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Synthesis and Biological Effects of Some 5-Heterocyclicmethyl-2'-deoxyuridines

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Abstract□The synthesis of 5-heterocyclicmethyl-2'-deoxyuridines (**4a-f**) has been accomplished by displacement reaction of 5-(bromomethyl)-3',5'-di-*O*-acetyl-2'-deoxyuridine with heterocyclic compounds, followed by removal of acetyl protecting group with methanolic ammonia. The compounds synthesized were evaluated the inhibitory effects on L1210 cell proliferation and antiviral activities against Herpes simplex virus type 1 (HSV-1). None of the compounds exhibited sufficient biological activities.

Keywords□5-Substituted 2'-deoxyuridine, thymidine derivatives.

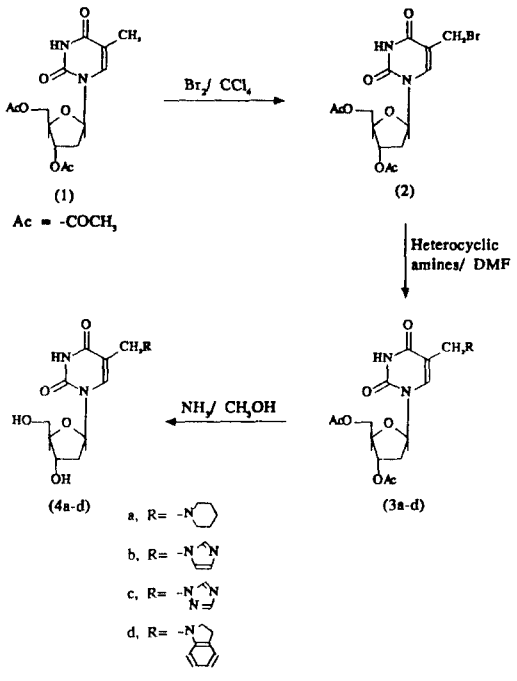
Modification at the C-5 position of deoxyuridine have produced a number of compounds with selective biological activities[1, 2]. Some of these derivatives were phosphorylated in cell culture with subsequent incorporation into DNA leading to a stable modified DNA. The findings that 5-(aminomethyl)-2'-deoxyuridine inhibited the growth of murine Sarcoma 180 and L1210 in culture by Shiau *et al.*[3] and many derivatives of 5-(aminomethyl)-2'-deoxyuridine containing various substituents on the amine function had some biological activities[5, 6] have led to extensive research into the preparation and evaluation of other 5-heterocyclic amine derivatives of thymidine as potent antitumor or antiviral agents. Several nucleotide analogues in this class have been explored by Mertes[7, 9] and Broom groups[8] as multisubstrate analogue inhibitors of Thymidylate synthetase. However, their nucleoside analogues have not been submitted to antitumor and antiviral studies.

We now report the synthesis of six 5-heterocyclicmethyl-2'-deoxyuridines and evaluate their biological activities.

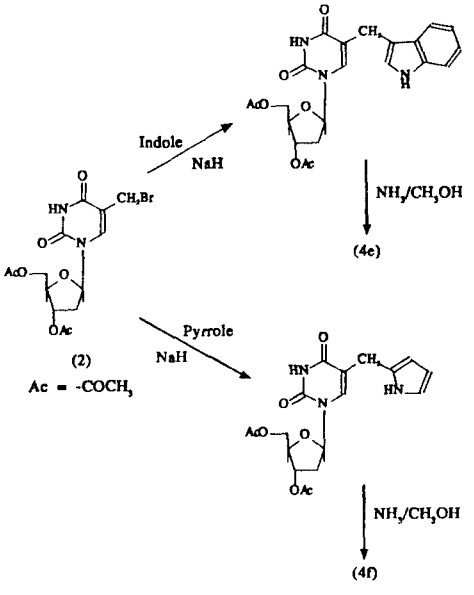
Bärwolff and Langen[4] have described the synthesis of 5-(bromomethyl)-3',5'-*O*-acetyl-2'-deoxyuridine via selective photochemical monobromination of the C-5 methyl group of 3',5'-di-*O*-acetyl-thymidine with bromine in carbon tetrachloride. When 70-75% of the starting material was converted to its monobromo derivative, the addition was stopped in order to avoid the formation of dibromo derivative. The unstable monobromo derivative has been shown to react with a variety of nucleophiles. Thus we have prepared compounds by displacement of the bromo function of compound (**2**) with heterocyclic amines in DMF at room temperature to give 5-heterocyclicmethyl-3',5'-di-*O*-acetyl-2'-deoxyuridines (**3a-d**) which were separated by column chromatography on silica gel. In all cases, the products were converted to the deblocked nucleosides (**4a-d**) with methanolic ammonia which were purified by column chromatography on silica, followed by fractional crystallization (Scheme 1).

