Synthesis, DNA binding studies of new pyrimidothiazepine and pyrimidobenzothiazepine derivatives

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Síntesis y estudios de unión de ADN de nuevos derivados de pirimidotiazepina y pirimidinobenzotiazepina.

Síntesi i estudis d'unió d'ADN de nous derivats de pirimidotiazepina i pirimidinobenzotiazepina.

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RESUMEN

Se ha sintetizado pirimidotiazepina y varios de sus derivados partiendo del tratamiento de 6-cloro-1-metiluracilo con glicolato de etilo seguido de reacción con hidrato de hidrazina a reflujo, lo cual conduce a la ciclacion con los aldehídos aromátios apropiados, o mediante tratamiento con 2-aminotiofenol seguido de reflujo con los aldehídos aromáticos adecuados respectivamente. La estructura de los compuestos sintetizados se confirma mediante datos de IR, 1H RMN, espectrometría de masas y análisis elemental. Además, se investigó la unión y fragmentación del ácido nucleico ADN de los nuevos derivados.

Palabras clave: 6-Chloro-1-metiluracilo, pirimidotiazepina, pirimidobenzotiazepina.

SUMMARY

Several pyrimidothiazepine and pyrimidobenzothiazepine were synthesized starting from 6-chloro-1-methyluracil by the treatment with ethyl thioglycolate followed by the reaction with hydrazine hydrate which cyclized through refluxing with appropriate aromatic aldehydes or by the treatment with 2-aminothiophenol followed by refluxing with appropriate aromatic aldehydes respectively. The structure of newly synthesized compounds was confirmed by IR, 1H NMR, mass spectral data and elemental analysis. Further the novel derivatives were investigated for their binding and fragmentation of the nucleic acid DNA.

Keywords: 6-Chloro-1-methyluracil, pyrimidothiazepine and pyrimidobenzothiazepine.

RESUM

S'ha sintetitzat pirimidotiazepina i varis dels seus derivats partint del tractament de 6-clor-1-metiluracil amb glicolat d'etil seguit de reacció amb hidrat d'hidrazina a reflux, la qual cosa condueix a la ciclació amb els aldehids aromàtics apropiats, o mitjançant tractament amb 2-aminotiofenol seguit de reflux amb els aldehids aromàtics adequats respectivament. L'estructura dels compostos sintetitzats es confirma mitjançant dades d'IR, 1H-RMN, espectrometria de masses i anàlisi elemental. A més, es va investigar la unió i fragmentació de l'àcid nucleic ADN dels nous derivats.

Paraules clau: 6-Chloro-1-metiluracil, pirimidotiazepina, pirimidobenzotiazepina.

INTRODUCTION

Great efforts have been directed towards the discovery of chemotherapeutic agents belong chemically to the nucleoside and nonnucleoside uracil derivatives. These compounds have both antimetabolites[1-4] such as the non-nucleoside derivatives 1-[(2-hydroxyethoxy) methyl]-6-(phenylsulfanyl)thymine (HEPT, 163) and its derivatives 162[5-7], methotrexate[8] and the antiviral activity especially against human immunodeficiency virus (HIV)[9-13], also the etiological agent of acquired immunodeficiency syndrome (AIDS)[14-16] like the non-nucleoside derivatives of acyclovir (159)[17], famciclovir (160), ganciclovir (161)[18], acridines, dihydroxyacridone[19] and nevirapine.[20-22] Uracil nucleo- sides have been studied as antimetabolites because they follow similar metabolic pathways as do the corresponding natural pyrimidines. This leads to inhibition of certain viral enzymes through their highly potent reverse transcriptase inhibitors by being incorporated into the viral DNA chain, resulting in obligatory viral DNA chain termination.[23-25]

In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents, we have synthesized some new pyrimidothiazepine and pyrimidobenzothiazepine which hopping to have antimetabolite and/or antiviral activity.

MATERIALS AND METHODS

Chemistry

All melting points were determined with an Electrothermal Mel.-Temp. II apparatus and were uncorrected. Elemental analyses were performed at the Micro Analytical Unit, Chemistry Department, Mansoura University, Egypt. 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, Egypt. 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, Egypt. All reactions were monitored by TLC using silica gel (Merck 60 F_{254}). The used solvent system was chloroform: methanol (9:1) & ethyl acetate: toluene (1:1).

