Pakistan J. Sci. Ind. Res., Vol. 21, Nos. 3-4, June - August 1978

ANTIVIRAL AGENTS

Part I. Indoles as Analogues of 2- (α -Hydroxybenzyl) benzimidazole

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(Received February 15, 1977)

Abstract. The synthesis and antiviral screening results of substituted indoles as analogues of 2-(α-hydroxybenzyl) benzimidazole have been described. Two of the compounds tested showed antiviral activity against two of the viruses used for screening.

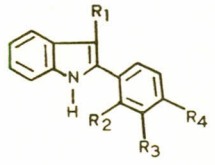
To improve upon the antiviral activity [1] of 2-(α -hydroxybenzyl) benzimidazole (HBB), many analogues [2-7] of HBB have been synthesised. It has been shown that α -hydroxybenzyl group as well as a fused bicyclic ring are important [8] for antiviral activity. The importance of intramolecular hydrogen bonding for antiviral activity of this series of compounds has been elucidated. In the present investigations a nitrogen atom of the imidazole ring of benzimidazole, which does not take part in the intramolecular hydrogen bonding, has been replaced by a carbon atom, thus giving rise to indoles (Tables 1 and 2).

BIOLOGICAL METHODS

All infectivity titrations, in triplicate, were performed in chick embryo fibroblast, using tube culture method. The compounds were tested for activity against five viruses: influenza, herpes simplex, parainfluenza, Semiliki Forest and vaccinia.

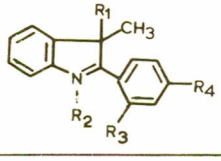
The highest concentration of each compound that could be tolerated by the cell culture without inducing obvious cellular damage (MTD) was determined(Table 3). Since some of the compounds had negligible water solubility, 3.0% (v/v) propylene glycol in water was used for dissolving those compounds. It had earlier been found that propylene glycol up to 4% (v/v) in water did not cause any damage to cells, when left in contact with cell culture for

Table 1



Compound	R_1	R_2	R_3	R_4	Ref
I	H	OH	H	H	9
II	H	H	OH	H	
III	H	H	H	OH	10
IV	CH_3	OCH_3	H	H	
V	CH_3	H	H	OH	

Table 2



Compound	R_1	R_2	R_3	R_4	Ref
VI	OH	-	OH	H	12
VII	CH_3	-	OH	H	12
VIII	CH_3	-	H	OH	
IX	CH_3	CH_3I	OH	H	
X	CH_3	CH_3I	-	OH	

Table 3

Compound	Cytotoxicity(g/ml)
I	1.0
II	1.0
III	1.0
IV	10.0
V	1.0
VI	5.0
VII	2.5
VIII	5.0
IX	10.0
X	10.0

7 days, and examined microscopically daily. Compounds I, II, III and V were found to be toxic at very low concentration, therefore,were not tested for their antiviral activity in tissue culture. For testing the antiviral activity, the highest nontoxic concentration of compounds in culture medium was added to tissue culture tubes. A predetermined dose of virus (about 100 $TCID_{50}$) in 0.1 ml medium was added to three tubes of each compound. The same number of remaining tubes received no virus and were observed as toxicity controls. An estimate of the activity of the test compound in reducing the cytopathic effect (CPE) was ascertained for each compound against each of the five test viruses. This was based on frequent microscopic examination of the tubes and any difference between the treated and untreated tubes were recorded on a numerical scale. In this way it was possible to determine all grades of antiviral activity of the compounds, ranging from 0 to 100%. Only two of the compounds VI against parainfluenza (Sendai) and VIII against vaccinia showed some (25%) activity.

EXPERIMENTAL

Melting points were recorded on a Kofler hot stage apparatus and are uncorrected. UV spectra were recorded in ethanolic solution (95%) on a Perkin-Elmer model 137 spectro photometer and IR spectra were recorded in Nujol on a Perkin-Elmer model 237 spectrophotometer. The solutions were dried over anhydrous magnesium sulphate and evaporations were carried out under reduced pressure (water pump). Solid analytical samples were dried over P_2O_5 at 80° / 0.1 mm for 4 hr.

