
4	H	$CH(CH_3)_2$	H	H	H	182–184	methanol	55	5	$10^{1.5}$	–	$C_{19}H_{18}O_4$	C,H
5	H	H	$CH(CH_3)_2$	H	H	157–160	chloroform/ petroleum ether	92	25	$10^{1.5}$	–	$C_{19}H_{18}O_4$	C,H
6	OCH_3	H	H	H	H	206–208	ethanol/water (1:4)	81	10	$10^{4.5}$	5	$C_{17}H_{14}O_5$	C,H
7	H	OCH_3	H	H	H	229–230	ethanol	82	50	$10^{2.5}$	–	$C_{17}H_{14}O_5$	C,H
8	H	H	OCH_3	H	H	224–226	methanol	68	50	$10^{3.5}$	2.5	$C_{17}H_{14}O_5$	C,H
9	H	H	H	OCH_3	H	226–227	methanol	72	50	10^5	2.5	$C_{17}H_{14}O_5$	C,H
10	H	OH	H	H	H	>300	methanol	72	10	10^4	5	$C_{16}H_{12}O_5$	C,H
11	H	H	OH	H	H	>300	methanol	76	5	10^4	4	$C_{16}H_{12}O_5$	C,H
12	H	Cl	H	H	H	254–255	ethanol	80	100	$10^{3.5}$	1.5	$C_{16}H_{11}O_4Cl$	C,H
13	H	H	Cl	H	H	266–267	ethanol/acetone (9:1)	83	100	10^3	20	$C_{16}H_{11}O_4Cl$	C,H
14	H	H	Br	H	H	288–289	acetone	83	30	10	–	$C_{16}H_{11}O_4Br$	C,H
15	H	H	I	H	H	243–245	acetone	53	10	10^3	10	$C_{16}H_{11}O_4I$	C,H
16	H	H	F	H	H	228–229	methanol	69	10	10^2	–	$C_{16}H_{11}O_4F$	C,H
17	H	Cl	H	CH_3	H	292–293	methanol/acetone (1:1)	57	25	1	–	$C_{17}H_{14}O_4Cl$	C,H
18	H	NO_2	H	H	H	259–260	methanol	75	50	$10^{2.5}$	–	$C_{16}H_{10}O_6N$	C,H
19	H	NH_2	H	H	H	205	methanol	35	10	10^3	10	$C_{16}H_{13}O_4N$	C,H
20	OH	H	H	H	H	200–202	ethanol	45	5	10^3	1.5	$C_{16}H_{12}O_5$	C,H
21	OH	H	OH	H	H	275–280	water/ethanol (1:3)	45	10	10^5	0.25	$C_{16}H_{12}O_6$	C,H
22	OH	H	OCH_3	H	H	243–245	ethanol/water (1:1)	69	100	10^5	0.3	$C_{17}H_{14}O_6$	C,H
23	OCH_3	H	OCH_3	H	H	273–275	ethanol/water (1:1)	84	100	10^4	5	$C_{18}H_{16}O_6$	C,H
24	CH_3	H	OH	H	H	270–273	ethanol/water (1:1)	60	10	10^4	0.05	$C_{17}H_{14}O_5$	C,H
25	CH_3	H	OCH_3	H	H	255–257	ethanol/water (1:1)	95	25	10^4	0.5	$C_{18}H_{16}O_5$	C,H
26	CH_3	CH_3	OH	H	H	272–273	methanol	83	>100	10^5	<2.5	$C_{18}H_{16}O_5$	C,H
27	OH	H	OH	H	OH				20	10^5	0.5		
28	OH	H	OCH_3	H	OH				10	10^5	0.5		
29	OH	H	OCH_3	H	OCH_3				20	10^5	0.5		
30	OH	OCH_3	OH	H	H				20	10^4	1		
31	OH	OCH_3	OCH_3	H	H				100	10^5	0.1		
32	OH	H	OCH_3	OCH_3	H				25	10^5	0.3		
33	OH	H	OCH_3	OCH_3	OH				10	10^4	0.5		
34	OH	H	OH	OCH_3	OH				20	10^5	2		
35	OH	OCH_3	OH	H	OCH_3				10	10^3	1		

[a] **1–26** are synthetic in origin; **27–35** are natural in origin. [b] Maximal nontoxic dose. [c] RF_{MNTD} = reduction factor at MNTD. [d] MD_{RF10^3} = minimal dose with a RF 10^3.

of the cells from CPE (Table III). This screening was carried out by K. Andries at the Janssen Research Foundation at Beerse, Belgium as described earlier.[28]

The mutagenicity experiments were performed with *Salmonella typhimurium* strains TA 98 and TA 100 with and without S9 mix from rat liver by using the plate incorporation assay.[29-31]

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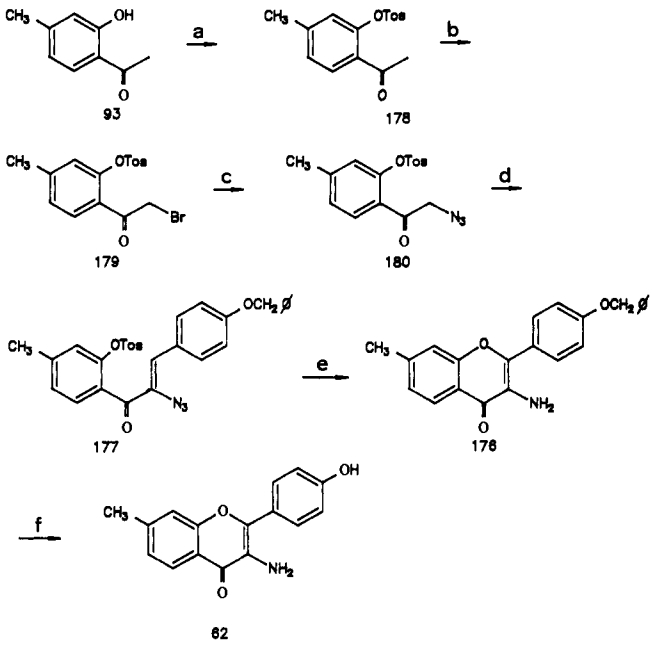
Scheme VI[a]



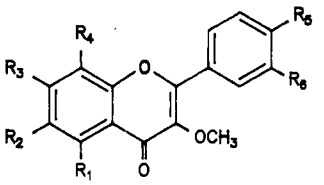
[a] (a) *p*-Tolylsulfonyl chloride, K_2CO_3, acetone; (b) dioxane bromide, dioxane; (c) NaN_3, DMF; (d) **108**, piperidinium acetate, EtOH; (e) NaOH, EtOH; (f) AcOH, HCl.

Results and Discussion

The results of the antiviral tests of 4′-hydroxy-3-methoxyflavones against poliovirus are presented in Tables I and II. They reveal 4′-hydroxy-3-methoxyflavones with a monosubstituted A ring to be less active than the corresponding compounds having a polysubstituted A ring. In the former series the hydroxylated analogues **10**, **11**, and **20** are the most active substances, whereas substitution of the parent compound **1** with a methoxyl, methyl, or chloro function greatly decreases cytotoxicity. The 5-hydroxy-, 7-methoxy-, and 6-chloro derivatives **20**, **8**, and **12**, respectively, combine a rather low cytotoxicity with a moderate antipolio activity giving more than 10 times higher TI_{99} values (ranging from 16.7 to 50) than that of compound **1**. Monosubstitution of the A ring of the latter with bromo, iodo, fluoro, amino, and nitro groups afford compounds having low antipolio properties, mostly within the range of the cytotoxic dose.

