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A Simple and Convenient Synthesis of Novel Thiopyrimidine Derivatives as Anticancer Agents

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ABSTRACT

The starting thiopyrimidine carbonitrile derivatives 1a, b were synthesized via the reaction of phenylthiourea, ethylcyanoacetate and appropriate aromatic aldehydes namely 2-thiophenaldehyde and verateraldehyde. Chlorination of compounds 1a,b by phosphorus oxychloride and phosphorus pentachloride gave compounds 2a, b, which were subjected to react with anthranilic acid, glycine and sodium azide afforded compounds 3a, b ,4a, b and 5a, b respectively. While reaction of compounds 2a, b with some aromatic amines or hydrazine hydrate furnished compounds 6a-d and 7a, b respectively. Structures of all synthesized compounds were elucidate using IR, ^{1}H-NMR and Mass spectroscopes. Some of the newly synthesized analogues were chosen to evaluate their in-vitro cytotoxic activity against human liver carcinoma cell lines (HEPG2), human breast mammary gland adenocarcinoma cell lines (MCF7), prostate cancer (PC3). The obtained data revealed that some of the tested derivatives especially 6d has high potency against MCF7, while on the other hand it has good activity against PC3, while 3a and 6b exhibited good activity against MCF7 and HepG2 respectively, on the other hand, 1a have moderate activity against MCF7.

Keywords: Thiopyrimidin, HEPG2, MCF7, PC3, cytotoxic activity.

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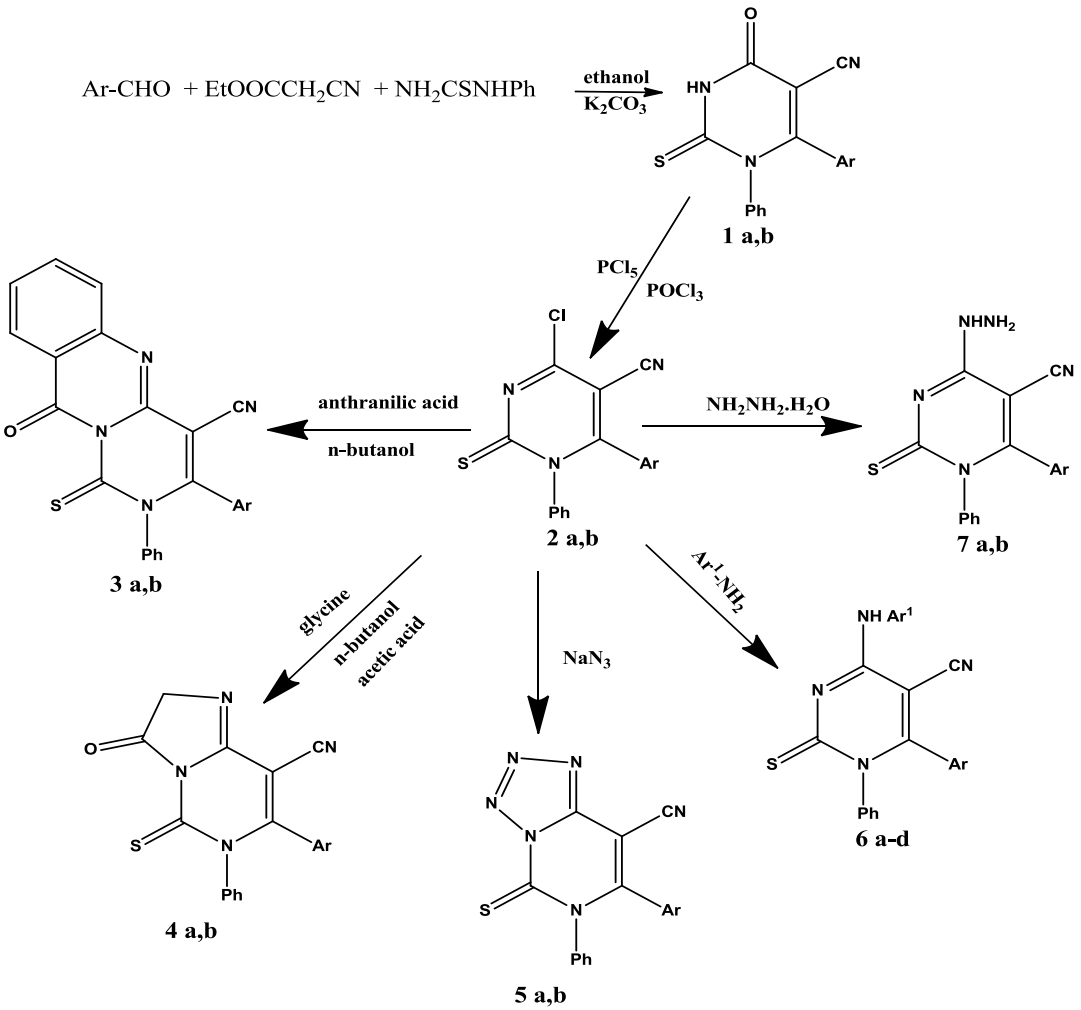
INTRODUCTION

