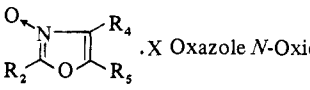
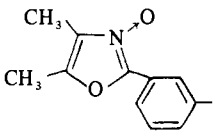
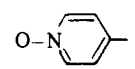
Table I.


.X Oxazole *N*-Oxides

Compd	R_2	R_4	R_5	X	Mp, °C	Formula[a]	Pmr, δ
1	C_6H_5	CH_3	CH_3	$4H_2O$	61-62[b]	$C_{11}H_{19}NO_6$	2.10(s,3), 2.35(s, 3), 3.52(s,6, shifts to 3.97 on addn of D_2O), 7.42-7.70(m, 3), 8.30-8.53(m,2)[g]
2	5-NO_2-2-C_4H_2O	CH_3	CH_3		162-163	$C_9H_8N_2O_5$	2.24(s,3), 2.48(s,3), 7.55(d,1,J = 4 Hz), 7.77(d,1,J = 4 Hz)[f]
3	5-NO_2-2-C_4H_2OCH=CH	CH_3	CH_3		197-198 dec	$C_{11}H_{10}N_2O_5$	2.18(s,3), 2.35(s,3), 6.70(d,1,J = 4 Hz), 7.18 (d,1,J = 16.25 Hz), 7.37(d,1,J = 4 Hz), 7.95(d,1,J = 16.25 Hz)[f]
4	2-C_4H_3S	CH_3	CH_3		135-137	$C_9H_9NO_2S$	2.20(s,3), 2.33(s,3), 7.11,7.18(pr d,1,J's = 5 Hz), 7.50(AMXq,1,J_{am} = 5 Hz, J_{ax} = 1.25 Hz), 7.79(AMXq,1,J_{am} = 5 Hz,J_{ax} = 1.25 Hz)[f]
5	C_6H_5	$CO_2C_2H_5$	CH_3	HCl	93-95[c]	$C_{13}H_{14}NO_4Cl$	1.30(t,3,J = 7 Hz), 2.52(s,3), 4.37(q,2,J = 7 Hz), 7.27-7.67(m,3), 7.73-8.00(m,2), 8.10(br s,1, disappears on addn of D_2O)[f]
6	3-$NO_2C_6H_4$	CH_3	CH_3		166-167[d]	$C_{11}H_{10}N_2O_4$	2.25(s,3), 2.47(s,3), 7.70(t,1,J = 8 Hz), 8.33 (d,1,J = 4 Hz), 8.93(d,1,J = 4 Hz), 9.27(s,1)[f]
7		CH_3	CH_3	$0.5H_2O$	211 dec	$C_{16}H_{17}N_2O_{4.5}$	2.48, 2.62(pr s,3), 8.65(s,1)[h]
8	4-$(CH_3)_2NC_6H_4$	CH_3	CH_3	H_2O	190-191 dec	$C_{13}H_{18}N_2O_3$	2.23,2.33(pr s,6), 3.07(s,6), 6.76(d,2,J = 9 Hz), 8.28(d,2,J = 9 Hz)[f]
9	$C_6H_5CH_2$	C_6H_5	C_6H_5	HCl	128	$C_{22}H_{18}NO_2Cl$	4.78(s,2), 7.22-7.88(m,15), 14.43(s,1,disappeared on addn of D_2O) peak at 4.78 splits to 4.63(s,0.35) and 5.27 (s,1.53)[f]
10	H	C_6H_5	C_6H_5	HCl	130-131 dec	$C_{15}H_{12}NO_2Cl$	7.55, 7.63(pr s,10), 9.75(s,1)[h,i]
11	$C_6H_5C_6H_4$	CH_3	C_6H_5		161-163	$C_{22}H_{17}NO_2$[k]	2.51(s,3), 7.28-7.88(m,12), 8.63(½ABq, 2,J = 9 Hz)[f]
	$C_6H_5C_6H_4$	CH_3	C_6H_5	HCl	190-193 dec	$C_{22}H_{18}NO_2Cl$[l]	2.72(s,3), 7.38-7.95(m + ½ABq, 12.7,J = 9 Hz), 8.48(½ABq, 2,J = 9 Hz)[h]
12	4-$NO_2C_6H_4$	H	C_6H_5		152, 184-186[e]	$C_{15}H_{10}N_2O_4$	7.50-8.07(m, 5), 8.30(s,1),8.47-8.92(m,4)[h]
13		CH_3	CH_3	HCl	210 dec	$C_{10}H_{11}N_2O_3Cl$	2.33(s,3), 2.60(s,3), 5.07(s,2), 8.66 + 8.70(d + s with sidebands, 4, J = 1 Hz)[j]
14	C_2H_5	$C_6H_5CH_2$	C_6H_5	HCl	122-123	$C_{18}H_{18}NO_2Cl$	1.53(t,3,J = 7.5 Hz), 3.40(q,2,J = 7.5 Hz), 4.40(s,2), 7.32(s,5), 7.57(s,5), 13.88(s,1, disappears on addn of D_2O)[f]
15	3-$NO_2C_6H_4$	H	C_6H_5		130, 168-171[e]	$C_{15}H_{10}N_2O_4$	7.50-8.23(m,6), 823(s,1), 8.60(d,1,J = 8 Hz), 8.87(d,1,J = 8 Hz), 9.25(s,1)
	3-$NO_2C_6H_4$	H	C_6H_5	HCl	165-166 dec	$C_{15}H_{11}N_2O_4Cl$	7.50-8.20(m,6), 8.30(s,1), 8.67(d,1,J = 8 Hz), 8.87(d,1,J = 8 Hz), 9.33(s,1)[h]
16	C_6H_5	H	C_6H_5	HCl	153-154	$C_{15}H_{12}NO_2Cl$	7.53-7.87(m,7), 7.92-8.15(m,2), 8.42-8.65(m,2), 8.92(s,1), 10.27(s,2)[g]