Within the series of compounds containing a polysubstituted A ring 4′,7-dihydroxy-3-methoxy-5,6-dimethylflavone (**26**) is the most interesting substance. The ED_{99} value against polio is 0.1 μg/mL, whereas it is not cytotoxic to Vero cells at the highest concentration tested (100 μg/mL), which results in a TI_{99} of >1000. Other highly active antipolio substances are the naturally occurring compounds, 4′,5-dihydroxy-3,6,7-trimethoxyflavone (penduletin, **31**), 4′,5-dihydroxy-3,7,8-trimethoxyflavone (**32**), 4′,5-dihydroxy-3,7-dimethoxyflavone (jaranol, **22**) with TI_{99} values of 500, >400, and 33.3, respectively. The synthetic compound 4,7-dihydroxy-3-methoxy-5-methylflavone (**25**) has the same high antipolio activity, but is more toxic, which results in a TI_{99} value of 150. These data indicate that substitution of a 5-hydroxylated or methylated A ring of the parent compound **1** with one methoxyl or hydroxyl function or preferably one hydroxyl and one methyl group or two methoxyl groups largely increases antipolio potency (at least 20 times) and likewise decreases cytotoxicity to Vero cells (at least 10 times), resulting in promising antipolio products. All other compounds of these series have TI_{99} values of 50 or less. It should be noticed that substitution of the 3′-position of the B ring with hydroxyl or methoxyl functions increases cytotoxicity of the corresponding substance without influencing much antipolio activity, indicating that the naturally occurring 3-*O*-methylkaempferol derivatives possess better TI_{99} values

Table II. Therapeutic Index of 3-Methoxyflavones against Polio- and Rhinovirus Infections



	polio			rhino		
no.	CyD_{50},[a] μg/mL	ED_{99},[b] μg/mL	TI_{99}[c]	CyD_{50},[a] μg/mL	ED_{99},[b] μg/mL	TI_{99}[c]
1	10	6	1.7	–[d]	–	–
2	75	5	15	75	RF < 10^2[e]	–
3	25	2	12.5	25	25	1
4	10	5	2	10	10	1
6	150	10	15	150	15	10
7	200	25	8	–	–	–
8	>50	2.5	>20	>50	10	>5
9	25	20	1.25	–	–	–
10	3	2	1.5	15	10	1.5
11	15	1	15	25	25	1
12	100	6	16.7	100	RF < 10^2	–
13	100	7	14.3	100	25	4
14	30	>30	<1	–	–	–
15	20	4	5	20	RF < 10^2	–
16	25	10	2.5	–	–	–
17	50	>50	<1	–	–	–
18	>0.1	NA[f]	–	–	–	–
19	10	5	2	–	–	–
20	25	0.5	50	5	1	5
21	10	0.2	50	–	–	–
22	100	0.3	333.3	100	2.5	40
23	100	10	10	100	20	5
24	15	0.1	150	15	1	15
25	50	1	50	50	25	2
26	>100	0.1	>1000	100	0.5	>200
27	5	0.3	16.7	–	–	–
28	10	0.3	33.3	–	–	–
29	25	2.5	10	–	–	–
30	25	0.5	50	–	–	–
31	100	0.2	500	10	10	1
32	>100	0.25	400	>100	0.5	>200
33	>5	0.2	25	20	2	10
34	10	1.5	6.6	–	–	–
35	1	NA	–	–	–	–
38	100	50	2	–	–	–
39	>200	NA	–	–	–	–
41	20	NA	–	–	–	–
42	>200	NA	–	–	–	–
43	100	RF < 10^2	–	–	–	–
44	20	NA	–	–	–	–
45	20	NA	–	–	–	–
47	50	5	10	–	–	–
58	25	>25	<1	25	NA	–
59	2	>2	<1	2	NA	–
60	50	10	5	50	20	2.5
62	10	NA	–	–	–	–
61	100	25	4	100	25	4

[a] CyD_{50} = 50% cytotoxic dose. [b] ED_{99} = 99% effective dose. [c] TI_{99} = therapeutic index 99. [d] – = not tested. [e] RF < 10^2 = viral titer reduction factor at CyD_{50} lower than 10^2, no ED_{99}. [f] Not active.

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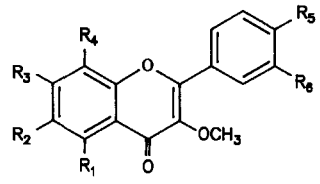
(31) Ong, T. M.; Mukhtar, M.; Wolf, C. R.; Zeiger, E. *J. Environm. Pathol. Toxicol.* **1980**, *4*, 55.

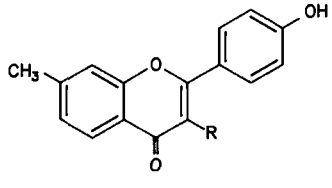
Table III. Antipoliovirus Activity of 4′-Chloro-, 4′-Methoxy-, and 4′-Acetoxyflavones **36–57**



no.[a]	R_1	R_2	R_3	R_4	R_5	R_6	mp, °C	crystn solvent	yield, %	MNTD,[b] μg/mL	RF_{MNTD}[c]	mD_{RF10^3},[d] μg/mL	anal.	
36	H	H	CH_3	H	OCH_3	H	157–158	acetone/water (2:3)	85	100	1	–	$C_{18}H_{16}O_4$	C,H
37	OCH_3	H	H	H	OCH_3	H	141–142	methanol	78	100	1	–	$C_{18}H_{16}O_5$	C,H
38	H	OCH_3	H	H	OCH_3	H	137–138	methanol	60	50	10^2	–	$C_{18}H_{16}O_5$	C,H
39	H	H	H	OCH_3	OCH_3	H	131–133	ethanol	58	100	1	–	$C_{18}H_{16}O_5$	C,H
40	H	OH	H	H	OCH_3	H	221–222	methanol/acetone (1:2)	80	100	1	–	$C_{17}H_{14}O_5$	C,H
41	H	Cl	H	H	OCH_3	H	135–136	methanol/acetone (1:1)	76	25	1	–	$C_{17}H_{13}O_4Cl$	C,H
42	H	H	Cl	H	OCH_3	H	171–172	methanol/acetone (1:1)	80	100	1	–	$C_{17}H_{13}O_4Cl$	C,H
43	H	H	CH_3	H	Cl	H	144–145	methanol/acetone (2:1)	72	25	1	–	$C_{17}H_{13}O_3Cl$	C,H
44	H	Cl	H	H	Cl	H	168–169	methanol/acetone (2:1)	88	25	1	–	$C_{16}H_{10}O_3Cl_2$	C,H
45	H	H	Cl	H	Cl	H	171–172	methanol/acetone (2:1)	78	25	1	–	$C_{16}H_{10}O_3Cl_2$	C,H
46	OH	H	OCH_3	H	OCH_3	H				>50	1	–		
47	OH	H	OH	H	OCH_3	H				10	10^5	5		
48	OH	H	OCH_3	H	OCH_3	OCH_3				>50	1	–		
49	OH	H	OH	H	OCH_3	OCH_3				50	10^5	2.5		
50	OH	OCH_3	OH	H	OCH_3	H				100	1	–		
51	OH	H	OH	OCH_3	OCH_3	H				>10	1	–		
52	OH	H	OCH_3	OCH_3	OCH_3	H				7.5	–	–		
53	H	OCH_3	OCH_3	H	OCH_3	OCH_3				50	10^3	50		
54	OH	OCH_3	OCH_3	OCH_3	OCH_3	H				5	1	–		
55	OH	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3				50	10^3	50		
56	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3				50	10^4	25		
57	$OCOCH_3$	H	$OCOCH_3$	H	$OCOCH_3$	$OCOCH_3$				>50	10^5	2		

