PYRIMIDINES AS POSSIBLE ONCOLYTIC AGENTS

Part I. 2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-alkyl-pyrimidines and 6-alkyl-5-(β-hydroxyethyl)-2-thiouracils

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ABSTRACT

Two series of 5-(β-hydroxyethyl)-6-alkyl pyrimidines have been synthesised and thirty new compounds reported. Two of the pyrimidines have been found to possess significant tumour inhil itory activity against a solid rat tumour. No relationship between the length of alkyl chain at position '6' in the compounds and their tumour inhibitory activity was observed.

INTRODUCTION

SUBSTITUTED pyrimidines have been under active investigation as oncolytic agents during the recent years.[3] 5-Fluorouracil[6] is being used in the treatment of cancer, while a number of pyrimidines,[2] which have been found to possess meaningful activity against animal tumours, are awaiting further investigation.

Although random synthesis of a few 5-(β-hydroxyethyl)-pyrimidines[4] has been reported, synthesis of 6-alkyl-5-(β-hydroxyethyl)-pyrimidines has not received the attention it deserves. Synthesis of the pyrimidines with a view to studying their oncolytic properties has, therefore, been undertaken by us.

DISCUSSION

α-Acyl-γ-butyrolactones, required for synthesising pyrimidines with different alkyl radicals in position '6', were obtained by interacting α-acetyl-γ-butyrolactone[8] and an appropriate aliphatic acid chloride, in presence of magnesium ethoxide. The lactones are high boiling liquids and being unstable in nature, have been characterised, by reacting with hydrazine hydrate, as 3-acyl-4-(β-hydroxyethyl)-2-pyrazoline-5-ones.[10] The derivatives are crystalline solids with sharp melting points. The butyrolactones on condensation

with guanidine nitrate in presence of sodium ethoxide, after the method of Schrage and Hitchings[4b] (Fig. 1), furnished a series of 2-amino-4-hydroxy-5-(β-hydroxyethyl)-6-alkyl-pyrimidines (IX–XV).

The amino group, in 2-amino-4-hydroxy-5-(β-hydroxyethyl)-6-methyl pyrimidine[4b] and in 2-amino-4-hydroxy-5-(β-hydroxyethyl)-6-*p*-chlorophenyl pyrimidine[4g] was converted into the hydroxyl through diazotization and decomposition of the diazonium salt, to obtain the corresponding dihydroxy pyrimidines (XVI and XVII).

The condensation of α-acyl-γ-butyrolactones and thiourea in the presence of sodium methoxide, after the method of Traube[11] (Fig. 1), have yielded a series of 6-alkyl-5-(β-hydroxyethyl)-2-thiouracils (XVIII–XXII).

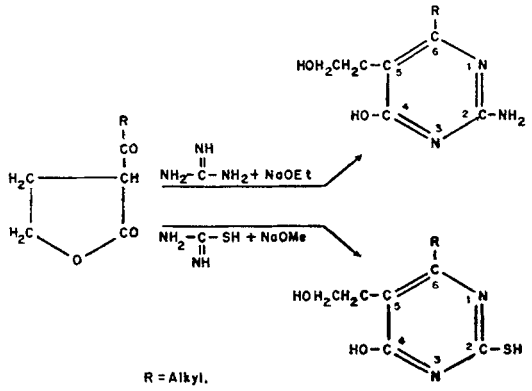

FIG. 1

2-Thio-pyrimidine (XX) on treatment with methyl iodide, in the presence of sodium ethoxide, yielded the 2-methyl-thio-pyrimidine (XXIII).

Pyrimidines described in this paper have been screened for their tumour inhibitory activity on experimental animal tumours. Two of the pyrimidines (XV and XVIII) have been found to possess high tumour inhibitory activity against a rat carcinosarcoma. No correlation between the length of the alkyl chain at position '6' in the pyrimidine moiety and tumour inhibitory activity has been observed. Detail results of the biological activity of the pyrimidines from both the series will be published elsewhere.