[a] All new compds were analyzed for C, H, and N and results are within ±0.4%. [b] Lit. mp 58-62. [c] Mp varies with rate of heating. [d] Lit mp 159-160 dec. [e] Double mp. [f] Run in $CDCl_3$. [g] Run in DMSO-d_6. [h] Run in CF_3CO_2H-$CDCl_3$. [i] The sample seems to decomp in DMSO-d_6 as evidenced by a change in spectrum; *e.g.*, immediate run 7.3-8.2 (m), 11.13 (s), 13.57 (broad m); on standing overnight 7.3-8.3 nature of m has changed), 13.57 (broad m). [j] Run in D_2O. [k] Calcd C, 80.71; found C, 81.27. [l] Calcd C, 72.62; found C, 71.28.

in H_2O and making basic (to about pH 8) with solid $NaHCO_3$, followed by standard work-up. See Table I for the results.

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Antiviral Agents. 2. Analogs of 2-(α-Hydroxybenzyl)benzimidazole

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A large number of analogs of the antiviral agent, 2-(α-hydroxybenzyl)benzimidazole (HBB), have been synthesized and evaluated for their effects on various viruses. One of the more recent publications[1] reported on the antiviral

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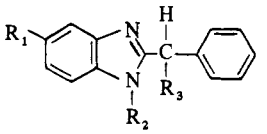
Table I.[a] Analogs of HBB

Compound	R_1	R_2	R_3	Mp, °C	Recryst solvent[b]	Yield, %	Formula[c]
							