[a] **36–45** are synthetic in origin. **46–57** are natural in origin. [b–d] See Table I.

Table IV. Antipoliovirus Activity of 3-Substituted 4′-Hydroxy-7-methylflavones **58–62**



no.[a]	R	mp, °C	crystn solvent	yield, %	MNTD,[b] μg/mL	RF_{MNTD}[c]	MD_{RF10^3},[d] μg/mL	anal.	
58	OC_2H_5	229	methanol	45	25	1	–	$C_{18}H_{16}O_4$	C,H
59	$OCH(CH_3)_2$	241	methanol	89	1	1	–	$C_{19}H_{18}O_4$	C,H
60	Cl	268	methanol	67	10	10^3	6	$C_{16}H_{11}O_3Cl$	C,H
61	CH_3	249	ethanol	83	100	10^3	50	$C_{17}H_{14}O_3$	C,H
62	NH_2	218	ethanol	48	20	1	–	$C_{16}H_{13}NO_3$	C,H

[a] Synthetic in origin. [b–d] See Table I.

Table V. Antirhinovirus Activity of 4',7-Dihydroxy-3-methoxy-5,6-dimethylflavone **26** and 4',7-Dihydroxy-3-methoxy-5-methylflavone **25** against 12 Strains; MIC_{50}, μg/mL

	rhinovirus serotype											
compd	2	29	39	85	9	15	59	63	89	41	14	70
26	0.044	0.078	0.088	0.076	0.156	0.088	0.016	0.074	0.5	0.088	0.072	0.097
25	2.4	0.175	12.0	-	0.2	0.4	3.0	-	0.2	0.375	10.8	5.0
guanidine	>64	>64	109	750	375	438	350	500	1000	1000	7	6
HBB[a]	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	16	50

[a] 2-(α-Hydroxybenzyl)benzimidazole.

than their corresponding 3-*O*-methylquercetin analogues.

These results are largely confirmed in the antiviral testing against rhinovirus type 15 and the corresponding cytotoxicity investigation on human skin fibroblasts as shown in Table II. For most substances the same degree of cytotoxicity on Vero cells and human skin fibroblasts is observed. On the contrary, the antirhino ED_{99} values are usually smaller than the corresponding antipolio ones which results in lower TI_{99} values. Therapeutic ratios of >200 are found for compounds **26** and **32**, whereas substances **22** and **25** are 10 times less active against rhinovirus than against poliovirus. Surprisingly penduletin (**31**) is only active against rhinovirus in its cytotoxic concentration. All other compounds tested have TI_{99} values of 10 or less.

From Table III it is clear that a 4'-hydroxyl group is important for antipolio activity. Comparison of the antiviral activities of e.g. 4'-hydroxy-3-methoxy-7-methylflavone (**3**), 5,4'-dihydroxy-3,7-dimethoxyflavone (**22**), and 5,7,4'-trihydroxy-3,6-dimethoxyflavone (**30**) with those of, respectively, 3,4'-dimethoxy-7-methylflavone (**36**), 5-hydroxy-3,7,4'-trimethoxyflavone (**46**), and 5,7-dihydroxy-3,6,4'-trimethoxyflavone (**50**) shows the superiority of the 4'-hydroxy analogues. Although some of the 4-methoxy and acetoxy derivatives show some degree of antipolio activity viz. compounds **47**, **49**, and **57**, their TI_{99} values are considerably lower than those of the corresponding 4'-hydroxy analogues. None of the 4-chloro derivatives shows any antipoliovirus activity. Table IV indicates the necessity of the 3-methoxyl group. Replacement of this function with different R substitutions **58–62**, results in compounds having no or lower antipolio activities and/or higher cytotoxicities than the corresponding 3-methoxy derivatives.

Substance **26**, being the most interesting compound in both antiviral test systems, was further screened by the EPTT against a series of 12 rhinovirus serotypes, which were grown on Ohio HeLa cells. Its activity was compared to that of a less active synthetic flavone viz. 4'-hydroxy-3,7-dimethoxy-5-methylflavone (**25**) and the reference substances guanidine and 2-α-(hydroxybenzyl)benzimidazole (HBB). The data of this screening are presented in Table V. Whereas the different rhinovirus serotypes widely vary in their susceptibility to the reference substances, the MIC's for 50% CPE reduction of **26** range from 0.016 to 0.5 μg/mL. The lower antiviral activity which is found for **25** against rhinovirus serotype 15 is confirmed for nearly all other rhinovirus serotypes tested. The MIC_{50}'s vary from 0.175 to 12 μg/mL. It should be pointed out that all rhinovirus serotypes investigated are sensitive to both synthetic flavones. It is well known that drug resistance against capsid-binding antiviral drugs such as flavans and chalcones, on the contrary, is a common feature so that often cross-resistance is exhibited by these antirhinovirus active canyon products.[32]

Since some 3-methoxyflavones, when administered intraperitoneally have shown to protect mice from lethal infections from coxsackie B_4,[9] the most antivirally active substance of this study viz. compound **26** should be considered as a promising candidate for antirhinovirus clinical studies in human volunteers. As quercetin and several familiar flavonoids have been reported to be mutagenic in a number of short-term microbial assays,[33,34] mutagenicity experiments were performed with compound **26** using *Salmonella typhimurium* strains TA 98 and TA 100 with and without S9 mix from rat liver according to the plate incorporation assay. The results of these tests are given in Table VI. It is clearly shown that the number of revertants is not significantly increased by **26** in concentrations up to 2.5 mg/plate, with or without S9 fraction. On the contrary, the reported mutagenicity of quercetin is confirmed.

Table VI. Mutagenicity Testing of 3-Methoxyflavones **26** and Quercetin

		revertants per plate			
	Salmonella typhimurium	strain TA 98		strain TA 100	
mutagen	mg/plate	no S9	with S9	no S9	with S9
-	-	12	73	96	87
26	0.25	12	20	110	103
26	2.5[a]	10	9	85	94
quercetin	0.25	1100	*b*	589	*b*
quercetin	2.5	*b*	*b*	*b*	*b*

[a] Precipitation occurs after addition of the S9 mix. [b] Toxic concentration.

