One pot Synthesis, DNA binding and fragmentation in vitro *of new fused uracil derivatives for anticancer properties*

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Síntesis en un solo recipiente, combinación ADN y fragmentación in vitro *de derivados condensados de uracilo con propiedades anticancerígenas*

Síntesi en un sol recipient, combinació ADN i fragmentació in vitro *de derivats condensats d'uracil amb propietats anticancerígenes*

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RESUMEN

Se ha sintetizado una nueva serie de pirimidopirimidinas partiendo de 6-amino-1-benzyl [o 1-(2-chlorobenzyl)] uracilo a través de la formación de un aducto de Michael no aislado seguido de adición nucleofílica y aromatización final. El mismo material de partida puede ser utilizado para la preparación de algunas nuevas pirimidodipirimidinas por reflujo con diferentes aldehídos en medio ligeramente básico. Finalmente se han sintetizado diferentes lumazinas vía reacción de tipo S_N^2 entre clorhidrato de diaminouracilo con bromuro de fenacilo seguida de ciclodeshidratación intramolecular y aromatización. Estos nuevos compuestos de síntesis mostraron propiedades de combinación, quelación y fragmentación del ácido nucleico ADN.

Palabras clave: 6-Amino-1-bencil [o 1-(2-clorobenzil)] uracilo, clorhidrato de diaminouracilo, pirimidopirimidinas, pirimidodipirimidinas y lumazinas.

SUMMARY

A new series of pyrimidopyrimidines are synthesized starting from 6-amino-1-benzyl[or1-(2-chlorobenzyl)]uracil via the formation of nonisolable acyclic Michael type adduct followed by nucleophilic addition and finally aromatization. The same starting material can be used for the preparation of some new pyrimidodipyrimidines by refluxing with different aldehydes in slightly basic medium. Finally different lumazines are synthesized via S_N^2 type reaction of diaminouracil hydrochloride with phenacyl bromides followed by intramolecular cyclodehydration then aromatization. The newly synthesized compounds showed binding, chelation and fragmentation of the nucleic acid DNA.

Keywords: 6-Amino-1-benzyl[or1-(2-chlorobenzyl)]uracil, diaminouracil hydrochloride, pyrimidopyrimidines, pyrimidodipyrimidines and lumazines.

RESUM

S'ha sintetitzat una nova sèrie de pirimidopirimidines partint de 6-amino-1-benzyl [o 1-(2-chlorobenzyl)] uracil a través de la formació d'un aducte de Michael no aïllat seguit d'addició nucleofílica i aromatització final. El mateix material de partida pot ser utilitzat per a la preparació d'algunes noves pirimidodipirimidines per reflux amb diferents aldehids en medi lleugerament bàsic. Finalment s'han sintetitzat diferents lumazines via reacció de tipus SN2 entre clorhidrat de diaminouracil amb bromur de fenacil seguida de ciclodeshidratació intramolecular i aromatització. Aquests nous compostos de síntesis van mostrar propietats de combinació, quelació i fragmentació de l'àcid nucleic ADN.

Paraules clau: 6-Amino-1-bencil [o 1-(2-clorobenzil)] uracil, clorhidrato de diaminouracil, pirimidopirimidines, pirimidodipirimidines i lumazines.

INTRODUCTION

Resistance of tumor cells to chemotherapeutic agents is a major problem in the treatment of human malignancy.[1-3] The causes of drug resistance fall into two groups; firstly, those leading to inadequate drug exposure and, secondly, alterations in the cancer cell itself that affect drug sensitivity.[4] Differences in the metabolic and structural properties of cells may lead to drug-resistant phenotypes.[5] The antimetabolite group of drugs blocks the *de novo* synthesis of the purines and pyrimidines required for DNA synthesis.[5-7] Resistance has been reported due to the increased activities of the enzymes in alternative pathways for purine and pyrimidine biosynthesis, the salvage pathways.[5,7,8] The inhibitory activity of an antimetabolite depends on its successful competition with the corresponding metabolite for the enzymes of which the metabolite is the natural sub-

