of 10 μg/ml against *Streptococcus pyogenes*, and this was the only derivative to show activity against a range of bacteria. Against *Entamoeba histolytica*,[6] 1 and 4 were slightly active, causing 90–99.9% inhibition at 10 and 40 μg/ml, respectively. It can be seen from the above results that replacement of the 5-NO_2 group by other electron-withdrawing groups eliminates the antibacterial and antiparasitic activity present in a variety of substituted 2-amino-5-nitrothiazoles.

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Antiviral Benzimidazoles. Direct 1-Substitution of 2-(α-Hydroxybenzyl)benzimidazole and Related Compounds

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Simple 1-alkyl derivatives of 2-(α-hydroxybenzyl)benzimidazole (HBB) and of its *O*-methyl derivative (MBB) are potent inhibitors of the multiplication of several small RNA viruses.[1-4] These derivatives were previously prepared by condensing the appropriately N-substituted *o*-phenylenediamine with mandelic acid[1] or with α-methoxyphenylacetic acid.[2] The diamines had to be synthesized from *o*-chloronitrobenzene. Reaction of the parent HBB or MBB with an alkyl halide and NaOEt in boiling PhMe provides an easier route to many such compounds and also permits synthesis of new highly active compounds, not otherwise readily accessible, and of compounds with ^{14}C- or ^{3}H-labeled 1-alkyl substituents of value in studies of the mechanism of the antiviral action.

Unsuccessful attempts were made to N-alkylate HBB by allowing its Ag salt to react with *n*-PrI using the method of Buchanan, *et al.*[5] Wagner and associates[6] N-methylated a derivative of HBB using MeI, but our attempts to produce a reaction between HBB and PrI under similar conditions failed to give the 1-Pr derivative (PHBB). The yield of PHBB was poor when the reaction was carried out in the presence of NaOEt in boiling EtOH, but was satisfactory in boiling PhMe (Table I).

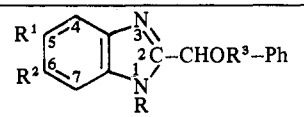
The presence of a substituent in position 4(7) or in position 5(6) of HBB could give rise to 2 isomeric products on N-alkylation. 1-Propylation of 5-chloro-HBB gave both, but the yield of the 5-chloro-1-propyl derivative was much better than that of its 6-chloro isomer. As the former had been previously synthesized by an unambiguous route,[7] the correct structures could be assigned.

Crotyl chloride of bp 84° was used to prepare 1-crotyl-HBB, the structure of the 1 substituent being confirmed by the nmr spectrum and by hydrogenation.

An attempt to prepare L-PHBB by treating L-HBB with PrI in the presence of NaOEt in PhMe gave a crystalline product of the expected formula, but with $[\alpha]^{21}D$ −24.7° (EtOH, *c* 0.77). As D-PHBB has $[\alpha]^{24}D$ + 144° (EtOH, *c* 0.83), the product contd 83% of racemate.

Reactivity of the NH Group. We found that HBB, unlike benzimidazole itself, does not react within 24 hr at 20° either with CH_2O in MeOH (with or without K_2CO_3) or with 2-chloroethanol in the presence of NaOEt in EtOH. The Mannich reaction produces ready substitution at the NH of benzimidazole, but 2-hydroxyphenyl- and 2-hydroxybenzyl-benzimidazoles are not attacked at this position.[8] Reaction of the chloromercury derivative of benzimidazole with triacetyl-α-D-ribofuranosyl chloride results in a Walden inversion and the formation, after hydrolysis, of 1-β-D-ribofuranosylbenzimidazole.[9] In the past, such *N*-glycosides have been considered to possess specific antiviral activity.[10] We used this method in attempts to prepare the 1-β-D-ribofuranoside of MBB, but always quantitatively recovered the MBB. This reduced reactivity of the NH group in HBB or in MBB might arise from steric or from bond-transmitted effects due to the 2 substituent. Ir and nmr spectra and ionization constants indicate that the latter effects are small.