Pyrimidines are of chemical and pharmacological interest [1, 2] and compounds containing the pyrimidine ring system have been shown to possess antitumor, antibacterial, antifungal, antimalarial and anticonvulsant activities[1-5]. Some are valuable drugs for the treatment of hyperthyroidism, acute leukemia children and adult granulocytic leukemia[5]. Furthermore, several pyrimidines are used in polymer and supramolecular chemistry]6, 7]. Conjugated molecules which have a pyrimidine core as the key unit have received much attention and they are prospective candidates for light emitting devices[8] and molecular wires[9]. After the invention of 5-fluorouracil as an antimetabolite of uracil[10] it has become one of the most widely used antineoplastic agents. Analogously, some 5-halogenated thiouracil[11] were synthesized and screened for anticancer activity. It has been reported that the tested compounds have comparable activity of that of uracil[12]. From 1961-1995 different research laboratories investigated the anticancer activity of some 5-substituted-2-thiouracil[13-16] reported that the tested compounds were found to inhibit DNA synthesis. We reported here the synthesis of novel thiouracil derivatives based on the diverse medicinal uses and biological activities of thiouracil as anticancer[17-19] Based on this finding it was of interest to synthesis and evaluation of chemotherapeutic activity of a number of 6-substituted-4-oxo-2-thio-1,2,3,4-tetrahydropyrimidines (2-thiouracils). In view of the biological significance of 2-thiouracils, we became interested in obtaining some new compounds derived by substitutions of 2-thiouracils at different positions.

Scheme:



Ar = 2-thiophenyl , 3,4-dimethoxyphenyl
Ar[1]= 2-furfuryl , 4-bromophenyl

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MATERIAL AND METHODS

Chemistry

All melting points are uncorrected and were recorded on an open glass capillary tubes using an Electrothermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out at Micro analytical Unit. Central Services Laboratory, National Research Centre, Dokki, Cairo. Egypt, using Vario Elementar and were found within ±0.5% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer (Japan) at cm^{-1} scale using KBr disc technique at Central Services Laboratory. National Research Centre, Dokki, Cairo, Egypt. ^{1}H-NMR spectra were determined in using a JEOI EX-270 NMR spectrometer (Japan) at Central Services Laboratory. National Research Centre, Dokki, Cairo, Egypt. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, Cairo University, Giza, and Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt Germany) and the spots were detected by exposure to UV analysis lamp at λ 254/366 nm for few seconds.

General procedure for synthesis of thiopyrimidin -5- carbonitrile derivatives 1a, b.

A mixture of phenyl thiourea (0.1 mol), ethyl cyanoacetate (0.1 mol) and the appropriate aromatic aldehydes namely 2-thiophenaldehyde and verateraldehyde was refluxed in absolute ethanol in presence of potassium carbonate for 48 hrs. The reaction mixture was poured onto ice cold-water. The precipitate was filtered off, dried then crystallized from the proper solvent to give compounds **Ia, b** respectively.