strate or cofactor. A series of nonnucleoside drugs as flavin analogues[9-11] e.g. alloxazine[9] have exhibited antitumor activity against different tumor cell lines. 5-Fluorouracil[12,13], methotrexate (MTX)[14,15] is the oldest antifolate anticancer drugs,[16] which is widely used as chemotherapeutic drug. It competes with the normal substrates, folic acid and dihydrofolate, for the active site on the enzyme dihydrofolate reductase (DHFR).[2,17,18] Mild reduction in reduced folates at low serum concentrations of MTX results in inhibition of pyrimidine (i.e. thymidine) synthesis.[19,20] Thus, by inhibiting DHFR, methotrexate prevents DNA synthesis and kills cells by depleting thymidylic acid.[21-23] We rationalized to synthesize new series of bicyclic fused uracil of pyridopyrimidines and lumazines as well as tricyclic derivatives of pyridodipyrimidines.

MATERIALS AND METHODS

Chemistry

All melting points were determined with an Electrothermal Mel.-Temp. II apparatus and were uncorrected. Element analyses were performed at the Micro Analytical Unit, Chemistry Department, Mansoura University. The infrared (IR) spectra were recorded using potassium bromide disc technique on Nikolet IR 200 FT IR at Pharmaceutical Analytical Unit, Faculty of Pharmacy, Al-Azhar University. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Varian Gemini 300 MHz Spectrometer using DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard (Chemical shift in δ, ppm), Faculty of Science, Chemistry Department, Cairo University. Mass spectra were recorded on DI-50 unit of Shimadzu GC/MS-QP 5050A at the Regional Center for Mycology and Biotechnology at Al-Azhar University. All reactions were monitored by TLC using precoted plastic sheets silica gel (Merck 60 F_{254}) and spots were visualized by irradiation with UV light (254 nm). The used solvent system was chloroform: methanol (9:1) & ethyl acetate:toluene (1:1).

7-Amino-5-aryl-1-(2-chlorobenzyl)-6-cyanopyrido[2,3-d]pyrimidine-2,4(1*H*,3*H*)-diones (2a-d)

Two methods were used to synthesize the target compounds:

A- A mixture of 6-amino-1-(2-chlorobenzyl)uracil (1b) (0.3 g, 1.2 mmol) and the appropriate benzylidenemalononitrile (1.2 mmol) in DMF (3 ml) in the presence of TEA (0.3 ml) was heated under reflux for 6-8 hours. The reaction mixture was evaporated under reduced pressure. The residue was treated with ethanol (10 ml), the formed precipitate was filtered, washed with ethanol and crystallized from DMF/ethanol (2:1) to afford 2a-d.

B- A mixture of 6-amino-1-(2-chlorobenzyl)uracil (1b) (0.3 g, 1.2 mmol) and the appropriate benzylidenemalononitrile (1.2 mmol) in sodium ethoxide (0.3 g Na in 15 ml absolute ethanol) was heated under reflux for 10 hours. Hydrochloric acid was added till pH=7, the formed precipitate was filtered, washed with ethanol and crystallized from DMF/ethanol (2:1) to afford 2a.

7-Amino-1-(2-chlorobenzyl)-5-(4-chlorophenyl)-6-cyanopyrido[2,3-d]pyrimidine-2,4(1*H*,3*H*)-dione (2a)

Yield: A 82%, B 86%, m.p.= >330°C. IR= 3335, 3191(NH_2& NH), 3069 (CH arom.), 2918, 2849 (CH aliph.), 2222 (CN), 1710, 1667 (2 C = O), 1627 (C = N), 1559 (C = C), 820 (*p*-substituted phenyl), 755 (*o*-substituted phenyl). ^{1}H-NMR (DMSO-d_6) δ 11.34 (bs, 1H, NH), 7.70 (bs, 2H, NH2), 7.51-7.48 (m, 2H, arom.), 7.34-7.25 (m, 4H, arom.), 7.06-7.04 (m, 2H, arom.), 5.35 (s, 2H, NCH2). MS: m/z 439 (M^++2, 0.9), 437 (M^+, 1.73), 404 (38), 402 (100). Anal. Calcd for $C_{21}H_{13}Cl_2N_5O_2$ (438.26), Calcd.: C, 57.55, H, 2.99, N, 15.98, Found: C, 57.25, H, 3.45, N, 15.79.