6-[((Hydrazinecarbonyl)methyl)sulfanyl]-1-methyluracil (IV)

A mixture of 6-[((ethoxycarbonyl)methyl)sulfanyl]-1-methyluracil (III) (0.29 g, 1.2 mmol) and hydrazine hydrate (0.12 g, 2.4 mmol) in ethanol (10 ml) was stirred at room temperature for 15 minutes. The formed precipitate was collected by filtration, washed with ethanol and crystallized from DMF into colourless crystals.

Yield: (84%), m.p. 258-260 °C. IR: 3260, 3157 (NH_2 & NH), 3029 (CH- arom.), 2910 (CH- aliph.), 1694 (br, C = O), 1560 (C = C). Anal. Calcd for $C_7H_{10}N_4O_3S$ (230.24): Calcd. C, 36.52, H, 4.38, N, 24.33, Found C, 36.90, H, 4.17, N, 24.79

6-[(Arylmethylidene)amino]-5-hydroxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4]thiazepine-2,4,7(1H,3H,8H)-triones (Va-f)

A mixture of 6-[((hydrazinecarbonyl)methyl)sulfanyl]-1-methyluracil (IV) (0.3 g, 1.3 mmol) and the appropriate aromatic aldehydes (1.3 mmol) in dry DMF (4 ml) was heated under reflux for 2 hours. After cooling, ethanol (7.0 ml) was added; the separated product was filtered, washed with ether and crystallized from DMF/ethanol (1:1) to afford V.

6-[(benzylidene)amino]-5-hydroxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4]thiazepine-2,4,7(1*H*,3*H*,8*H*)-triones Va

Yield: 80%, m.p. 252-254°C. IR: 3431 (OH), 3141 (NH), 3067 (CH arom.), 2989, 2863 (CH aliph.), 1665 (C = O), 1554 (C = C), 690, 759 (monosubstituted phenyl). Anal. Calcd for $C_{15}H_{14}N_4O_4S$ (346.36): Calcd. C, 52.02, H, 4.07, N, 16.18, Found C, 51.99, H, 4.09, N, 16.13.

6-[(4-chlorobenzylidene)amino]-5-hydroxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4]thiazepine-2,4,7-(1*H*,3*H*,8*H*)-triones Vb

Yield: 86%, m.p. 285-287°C. IR: 3222 (OH), 3149 (NH), 3012 (CH arom.), 2833 (CH aliph.), 1669 (br, C = O), 1551 (C = C), 829 (*p*-substituted phenyl).^{1}H-NMR (DMSO-d_6) δ ppm: 11.83 (s, 1H, NH, exchangeable), 11.22, 11.24 (2 unequal s, 1H, OH, exchangeable), 8.21, 8.03 (2 unequal s, 1H, CH=N), 7.77-7.72 (d, 2H, arom.), 7.52-7.49 (d, 2H, arom.), 5.52-5.48 (2 unequal s, 1H, CH-5), 4.39, 3.98 (2 unequal s, 2H, CH_2-8), 3.34 (s, 3H, NCH_3). MS m/z (%): 380 (M, 0.04), 366 (0.05), 278 (14), 269 (0.47), 243 (0.75), 214 (28), 199 (100), 158(35). Anal. Calcd for $C_{15}H_{13}ClN_4O_4S$ (380.80): Calcd. C, 47.31, H, 3.44, N, 14.71, Found C, 47.41, H, 3.46, N, 14.43.

6-[(4-Fluorobenzylidene)amino]-5-hydroxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4]thiazepine-2,4,7(1*H*, 3*H*, 8*H*)-triones Vc

Yield: 81%, m.p. 254-256°C. IR: 3426 (OH), 3144 (NH), 3023 (CH arom.), 2922, 2849 (CH aliph.), 1708, 1672, 1650 (C = O), 1548 (C = C), 820 (p-substituted phenyl). Anal. Calcd for $C_{15}H_{13}N_4O_4S$ (364.35): Calcd. C, 49.45, H, 3.60, N, 15.38, Found C, 49.56, H, 3.73, N, 15.51.