1,2,3,4-Tetrahydro-4-oxo-1-phenyl-6-(thiophen-2-yl)-2-thioxopyrimidin-5-carbontrile (1a)

Yield (79%); mp 142-145˚C; Cryst.(Ethanol); IR (KBr) V_{max}/cm^{-1}: 3280(NH), 3067,3002 (CH aromatic), 2218(C≡N), 1713(C=O), 1585(C=C), 1266(C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 7.10-8.13 (m,8H,H aromatic and H thiophen), 10.40 (s,1H,NH exchangeable with D_2O); MS *m/z* (%): 311 (M^+, 83.15), 308(M^+-3, 58.43). Anal. Calcd for $C_{15}H_9N_3OS_2$ (311.38): C, 57.86; H, 2.91; N, 13.49; Found: C, 57.93; H, 3.21; N, 13.60

1,2,3,4-Tetrahydro-4-oxo-1-phenyl-6-(3,4-dimethoxyphenyl)-2-thioxopyrimidin-5 carbontrile (1b).

Yield (81%); mp 179-181˚C; Cryst.(Iso-propanol); IR (KBr) V_{max}/cm^{-1}: 3423 (NH), 3072,3035 (CH aromatic),2997,2964 (CH aliph), 2221 (C≡N), 1685.79 (C=O), 1610.56 (C=N), 1155 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.70 (s,3H,OCH_3), 3.80 (s,3H,OCH_3), 6.85-7.73 (m,8H,H aromatic), 11.20(s,1H,NH exchangeable with D_2O); MS *m/z*(%): 366 (M^++1, 5.7), 365 (M^+, 11.), 193(100). Anal. Calcd for $C_{19}H_{15}N_3O_3S$ (365.45): C, 62.45; H, 4.14; N, 11.53; Found: C, 62.52; H, 4.30; N, 11.76.

General procedure for synthesis of 4-chlorothiopyrimidine derivatives 2a, b.

A mixture of compounds **1a, b** (0.01 mol) and phosphorus pentachloride (0.01 mol) in phosphorusoxychloride (20 ml) was heated on a steam bath for 3 hrs. Then reaction mixture poured gradually onto crushed ice. The precipitate was filtered off, dried then crystallized from the proper solvent to give the corresponding compounds **2a, b,** respectively.

4-Chloro-1,2-dihydro-1-phenyl-6-(thiophen-2-yl)-2-thioxopyrimidin-5-carbonitrile(2a).

Yield (68%); mp 90-93˚C; Cryst.(Acetone); IR (KBr) V_{max}/cm^{-1}: 3066 (CH aromatic), 2215 (C≡N), 1645 (C=N), 1157 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 7.14-7.99 (m,8H, H aromatic and H thiophen).; MS *m/z*(%):330(M^++1, 15.70), 329 (M^+, 20), 179(100). Anal. Calcd for $C_{15}H_8ClN_3S_2$ (329.83): C, 54.62; H, 2.44; N, 12.74; Found: C, 54.35; H, 2.01; N, 12.21.

4-Choloro-1,2-dihydro-1-phenyl-6-(3,4-dimethoxyphenyl)-2-thioxopyrimidin-5-carbonitrile (2b).

Yield (70%); mp 119-121˚C; Cryst.(Chloroform); IR (KBr) V_{max}/cm^{-1}: 3066(CH aromatic),2935,2839 (CH aliph), 2220 (C≡N), 1630 (C=N), 1265 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.85 (s,6H,2OCH_3), 6.73-7.75 (m,8H,H

aromatic); MS *m/z*(%):385(M^++2, 10.9),383.87 (M^+, 10.1). Anal. Calcd for $C_{19}H_{14}ClN_3O_2S$ (383.85): C, 59.45; H, 3.68; N, 10.95; Found: C, 59.12; H, 3.34; N, 10.53.

General procedure for synthesis of thiopyrimidoquinazoline derivatives 3a, b.

A mixture of compounds **2a, b** (0.01 mol) and anthranilic acid (0.015 mol) in (30 ml) n-butanol was heated under reflux for 12 hrs. The solid obtained after cooling was crystallized from the suitable solvent to give compounds **3a, b**.

2,10-Dihydro-10-oxo-2-phenyl-3-(thiophen-2-yl)-1-thioxo-1*H*-pyrimido[6,1-*b*] quinazolin-4-carbonitrile (3a).