7-Amino-1-(2-chlorobenzyl)-5-(4-fluorophenyl)-6-cyanopyrido[2,3-d]pyrimidine-2,4(1*H*,3*H*)-dione (2b)

Yield: A 81%, m.p.= 312-314° C. IR= 3311, 3226 (NH_2& NH), 3072 (CH arom.), 2923, 2844 (CH aliph), 2219 (CN), 1701, 1665 (2 C = O), 1637 (C = N), 1560 (C = C), 803 (p-substituted phenyl), 754 (*o*-substituted phenyl). Anal. Calcd for $C_{21}H_{13}ClFN_5O_2$ (421.81), Calcd.: C, 59.80, H, 3.11, N, 16.60, Found: C, 59.37, H, 3.10, N, 16.51.

7-Amino-1-(2-chlorobenzyl)-5-(2-methoxyphenyl)-6-cyanopyrido[2,3-d]pyrimidine-2,4(1*H*, 3*H*)-dione (2c)

Yield: A 89%, m.p.= 316-318° C. IR= 3310, 3227 (NH_2& NH), 3074 (CH arom.), 2953, 2838 (CH aliph), 2217 (CN), 1702, 1665 (2 C = O), 1635 (C = N), 1557 (C = C), 755 (*o*-substituted phenyl). ^{1}H-NMR (DMSO-d_6) δ 11.32 (s, 1H, NH, exchangeable), 7.70 (bs, 2H, NH_2, exchangeable), 7.51-7.48 (m, 2H, arom.), 7.48-7.40 (m, 2H, arom.), 7.40-6.98 (m, 4H, arom.), 5.43-5.29 (two d, 2H, NCH_2), 3.72 (s, 3H, OCH_3). Anal. Calcd for $C_{22}H_{16}ClN_5O_3$ (433.84), Calcd.: C, 60.91, H, 3.72, N, 16.14, Found: C, 61.22, H, 4.21, N, 15.87.

7-Amino-1-(2-chlorobenzyl)-5-(4-hydroxyphenyl)-6-cyanopyrido[2,3-d]pyrimidine-2,4(1*H*,3*H*)-dione (2d)

Yield: A 63%, m.p.= 293-295° C. IR= 3441 (OH), 3336, 3227 (NH_2& NH), 3070 (CH arom.), 2980 (CH aliph), 2224 (CN), 1690-1641 (br, C = O), 1569 (C = C &/or C = N), 821 (*p*-substituted phenyl), 754 (*o*-substituted phenyl). Anal. Calcd for $C_{21}H_{14}ClN_5O_3$ (419.82), Calcd.: C, 60.08, H, 3.36, N, 16.68, Found: C, 60.15, H, 3.81, N, 17.00.

5-Aryl-1-benzyl[or 1-(2-chlorobenzyl)]pyrido[2,3-d] pyrimidine-2,4,7(1*H*,3*H*,8*H*)-triones (3a-d)

A mixture of the appropriate 6-amino-1-benzyluracil 1a or b (1.2 mmol) and ethyl arylidenacetoacetate (1.2 mmol) in DMF (3 ml) in the presence of TEA (0.3 ml) was heated under reflux for 6-8 hours. The reaction mixture was evaporated under diminished pressure. The residue was treated with ethanol (10 ml), the formed precipitate was filtered, washed with ethanol and crystallized from DMF/ethanol (3:1) to afford the target pyridopyrimidines 3a-d.