Experimental Section

Melting points were determined with a Büchi SMP-20 apparatus and were not corrected. ^1H NMR and ^{13}C NMR spectra (of the chalcones and flavones derivatives) were recorded on a JEOL JNM-FX 200, IR spectra were recorded on a Beckman Acculab 4 spectrophotometer.

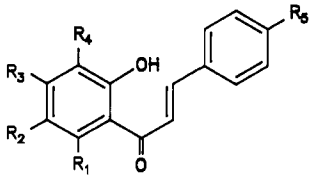
The purity of the compounds was verified by TLC ($CHCl_3$/MeOH 9:1), using fluorescent silica gel plates (Merck). Components were visualized by UV fluorescence properties.

2'-Hydroxychalcones 63–90. 2'-Hydroxyacetophenone **91–107** (0.02 mol) and 0.02 mol aldehyde **108–110** were dissolved in EtOH (15 mL) under stirring, and aqueous NaOH (50%, 12 mL) was added dropwise. The reaction mixture was stirred at room temperature during the period mentioned in Table VII. The reaction mixture was diluted with H_2O and acidified with HCl (10%). The precipitate was filtered off and recrystallized from CH_3OH, EtOH, or a mixture with acetone (Table VII).

3-Hydroxyflavones 111–138. 2'-Hydroxychalcone (1 mmol) **63–90** was dissolved in CH_3OH (15 mL). Sodium hydroxide solution (10%, 15 mL) was added with stirring to the warm solution, followed by H_2O_2 solution (30%, 10 mL). After 15 min the reaction mixture was diluted with H_2O and acidified with HCl (10%). The precipitate was extracted with CH_2Cl_2. The organic layer was isolated and washed with $NaHCO_3$ solution (5%) and H_2O. The CH_2Cl_2 layer was dried on Na_2SO_4, and the solvent was evaporated. The precipitate was recrystallized from EtOH, CH_3OH, or a mixture with acetone (Table VIII).

(32) Tyrrell, D. A. J. *Ann. Rev. Microbiol.* **1988**, *42*, 35.
(33) Mac Gregor, J. T.; Jurd, L. *Mutation Res.* **1978**, *54*, 297.
(34) Elliger, C. A.; Henika, P. R.; Mac Gregor, J. T. *Mutation Res.* **1984**, *135*, 77.

Table VII. 2'-Hydroxychalcones



no.	R_1	R_2	R_3	R_4	R_5	react time	mp, °C	crystn solvent	yield, %	anal.	
63	H	H	H	H	$OCH_2C_6H_5$	4 days	112–113	ethanol	59	$C_{22}H_{18}O_3$	C,H
64	H	CH_3	H	H	$OCH_2C_6H_5$	12 h	139	methanol	53	$C_{23}H_{20}O_3$	C,H
65	H	H	CH_3	H	$OCH_2C_6H_5$	2 days	144–145	acetone/methanol (1:1)	52	$C_{23}H_{20}O_3$	C,H
66	H	$CH(CH_3)_2$	H	H	$OCH_2C_6H_5$	6 days	85–86	acetone	31	$C_{25}H_{24}O_3$	C,H
67	H	H	$CH(CH_3)_2$	H	$OCH_2C_6H_5$	4 days	90–93	ethanol	89	$C_{25}H_{24}O_3$	C,H
68	OCH_3	H	H	H	$OCH_2C_6H_5$	6 days	111–112	ethanol	50	$C_{23}H_{20}O_4$	C,H
69	H	OCH_3	H	H	$OCH_2C_6H_5$	4 days	142–143	acetone	50	$C_{23}H_{20}O_4$	C,H
70	H	H	OCH_3	H	$OCH_2C_6H_5$	4 days	123–124	methanol	35	$C_{23}H_{20}O_4$	C,H
71	H	H	H	OCH_3	$OCH_2C_6H_5$	4 days	152–153	methanol	45	$C_{23}H_{20}O_4$	C,H
72	H	$OCH_2C_6H_5$	H	H	$OCH_2C_6H_5$	6 days	121–122	acetone/ethanol (1:1)	59	$C_{29}H_{25}O_4$	C,H
73	H	H	$OCH_2C_6H_5$	H	$OCH_2C_6H_5$	4 days	134–136	ethanol	55	$C_{29}H_{25}O_4$	C,H
74	H	Cl	H	H	$OCH_2C_6H_5$	4 days	100–102	acetone	88	$C_{22}H_{17}O_3Cl$	C,H
75	H	H	Cl	H	$OCH_2C_6H_5$	5 days	140–141	ethanol/acetone (3:1)	40	$C_{22}H_{17}O_3Cl$	C,H
76	H	H	Br	H	$OCH_2C_6H_5$	2 days	158–159	acetone	68	$C_{22}H_{17}O_3Br$	C,H
77	H	H	I	H	$OCH_2C_6H_5$	16 h	119–120	acetone	83	$C_{22}H_{17}O_3I$	C,H
78	H	H	F	H	$OCH_2C_6H_5$	2 days	119–120	methanol/acetone (3:2)	78	$C_{22}H_{17}O_3F$	C,H
79	H	NO_2	H	H	$OCH_2C_6H_5$	24 h	176–177	acetone	86	$C_{22}H_{17}O_5N$	C,H
80	H	Cl	H	CH_3	$OCH_2C_6H_5$	2 days	153–154	acetone	85	$C_{23}H_{19}O_3Cl$	C,H
81	H	H	CH_3	H	OCH_3	2 days	103–104	ethanol	59	$C_{17}H_{16}O_3$	C,H
82	OCH_3	H	H	H	OCH_3	6 days	98–100	ethanol	58	$C_{17}H_{16}O_4$	C,H
83	H	OCH_3	H	H	OCH_3	5 days	63–64	ethanol	78	$C_{17}H_{16}O_4$	C,H
84	H	H	H	OCH_3	OCH_3	18 h	118	ethanol	74	$C_{17}H_{16}O_4$	C,H
85	H	$OCH_2C_6H_5$	H	H	OCH_3	22 h	89–92	acetone/petroleum ether (3:1)	72	$C_{23}H_{20}O_4$	C,H
86	H	Cl	H	H	OCH_3	2 days	191–192	ethanol	40	$C_{16}H_{13}O_3Cl$	C,H
87	H	H	Cl	H	OCH_3	2 days	128–129	acetone	68	$C_{16}H_{13}O_3Cl$	C,H
88	H	H	CH_3	H	Cl	2 days	140–141	ethanol/acetone (1:1)	55	$C_{16}H_{13}O_2Cl$	C,H
89	H	Cl	H	H	Cl	2 days	188–189	ethanol	60	$C_{15}H_{10}O_2Cl_2$	C,H
90	H	H	Cl	H	Cl	2 days	150–151	ethanol/acetone (1:4)	80	$C_{15}H_{10}O_2Cl_2$	C,H

3-Methoxyflavones 36–45 and 139–156. 3-Hydroxyflavone **111–138** (1 mmol) was dissolved in dry acetone. The solution was refluxed for 16 h with anhydrous K_2CO_3 (3 mmol) and dimethyl sulfate (2 mmol). The acetone was evaporated and H_2O was added to the residue. The precipitate was filtered off and recrystallized from EtOH, CH_3OH, or a mixture with acetone (Tables III and IX).