Yield (69%); mp 210-213˚C; Cryst.(Methanol); IR (KBr) V_{max}/cm^{-1}: 3093,3039 (CH aromatic), 2216 (C≡N),1685(C=O),1620 (C=N), 1234 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 6.50-8.05 (m,12H, H aromatic and H thiophene); MS *m/z*(%): 412.49 (M^+, 0.11), 162(100). Anal. Calcd for $C_{22}H_{12}N_4OS_2$ (412.49): C, 64.06; H, 2.93; N, 13.58; Found: C, 64.31; H, 3.21; N, 13.79.

2,10-Dihydro-10-oxo-2-phenyl-3-(3,4-dimethoxyphenyl)-1-thioxo-1*H*-pyrimido[6,1-*b*]quinazolin-4-carbonitrile (3b).

Yield (67%); mp 137-139˚C;Cryst.(Methanol); IR (KBr) V_{max}/cm^{-1}: 3140 (CH aromatic),2917,2845 (CH aliph), 2218 (C≡N), 1692 (C=O), 1635 (C=N), 1245 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.87, 3.88 (2s,6H,2OCH_3), 7.27-7.92 (m,12H,H aromatic); MS *m/z*(%): 466 (M^+, 5.96), 468(M^{+2}, 1.20). Anal. Calcd for $C_{26}H_{18}N_4O_3S$ (466.51): C, 66.94; H, 3.89; N, 12.01; Found: C, 66.38; H, 3.28; N, 11.73.

General procedure for synthesis of imidazopyrimidincarbonitrile derivatives 4a, b.

A mixture of compounds **2a, b** (0.01 mol) and glycine (0.01 mol) in n-butanol (30 ml) was heated under refluxed for 3 hrs. The solid separated was refluxed with glacial acetic acid (5 ml) for 2 hrs. The precipitate was filtered off, dried then crystallized from the proper solvent to give compounds **4a, b,** respectively.

2,3,5,6-Tetrahydro-3-oxo-6-phenyl-7-(thiophen-2-yl)-5-thioxoimidazo[1,2-*F*]pyrimidin-8-carbonitrile (4a).

Yield (73%); mp 68-70˚C; Cryst.(Acetic acid); IR (KBr) V_{max}/cm^{-1}: 3095 (CH aromatic),2925(CH aliph), 2212 (C≡N), 1695 (C=O), 1630 (C=N), 1160 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 4.10 (s,2H,N-CH_2), 7.55-8.30 (m,8H,H aromatic and H thiophene); MS *m/z*(%): 350.42(M^+, 0.48), 64(100). Anal. Calcd for $C_{17}H_{10}N_4OS_2$ (350.42): C, 58.27; H, 2.88; N, 15.99; Found: C, 58.42; H, 2.93; N, 16.06.

2,3,5,6-Tetrahydro-3-oxo-6-phenyl-7-(3,4-dimethoxyphenyl)-5-thioxoimidazo[1,2-*f*]pyrimidin-8-carbonitrile(4b).

Yield (76%); mp 100-102˚C; Cryst.(Dioxane); IR (KBr) V_{max}/cm^{-1}: 3095 (CH aromatic),2920,2851 (CH aliph), 2215 (C≡N), 1688 (C=O), 1639 (C=N), 1165 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.76, 3.84 (2s,6H,2OCH_3), 4.25 (s,2H,N-CH_2), 6.55-7.98 (m,8H,H aromatic); MS *m/z*(%): 404.44 (M^+, 2.55), 406.40(M^{+2}, 3.10). Anal. Calcd for $C_{21}H_{16}N_4O_3S$ (404.44): C, 62.36; H, 3.99; N, 13.85; Found: C, 62.49; H, 4.23; N, 13.99.

General procedure for synthesis of tetrazolopyrimidine derivatives 5a, b.

A mixture of compounds **2a or b** (0.01 mol) and sodium azide (0.05 mol) in (30 ml) glacial acetic acid was refluxed for 3 hrs. The solid obtained after cooling was crystallized from the proper solvent to give compounds **5a, b.**

5,6-Dihydro-6-phenyl-7-(thiophen-2-yl)-5-thioxotetrazolo[1,5-*f*]pyrimidin-8-carbonitrile (5a).