1-Benzyl-5-(4-chlorophenyl)pyrido[2,3-d]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (3a)

Yield: 58%, m.p.= 250-252 °C. IR= 3160 (NH), 3028 (CH arom.), 2920, 2848 (CH aliph.), 1693 (C = O), 1596 (C = C), 829 (*p*-substituted phenyl). ^{1}H-NMR (DMSO-d_6) δ 11.79 (bs, 1H, NH), 8.40-8.37 (d, 1H, arom.), 8.11-8.08 (d, 2H, arom.), 7.87-7.85 (d, 1H, arom.), 7.56-7.53 (d, 2H, arom.), 7.38- 7.22 (m, 4H, arom.), 7.20 (bs, 1H, NH &/or OH), 5.45 (s, 2H, NCH_2). MS: m/z 379 (M^+, 0.62), 91 (100). Anal. Calcd for $C_{20}H_{14}ClN_3O_3$ (379.79), Calcd.: C, 63.25, H, 3.72, N, 11.06, Found: C, 63.00, H, 3.68, N, 10.88.

1-(2-chlorobenzyl)-5-(4-nitrophenyl)pyrido[2,3-d]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (3b)

Yield: 62%, m.p.= 318-320 °C. IR= 3224 (NH), 3073 (CH arom.), 2964, 2846 (CH aliph), 1710 (C = O), 1564 (C = C), 1519, 1347 (NO_2), 826 (*p*-substituted phenyl), 751 (*o*-substituted phenyl). Anal. Calcd for $C_{20}H_{13}ClN_4O_5$ (424.79), Calcd.: C, 56.55, H, 3.08, N, 13.19, Found: C, 56.18, H, 3.13, N, 13.46.

5-(4-Bromophenyl)-1-(2-chlorobenzyl)pyrido[2,3-d]pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (3c)

Yield: 67%, m.p.= 268-270 °C. IR= 3238 (NH), 3089 (CH arom.), 2829 (CH aliph), 1700, 1631 (C = O), 1500 (C = C), 820 (*p*-substituted phenyl), 753 (*o*-substituted phenyl). Anal. Calcd for $C_{20}H_{13}BrClN_3O_3$ (458.69), Calcd.: C, 52.37, H, 2.86, N, 9.16, Found: C, 52.30, H, 2.88, N, 8.85.

1-(2-chlorobenzyl)-5-(4-hydroxyphenyl)pyrido[2,3-d] pyrimidine-2,4,7(1*H*,3*H*,8*H*)-trione (3d)

Yield: 51%, m.p.= > 330 °C. IR= 3416 (OH), 3254 (NH), 3087 (CH arom.), 2838 (CH aliph.), 1694, 1621 (C = O), 1506 (C = C), 830 (*p*-substituted phenyl), 756 (*o*-substituted phenyl). Anal. Calcd for $C_{20}H_{14}ClN_3O_4$ (395.79), Calcd.: C, 60.69, H, 3.57, N, 10.62, Found: C, 60.52, H, 3.44, N, 10.34.

5,5'-Methylene bis(6-amino-1-benzyluracil) (4)

A mixture of 6-amino-1-benzyluracil (1a) (0.3 g, 1.4 mmol) and excess formalin (2 ml) in ethanol (20 ml) in the presence of acetic acid (3 ml) was stirred at room temperature for 1 hour. The formed precipitate was filtered, washed with ethanol and crystallized from DMF/ethanol (4:1) into colourless crystals.

Yield: 0.27g (87.7%), m.p.: 315-317 °C. IR = 3460, 3381 (NH_2), 3134 (NH), 3067 (CH arom.), 2987 (CH aliph.), 1707, 1640 (C = O), 1572 & 1502 (C = C). ^{1}H-NMR (DMSO-d_6) δ 10.88 (s, 2H, 2NH, exchangeable), 7.52 (s, 4H, $2NH_2$, exchangeable), 7.36-7.19 (m, 10H, arom.), 5.06 (s, 4H, $2NCH_2$), 3.18 (s, 2H, CH_2). Anal. Calcd for $C_{23}H_{22}N_6O_4$ (446.45), Calcd.: C, 61.87, H, 4.97, N, 18.82, Found: C, 61.60, H, 5.34, N, 18.52.