EXPERIMENTAL

Intermediates in the Synthesis of α-Acyl-γ-butyrolactones

(*a*) The α-acetyl-γ-butyrolactone (ABL), which formed the starting material for the present work, was prepared according to the method of

Knunyantz *et al.*[8] The compound was obtained in 60% yield and had a b.p. 142–143°/30 mm. (Knunyantz,[8] 142–143°/30 mm.).

(*b*) Acid chlorides, which formed the second intermediate in the preparation of the various lactones, were obtained in general by the action of thionyl chloride on the appropriate aliphatic acid. Propionyl chloride was obtained by the action of phosphorus pentachloride on the acid.

General Method for the Preparation of α-Acyl-γ-butyrolactones

In a one litre three-necked flask equipped with a mechanical stirrer, dropping funnel, thermometer and reflux condenser carrying a calcium chloride guard tube, were placed magnesium turnings (2·14 g.; 0·88 mole) absolute ethyl alcohol (2 ml.) and dry carbon tetrachloride (0·4 ml.). The reaction between magnesium and ethyl alcohol was allowed to proceed for a few minutes and then dry chlorobenzene (20 ml.) was cautiously added. The resulting mixture was placed in a water-bath at 65–70° C. and a mixture of ABL (11·52 g.; 0·88 mole), absolute ethyl alcohol (8 ml.) and dry chlorobenzene (10 ml.) was then added to it under stirring, at such a rate that rapid refluxing was maintained, heat being applied if necessary. Finally, the reaction was maintained at 75° C. for 3 hours.

The contents of the flask were cooled at 15–25° C. and a solution of the appropriate acid chloride (0·1 mole) in dry chlorobenzene (15 ml.) was added to it under vigorous stirring. The reaction mixture was then slowly warmed to 35° C., agitated for three more hours, cooled and acidified with dilute sulphuric acid (15%). The aqueous layer of the solution was discarded and the chlorobenzene layer was washed with sodium bicarbonate solution and then with water. The non-aqueous layer was dried over anhydrous sodium sulphate, concentrated and distilled under reduced pressure.

The properties and physical data of the individual α-acyl-γ-butyrolactones prepared by the above method are given below:

α-Propionyl-γ-butyrolactone (*I*).—Interaction of ABL (11·52 g.; 0·88 mole) and propionyl chloride[12] (9·2 g.; 0·1 mole) gave (I), b.p. 145–147°/30 mm. (5·1 g.; 36% yield). (I) was characterised by preparing 3-ethyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (I–*a*) derivative, which crystallized from alcohol in colourless plates, m.p. 155–156° C. (Found: N, 17·20; $C_7H_{12}N_2O_2$ requires N, 17·94%).

α-n-*Butyryl-γ-butyrolactone* (*II*).—Interaction of ABL (11·52 g.; 0·88 mole) and *n*-butyryl chloride[12] (10·6 g.; 0·1 mole) gave (II), b.p.

142–146° C./20 mm. (5·3 g.; 34% yield). (II) was characterised through its 3-*n*-propyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (II–*a*) derivative, which crystallized from alcohol in colourless plates, m.p. 165–66° C. (Found: N, 16·22; $C_8H_{14}N_2O_2$ requires N, 16·46%).

α-n-*Valeryl-γ-butyrolactone* (*III*).—Interaction of ABL (11·52 g.; 0·88 mole) and *n*-valeryl chloride[12] (12·0 g.; 0·1 mole) gave (III), b.p. 168–170° C./22 mm. (6·0 g.; 35% yield). (III) was characterised by preparing the 3-*n*-butyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (III–*a*) derivative, which crystallized from alcohol in colourless plates, m.p. 130–131° C. (Found: N, 14·72; $C_9H_{16}N_2O_2$ requires N, 15·21%).

α-n-*Caproyl-γ-butyrolactone* (*IV*): Interaction of ABL (11·52 g. ; 0·88 mole) and *n*-caproyl chloride[12] (13·4 g. ; 0·1 mole) gave (IV), b.p. 180–182° C./35 mm. (8·3 g. ; 45% yield). (IV) was characterised by preparing the 3-*n*-amyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (IV–*a*) derivative which crystallized from 60% alcohol in colourless needles, m.p. 179–180° C. (Found: N, 14·67; $C_{10}H_{18}N_2O_2$ requires N, 14·13%).