1	H	H	Cl	158-160	A	75	*d*
2	H	H	SH	233-234	B	49	$C_{14}H_{12}N_2S$
3	H	H	NH_2	201-202	C	49	*e*
4	H	H	OCH_3	159-161	D	83	*f*
5	H	H	OC_2H_5	209-210	E	73	$C_{16}H_{16}N_2O$
6	H	H	OC_3H_7	216-217	E	73	$C_{17}H_{18}N_2O$
7	H	H	$NHCH_3$	158-160	C	72	$C_{15}H_{15}N_3$
8	H	H	$N(CH_3)_2$	202-204	E	90	$C_{16}H_{17}N_3$
9	H	H	$NHCOCH_3$	251-252	C	24	$C_{16}H_{15}N_3O$
10	H	$COCH_3$	$NHCOCH_3$	207-209	F	43	*g*
11	Cl[h]	H	SH	211-213	E	15	$C_{14}H_{11}ClN_2S$
12	Cl[h]	H	OCH_3	184-185	G	57	$C_{15}H_{13}ClN_2O$
13	Cl[h]	H	$NHCH_3$	178-179	C	62	$C_{15}H_{14}ClN_3$
14	CH_3[h]	H	SH	205-207	E	43	$C_{15}H_{14}N_2S$
15	CH_3[h]	H	OCH_3	166-168	C	70	$C_{16}H_{16}N_2O$
16	CH_3[h]	H	$NHCH_3$	152-154	C	74	$C_{16}H_{17}N_3$
17	CH_3[h]	H	$N(CH_3)_2$	164-166	E	80	$C_{17}H_{19}N_3$
18	CH_3[h]	H	SCH_3	184-186	E	58	$C_{16}H_{16}N_2S$
19	H	CH_3	SH	184-186	C	34	*i*
20	H	CH_3	OCH_3	72-73	*j*	70	$C_{16}H_{16}N_2O$
21[k]	H	CH_3	$NHCH_3$	218-220	E	39	$C_{16}H_{17}N_3 \cdot Pic$
22[k]	H	CH_3	$N(CH_3)_2$	170-172	E	77	$C_{17}H_{19}N_3 \cdot 2Pic$
23	H	CH_3	SCH_3	78-79	H	51	$C_{16}H_{16}N_2S$
24	H	C_6H_5	OCH_3	93-94	H	87	$C_{21}H_{18}N_2O$
25	H	C_6H_5	NH_2	88-89	I	25	$C_{20}H_{17}N_3$
26	H	C_6H_5	$NHCOCH_3$	216-218	E	79	$C_{22}H_{19}N_3O$
27	H	C_6H_5	$NHCH_3$	143-145	C	54	$C_{21}H_{19}N_3$

[a] Ir and nmr spectra of the compounds of this table are consistent with the proposed structure. [b] A = acetone; B = AcOH; C = $MeNO_2$; D = EtOAc; E = EtOH; F = Ac_2O; G = Me_2CO–H_2O; H = hexane; I = PhH-petr ether. [c] *Anal.* C, H, N (**2**, **14**, **18**, **19**, and **23** were also analyzed for S). [d] W. R. Siegart and A. R. Day, *J. Amer. Chem. Soc.*, **79**, 4391 (1967). [e] M. Mengelberg, *Chem. Ber.*, **92**, 977 (1959). [f] D. G. O'Sullivan, D. Pantic, and A. K. Wallis, *Nature (London)*, **205**, 262 (1962). [g] *Anal.* Calcd: C, 70.34; H, 5.58; N, 13.67. Found: C, 69.44; H, 5.64; N, 13.97. [h] Tautomeric forms exist, 5 or 6 position. [i] *Anal.* Calcd: C, 70.85; H, 5.55; N, 11.01; S, 12.58. Found: C, 70.69; H, 5.05; N, 11.17; S, 12.65. [j] Purified by column chromatog (SiO_2; EtOAc–cyclohexane 20:30 as eluant). [k] Oily; identified as picrates.

activity of analogs of HBB against ECHO 6 virus in monkey kidney cells. Of the 42 compounds evaluated, only 2-(α-methyl-α-hydroxybenzyl)benzimidazole was more active and more selective than HBB. The α-ethyl derivative was more active but less selective than HBB.

An earlier report[2] indicated that with polio viruses, the α-methyl and related derivatives gave less protection of infected cells than did HBB. This is in agreement with the known differences between polio and other enteroviruses in sensitivity to HBB.[3] Gwaltney[4] investigated the activity of HBB against rhinovirus strains representative of the 55 numbered serotypes and one subtype. Half the strains showed inhibition at a 447 μ*M* concentration of HBB and strains of all types except 1A and subtype 1B showed some degree of inhibition at a concentration of 574 μ*M* or less.

As a continuation of our studies on structure-activity relations in antiviral agents of the HBB type[5] we synthesized a group of benzimidazoles as shown in Table I. These compounds were evaluated in tissue culture for their antiviral activity (Table II). Standard reference compounds were previously reported[5] using this assay method.

The synthesis of these HBB analogs was *via* the 2-(α-chlorobenzyl)benzimidazole which was synthesized from the 2-(α-hydroxybenzyl)benzimidazole with $SOCl_2$. The active Cl was then replaced with the appropriate grouping to yield the desired 2-(α-substituted benzyl)benzimidazole.