6-[(4-Bromobenzylidene)amino]-5-hydroxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4]thiazepine-2,4,7 (1*H*, 3*H*, 8*H*)-triones Vd

Yield: 79%, m.p. 259-260 °C. IR: 3449 (OH), 3137 (NH), 3067 (CH arom.), 2858 (CH aliph.), 1715, 1668 (C = O), 1553 (C = C), 838 (p-substituted phenyl). ^{1}H-NMR (DMSO-d_6) δ ppm: 11.83 (s, 1H, NH), 11.22, 11.24 (2 unequal s, 1H, OH), 8.20, 8.02 (2 unequal s, 1H, CH=N), 7.71-7.63 (m, 4H, arom.), 5.52-5.48 (2 unequal s, 1H, CH-5), 4.39, 3.98 (2 unequal s, 2H, CH_2-8), 3.34 (s, 3H, NCH_3). Anal. Calcd for $C_{15}H_{13}BrN_4O_4S$ (425.25): Calcd. C, 42.37, H, 3.08, N, 13.17, Found C, 42.39, H, 3.10, N, 13.08.

6-[(4-Hydr oxybenzylidene)amino]-5-hydr oxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4]thiazepine-2,4, 7(1*H*,3*H*,8*H*)-triones Ve

Yield: 67%, m.p. 298-300 °C. IR: 3238 (OH), 3163 (NH), 3049 (CH arom.), 2997, 2852 (CH aliph.), 1719, 1657 (C = O), 1553 (C = C), 833 (*p*-substituted phenyl). Anal. Calcd for $C_{15}H_{14}N_4O_5S$ (362.36): Calcd. C, 49.72, H, 3.89, N, 15.46, Found C, 49.42, H, 3.60, N, 15.27.

6-[(4-nitrobenzylidene)amino]-5-hydroxy-1-methyl-5,6-dihydropyrimido[5,4-*f*][1,4] thiazepine-2,4,7(1*H*, 3*H*, 8*H*)-triones Vf

Yield: 62%, m.p. 295-296 °C. IR: 3402 (OH), 3129 (NH), 3005 (CH arom.), 2841 (CH aliph.), 1714, 1682, 1665 (C = O), 1565 (C = C), 1518, 1348 (NO_2), 845 (p- substituted phenyl). Anal. Calcd for $C_{15}H_{13}N_5O_6S$ (391.35): Calcd. C, 46.03, H, 3.35, N, 17.89, Found C, 46.40, H, 3.44, N, 17.72.

6-[(2-Aminophenyl)sulfanyl]-1-methyluracil (VI)

To a solution of 6-chloro-1-methyluracil (II) (1.0 g, 6.23 mmol) in hot chloroform (10 ml), few drops of TEA (0.4 ml) and 2-aminothiophenol (0.8 g, 6.23 mmol) were added. After cooling, the reaction mixture was stirred at room temperature for 30 minutes. The formed precipitate was filtered, washed with ethanol and crystallized from DMF/ethanol (2:1) into colourless crystals.

Yield: (81%), m.p.: 270-272 °C. IR: 3370, 3297 (NH2 & NH), 3060 (CH arom.), 2994, 2856 (CH aliph.), 1700, 1673 (2 C = O), 1564 (C = C), 760 (o-substituted phenyl). Anal. Calcd for $C_{11}H_{11}N_3O_2S$ (249.28): Calcd. C, 53.00, H, 4.45, N, 16.86, Found: C, 53.00, H, 4.53, N, 16.38.

5-Aryl-1-methyl-5,6-dihydropyrimido[4,5-*b*][1,5]benzothiazepine-2,4(1*H*,3*H*)-diones (VIIa-d)

A mixture of 6-[(2-aminophenyl)sulfanyl]-1-methyluracil (VI) (0.3 g, 1.2 mmol) and the appropriate aromatic aldehyde (1.2 mmol) in acetic acid (3 ml) was heated under reflux for 8 hours. After cooling, ethanol (8.0 ml) was added; the formed precipitate was filtered, washed with ethanol and crystallized from DMF/ethanol (1:1) to afford VIIa-d.

5-(4-Chloro)-1-methyl-5,6-dihydropyrimido[4,5-*b*][1,5] benzothiazepine-2,4(1*H*,3*H*)-diones VIIa

Yield: 78%, m.p.: 190-192 °C. IR: 3265, 3162 (NH), 3100(CH arom.), 2985 (CH aliph.), 1716 (br, 2 C = O), 1557 (C = C), 820 (p-substituted phenyl), 754 (o-substituted phenyl). ^{1}H-NMR (DMSO-d_6) δ ppm: 11.04 (s, 1H, NH), 8.26 (s, 1H, NH), 8.11-8.09 (d, 1H, arom.), 8.01-7.99 (d, 1H, arom.), 7.60-7.25 (m, 7H, arom. CH-5), 3.47 (s, 3H, NCH_3). MS m/z (%): 373 (M+2 , 12), 371 (M , 44), 339 (23), 313 (54), 297 (12), 270 (92), 260 (64), 235 (74), 203 (19), 58 (100). Anal. Calcd for $C_{18}H_{14}ClN_3O_2S$ (371.84): Calcd. C, 58.14, H, 3.79, N, 11.30, Found C, 58.68, H, 4.13, N, 11.02.