Yield (72%); mp 170-173˚C; Cryst.(Methanol); IR (KBr) V_{max}/cm^{-1}: 3035 (CH aromatic), 2214 (C≡N), 1596 (C=C), 1255 (C=S); MS *m/z*(%): 336 (M^+, 0.58), 337(M^{+1}, 0.44), 64(100). Anal. Calcd for $C_{15}H_8N_6S_2$ (336.39): C, 53.56; H, 2.40; N, 24.98; Found: C, 53.72; H, 2.63; N, 25.21.

5,6-Dihydro-6-phenyl-7-(3,4-dimethoxyphenyl)-5-thioxotetrazolo[1,5-*f*]pyrimidin-8-carbonitrile (5b).

Yield (78%); mp 171-172˚C; Cryst.(Acetic Acid); IR (KBr) V_{max}/cm^{-1}: 3055 (CH aromatic), 2922,2855(CH aliph),2221 (C≡N), 1625 (C=C), 1595 (C=N), 1260(C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.65 (s,3H,OCH_3), 3.83 (s,3H,OCH_3), 7.10-7.98 (m,8H,H aromatic); MS *m/z* (%): 391 (M^++1, 11.50), 64(100). Anal. Calcd for $C_{19}H_{14}N_6O_2S$ (390.42): C, 58.45; H, 3.61; N, 21.53; Found: C, 58.31; H, 3.36; N, 21.29.

General procedure for synthesis of 4-substituted amino pyrimidine derivatives (6a-d).

A mixture, of compounds **2a, b** (0.01 mol) and the primary aromatic amines namely 4-bromoaniline and 2-furfurylamine (0.02 mol) in methanol (30 ml) was refluxed for 8-12 hrs. The solid obtained after cooling was crystallized from the proper solvent to give the titled compounds.

4-((4-bromophenyl)amino)-1-phenyl-6-(thiophen-2-yl)-2-thioxo-1,2-dihydropyrimidin-5-carbonitrile(6a).

Yield (69%); mp 180-183˚C; Cryst.(Ethanol); IR (KBr) V_{max}/cm^{-1}: 3336 (NH), 3043 (CH aromatic), 2213 (C≡N), 1639 (C=N), 1590 (C=N), 1154(C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 6.90-8.15 (m,12H,H aromatic and H thiophene),11.79(s,1H,NH exchangeable with D_2O); MS *m/z*(%): 464 (M^+-1, 55.03), 149(100). Anal. Calcd for $C_{21}H_{13}BrN_4S_2$ (465.39): C, 54.20; H, 2.82; N, 12.04; Found: C, 54.83; H, 3.05; N, 12.65.

4-((4-bromophenyl)amino)-6-(3,4-dimethoxyphenyl)-1-phenyl-2-thioxo-1,2-dihydropyrimidin-5-carbonitrile (6b).

Yield (73%); mp 119-121˚C; Cryst.(Methanol); IR (KBr) V_{max}/cm^{-1}: 3420 (NH), 3060 (CH aromatic),2935,2839 (CH aliph), 2217 (C≡N), 1625 (C=N), 1605(C=N), 1157 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.75 (s,6H,2OCH_3), 6.75-7.90 (m,12H,H aromatic),12.00(s,1H,NH exchangeable with D_2O), ; MS *m/z*(%): 519(M^{+1}, 34.31), 149(100). Anal. Calcd for $C_{25}H_{19}BrN_4O_2S$ (518.04): C, 57.80; H, 3.69; N, 10.79; Found: C, 57.03; H, 3.14; N, 10.27.

4-((furan-2-ylmethyl)-1-phenyl-6-(thiophen-2-yl)-2-thioxo-1,2-dihydropyrimidin-5-carbonitrile (6c).

Yield (70%); mp 170-173˚C; Cryst.(Methanol); IR (KBr) V_{max}/cm^{-1}: 3371 (NH), 3065 (CH aromatic), 2211 (C≡N), 1633 (C=N), 1595 (C=C), 1165(C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 4.92 (s,2H,$\underline{CH_2}$-NH), 6.55-7.80 (m,11H,H aromatic, H furan and H thiophene), 11.34 (s,1H,NH exchangeable with D_2O); MS *m/z*(%):392 (M^++2,19.08), 390 (M^+, 31.03), 64(100). Anal. Calcd for $C_{20}H_{14}N_4OS_2$ (390.48): C, 61.52; H, 3.61; N, 14.35; Found: C, 61.93; H, 3.87; N, 14.79.