Due to the extreme reactivity of the α-chlorobenzyl compound, it was not purified in most cases prior to the final reaction. The mercapto derivatives were particularly susceptible to oxidation and in the case of **2** the decomposition product of heating was isolated and identified as 2-benzoylbenzimidazole.

Antiviral Activity. Initially, HBB was evaluated by the method previously reported,[5] against the following viruses in tissue culture using primary culture of rhesus monkey kidney: ECHO-12, polio 1, vaccinia, influenza A2, vesicular stomatitis virus (VSV), and adenovirus-7. Activity was found only against ECHO-12 and polio 1. Activity was highest against ECHO-12. None of the analogs synthesized were active against polio 1 but some were quite active against ECHO-12.

The most interesting findings in this series of HBB analogs were the activities of the amino analogs of HBB, 2-(α-aminobenzyl)benzimidazole (**3**) and 1-phenyl-2-(α-aminobenzyl)-benzimidazole (**25**). Against ECHO-12, **3** gave 100% reduction of the cytopathic effect at 125 μg/ml test concn with the cytotoxic level at 1000 μg/ml.

To determine cytotoxicity, each compd is tested to determine the highest concn that will be tolerated by the cell culture without inducing obvious cellular damage. To make this determination, approximately 20 mg of compd is dissolved in an amount of tissue culture medium to give a concn of 500 μg/ml. If the compd does not go into solution, it is subjected to sonic vibration to produce a homogeneous suspension. Serial twofold dilutions down to 1 μg/ml are then made, using tissue culture medium as diluent; 2 ml of each dilution serving as sole source of medium is then added to four monkey kidney tubes. The tubes are then placed in a

Table II. Antiviral Activity against ECHO-12 Virus

Compound	Concentration, μg/ml: Cytotoxic	Lowest level causing 100% reduction in cytopathic effect of the virus	EC_{50}[a]	Therapeutic index[b]
HBB-*dl*·Base	250	62	28	8.9
HBB-*dl*·HCl	125	62	27	4.6
HBB-*d*(−)·HCl	250	31	20	12.5
2	16	No activity		
3	1000	125	72	13.9
4	125	No activity		
5	31	No activity		
6	31	No activity		
7	62	No activity		
8	62	No activity		
9	125	No activity		
10	125	No activity		
11	31	No activity		
12	16	No activity		
13	16	No activity		
14	250	No activity		
15	62	No activity		
16	62	31[c]		
17	250	No activity		
18	125	No activity		
19	31	No activity		
20	62	No activity		
21	62	31	14	4.4
22	16	No activity		
23	16	No activity		
24	31	8	4	7.7
25	62	4	3	20.7
26	16	No activity		

[a] Concn in μg/ml resulting in 50% reduction in cytopathic effect of the virus; estimated using the log probit method.[8] [b] Therapeutic index is the ratio of the cytotoxic concn to the EC_{50}. [c] 100% active at 31 μg/ml when the dose of virus was 20 ID_{50}; at a virus dose of 200 ID_{50}, only 27% activity was noted.

roller drum at 35° and incubated for 7 days. The cultures are examined microscopically at intervals to determine the highest concn of the compd that is nontoxic.

Compd **25** gave 100% reduction of the cytopathic effect at 4 μg/ml with the cytotoxic level at 62 μg/ml. Alkylation or acetylation of the amino function resulted in loss of antiviral activity with the aromatically unsubstituted benzimidazole moiety. The presence of a 5- or 1-Me group in the *N*-methylamino series increased activity against ECHO-12. Substitution in the 1 position by Ph increased the activity against ECHO-12 of the 2-(α-methoxybenzyl)benzimidazole **(4)** as well as the α-amino derivative **(3)**. Although the concn of the 1-Ph derivative of **4 (24)** causing 100% reduction of the cytopathic effect was much lower than HBB, the increased cytotoxicity of the compound resulted in a therapeutic index of 7.7 as compared to 8.9 for HBB. The therapeutic index of **25** was, however, 20.7; considerably better than that of HBB. This compd, in contrast to HBB, was found to be inactive against polio 1.