α-n-*Heptylic-γ-butyrolactone* (*V*).—Interaction of ABL (11·52 g. ; 0·88 mole) and *n*-heptylic chloride[12] (14·8 g.; 0·1 mole) gave (V), b.p. 175–178° C./16 mm. (5·5 g.; 28% yield). The 3-*n*-hexyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (V–*a*) obtained from (V) crystallized in colourless plates (Benzene), m.p. 103–104° C. (Found: N, 13·40; $C_{11}H_{20}N_2O_2$ requires N, 13·20%).

α-n-*Caprylyl-γ-butyrolactone* (*VI*).—Interaction of ABL (11·52 g.; 0·88 mole) and *n*-caprylyl chloride[12] (16·2 g.; 0·1 mole) gave (VI), b.p. 202–205° C./30 mm. (4·8 g.; 23% yield). The 3-*n*-heptyl-4-(β-hydroxy ethyl)-2-pyrazoline-5-one (VI–*a*) derivative of (VI) crystallized (Benzene) in pink needless, m.p. 97–98° C. (Found: N, 12·71; $C_{12}H_{22}N_2O_2$ requires N, 12·38%).

α-n-*Pelargonyl-γ-butyrolactone* (*VII*).—Interaction of ABL (11·52 g. ; 0·88 mole) and *n*-pelargonyl chloride[1] (17·6 g.; 0·1 mole) gave (VII), b.p. 188·190° C./5 mm. (7·3 g.; 32% yield). The 3-*n*-octyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (VII–*a*) derived from (VII) crystallized from alcohol in colourless needles, m.p. 106–107° C. (Found: N, 11·50; $C_{13}H_{24}N_2O_2$ requires N, 11·66%).

α-*Lauryl-γ-butyrolactone* (*VIII*).—Interaction of ABL (11·52 g.; 0·88 mole) and the lauryl chloride[5] (19·0 g.; 0·1 mole) gave (VIII), b.p. 232–236° C./20 mm. (6·2 g.; 23% yield). The compound (VIII) was characterised through

its 3-undecyl-4-(β-hydroxyethyl)-2-pyrazoline-5-one (VIII–*a*) derivative, which crystallized from benzene in colourless needles, m.p. 109–110° C. (Found: N, 10·00; $C_{16}H_{30}N_2O_2$ requires N, 9·92%).

General Method for the Preparation of 2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-acyl pyrimidines

A mixture of α-acyl-γ-butyrolactone (0·01 mole) guanidine nitrate (GN) (0·01 mole) and sodium ethoxide (0·23 g. sodium in ethanol 30 ml.) was heated under reflux on a boiling water-bath for 10 hours and kept overnight at room temperature. Next day excess of alcohol was removed under reduced pressure and the residue washed with petroleum ether (20 ml.) and ether (10 ml.). The insoluble material was suspended in water (10 ml.), cooled and acidified with con. hydrochloric acid to pH 6. The resulting solid was filtered, thoroughly washed with water and crystallized from alcohol. The pyrimidines synthesised by the above method are given below:

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-ethyl-pyrimidine (*IX*):—Interaction of (I) (1·42 g.; 0·01 mole) and (GN) (1·22 g.; 0·01 mole) gave (IX) which crystallized from alcohol in colourless needles, m.p. 239–240°C. (0·2 g.; 11% yield) (Found: N, 22·50; $C_8H_{13}N_3O_2$ requires N, 22·95%).

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-n-propyl-pyrimidine (*X*).—Interaction of (II) (1·56 g. ; 0·01 mole) and (GN) (1·22 g. ; 0·01 mole) gave (X), which crystallized from water in colourless plates, m.p. 245–246° C. (0·88 g.; 46% yield) (Found: N, 20·90; $C_9H_{15}N_3O_2$ requires N, 21·31%).