A number of heterocyclic amines which behave as aliphatic amines may also be used in similar displacement reactions. Extremely weak bases such as pyrrole or indole, however, does not undergo nucleophilic displacement reaction under the above mild conditions. When sodium hydride was used to promote the displacement reaction with bromo function, the reaction gave a mixture due to several orientation of electrophilic substitution. Major product was separated by the column chromatography and identified by ^{1}H NMR. It was determined that indole undergoes electrophilic substitution chiefly at position 3, but pyrrole at position 2 (Scheme 2)[11, 12].

The nucleosides synthesized were examined for their inhibitory effects on L1210 cell proliferation and antiviral activity against HSV-1 by Korean Research Institute of Chemical Technology. They didn't decrease the growth rate of L1210 cell significantly and were inactive against HSV-1. The lack of cytotoxic activity may be due to the inability of cellular enzymes to phosphorylate them or an insensitivity of the cellular enzyme targets to their phosphorylated derivatives.



Scheme 1.



Scheme 2.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover Capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra were determined in Me_2-SO-d_6 with Me_4Si as internal standard with a Brucker AM-300 spectrometer; chemical shifts are recorded in parts per million. Mass spectra were measured on a JEOL JMS-DX 303 spectrophotometer, UV spectra with a Shimazu UV-240. Silica gel column chromatography was carried out with SF (60-240 mesh) silica gel. Microanalysis were within ±0.4% of theoretical values for all elements listed. 3',5'-di-*O*-acetyl-2'-deoxyuridine (**1**) and 5-(bromomethyl)-3', 5'-di-*O*-acetyl-2'-deoxyuridine (**2**) were prepared by using the literature procedures[4, 10].

General procedure for alkylation of heterocyclic compounds by 5-bromomethyl-2'-deoxyuridine derivatives (3a-d)

A solution of bromo compound **2**, (70-75%) (from 1.4 mmol of 3',5'-di-*O*-acetyl-2'-deoxyuridine) in 20 ml of dry DMF containing 1 mmol of heterocyclic amines was stirred at room temperature for 2 hrs. The solvent was completely removed, residue was suspended in water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over anhydrous Na_2SO_4. The solid was removed by filtration, the filtrate was concentrated to a small volume and applied to silica gel column, which was eluted with $CHCl_3$: MeOH (97:3) to remove the unreacted bromo derivative.

N-[1-(2'-deoxy-3',5'-di-O-acetyl- β-D-ribofuranosyl)thyminyl]piperidine (3a)

40% Yield; MS: *m/z* 409 (M^+); ^{1}H-NMR: δ 1.36-1.47 (6H, m, piperidine H), 2.03 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.33-2.40 (4H, m, piperidine H, overlapped with H-2'), 3.14 (2H, d, H-5'), 4.20 (1H, m, H-4'), 4.22 (2H, s, 5-CH_2), 5.21 (1H, t, H-3'), 6.19 (1H, t, H-1'), 7.53 (1H, s, H-6), 11.40 (1H, br s, Ur NH); UV λ_{max} (MeOH): 282 nm (ε 2,000); Anal. ($C_{19}H_{27}N_3O_7$) C,H,N.