1,9-Dibenzyl-5,10-dihydropyrido[2,3-d:6,5-d']dipyrimidine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraones (5a,b)

Two methods were used to synthesize the target compounds:

A- A mixture of 6-amino-1-benzyluracil (1a) (0.26 g, 1.2 mmol), and excess of formalin (2 ml) or *p*-chlorobenzaldehyde (1.2 mmol) in the presence of TEA (0.3 ml) in DMF (3.0 ml) was heated under reflux for 4-6 hours. The reaction mixture was evaporated under reduced pressure. The residue was treated with ethanol (10 ml), the formed precipitate was filtered, washed with ethanol and crystallized from DMF to afford 5a,b.

B- A mixture of 5,5'-methylene bis(6-amino-1-benzyluracil) (4) (0.1 g, 0.22 mmol) and DMF (2 ml) in the presence of TEA (0.3 ml) was heated under reflux for 1 hour. The reaction mixture was evaporated under reduced pressure. The residue was treated with ethanol (10 ml), the formed precipitate was filtered, washed with ethanol and crystallized from DMF to afford 5a.

1,9-Dibenzyl-5,10-dihydropyrido[2,3-d:6,5-d']dipyrimidine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (5a)

Yield: A 37%, B 62%, m.p.= > 330 °C. IR= 3187 (NH), 3062 (CH arom.), 2917, 2848 (CH aliph.), 1697-1650 (C = O), 1595 (C = C), 735, 700 (monosubstituted phenyl). Anal. Calcd for $C_{23}H_{19}N_5O_4$ (429.42), Calcd.: C, 64.33, H, 4.46, N, 16.31, Found: C, 64.30, H, 4.34, N, 15.97.

5-(4-chlorophenyl)-1,9-dibenzyl-5,10-dihydropyrido[2,3-d:6,5-d']dipyrimidine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)-tetraone (5b)

Yield: A 58%, m.p.= > 330 °C. IR= 3134 (NH), 3054 (CH arom.), 2922, 2952 (CH aliph.), 1697-1616 (C = O), 1567 (C = C), 844 (*p*-substituted phenyl), 724, 696 (monosubstituted phenyl). ^{1}H-NMR (DMSO-d_6) δ 10.96 (s, 2H, 2NH, exchangeable), 7.40 (bs, 1H, NH, exchangeable), 7.37-7.05 (m, 14H, arom.), 5.43 (s, 1H, CH-5), 5.12 (s, 4H, $2NCH_2$). Anal. Calcd for $C_{29}H_{22}ClN_5O_4$ (539.96), Calcd.: C, 64.51, H, 4.11, N, 12.97, Found: C, 64.75, H, 4.31, N, 13.21.

7-Aryl-1,3-dimethylpteridine-2,4(1*H*,3*H*)-diones (7a-d)

A mixture of 5,6-diamino-1,3-dimethyluracil hydrochloride (6) (0.29 g, 1.4 mmol) and the respective phenacyl bromide derivative (1.4 mmol) in ethanol (10 ml) in the presence of TEA (2.0 ml) was heated under reflux for 4-6 hours. After cooling, the formed precipitate was filtered, washed with ethanol and crystallized from ethanol.

1,3-Dimethyl-7-phenylpteridine-2,4(1*H*,3*H*)-dione (7a)

Yield: 86%, m.p. lit.[24]= 308-309 °C, m.p.= 310-312 °C. IR= 3065 (CH arom.), 2920, 2852 (CH aliph.), 1715, 1668 (2 C = O), 1547 (C = C), 750, 693 (monosubstituted benzene ring). ^{1}H-NMR (DMSO-d_6) δ 9.18 (s, 1H, CH-6), 8.30- 8.27 (m, 2H, arom.), 7.61- 7.59 (m, 3H, arom.), 3.39 (s, 3H, NCH^3), 3.33 (s, 3H, NCH^3). Anal. Calcd for $C_{14}H_{12}N_4O_2$ (268.27), Calcd.: C, 62.68, H, 4.51, N, 20.88, Found: C, 62.68, H, 4.10, N, 20.70.

7-(4-Methoxyphenyl)-1,3-dimethylpteridine-2,4(1*H*,3*H*)-dione (7b)

Yield: 81%, m.p.= 288-290 °C. IR= 3062 (CH arom.), 2931, 2849 (CH aliph), 1710, 1668 (2 C = O), 1538 (C = C), 841 (*p*-substituted phenyl). MS: m/z 298 (M+, 99.57), 186 (100). Anal. Calcd for $C_{15}H_{14}N_4O_3$ (298.29), Calcd.: C, 60.40, H, 4.73, N, 18.78, Found: C, 60.15, H, 4.04, N, 18.55.