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-n-amyl (or pentyl) pyrimidine (*XI*).—Interaction of (IV) (1·84 g.; 0·01 mole) and (GN) (1·22 g.; 0·01 mole) gave (XI) which crystallized from alcohol in colourless needles, m.p. 180–181° C. (1·1 g.; 50% yield) (Found: N, 19·30; $C_{11}H_{19}N_3O_2$ requires N, 18·66%).

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-n-hexyl pyrimidine (*XII*).—Interaction of V (1·98 g.; 0·01 mole) and (GN) (1·22 g. ; 0·01 mole) gave (XII) which crystallized from water in colourless plates, m.p. 210–211° C. (0·9 g.; 38%) (Found: N, 18·00; $C_{12}H_{21}N_3O_2$ requires N, 17·57%).

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-n-heptyl pyrimidine (*XIII*).—Interaction of VI (2·12 g.; 0·01 mole) and (GN) (1·22 g. ; 0·01 mole) gave (XIII) which crystallized from alcohol in colourless needles, m.p. 182–183° C. (0·91 g.; 36% yield) (Found: C, 61·84, H, 9·30, N, 17·10; $C_{13}H_{23}N_3O_2$ requires C, 61·63, H, 9·15, N, 16·60%).

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-n-octyl pyrimidine (XIV).—Interaction of VII (2·26 g.; 0·01 mole) and (GN) (1·22 g.; 0·01 mole) gave (XIV) which crystallized from alcohol in colourless needles, m.p. 206–207° C. (0·48 g.; 18% yield) (Found: C, 62·55, H, 9·07, N, 15·50; $C_{14}H_{25}N_3O_2$ requires C, 62·89, H, 9·43, N, 15·73%).

2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-undecyl pyrimidine (XV).—Interaction of VIII, (2·68 g.; 0·01 mole) and (GN) (1·22 g.; 0·01 mole) gave (XV) which crystallized from alcohol in colourless needles, m.p. 202–203° C. (1·7 g.; 57% yield) (Found: C, 66·39, H, 9·90, N, 13·30; $C_{17}H_{31}N_3O_2$ requires C, 65·98, H, 10·10, N, 13·59%).

6-Methyl-5-(β-hydroxyethyl)-uracil (XVI). — 2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-methyl pyrimidine[4b] (2·0 g.; 0·0114 mole) was taken in 40 ml. of 25% acetic acid and warmed on a water-bath for 20 minutes. The suspension of the compound was cooled to 0° C. and a solution of 0·96 g. (0·014 mole) sodium nitrite in water (15 ml.) was added under stirring. The reaction mixture was then heated on a boiling water-bath for 5 hours, when the solution turned dark yellow. The solution on refrigeration overnight yielded a solid. It was filtered and dried in air. On crystallization from alcohol it melted at 263–265° C. (m.p. 264–265° C.)[8] (Found: C, 59·30, H, 5·62, N, 16·10; $C_7H_{10}N_2O_3$ requires C, 59·41, H, 5·88, N, 16·46%) (0·36 g.; 60% yield).

6-p-Chlorophenyl-5-(β-hydroxyethyl)-uracil (XVII).—2-Amino-4-hydroxy-5-(β-hydroxyethyl)-6-*p*-chlorophenyl pyrimidine[4g] (3·0 g.; 0·0114 mole) was taken in 40 ml. of 25% acetic acid and warmed on a water-bath for 20 minutes. The mixture was cooled to 0° C. and a solution of 0·96 g. (0·014 mole) of sodium nitrite in water was added under stirring. The reaction mixture was then heated on boiling water-bath for 4 hours during which time the solution turned yellow and deposited a yellow solid. On refrigeration overnight, the solid was filtered and dried in air. On crystallization from alcohol the compound melted at 235–236° C. (1·3 g.; 49% yield) (Found: C, 54·07, H, 4·12, N, 10·40; $C_{13}H_{11}N_2O_3Cl$ requires C, 54·13, H, 4·13, N, 10·52%).