Table I. Compounds Prepared by Direct 1-Alkylation



R	R^1	R^2	R^3	Mp, °C	Reactant	Yield, %	Yield, %[a]	Analyses
Pr	H	H	H	141–142	RI	50.5	51.8	
Bu	H	H	H	135–136	RI	68.5	39.7	
Benzyl	H	H	H	166–167	RBr	30.0	16.7	
Pr	Cl	H	H	172	RI	27.8	12.6	
Pr	H	Cl	H	143–144	RI	7.9[b]	–	C, H, Cl
Et	H	H	Me	Oil	RI	48.0[c]	45.4	
Allyl	H	H	H	144.5–145.5	RI	27.2[d]	–	C, H, N
Crotyl	H	H	H	155–156	RCl	19.6[e]	–	C, H, N

[a]Overall yield from the appropriate *o*-nitrochlorobenzene by previous method of D. G. O'Sullivan and A. K. Wallis [*Nature (London)*, 198, 1270 (1963)]. [b]White prisms from $MeNO_2$. [c]Purified *via* the picrate, mp 181–182°. [d]White prisms from aqueous MeOH. [e]White plates from EtOH.

Electron-repelling substituents raise and electron-withdrawing substituents lower the non-H-bonded NH stretching frequency of benzimidazole.[11] As the ν(NH) values in chloroform for HBB (3452 cm^{-1}) and MBB (3451-3455 cm^{-1}) are only slightly less than that of benzimidazole (3465 cm^{-1}), this suggests only small electron-withdrawing effects and little difference in the strengths of the NH bonds. Confirmation is given by τ(NH) values (in DMSO) of −2.45 for benzimidazole and −2.33 for HBB under comparable conditions.

In benzimidazoles, pK_a values decrease markedly as the electron-withdrawing effect of a substituent on the NH group increases.[12] We found the pK_a's of benzimidazole, HBB, and MBB to be 5.25, 5.00, and 4.80, respectively, showing that electron-withdrawing effects of the 2 substituents must be small.

Molecular models show that steric hindrance by the 2 substituents exists, is of similar magnitude in HBB and MBB, and increases for potential substituting groups in the order: Me < Et, Pr < benzyl, Ph, *i*-Pr < β-ribosides < α-ribosides. Steric hindrance makes the formation of 1-ribosides of HBB and MBB unlikely and this strengthens the belief that incorporation into abnormal nucleic acids plays no part in the antiviral activities of HBB and MBB.

Antiviral Activities. Compds in Table I all inhibit picornaviral multiplication. Toxicities to ERK cultures[1] and the pattern of inhibiting actions of subtoxic concns on cytopathic effects[1] of polioviruses 1 and 2 and coxsackievirus A9 are of a similar order for the 1-Pr, 1-allyl, and 1-crotyl derivatives of HBB (Table II). The activities of the 1-alkyl derivatives are dependent on the length of the side chain with maximum activities when C = 3 or 4.[1,3] Table II suggests that unsaturation in this side chain does not greatly influence activities. However, the allyl derivative appears to be a better inhibitor than PHBB of polioviruses but this order of these activities is reversed for coxsackievirus A9. 6-Chloro-1-propyl-HBB (80 μM) is better than its 5-Cl isomer at the same concn at inhibiting the cytopathic effect of polioviruses (Table II). Sensitivity of the viral replication to the compounds is in the order: coxsackievirus A9 > poliovirus 2 > poliovirus 1.

Experimental Section

Table I contains information on the compds.

Ionization Constants. A 10^{-3} *M* soln (10 ml) of the benzimidazole in EtOH–H_2O (1:4/v) was titrated with 0.02 *M* aq HCl at 22°. The effective EtOH concn (19.5% by vol) prevented crystn. H_2O with low CO_2 content was used throughout. The pH curves were recorded using a Radiometer Automatic Titrator with calomel and glass electrodes and the pK_a values (to ±0.03 unit) were obtained by repeated application of the eq pK_a = pH + log ($[BH^+]/[B]$) to data from the curves. An example of the 1-alkylation process follows.