1,3-Dimethyl-6,7-diphenylpteridine-2,4(1*H*,3*H*)-dione (7c)

Yield: 89%, m.p.= 227-229 °C. IR= 3060 (CH arom.), 2959 (CH aliph), 1718, 1676 (2 C = O), 1549 (C = C), 749, 697 (phenyl group). Anal. Calcd for $C_{20}H_{16}N_4O_2$ (344.36), Calcd.: C, 69.76, H, 4.68, N, 16.27, Found: C, 69.66, H, 4.31, N, 16.00.

1,3-Dimethyl-7-(4-nitrophenyl)pteridine-2,4(1*H*,3*H*)-dione (7d)

Yield: 63%, m.p.= > 330 °C. IR= 3069 (CH arom.), 2962, 2859 (CH aliph.), 1715, 1668 (2 C = O), 1545 (C = C), 1520, 1343 (NO_2), 852 (*p*-substituted phenyl). Anal. Calcd for $C_{14}H_{11}N_5O_4$ (313.26), Calcd.: C, 53.68, H, 3.54, N, 22.36, Found: C, 54.00, H, 3.95, N, 22.08.

Biological evaluation

Nucleic acids preparation

For extraction of genomic DNA, yeast cells were washed with cold phosphate borate sodium chloride (PBS) buffer and lysed in a buffer containing 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.2% Triton X-100 for 20 min at 4 ºC. After centrifugation at 14,000 rpm for 15 min, the supernatant was treated with proteinase K (0.5 mg/ml) and 1% SDS for 1 h at 50 ºC. DNA was extracted twice with buffered phenol/chloroform and precipitated with 140 mM NaCl and 2 volumes of ethanol at - 20 ºC overnight. DNA precipitates were washed twice with 70% ethanol, air-dried and dissolved in TE buffer, and treated for 1 h at 37 °C with RNase A according to reported method.[25] Finally, DNA preparations were electrophoresed in 1% agarose gels.

Agarose gel preparation and visualization of DNA

1% Agarose gel was prepared by adding 1gm ultra agarose to 100 ml Tris-Acetate-EDTA (TAE) buffer and heated in a microwave oven then cooled to ~60°C before pouring in gel tray.

Examination of the gel was carried out using ultraviolet illuminated box. Ethidium bromide (0.1 mg/ml) solution was used to stain the nucleic acid (DNA bands) in the gel as it intercalates between DNA bases and give florescence. The gel was photographed using polarized camera.

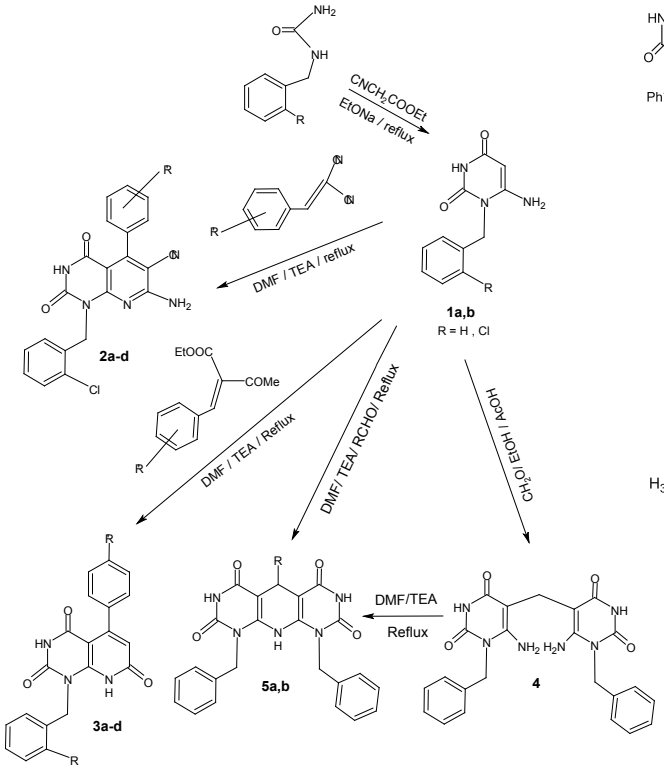
Nucleic acid affinity, binding and fragmentation assay