5-(4-Bromo)-1-methyl-5,6-dihydropyrimido[4,5-*b*][1,5] benzothiazepine-2,4(1*H*,3*H*)-diones VIIb

Yield: 71%, m.p.: 250-252 °C. IR: 3260, 3157 (NH), 3086 (CH arom.), 2959 (CH aliph.), 1715, 1674 (2 C = O), 1557 (C = C), 818 (p-substituted phenyl), 755 (o-substituted phenyl). Anal. Calcd for $C_{18}H_{14}BrN_3O_2S$ (416.84): Calcd. C, 51.93, H, 3.39, N, 10.09, Found C, 51.70, H, 3.40, N, 10.51.

5-(4-Fluoro)-1-methyl-5,6-dihydropyrimido[4,5-*b*][1,5] benzothiazepine-2,4(1*H*,3*H*)-diones VIIc

Yield: 82%, m.p.: 293-295 °C. IR: 3240 (NH), 3117 (CH arom.), 2986, 2819 (CH aliph.), 1704, 1637 (2 C = O), 1564 (C = C), 827 (p-substituted phenyl), 754 (o-substituted phenyl). Anal. Calcd for $C_{18}H_{14}FN_3O_2S$ (355.38): Calcd. C, 60.83, H, 3.97, N, 11.82, Found C, 60.45, H, 3.81, N, 11.79.

5-(4-nitro)-1-methyl-5,6-dihydropyrimido[4,5-*b*][1,5] benzothiazepine-2,4(1*H*,3*H*)-diones VIId

Yield: 59%, m.p.: 218-220 °C. IR: 3242 (NH), 3116 (CH arom.), 2989, 2849 (CH aliph.), 1703, 1636 (2 C = O), 1564 (C = C), 1525, 1340 (NO2), 821 (p-substituted phenyl), 754 (o-substituted phenyl). Anal. Calcd for $C_{18}H_{14}N_4O_4S$ (382.39): Calcd. C, 56.54, H, 3.69, N, 14.65, Found C, 56.50, H, 3.91, N, 15.04.

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 ºC with RNase A according to reported method.[26] 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 concen-trations, 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 gel was stained with ethidium bromide and visualized using polarized camera.