N-[1-(2'-deoxy-3',5'-di-O-acetyl- β -D-ribofuranosyl)thyminyl]imidazole (3b)

42% Yied; mp. 187-189°C; MS: *m/z* 392 (M^+); ^{1}H-NMR: δ 2.03 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.13 (2H, m, H-2'), 3.76 (2H, m, 5'-H), 4.25 (1H, m, 4'-H), 4.78 (2H, s, 5-CH_2), 6.12 (1H, t, 1'-H), 6.86, 7.11. 8.03 (3H, imidazole H), 7.61 (1H, s, H-6), 11.50 (1H, s, Ur NH); UV λ_{max} (MeOH): 287 nm (ε 1,200); Anal. ($C_{17}H_{20}N_4O_7$) C,H,N.

1-[1-(2'-deoxy-3',5'-di-O-acetyl- β -D-ribofuranosyl)thyminyl]triazole (3c)

45% Yield; MS: *m/z* 393 (M^+); ^{1}H-NMR: δ

2.05 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 5.04 (2H, s, 5-CH_2), 6.16 (1H, t, H-1'), 7.91 (1H, s, 6-H), 7.94 (1H, s, triazole H), 8.49 (1H, s, triazole H), 11.62 (1H, s, Ur NH); UV λ_{max} (MeOH): 289 nm (ε 800); Anal ($C_{16}H_{19}N_5O_7$. 2/3 H_2O) C,H,N.

N-[1-(2'-deoxy-3',5'-di-O-acetyl-β-D-ribofuranosyl)thyminyl]indoline (3d)

32% yield; mp: 139°C; MS: *m/z* 443 (M^+); ^{1}H-NMR: δ 1.95 (3H, s, $COCH_3$), 2.07 (3H, s, $COCH_3$), 2.86 (2H, t, indoline H), 3.29 (1H, s, indoline H), 3.90 (1H, s, indoline H), 4.18 (2H, s, 5-CH_2), 6.18 (1H, t, H-1'), 6.59, 7.00 (2H, q each, indoline H), 7.62 (1H, s, 6-H), 11.52 (1H, s, Ur NH); UV λ_{max} (MeOH); 289 nm (ε 2,900); Anal. ($C_{22}H_{25}N_3O_7$. 1/8 H_2O) C,H,N.

General procedure of removal of the diacetyl group

To a solution of **(3a-d)** (0.25 mmol) was added 1 m*l* of methanolic ammonia, and the solution was stirred for 24 hrs. The solvent was removed in vacuo. The residue was dissolved in the minimum amount of $CHCl_3$ and added to the top of silica column packed with $CHCl_3$. Elution with $CHCl_3$-MeOH (95:5) resolved the product. A small amount was crystallized from EtOH.

N-[1-(2'-deoxy-β-D-ribofuranosyl)thyminyl] piperidine (4a)

55% Yield; mp 168-170°C; MS: *m/z* 325 (M^+); ^{1}H-NMR: δ 1.36, 1.48 (6H, m, piperidine H), 2.11 (2H, m, H-2'), 2.31 (4H, m, piperidine H), 3.16 (2H, s, 5-CH_2), 3.77 (1H, m, H-2'), 4.25 (1H, m, H-4'), 5.04 (1H, t, 5'-OH), 5.30 (1H, d, 3'-OH), 6.18 (1H, t, H-1'), 7.74 (1H, s, 6-H), 11.31 (1H, br s, Ur NH); UV λ_{max} (MeOH): 290 nm (ε 1,200). Anal. ($C_{15}H_{23}N_3O_5$. 3/4 H_2O), C,H,N.

N-[1-(2'-deoxy-β-D-ribofuranosyl)thyminyl] imidazole (4b)

52% Yield; mp. 185°C; MS: *m/z* 308 (M^+); ^{1}H-NMR: δ 2.09 (2H, m, H-2'), 3.54 (2H, m, H-5'), 4.24 (1H, m, 4'-H), 4.78 (2H, s, 5-CH_2), 5.07 (1H, t, 5'-OH), 5.28 (1H, d, 3'-OH), 6.86, 7.11, 8.03 (3H, imidazole H), 7.61 (1H, s, H-6), 11.53 (1H, s, Ur NH); UV λ_{max} (MeOH): 207 nm, 266 nm (ε 2,200, 3,500); Anal. ($C_{13}H_{16}N_4O_5$. 1/2 H_2O) C,H,N.