2- (3-Hydroxyphenyl) indole (II). Phenyl hydrazine (10.8 g) and 3-hydroxyphenyl methyl ketone (13.6 g) were mixed [13] and heated at 110-115° (oil bath) until the evolution of steam ceased (ca 1½ hr). The resulting crystalline phenyl hydrazone (20 g) was dried under reduced pressure overnight over phosphorus pentoxide, and a mixture of it with anhydrous zinc chloride (140 g) was finely powdered and then heated at 180° for 10 minute. The molten viscous oil was then poured into stirred 0.3N HCl (600 ml) and the mixture was heated on a steam bath for 1 hr. The liberated oil was extracted with ether (4 ×100 ml) and the combined extracts were dried and the solvent evaporated to afford crude indole (15.2 g, 82%),which was recrystallised from benzene to yield II, as cream coloured needles, m.p. 162-163° (12.7 g, 69%). (Found: C,80.7; H,5.6; N, 6.8. $C_{14}H_{11}NO$ requires; C,80.3; H,5.3; N, 6.8%). λ_{max} 221 and 317 nm (log ∈ 4.20 and 4.05); λ_{infl} 335 nm (log ∈ 3.73);υ_{max} 3435 and 3495 cm^{-1}.

2- (2-Methoxyphenyl)-3-methylindole (IV). To a heated (steam bath) solution of 2- (2-hydroxyphenyl)-3-methylindole (0.05 g) in 5% KOH (6 ml aq)was added dimethyl sulphate (0.40 g) dropwise, with stirring. The heating and stirring were continued a further ½ hr during which time the reaction mixture was kept alkaline by the addition of further quantity of the KOH solution. During the reaction *o*-methyl derivative separated out as an oil, which gradually completely crystallized. Recrystallisation from cyclohexane afforded white needles m.p. 119-120° (0.40 g, 81%). (Found: C,81.5; H,6.5; N,5.5. $C_{16}H_{15}NO$ requires: C,81.0;H,6.3; N, 5.9%)λ_{max} 221 and 307 nm (log ∈ 4.27 and 3.96); υ_{max} 3377 cm.

2-(4-Hydroxyphenyl)-3,3-dimethyl-3H-indole (VIII). To an ethereal solution of methylmagnesium iodide [prepared from magnesium (1.76 g) and methyl iodide (12.96 g) in ether (10 ml)] was added a solution of 2-(4- hydroxyphenyl)-3-methylindole (6.48 g) in ether (4 ml); the mixture was boiled under reflux for ½ hr. Ether was evaporated off and dry benzene (60 ml) was added to the residue followed by methyl iodide (26 g); the mixture was boiled under reflux for 4 hr. The cooled mixture was decomposed with water and the benzene layer was separated off; the

white precipitate was washed with benzene (3 X 10 ml) and the combined benzene solutions were extracted into 3*N* HCl (4 X 30 ml). The combined acidic extracts were neutralised with aqueous sodium bicarbonate solution and the 3-*H* indole separated as yellow precipitate, which on recrystallisation from acetone afforded yellow prisms, m.p. 253-255° (1.34 g, 63%) (Found: C,80.9; H,6.3, N,5.5. C_{16} H_{15} N O requires: C,81; H, 6.3; N,5.9 %.) λ_{max} 233 and 326 nm (log ϵ 4.18, 4.36); λ_{infl} 240 and 249 mμ (log ϵ = 4.13 and 3.86).

2-(4-Hydroxyphenyl)-3,3-dimethyl-3H-indole methiodide (X). The methiodide (X) was prepared by heating 2-(4-hydroxyphenyl)-3,3-dimethyl-3H-indole (VIII) (0.25g) and methyl iodide (1.0 g) in a sealed tube at 100° for 12 hr. The dark brown solid (0.30 g, 76%) which precipitated was crystallised from acetone/ether to afford yellow prisms, m.p. 215-217° (0.22 g, 56%) (Found; C,54.4; H,5.0; N,3.4 C_{17} H_{18} I N O requires: C,53.8; H,4.7; N,3.7 %). λ_{max} 220 and 355 nm (log ϵ 4.28 and 4.10); λ_{infl} 238 and 282 nm (log ϵ 3.94 and 3.47).

2-(2-Hydroxyphenyl)-3,3-dimethy-3H-indole methiodide (IX). The methiodide (IX) was prepared by heating 2-(2-hydroxyphenyl)-3,3-dimethyl-3H-indole (VII) (0.25 g) and methyl iodide (1.0 g) in a sealed tube at 100° for 5 hr. The dark brown solid which separated was crystallised from acetone/ether to afford yellow platelets, m.p. 196-198° (0.26 g, 65 %) (Found: C,53.7; H,4.9; N,3.9 C_{17} H_{18} I N O requires: C, 53.8; H,4.7; N, 3.7 %). λ_{max} 286 nm (log ϵ 3.89); λ_{infl} 233, 242 and 325 nm (log ϵ 4.07,3.91 and 3.53).

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