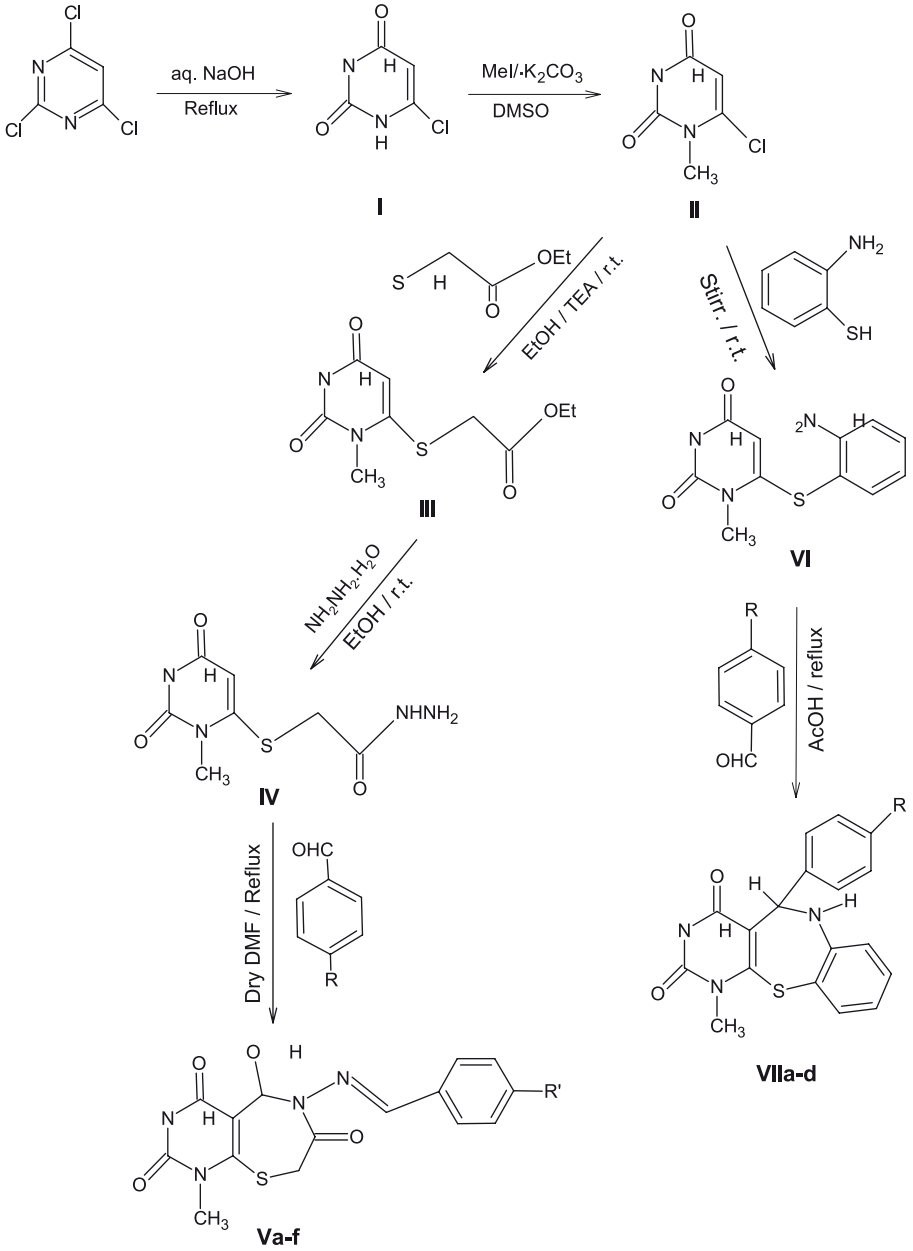
RESULTS AND DISCUSSION

Chemistry

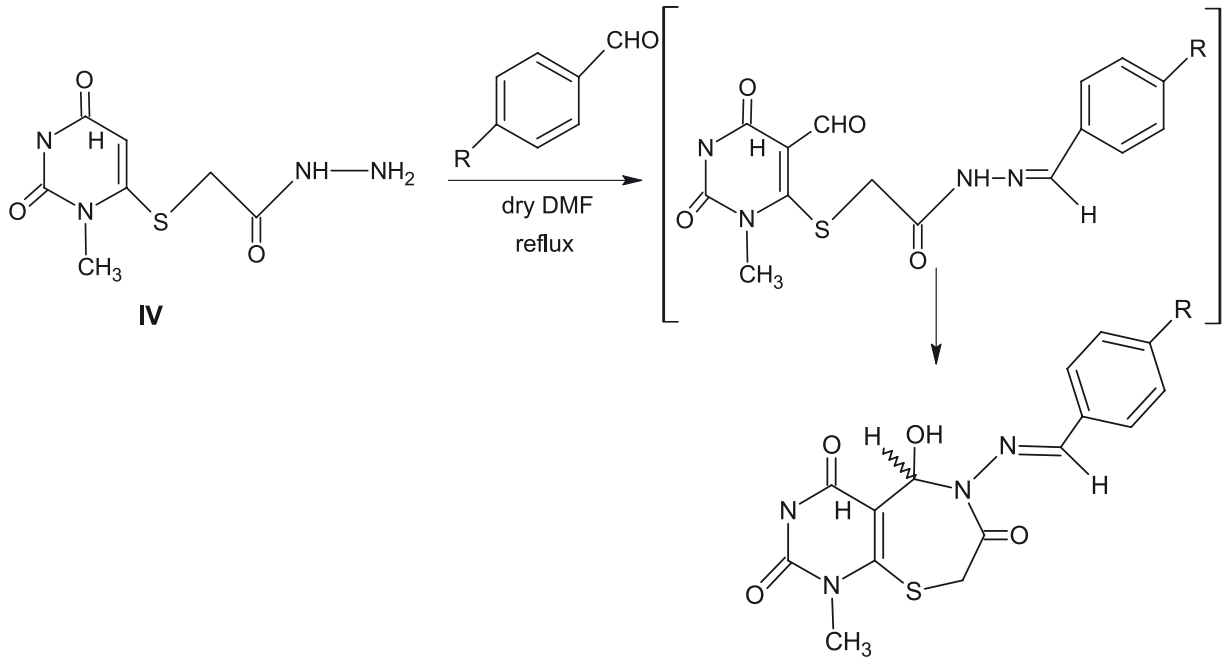
6-Chlorouracil[27] was prepared by the alkaline hydrolysis of 2,4,6-trichloropyrimidine.[28,29] This compound was methylated with methyl iodide in the presence of potassium carbonate applying a reported procedure.[30] The direct reaction of 6-chloro-1-methyluracil (II) with ethyl thioglycolate in the presence of triethylamine (TEA) produce the target ester III in a good yield.[31] In the course of this investigation, the ester III was allowed to react with hydrazine hydrate at room temperature to yield the acid hydrazide IV which on reaction with certain aromatic aldehydes namely, benzaldehyde, 4-hydroxy-, 4-nitro-, 4-chloro-, 4-bromo-, and 4-fluorobenzaldehydes in dry DMF, gave rise to the corresponding pyrimidothiazepines Va-f and no hydrazones could be isolated as shown in **(scheme 1)**. Literature[32,33] survey directed the attention to explain