4'-Hydroxyflavones 1–18, 21–26, and 58–61. 4-Benzyloxyflavone (**139–162, 170, 171,** and **173–175**) (1 mmol) was dissolved in AcOH (10 mL) and HCl (5 mL). The solution was heated on a waterbath for 3 h. After cooling, H_2O was added and the precipitate was filtered off. The precipitate was washed with H_2O. The product was recrystallized from EtOH, CH_3OH, or mixtures with acetone (Tables IV and IX).

6'-Amino-4'-hydroxy-3-methoxyflavone (19). 4'-Hydroxy-3-methoxy-6-nitroflavone (0.5 g, 1.24 mmol) (**18**) and cyclohexene (0.5 g, 6.2 mmol) were dissolved in EtOH (25 mL). Palladium on carbon (10%, 0.12 g) was added. The reaction mixture was refluxed for 3 h. The solution was filtered warm, and the EtOH was evaporated. The residue was dissolved in AcOH (10 mL) and HCl (5 mL). The solution was heated for 2 h on a waterbath. After cooling H_2O was added and the solution was neutralized with 1 M NaOH. The yellow precipitate was filtered off and recrystallized from CH_3OH (Table I).

4'-(Benzyloxy)-5,7-dihydroxy-3-methoxyflavone (157), 4'-(Benzyloxy)-7-hydroxy-3-methoxy-5-methylflavone (160), 4'-(Benzyloxy)-7-hydroxy-5,6-dimethylflavone (162). Acetophenones **163–165** (4.76 mmol), *p*-(benzyloxy)benzoic acid anhydride (3.89 g, 8.9 mmol) and potassium *p*-(benzyloxy)benzoate (0.68 g, 2.54 mmol) were combined. The reaction mixture was heated at 160 °C at reduced pressure for 8 h. Afterward it was refluxed in methanolic NaOH (4%, 100 mL) solution for 30 min. The solution was filtered. The precipitate was refluxed again with the NaOH solution and filtered off. The filtrates were collected and CH_3OH was evaporated. H_2O was added to the residue. The precipitate was filtered off and washed several times with $NaHCO_3$ solution (4%). The products were recrystallized (Table IX).

4'-(Benzyloxy)-5-hydroxy-3,7-dimethoxyflavone (158). 4'-(Benzyloxy)-5,7-dihydroxy-3-methoxyflavone (**157**) (1 g, 2.56 mmol) was dissolved in dry acetone. The solution was refluxed with anhydrous K_2CO_3 (0.7 g, 5.12 mmol) and dimethyl sulfate (0.36 g, 2.82 mmol). The acetone was evaporated and H_2O was added to the residue. The precipitate was filtered off and recrystallized from EtOH (Table IX).

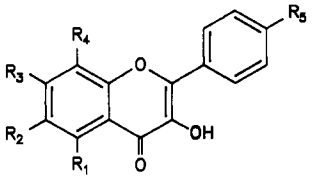
4'-(Benzyloxy)-3,5,7-trimethoxyflavone (159). 4'-(Benzyloxy)-5,7-dihydroxy-3-methoxyflavone (**157**) (0.8 g, 2.05 mmol) was dissolved in dry acetone. The solution was refluxed with anhydrous K_2CO_3 (0.7 g, 5.12 mmol) and dimethyl sulfate (0.52 g, 4.10 mmol). The acetone was evaporated and H_2O was added to the residue. The precipitate was filtered off and recrystallized from EtOH (Table IX).

4'-(Benzyloxy)-3,7-dimethoxy-5-methylflavone (161). 4'-(Benzyloxy)-7-hydroxy-3-methoxy-5-methylflavone (**160**) (0.25 g, 0.55 mmol) was dissolved in dry acetone (50 mL). The solution was refluxed with anhydrous K_2CO_3 (0.22 g, 1.60 mmol) and dimethyl sulfate (0.22 g, 1.70 mmol). The acetone was evaporated and H_2O was added to the residue. The precipitate was filtered off and recrystallized from $EtOH/H_2O$ (Table IX).

4'-(Benzyloxy)-5-hydroxy-3-methoxy-7-[(1-phenyltetrazol-5-yl)oxy]flavone (169). 4'-(Benzyloxy)-5,7-dihydroxy-3-methoxyflavone (1 g, 2.6 mmol) was dissolved in dry DMF (20 mL). Potassium *tert*-butoxide (0.33 g, 2.9 mmol) was added. When the base was dissolved, 5-chloro-1-phenyltetrazole (0.47 g, 2.6 mmol) in 5 mL of dry DMF (5 mL) was added to the solution. The mixture was stirred at room temperature for 30 min and poured into ice water. The precipitate was filtered off and recrystallized from EtOH (0.48 g, 35%), mp 145–147 °C. Anal. ($C_{30}H_{22}N_4O_6$) C, H.

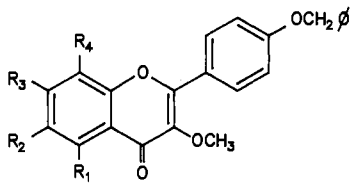
4',5-Dihydroxy-3-methoxyflavone (20). To a vigorously stirred solution of 4'-(benzyloxy)-5-hydroxy-3-methoxy-7-(1-