During the preparation of this manuscript a paper appeared in which the 2-(α-aminobenzyl)benzimidazole was reported[6] to be active against poliovirus types 1, 2, and **3**. However, it was not found to be as effective as HBB in protecting ERK cells against the cytopathic effects of types 1, 2, and 3 polioviruses.

Experimental Section

2-(α-Chlorobenzyl)benzimidazoles. A soln of $SOCl_2$ (0.06 mole) in 15 ml of $CHCl_3$ was added with stirring and cooling to the suitable 2-(α-hydroxybenzyl)benzimidazole (0.02 mole) and the mixt heated to reflux for 4 hr. The soln was evapd to dryness *in vacuo* and the solid washed with anhyd Et_2O. The solid crude product was used for further reactions except in the case of 1-phenyl-(α-chlorobenzyl)benzimidazole hydrochloride, recrystd as the hydrochloride, and 2-(α-chlorobenzyl)benzimidazole which was isolated as the free base.

2-(α-Methoxybenzyl)benzimidazole (4). 2-(α-Chlorobenzyl)-benzimidazole (0.01 mole) and NaOMe (0.01 mole) were dissolved in 50 ml of MeOH and left at room temp overnight. The soln was evapd *in vacuo* and the resultant solid was washed with H_2O, dried, and recrystd from EtOAc, mp 159–161°. All other alkoxy deriv were prepd in the same way.

2-(α-Methylaminobenzyl)benzimidazole (7). 2-(α-Chlorobenzyl)-benzimidazole (0.01 mole) was dissolved in anhyd dioxane (50 ml) and an excess of $MeNH_2$ (40% H_2O sol; 10 ml) was added. After standing overnight at room temp, the soln was evapd to dryness and the gummy solid was washed with H_2O, dried, and crystd from $MeNO_2$, mp 158–160°. All other alkylamino derivs were obtained in the same manner. Oily derivs were purified by alumina chromatog using EtOAc–cyclohexane (80:20) as elutant. They were characterized as picrates.

2-(α-Methylthiobenzyl)-5(6)-methylbenzimidazole (18). 2-(α-Chlorobenzyl)-5(6)-methylbenzimidazole hydrochloride (0.01 mole) was dissolved in 50 ml of anhyd dioxane and the soln added to 10 ml of a 2 *M* soln of $NaSCH_3$. After standing overnight at room temp, the dioxane was removed *in vacuo* and the residue extd with $CHCl_3$ and dried (Na_2SO_4). The solid obtained upon evaporation was crystd from EtOH–H_2O, mp 184–186°.

2-(α-Mercaptobenzyl)benzimidazole (2). 2-(α-Chlorobenzyl)-benzimidazole (0.01 mole) was dissolved in dioxane (20 ml) and the soln added to a soln of 0.04 *M* KOH in 50 ml of H_2O previously satd at 0° with H_2S. After standing overnight at room temp, a solid was pptd, filtered, and washed with H_2O, EtOH, and Et_2O. The solid was crystd from AcOH, mp 233–234°. From the mother liquor a cryst solid was obtained that was identified as 2-benzoylbenzimidazole. Other α-mercaptobenzyl derivatives were obtained in the same way except that occasionally acidification after the reaction was necessary to ppt the product.

2-(α-Aminobenzyl)benzimidazole (3). 2-(α-Chlorobenzyl)-benzimidazole (0.01 mole) was dissolved in 50 ml of dioxane and added to 10 ml of concd NH_4OH. After 12 hr at room temp the soln was evapd to dryness and the solid extd with 2 *M* HCl. The acidic ext was made alk with 2 *N* NH_4OH and a solid was obtained which was washed with H_2O and dried *in vacuo*. Purification was by column chromatog (Al_2O_3) using EtOAc–cyclohexane (1:1) as eluant. Crystn was from $MeNO_2$, mp 201–203° (lit.[7] mp 202–203°).

2-(α-Acetamidobenzyl)benzimidazole (9). 2-(α-Aminobenzyl)-benzimidazole **(3)** (0.5 g) was dissolved in Ac_2O and left at room temp for 48 hr. A white solid was obtained that could be crystd from Ac_2O. This was found to be the extremely unstable diacetyl deriv, hydrolyzing to the mono-Ac deriv readily. This compd can be crystd from $MeNO_2$, mp 251–252°.

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