General Method for the Preparation of 6-Acyl-5-(β-hydroxyethyl)-2-thiouracils

Thiourea (T) (0·015 mole) was added to a mixture of α-acyl-γ-butyrolactone (0·01 mole) and sodium methoxide (prepared from 0·46 g. sodium in methanol 40 ml.). The reaction mixture was refluxed on a boiling water-bath for 15 hours and kept overnight at room temperature. Next day excess of the solvent was removed under reduced pressure and at a temperature not

exceeding 50° C. The residue obtained was cooled, washed with petroleum ether (25 ml.) and suspended in water (15 ml.). The suspension of the material was acidified with dilute hydrochloric acid to pH 4, under cooling. The resulting solid was collected at the pump, thoroughly washed with water and crystallized from dilute alcohol.

The thiouracils synthesised by the foregoing method are given below:

6-*Methyl*-5-(*β-hydroxyethyl*)-2-*thiouracil* (*XVIII*).—Interaction of ABL (1·28 g.; 0·01 mole) and (T) (1·14 g.; 0·015 mole) gave (XVIII) which crystallized from water in colourless needles, m.p. 257–258° C. (0·6 g.; 32% yield) (Kawanishi,[7] 257–258° C. and Kuwayama,[9] 260–265° C., Decomp.).

6-n-*Propyl*-5-(*β-hydroxyethyl*)-2-*thiouracil* (*XIX*). —Interaction of II (1·56 g.; 0·01 mole) and (T) (1·14 g.; 0·015 mole) gave (XIX) which crystallized from 50% alcohol in colourless needles, m.p. 267–268° C. (1·0 g.; 50% yield) (Found: N, 13·50; $C_9H_{14}N_2O_2S$ requires N, 13·08%).

6-n-*Amyl*-5-(*β-hydroxyethyl*)-2-*thiouracil* (*XX*).—Interaction of IV (1·84 g; 0·01 mole) and (T) (1·14 g.; 0·015 mole) gave (XX) which crystallized from 60% alcohol in colourless needles, m.p. 194–195° C. (1·0 g.; 42% yield) (Found: N, 11·30; $C_{11}H_{18}N_2O_2S$ requires N, 11·57%).

6-n-*Hexyl*-5-(*β-hydroxyethyl*)-2-*thiouracil* (*XXI*).—Interaction of V (1·98 g.; 0·01 mole) and (T) (1·14 g.; 0·015 mole) gave (XXI) which crystallized from 50% alcohol in colourless needles m.p. 145–146° C. (1·25 g.; 50% yield) (Found: N, 11·27; $C_{12}H_{20}N_2O_2S$ requires N, 10·93%).

6-*Undecyl*-5-(*β-hydroxyethyl*)-2-*thiouracil* (*XXII*).—Interaction of VIII (2·68 g.; 0·01 mole) and (T) (1·14 g.; 0·015 mole) gave (XXII) which crystallized from 50% alcohol in colourless needles, m.p. 171–172° C. (1·3 g.; 40% yield) (Found: N, 8·37; $C_{17}H_{30}N_2O_2S$ requires N, 8·58%).

6-n-*Amyl*-5-(*β-hydroxyethyl*)-2-*methylthiouracil* (*XXIII*).—To a solution of sodium ethoxide (0·23 g. sodium in ethyl alcohol 15 ml.) were added 6-*n*-amyl-5-(*β*-hydroxyethyl)-2-thiouracil (2·42 g.; 0·01 mole) and methyl iodide (1·42 g.; 0·01 mole). The mixture was refluxed on a water-bath for 4 hours and kept overnight at the room temperature. The solvent was then removed under reduced pressure and at a temperature of 45° C. The residue was cooled, about 2 ml. water was added and acidified to pH 4, with dilute hydrochloric acid. The resulting solid was filtered and thoroughly washed with water. (XXIII) crystallized from alcohol in colourless needles, m.p. 126–127° C. (Found: N, 10·80; $C_{12}H_{20}N_2O_2S$ requires N, 10·93%). Yield 1·50 g.; 60% of theory.

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