1-Propyl-2-(α-hydroxybenzyl)benzimidazole (PHBB). 2-(α-Hydroxybenzyl)benzimidazole (HBB) (11.2 g, 0.05 mole) was dissolved in a soln of 1.15 g (0.05 g-atom) of Na in 110 ml of EtOH, which was then concd *in vacuo* to a small vol. Dried PhMe (100 ml) was added, and the mixt was stirred and distd at atm pressure until the boiling temp reached 110°. The residue was dild to 100 ml with dried PhMe and 11.3 g (0.067 mole) of PrI was added. The mixt (still protected from moisture) was stirred and heated under reflux for 3 hr. After filtering the hot reaction mixt and washing the salt deposit with hot PhMe, the PhMe was removed completely under reduced pressure. The dry residue was dissolved in 180 ml of hot 1 *M* HCl and treated with charcoal, and the cooled soln was neutralized with 3 *M* K_2CO_3. The sepd base was collected and crystd from MeOH–H_2O. Washing the crystals with MeOH–H_2O (1:1) at 0° gave the pure 1,2-disubstituted benzimidazole as white prisms; mp 141-142°, yield 6.7 g.

Table II. Protection of Virus-Infected Cells[a] by 2-(α-Hydroxybenzyl)benzimidazoles

Substituents	MTC[b] μM	Test concn, μM	Percentage remission time[c] Polio 1[d] (ERK cells)	Polio 2[e] (ERK cells)	Coxsackie A9 (MK cells)
1-Pr	80	40	43 (17[f])	100 (21[g])	100 (64[g])
1-Allyl	75	37.5	52 (22[f])	100 (29[g])	100 (57[g])
1-Crotyl	80	40	48 (17[f])	100 (21[g])	100 (86[g])
5-Cl-1-Pr	100	80	9	50	
6-Cl-1-Pr	160	80	22	79	

[a] Virus inocula sufficient to infect half the cells in monolayers in tubes and added simultaneously with compd. [b] Maximum concn of compd giving no well-defined cytotoxic effect in 4.5 days. [c] Defined as $100\Delta t/\Delta t_0$, where Δt is the interval between 50% cytopathic effect for compd-treated infected cells and for untreated infected controls and Δt_0 is the interval between 50% cell death for noninfected and 50% cytopathic effect for infected controls. [d] Strain: *L Sc 2 ab*. [e] Strain: *P 712 Ch 2 ab*. [f] Percentage remission time using 25% of MTC of compd. [g] Percentage remission time using 12.5% of MTC of compd.

5- and 6-Chloro-1-propyl-2-(α-hydroxybenzyl)benzimidazoles. Reaction of 5(6)-chloro-HBB with propyl iodide, as described above, gave a mixt that was crystd from MeOH–H_2O giving white plates of 5-chloro-1-propyl-HBB. Seeding with a crystal of the product helps this crystn. The filtrate was evapd to dryness, and the residue was crystd from MeOH–H_2O and then from $MeNO_2$ to give the 6-Cl isomer.

1-Crotyl-2-(α-hydroxybenzyl)benzimidazole. The structure of the 1 substituent of this compd was confirmed by nmr [τ($CDCl_3$) 2.30 (1 H, m, H-4), 2.60–2.90 (8 H, m, arom), 3.94 (1 H, s, CHPh), 4.5–5.1 (3 H, m, CH = CH and OH), 5.55 (2 H, d, J = 6 Hz, NCH_2), 8.43 (3 H, d, J = 6 Hz, CH_3)] and by hydrogenation. The compd (0.08 g) in 25 ml of MeOH absorbed 8.0 ml (≡1.1 double bond) of H_2 at atm pressure in the presence of PtO_2 catalyst at 25°. Filtration, concn, crystn from MeOH–H_2O and then from $MeNO_2$ gave 0.045 g of crystals, mp 132–133°, undepressed on mixing with an authentic specimen of 1-butyl-2-(α-hydroxybenzyl)benzimidazole prepared by the alternative nonequivocal synthesis.[1]

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