that the reaction pathway for the formation of compounds **Va-f** may be through formylation of the pyrimidine ring **IV** at the active 5-position. At the same time, condensation between the acid hydrazide and the used aldehyde is achieved. Simultaneous cyclization via intramolecular addition of the hydrazone NH to the formyl group is affected to bring about target com- pounds **Va-f**. ^{1}H-NMR (DMSO-d_6) for **Vb** (R = Cl) showed a singlet exchangeable signal at δ = 11.83 ppm indicating NH at 3 position, two unequal exchangeable singlets at δ = 11.22 & 11.24 ppm corresponding to OH at 5-position and two unequal singlets corresponding to one H at 5-position at δ = 5.52 & 5.48 ppm indicating the asymmetric center (nonequal mixture of R & S isomers). Also, the methylidene group CH appeared as 2 unequal singlets at δ = 8.21 & 8.03 ppm indicating two isomeric forms (Z & E). Similarly, CH2 at 8-position appeared as two unequal signals at δ = 4.39 & 3.98 ppm. Compound **Vd** showed the same observations.

On the other hand, a mixture of 6-chloro-1-methyluracil (II) and 2-aminothiophenol was allowed to react at room temperature in the presence of TEA produced the thioether **VI**. Condensation of **VI** with aromatic aldehydes in acid medium resulted in intramolecular cyclisation of the formed arylidene derivatives to furnish pyrimidobenzothiazepines **VIIa-d** as shown in **(scheme 1)**. ^{1}H-NMR (DMSO-d_6) for **VIIa** (R= Cl) showed two broad singlets at δ = 11.04 & 8.26 ppm characteristic for two NH groups, two doublets at δ = 8.11 & 8.01 ppm (2H) at 3 & 5 positions of 4-chlorophenyl group, a multiplet at δ = 7.60-7.25 ppm characteristic for 7 protons (6 aromatic and one at 5-position), and a singlet at δ = 3.47 ppm characteristic for NCH_3. The appearance of signals of two protons at 3 & 5 position of the phenyl group as two separate doublets indicated that the compound is present in two isomeric forms, R & S.



scheme 1



IV

Va-f

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 agarose 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 4-14 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.

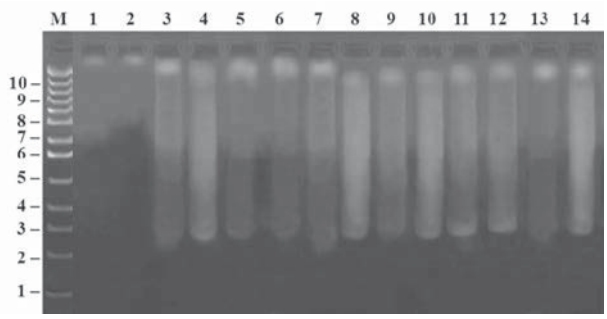


Figure 1. *Gel electrophoresis 1%w/v agarose of untreated and treated genomic DNA. Lane M: Molecular weight marker (left side); Lane 1: Untreated nucleic acid; Lane 2: DMSO treated nucleic acid (negative control); Lane 3: Methotrexate treated nucleic acid (positive control); Lanes 4-14: Compounds (VIIa-d, IV, Va-f) treated nucleic acid.*

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