6-(3,4-dimethoxyphenyl)-4-((furan-2-ylmethyl)amino)-1-phenyl-2-thioxo-1,2-dihydropyrimidin-5-carbonitrile (6d).

Yield (75%); mp 100-103˚C; Cryst.(Iso-propanol); IR (KBr) V_{max}/cm^{-1}: 3327 (NH), 3062 (CH aromatic), 2927,2841 (CH aliph),2209 (C≡N), 1635(C=N), 1593 (C=N), 1156 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.70, 3.81 (2s,6H,2OCH_3), 4.45 (s,2H,CH_2-NH), 6.70-7.90 (m,11H,H aromatic and H furan), 11.00(s,1H,NH exchangeable with D_2O); MS *m/z*(%): 444 (M^+, 33.15), 81(100). Anal. Calcd for $C_{24}H_{20}N_4O_3S$ (444.51): C, 64.85; H, 4.54; N, 12.60; Found: C, 64.52; H, 4.26; N, 12.01.

General procedure for synthesis of hydrazino pyrimidine derivatives 7a, b.

A mixture of compounds **2a, b** (0.01 mol) and hydrazine hydrate (0.015 mol) in methanol (10 ml) was stirred for 8 hrs. The precipitate was filtered off, dried then crystallized from the proper solvent to give the titled compounds.

4-Hydrazinyl-l,2-dihydro-1-phenyl-6-(thiophen-2-yl)-2-thioxopyrimidin-5-carbonitrile (7a).

Yield (68%); mp 110-113˚C; Cryst.(Benzene/pet.ether); IR (KBr) V_{max}/cm^{-1}: 3352,3310,3205 (NH), 3062 (CH aromatic), 2206 (C≡N), 1601 (C=C), 1170 (C=S).^{1}H NMR (DMSO-d_6, δ ppm): 5.10 (S,2H,NH_2exchangeable with D_2O), 7.10-7.95 (m,8H, H aromatic, H thiophene), 11.70 (S,H,NH exchangeable with D_2O); MS

m/z(%):327(M^++2, 3.21), 325 (M^+, 5.03), 64(100). Anal. Calcd for $C_{15}H_{11}N_5S_2$ (325.41): C, 55.36; H, 3.41; N, 21.52; Found: C, 55.02; H, 3.17; N, 21.36.

4-Hydrazinyl-l,2-dihydro-1-phenyl-6-(3,4-dimethoxyphenyl)-2-thioxopyrimidin-5-carbonitrile (7b)

Yield (75%); mp 160-163˚C; Cryst.(Methanol); IR (KBr) V_{max}/cm^{-1}: 3430, 3340 (NH_2), 3185(NH), 3074(CH aromatic), 2921,2866 (CH aliph),2221 (C≡N), 1633 (C=N), 1265 (C=S).^{1}H-NMR (DMSO-d_6, δ ppm): 3.80,3.91 (2s,6H,2OCH_3),5.22 (s,2H,NH_2, exchangeable with D_2O), 6.85-7.92 (m,8H,H aromatic), 13.20(s,H,NH exchangeable with D_2O); MS *m/z*(%): 380 (M^++1, 4.01), 164(100). Anal. Calcd for $C_{19}H_{17}N_5O_2S$ (379.44): C, 60.14; H, 4.52; N, 18.46; Found: C, 60.39; H, 4.85; N, 18.63.

In-vitro cytotoxic screening

Cell growth inhibition assay

Newly synthesized compounds were subjected to invitro antitumor screening against human cancer cell lines using cell based approach[20-23]. Tested compounds were used to evaluate their antitumor potency on three human tumor cell lines namely: hepato cellular carcinoma HepG2, prostatic carcinoma PC3, and breast carcinoma MCF7. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide] to purple Formosan[24,25]. Aprobit analysis was carried for LC50determination using SPSS 11 program the antitumor drug doxorubicin was used as a positive control.