The test compounds were dissolved in DMSO at 20 µg/µl concentrations, mixed with 2 µg/µl DNA and incubated at room temperature for 2 hrs. The mixtures were mixed with the gel loading buffer and then electrophoresed in the agarose gel (1% w/v) at 80 V for 1.5 hrs. As positive control for affinity, binding and fragmentation, methotrexate (20 µg/µl) was mixed with DNA and as negative control DMSO was mixed with equal amount of DNA. After running, agarose gels were stained with ethidium bromide and visualized using polarized camera.

RESULTS AND DISCUSSION

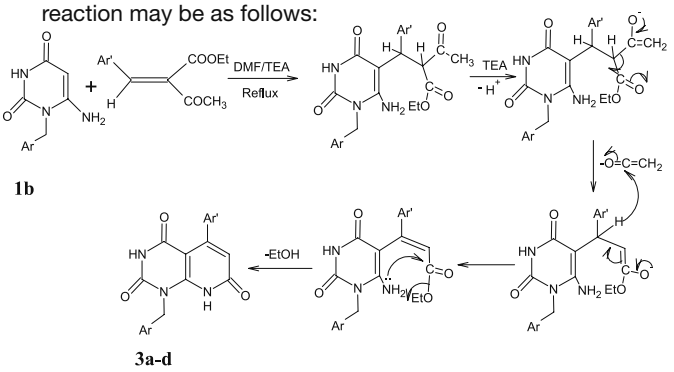
Chemistry

6-Amino-1-benzyl-[or 1-(2-chlorobenzyl)]uracil **1a,b** were synthesized by the condensation of ethyl cyanoacetate with N-benzyluea[26] or N-(2-chlorobenzyl)urea[27] in sodium ethoxide or methoxide in good yields accoding to the reported method. The synthetic strategy towards the desired pyridopyrimidines involves the addition of the nucleophilic enaminic carbon of aminouracil to the electrophilic carbon of an activated olefin. Thus, refluxing of 6-aminouracil **(1)** with arylidenemalononitrile in dimethylformamide (DMF)[28] in the presence of triethylamine (TEA) as a base afforded pyridopyrimidines **2a-d** in good yields. The formation of **2a-d** were carried out presumably via the formation of non-isolable acyclic Michael type adduct that undergo cyclization via the addition of nucleophilic nitrogen of amino group to the cyano- function and finally aromatization affording the target pyridopyrimidines **2a-d**. As illustrated in the synthesis of **2a-d**, the reaction of aminouracil with ethyl arylideneacetoacetate was also investigated. Thus, refluxing 6-amino-1-benzyl-[or 1-(2-chlorobenzyl)]uracil **(1a or b)** with ethyl arylidene-acetoacetate in DMF and in the presence of TEA as a base brought about the pyridopyrimidines **3a-d**.



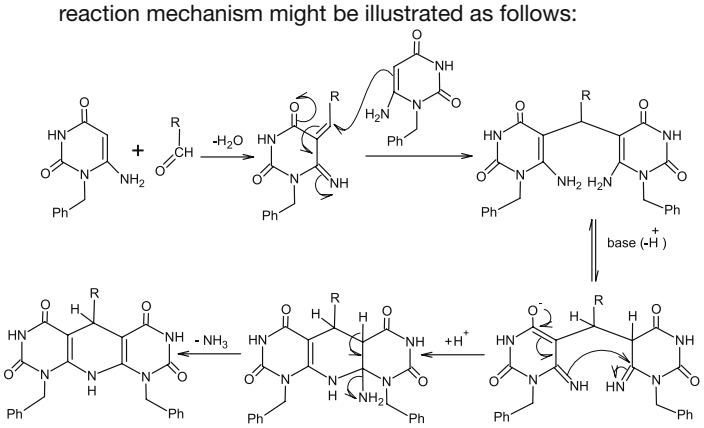
Scheme 1

The reaction takes place presumably via the formation of nonisolable acyclic Michael type adduct that undergo subsequent elimination of $COCH_3$ group[29] and finally cyclocondensation via the elimination of ethanol affording the final product **3a-d**. An expected mechanism for this reaction may be as follows:

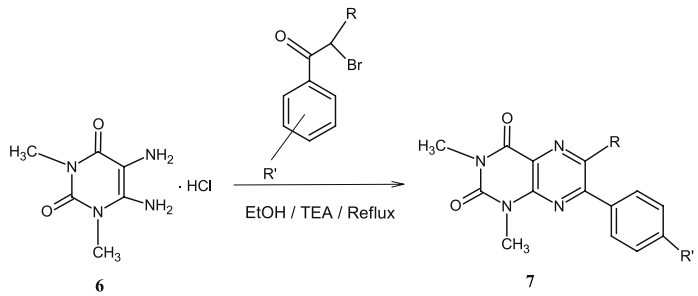


1b **3a-d**

On the other hand, 5'-Methylene bis(6-amino-1-benzyluracil) **(4)** was obtained through the condensation of 6-amino-1-benzyluracil **(1a)** with formalin in ethanol and acetic acid at room temperature. A simple method for the synthesis of pyridodipyrimidine **5a,b** in good yields was applied through the reaction of 6-amino-1-benzyluracil **(1a)** with formalin or 4-chlorobenzaldehyde in DMF in the presence of TEA as a base at the reflux temperature. A plausible reaction mechanism might be illustrated as follows:



This mechanism was supported by the ease of cyclization of compound **4** to **5a** when heated under reflux in the same medium (DMF/TEA) for one hour. The project now directed towards the possible utility of diaminouracils for the synthesis of lumazines. Thus, refluxing diaminouracil hydrochloride[26, 30-32] **6** and phenacyl bromides in the presence of triethylamine afforded lumazines **7a-d.**



6 **7**

Scheme 2

This may be presumably via the formation of nonisolable alkylated intermediate through S_N^2 type reaction, followed by intramolecular cyclodehydration and final aromatization to lumazines.

Biological evaluation

The newly synthesized compounds were subjected to nucleic acid binding assay using agarose gel electrophoresis method.

Nucleic acids binding assay:

Different synthetic drugs induced DNA damage was evaluated by measuring the level of genomic DNA fragmentation and detecting DNA ladders on agrose gel electrophoresis (Fig. 1). Compared to the vehicle control group (lane 2 negative control and 3 positive control), there was no significant change in genomic DNA fragmentation in some treated groups. There were major differences in the response of extracted DNA (from Lanes 4-13 in figure 1). It is possible that drugs exerted its effect solely by indirect mechanisms. This contrast may have been due to different enzyme(s) being with differing susceptibilities to drugs.

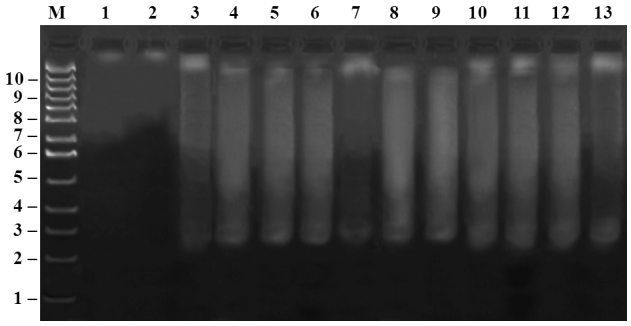


Figure 1. *Gel electrophoresis 1%w/v agrose of untreated and treated DNA. Lane M: Molecular weight marker (left side); Lane 1: Untreated nucleic acid; Lane 2: DMSO treated nucleic acid (negative control); Lane 3: Methotrexate treated nucleic acid (positive control); Lanes 4-13: Compounds* **(2a-d, 3c, d, 7a-d)** *treated nucleic acid.*

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