Table VIII. 3-Hydroxyflavones



no.	R_1	R_2	R_3	R_4	R_5	mp, °C	crystn solvent	yield, %	anal.	
111	H	H	H	H	$OCH_2C_6H_5$	176	ethanol	42	$C_{22}H_{16}O_4$	C,H
112	H	CH_3	H	H	$OCH_2C_6H_5$	213	ethanol/acetone (1:1)	62	$C_{23}H_{18}O_4$	C,H
113	H	H	CH_3	H	$OCH_2C_6H_5$	157–158	ethanol/acetone (1:1)	50	$C_{23}H_{18}O_4$	C,H
114	H	$CH(CH_3)_2$	H	H	$OCH_2C_6H_5$	165	ethanol	37	$C_{25}H_{22}O_4$	C,H
115	H	H	$CH(CH_3)_2$	H	$OCH_2C_6H_5$	125–130	ethanol	18	$C_{25}H_{22}O_4$	C,H
116	OCH_3	H	H	H	$OCH_2C_6H_5$	165–166	methanol	30	$C_{23}H_{18}O_5$	C,H
117	H	OCH_3	H	H	$OCH_2C_6H_5$	180–181	methanol/acetone (1:1)	60	$C_{23}H_{18}O_5$	C,H
118	H	H	OCH_3	H	$OCH_2C_6H_5$	169	ethanol	34	$C_{23}H_{18}O_5$	C,H
119	H	H	H	OCH_3	$OCH_2C_6H_5$	203	methanol	38	$C_{23}H_{18}O_5$	C,H
120	H	$OCH_2C_6H_5$	H	H	$OCH_2C_6H_5$	209	methanol/acetone (1:1)	42	$C_{29}H_{22}O_5$	C,H
121	H	H	$OCH_2C_6H_5$	H	$OCH_2C_6H_5$	210–212	methanol/acetone (1:1)	44	$C_{29}H_{22}O_5$	C,H
122	H	Cl	H	H	$OCH_2C_6H_5$	203–204	acetone	72	$C_{22}H_{15}O_4Cl$	C,H
123	H	H	Cl	H	$OCH_2C_6H_5$	>250	ethanol/acetone (1:9)	62	$C_{22}H_{15}O_4Cl$	C,H
124	H	H	Br	H	$OCH_2C_6H_5$	203–207	methanol	52	$C_{22}H_{15}O_4Br$	C,H
125	H	H	I	H	$OCH_2C_6H_5$	175–180	chloroform/methanol (1:3)	10	$C_{22}H_{15}O_4I$	C,H
126	H	H	F	H	$OCH_2C_6H_5$	178–179	methanol	45	$C_{22}H_{15}O_4F$	C,H
127	H	NO_2	H	H	$OCH_2C_6H_5$	237–239	methanol/acetone (1:1)	64	$C_{22}H_{15}O_6N$	C,H
128	H	Cl	H	CH_3	$OCH_2C_6H_5$	218–219	methanol/acetone (1:1)	50	$C_{23}H_{17}O_4Cl$	C,H
129	H	H	CH_3	H	OCH_3	177–178	methanol/acetone (1:1)	52	$C_{17}H_{14}O_4$	C,H
130	OCH_3	H	H	H	OCH_3	168–169	methanol	16	$C_{17}H_{14}O_5$	C,H
131	H	OCH_3	H	H	OCH_3	182–183	ethanol	65	$C_{17}H_{14}O_5$	C,H
132	H	H	H	OCH_3	OCH_3	212–214	ethanol	34	$C_{17}H_{14}O_5$	C,H
133	H	$OCH_2C_6H_5$	H	H	OCH_3	162–164	ethanol/acetone (1:1)	70	$C_{23}H_{18}O_5$	C,H
134	H	Cl	H	H	OCH_3	135–136	methanol/acetone (1:1)	76	$C_{16}H_{11}O_4Cl$	C,H
135	H	H	Cl	H	OCH_3	190–191	acetone/ethanol (1:1)	55	$C_{16}H_{11}O_4Cl$	C,H
136	H	H	CH_3	H	Cl	197–198	ethanol/acetone (1:1)	61	$C_{16}H_{11}O_3Cl$	C,H
137	H	Cl	H	H	Cl	216–217	ethanol/acetone (1:1)	58	$C_{15}H_8O_3Cl_2$	C,H
138	H	H	Cl	H	Cl	199–200	acetone	50	$C_{15}H_8O_3Cl_2$	C,H

Table IX. 4′-(Benzyloxy)-3-methoxyflavones



no.	R_1	R_2	R_3	R_4	mp, °C	crystn solvent	yield, %	anal.	
139	H	H	H	H	112	ethanol	63	$C_{23}H_{18}O_4$	C,H
140	H	CH_3	H	H	133–134	methanol/acetone (1:1)	62	$C_{24}H_{20}O_4$	C,H
141	H	H	CH_3	H	123–124	methanol/water (1:3)	78	$C_{24}H_{20}O_4$	C,H
142	H	$CH(CH_3)_2$	H	H	121–122	methanol/acetone (1:1)	50	$C_{26}H_{24}O_4$	C,H
143	H	H	$CH(CH_3)_2$	H	102–105	ethylacetate/petroleum ether (2:1)	89	$C_{26}H_{24}O_4$	C,H
144	OCH_3	H	H	H	120–122	methanol	75	$C_{24}H_{20}O_5$	C,H
145	H	OCH_3	H	H	135–136	methanol	60	$C_{24}H_{20}O_5$	C,H
146	H	H	OCH_3	H	149–151	methanol	72	$C_{24}H_{20}O_5$	C,H
147	H	H	H	OCH_3	170–171	methanol/acetone (2:1)	60	$C_{24}H_{20}O_5$	C,H
148	H	$OCH_2C_6H_5$	H	H	102	methanol/acetone (1:1)	72	$C_{30}H_{24}O_5$	C,H
149	H	H	$OCH_2C_6H_5$	H	113–114	methanol/acetone (1:1)	75	$C_{30}H_{24}O_5$	C,H
150	H	Cl	H	H	142–143	methanol	92	$C_{23}H_{17}O_4Cl$	C,H
151	H	H	Cl	H	131–132	methanol/acetone (1:1)	70	$C_{23}H_{17}O_4Cl$	C,H
152	H	H	Br	H	143–144	acetone	82	$C_{23}H_{17}O_4Br$	C,H
153	H	H	I	H	124–126	acetone	86	$C_{23}H_{17}O_4I$	C,H
154	H	H	F	H	132–133	methanol	87	$C_{23}H_{17}O_4F$	C,H
155	H	NO_2	H	H	148–149	methanol	75	$C_{23}H_{17}NO_6$	C,H
156	H	Cl	H	CH_3	126–127	methanol/acetone (1:1)	92	$C_{24}H_{19}O_4Cl$	C,H
157	OH	H	OH	H	295	DMF/water (2:1)	82	$C_{23}H_{18}O_6$	C,H
158	OH	H	OCH_3	H	125–127	ethanol	74	$C_{24}H_{20}O_6$	C,H
159	OCH_3	H	OCH_3	H	145–147	ethanol	63	$C_{25}H_{22}O_6$	C,H
160	CH_3	H	OH	H	265–268	ethanol	92	$C_{24}H_{20}O_6$	C,H
161	CH_3	H	OCH_3	H	147–149	ethanol/water	89	$C_{25}H_{22}O_5$	C,H
162	CH_3	CH_3	OH	H	258	methanol	74	$C_{25}H_{22}O_5$	C,H

phenyltetrazol-5-yl)flavone (**169**) (0.5 g, 0.9 mmol) in benzene (20 mL), H_2O (25 mL), and EtOH (40 mL) was added Pd/C (10%, 0.6 g). HCOOH (98%, 10 mL) was then added and the mixture was refluxed at 100 °C for 1/2 h. The solution was cooled and filtered. After filtration, the benzene layer is washed several times with aqueous NaOH (10%). Finally the alkaline solution was

neutralized with HCl (10%). The precipitate was filtered off, washed with H_2O, dried, and crystallized from EtOH (Table I).

4'-(Benzyloxy)-3-ethoxy-7-methylflavone (170). 4'-(Benzyloxy)-3-hydroxy-7-methylflavone (**113**) (1 g, 2.79 mmol) was dissolved in dry acetone. The solution was refluxed with anhydrous K_2CO_3 (0.77 g, 5.58 mmol) and diethyl sulfate (0.86 g, 15.58 mmol) for 16 h. The acetone was evaporated and H_2O was added to the residue. The precipitate was filtered off and recrystallized from CH_3OH (0.38 g, 35%), mp 127–128 °C. Anal. ($C_{25}H_{23}O_4$) C, H.