N-[1-(2'-deoxy-β-D-ribofuranosyl)thyminyl] triazole (4c)

40% Yield; mp. 188°C; MS: *m/z* 309 (M^+); ^{1}H-NMR: δ 2.12 (2H, m, 2'-H), 3.57 (2H, m, 5'-H), 5.00 (2H, s, 5-CH_2), 6.14 (1H, t, 3'-OH), 7.93 (1H, s, H-6), 8.09, 8.48 (2H, s each, triazole H), 11.42 (1H, br s, Ur NH); UV λ_{max} (MeOH): 285 nm (ε 2,100); Anal. ($C_{12}H_{15}N_5O_5$. 1/2 H_2O) C,H,N.

N-[1'-(2'-deoxy-β-D-ribofuranosyl)thyminyl] indoline (4d)

40% Yield; mp. 141°C; MS: *m/z* 359 (M^+); ^{1}H-NMR: δ 2.09 (2H, m, H-2'), 2.85, 3.29, 3.78 (4H, m, indoline H), 5.06 (1H, t, 5'-OH), 5.26 (1H, d, 3'-OH), 6.18 (1H, t, H-1'), 6.59 (2H, q, indoline H), 7.00 (2H, q, indoline H), 7.93 (1H, s, 6-H), 11.42 (1H, br s, Ur NH); UV λ_{max} (MeOH): 288 nm (ε 4,000). Anal. ($C_{18}H_{21}N_3O_5$. 1/4 H_2O) C,H,N.

3-[1'-(2''-deoxy-β-D-ribofuranosyl)thyminyl] indole (4e)

The same procedure as described in the general synthesis of compounds **(3a-d)** was used, with the exception that 5 mmol of NaH was slowly added to the solution of 20 m*l* of dry DMF containing 2.5 mmol of indole. The mixture was stirred for 1 hr. at 0°C and then poured into the solution of bromo compound (70-75%) (from 2.8 mmol of 3',5'-di-*O*-acetyl-2'-deoxyuridine). The reaction mixture was stirred for 5 hrs at room temperature. The product **4e** was obtained after resolution on silica gel column as a yellow sticky solution, which was treated with 1 m*l* of methanolic ammonia for removal of the diacetyl group. After 24 hrs the solution was removed in vacuo. The residue was purified by silica column. 28% yield from diacetyl thymidine. MS: *m/z* 357 (M^+); ^{1}H-NMR: δ 1.95-2.1 (2H, m, H-2'), 3.4 (2H, m, H-5'), 2.65 (2H, s, 5-CH_2), 3.75 (1H, m, H-3'), 4.15 (1H, m, H-4'), 5.03 (1H, t, 5'-OH), 5.30 (1H, d, 3'-OH), 7.0-7.6 (5H, m, indole H), 7.7 (1H, s, 6-H), 10.82 (1H, s, indole NH), 11.33 (1H, s, Ur NH).

2-[1'-(2''-deoxy-β-D-ribofuranosyl)thyminyl] pyrrole (4f)

This compound was prepared as described in the compound **4e**. 22% Yield; MS: *m/z* 307 (M^+). ^{1}H-NMR: δ 2.00 (2H, m, 2'-H), 3.53 (2H, s, 5-CH_2), 3.78 (1H, m, 3'-H), 4.2 (1H, m, 4'-H), 4.98 (1H, t, 5'-OH), 5.30 (1H, d, 3'-OH), 5.72, 5.90 6.6 (3H, m each, pyrrole H), 7.5 (1H, s, 6-H), 10.48 (1H, s, pyrrole NH), 11.35 (1H, s, Ur NH).

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