4'-(Benzyloxy)-3-isopropoxy-7-methylflavone (171). 4'-(Benzyloxy)-3-hydroxy-7-methylflavone (**113**) (1 g, 2.79 mol) was dissolved in dry acetone. The solution is refluxed with anhydrous K_2CO_3 (0.77 g, 5.58 mmol) and isopropyl iodide (0.95 g, 5.58 mmol) for 16 h. The acetone was evaporated and the residue recrystallized from CH_3OH (0.56 g, 51%), mp 113–115 °C. Anal. ($C_{26}H_{25}O_4$), C, H.

1-[4-(Benzyloxy)phenyl]-3-(2-hydroxy-4-methylphenyl)-1,3-dioxopropane (172). A solution of 4'-methyl-2'-hydroxyacetophenone (1.16 g, 7.7 mmol) in dry THF (15 mL) was added to a stirred solution of lithium diisopropylamide (7.7 mmol, from diisopropylamine and butyllithium) in dry THF at −25 °C. The mixture was stirred for 1 h at −25 °C and then cooled to −78 °C, and a solution of benzoyl chloride (2 g, 8.1 mmol) in dry THF (15 mL) was added. Stirring was continued for 3 h at −78 °C. The mixture was allowed to warm to room temperature and was then diluted with ethyl acetate and acidified to pH 3 with 1 N HCl. The organic layer was dried and the ethyl acetate was evaporated. The product was recrystallized from ethyl acetate (1.2 g, 45%), mp 134 °C. Anal. ($C_{23}H_{20}O_4$) C, H.

4'-(Benzyloxy)-3-chloro-7-methylflavone (173). 1-[4-(Benzyloxy)phenyl]-3-(2-hydroxy-4-methylphenyl)-1,3-dioxopropane (**172**) (1 g, 2.78 mmol) was suspended in dioxane (20 mL). SO_2Cl_2 (0.25 mL, 3 mmol) was added, and the mixture was refluxed slowly for 1 h. After cooling, it was diluted with a large excess of H_2O and extracted with CH_2Cl_2. The organic layer was dried, and the solvent was evaporated. The product was recrystallized from CH_3OH (5.8 g, 55%), mp 168–170 °C. Anal. ($C_{23}H_{18}O_3Cl$) C, H.

1-[4-(Benzyloxy)phenyl]-3-(2-hydroxy-4-methylphenyl)-2-methyl-1,3-dioxopropane (174). 1-[4-(Benzyloxy)-phenyl]-3-(2-hydroxy-4-methylphenyl)-1,3-dioxopropane (**172**) (1 g, 2.78 mmol) was dissolved in dry acetone (50 mL, 12.8 mmol). CH_3I (0.49 g, 3.42 mmol) and anhydrous K_2CO_3 (0.38 g) was added. The reaction mixture was refluxed for 18 h. After cooling, the acetone was evaporated. H_2O was added to the residue, and the solution was acidified. The H_2O layer was extracted with ether. The ether layers were collected, dried, and evaporated. The yellow residue was used in the reaction without further purification (0.26 g, 25%). Anal. ($C_{24}H_{22}O_4$) C, H.

4'-(Benzyloxy)-3,7-dimethylflavone (175). 1-[4-(Benzyloxy)phenyl]-3-(2-hydroxy-4-methylphenyl)-2-methyl-1,3-dioxopropane (**174**) (0.2 g, 0.6 mmol) was refluxed for 5 h in a mixture of sodium acetate (8 g) and AcOH (80 mL). After the reaction, the mixture was diluted with H_2O and extracted with ethyl acetate. The organic layer was washed with Na_2CO_3 solution (4%), dried, and evaporated. The product was crystallized from ethyl acetate–petroleum ether (0.14 g, 65%), mp 108–110 °C. Anal. ($C_{24}H_{21}O_3$) C, H.

4'-Methyl-2'-[(p-tolylsulfonyl)oxy]acetophenone (178). 2'-Hydroxy-4'-methylacetophenone (**93**) (12 g, 0.081 mol), *p*-tolylsulfonyl chloride (16.53 g, 0.087 mol) and anhydrous potassium carbonate (13.8 g, 0.1 mol) is refluxed for 3 h in dry acetone (50 mL). After cooling, the reaction mixture was poured into ice water. The precipitate was filtered off and washed with NaOH (1%) solution and H_2O. The product was recrystallized from EtOH (20.7 g, 84%), mp 79 °C. Anal. ($C_{16}H_{16}O_4S$) C, H.

2-Bromo-4'-methyl-2'-[(p-tolylsulfonyl)oxy]acetophenone (179). 4'-Methyl-2'-[(*p*-tolylsulfonyl)oxy]acetophenone (**178**) (4 g, 0.013 mol) was dissolved in dioxane (15 mL). Dioxane bromide (3.5 g, 0.015 mol), dissolved in a mixture of ether (10 mL) and dioxane, (10 mL) was added dropwise. The reaction mixture was refluxed overnight. After cooling, it was poured into ice water and extracted with ether. The ether layer was washed with water, dried, and evaporated. The residue was recrystallized from CH_3OH (3.5 g, 71%), mp 88 °C. Anal. ($C_{16}H_{15}O_4SBr$) C, H.

2-Azido-4'-methyl-2'-[(p-tolylsulfonyl)oxy]acetophenone (180). 2-Bromo-4'-methyl-2'-[(*p*-tolylsulfonyl)oxy]acetophenone (**179**) (1 g, 2.59 mmol) and NaN_3 (0.25 g, 2.6 mmol) was dissolved in dry DMF (20 mL). The solution was stirred for 2 h at 0 °C. The reaction mixture was poured into H_2O. The oil, which separated from the H_2O, was extracted with ether. The ether layer was washed with H_2O, dried, and evaporated. The product was an orange-yellow oil which was used without further purification (4.9 g, 55%). Anal. ($C_{16}H_{15}N_3O_4S$) C, H.

α-Azido-4-(benzyloxy)-4'-methyl-2'-[(p-tolylsulfonyl)oxy]chalcone (177). 2-Azido-4'-methyl-2'-[(*p*-tolylsulfonyl)-oxy]acetophenone (**180**) and (1.57 g, 4.5 mmol) 4-(benzyloxy)-benzaldehyde (4.5 mmol) were dissolved in EtOH (95%–25 mL). Piperidinium acetate (4 g) was added, and the solution was stirred for 24 h at room temperature. H_2O was added, followed by extraction with CH_2Cl_2. The organic layer was dried, and the solvent was evaporated. The residue was recrystallized from ethyl acetate (1.25 g, 52%), mp 59 °C. Anal. ($C_{30}H_{25}N_3O_5S$) C, H.

3-Amino-4'-(benzyloxy)-7-methylflavone (176). Chalcone (**177**) (0.5 g, 1.3 mmol) and NaOH (0.1 g, 1.3 mmol) in EtOH (95%, 20 mL) was stirred for 1 h at room temperature under N_2. After dilution with H_2O, the precipitate was filtered off and recrystallized from EtOH (0.39 g, 80%), 142–143 °C. Anal. ($C_{23}H_{20}NO_4$) C, H.

3-Amino-4'-hydroxy-7-methylflavone (62). 3-Amino-4'-(benzyloxy)-7-methylflavone (**176**) (0.4 g, 1.1 mmol) was dissolved in AcOH (10 mL) and HCl (5 mL). The solution was heated on a water bath for 2 h. After cooling, H_2O was added and the solution was brought to pH 8 with 0.1N NaOH solution. The precipitate was filtered off and recrystallized from EtOH (Table IV).

Antiviral and Cytotoxicity Testing. Culture Media. Poliomyelitis type 1 and rhinovirus type 15 were grown in Vero cells and human skin fibroblasts respectively. The tissue culture medium used was that described by Hronovsky supplemented as described previously.[27] Viral titers were estimated by the 50% end-point titration technique as described earlier.[27] The viral titers were 10^7 TCD_{50}/mL for polio and 10^3 TCD_{50}/mL for rhinovirus.

Preparation of Samples. Stocks of compounds were prepared at 1 mg/mL in a maximal volume of 0.1 mL DMSO and by adding maintenance medium (M-2) up to 1 mL. Further dilutions were made in maintenance medium.

Antiviral Testing (End-Point Titration Technique). Monolayers of cells in microtiter plates (Nunc, Denmark) were infected with 0.1 mL of serial 10-fold dilutions of the virus suspension. The virus was allowed to absorb for 1 h at 37 °C after which 0.1 mL of serial 2-fold dilutions of samples were added. The cultures were incubated at 37 °C for poliovirus and 33 °C for rhinovirus and examined daily for cytopathogenic effects by light microscopy for at least 1 week. Virus control, tissue culture control, and product control were included in the test in order to determine the toxicity of the samples at each dilution. The antiviral activity was determined as the reduction factor of the viral titer i.e. the ratio of the virus titer in and the absence and the presence of the maximal nontoxic dose of the test compound.

Evaluation of Cytotoxicity by Measurement of Cell Growth. Stock Vero cells or human skin fibroblasts were grown in 25-cm^3 plastic flasks (Nunc) until they formed a confluent monolayer. After trypzinization with 0.25% trypsin solution for 3 min at 37 °C, the cells were centrifuged and suspended in 10 mL of M-199 medium (Flow) supplemented with 6% new born calf serum and 20 μg/mL of gentamycin. Stock solutions of test compounds were diluted in medium and 1 mL of serial 2-fold dilutions were applied in quadruplicate to flasks containing 10^6 cells. The flasks were incubated for 6 days at 37 °C, which allowed control cells in medium without test compound to reach a normal monolayer. Medium was discarded and 1 mL of 0.25% trypsin solution was added for trypzinization during 3 min at 37 °C. Cells were counted in a glass Burke count chamber, centrifuged, and resuspended in medium containing 2-fold dilutions of compound. New cultures were tested with 10^6 cells for further growth. Cultures with compounds suppressing completely the growth of cells were not investigated further. The previous procedure was repeated five times. Cell-generation time was calculated for each concentration of compound. The LD_{50} was expressed as the lowest concentration of compound which was able to double at least the

generation time of the cells compared to control cells without compound.

Mutagenicity Testing. The mutagenicity experiments were performed with *Salmonella typhimurium* strains TA 98 and TA 100 with and without S9 mix from rat liver by using the plate incorporation assay. Liver S9 fractions were prepared from male Wistar rats after induction with a combination of sodium phenobarbital (0.1% in the drinking water) and β-naphtoflavone (dissolved in corn oil, 12 mg/mL, and injected intraperitoneally on the fifth day of induction at a dosage of 80 mg/kg, or 0.666 mL corn oil solution/100 mg). The animals were fasted for 24 h immediately preceding death, and they were killed on the seventh day of induction by cervical dislocation. The efficacy of the S9 fraction was confirmed by a control experiment with strain TA 100 and benzo[a]pyrene. For mutagenicity testing a concentration of 20 μL of S9 per plate was used. Quercetin was included as a standard mutagen. Duplicate plates were poured for each dose of mutagen.[29-31]

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Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one (TIBO) Derivatives

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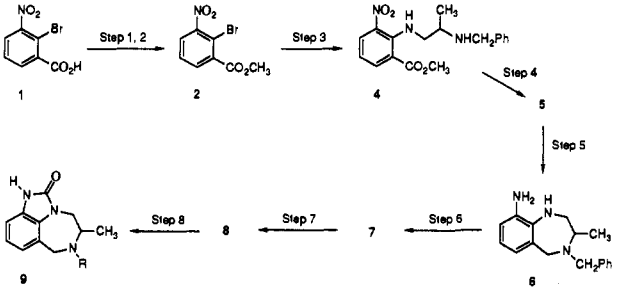
A series of 6-substituted 4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-ones (**9**) have been synthesized and tested for their ability to inhibit the replication of the HIV-1 virus in MT-4 cells. Two synthetic methods are described, one of which allows the synthesis of single enantiomers of the final products. A structure–activity study was done within the series of compounds to determine the optimum group for the 6-position substitution and to determine whether the activity was enantiospecific at the 5-position, which was substituted with a methyl group. The best analogue, **9jj**, inhibited HIV-1 with an IC_{50} of 4 μM, which is comparable to the activity level of DDI, a 2′,3′-dideoxynucleoside-type structure undergoing clinical trials as an anti-AIDS therapy.

Introduction

Therapeutic intervention for the treatment of HIV-1 infection which eventually results in AIDS (acquired immune deficiency syndrome) is limited to AZT (Zidovudine), the only approved drug for this disease. Unfortunately, it suffers from a number of limitations including limiting side effects and the revelation of the possible immergence of drug-resistant mutants of the virus.[1] Thus the need for new drugs to combat this disease is obvious, and several others are being evaluated for their effectiveness.[2] From the list of potential future therapeutic agents one is struck by the lack of good leads available for medicinal chemical optimization. The nucleoside analogues of which AZT is included are by far the most prevalent. However, the common mechanism of action of this group gives good reason to believe that all members of this structural class could suffer from some or all of the same limitations of AZT. Thus there is clearly a need for structures from other chemical families which inhibit the HIV-1 virus, possibly by alternative mechanisms.

Our approach to this problem was to choose a large group of representative structures from our collection of compounds for screening as inhibitors of HIV-1 replication. The limitations placed in choosing the compounds for initial screening were that they had little or no known pharmacological effect, including toxicity, from previous testing. Indeed, from 600 compounds that were evaluated in vitro for their ability to inhibit HIV-1 replication, **9a**

Scheme I



Step	Reagents and Conditions	Yield
1	$SOCl_2$, reflux, 2h	
2	CH_3OH, reflux, 1h	92% overall
3	$H_2NCH_2CH(CH_3)NHCH_2Ph$ (3), *n*-BuOH, Na_2CO_3, reflux, 1.5h	95%
4	2-hydroxypyridine, xylenes, reflux, 19h	93%
5	AlH_3, THF	87%
6	$Cl_3COCOCl$, N-methylmorpholine, CH_2Cl_2, 0°C	71%
7	H_2 (45 psi); 10% Pd/C, glacial HOAc	87%
8	Method: A; R-X, KI, Na_2CO_3, DMF	
	B; R-CHO, $NaCNBH_3$, CH_3CH	
	C; R-COOH, 1-hydroxybenzotriazole, DCC	

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