The in vitro antitumor screening was performed adopting previously reported procedures[2 3- 25]. Cells were suspended in RPMI 1640 medium for HepG2 and DMHM for MCI7 and PC3,1% antibiotic-antimycotic mixture (10.000 μ/ml potassium penicillin, 10,000 mg/ml streptomycin sulfate and 25 mg/ml amphotericin B) and 1% L-glutamine at 37°C, under 5% CO_2 and 95% humidity. Cells were seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiterplates for 24 h. Media was aspirated, fresh medium (without serum) was added and cells were incubated with different concentrations of sample to give a final concentration of (100, 50, 25 and 12.5 ppm.) 0.5% DMSO was used as negative control and doxorubicin was used as positive control. MTT assay was used for assessment of cytotoxicity[23 – 25]. After 72 hof incubation, medium was aspirated, 40 μl MTT salt (2.5 mg/ml) were added to each well and incubated for further 4 h. To stop there action and dissolve the formed crystals, 200 μl of 10% sodiumdodecyl sulfate (SDS) in deionized water were added to each well and incubated over night at 37°C. The absorbance was then measured at 595 nm and a reference wave length of 620 nm. The % cytotoxicitywas calculated according to the formula:

[1-(OD compound/OD negative control)] x100.

A probit analysis was carried for LC_{50} determination usingSPSS11 program.

RESULTS AND DISCUSSION

Chemistry

The prepation of our target compounds were started by the reaction phenylthiourea and ethylcyanoacetate[26] with the appropriate aromatic aldehydes, namely, 2-thiophenaldehyde and verateraldehyde in absolute ethanol in presence of anhydrous potassium carbonate[27] to get thioxopyrimidine carbonitriles **1a, b** respectively. Structures of compounds **1a, b** were confirmed by spectral and analytical data, where appearance of signals at δ = 10.40 ppm and 11.20 ppm due to NH groups of **1a, b** respectively. Chlorination of compounds **1a, b** by using phosphorusoxychloride and phosphoruspentachloride[28,29]affordedchloroderivatives **2a, b**, where the structures were elucidate by the disappearance of peaks at 1670,1685 cm^{-1}in IR and signals at δ = 10.40 and 11.20 ppm in H-NMR due to carbonyl and NH groups. Which was then react with anthranilic acid[29] gave compounds **3a, b**, which showed appearance of a peaks at 1685,1692cm^{-1} were assigned to C=O groups. In addition increased number of aromatic protons in H-NMR spectrum confirmed compounds **3a, b** structure. Compounds **4a, b** were achieved upon reactions of compounds **2a, b** with glycine[29] in n-butanol, structures of **4a, b** showed peaks at 2925, 2920

and 1695, 1688 cm-^{1} were assigned to CH_2 and C=O groups, in addition appearance singlet signal at 4.1,4.25 ppm of CH2 confirmed structure.Subjecting2a,b to react with sodium azide[29]in glacial acetic acid furnished thioxotetrazolopyrimidin derivatives **5a, b**, were their structures confirmed by mass spectra. Reaction of **2a,b** with 2-furfuralamine and 4-bromoaniline yielded compounds **6a-d**, their structure elucidate by H-NMR which showed appearance of singlet signals of NH groups at δ =11.79,12.00,11.34,11.00 ppm for **6a-d** respectively. Stirring of **2a,b** with hydrazine hydrate afforded **7a, b** ,the structure of **7a, b** confirmed by IR and H-NMR spectra which showed peaks at 3340-3352 cm-1 in IR and signals 4.92-5.22 ppm (NH) and 10.80-13.65 ppm (NH2) in H-NMR due to hydrazine moiety.

Cytotoxic activity evaluation

In the present work, the compounds 1a, 1b, 2b, 3a, 3b, 4a, 5b, 6a, 6b, 6c, 6d were selected to evaluate their in-vitro growth inhibitory activities against human cultured liver carcinoma cell line (HEPG2), human breast mammary gland (MCF7), prostate cancer (PC3) in comparison to Doxorubicin which is one of the most effective antitumor agents. According to (table 1), it is found that compound 1a shows moderate on HepG2, PC3 and MCF7 on the other hand compounds 6d show high potency on both PC3 and MCF7,while 6b showed high activity against HepG2 and compound 3a has activity against MCF7 in comparison with the used reference drug Doxorubicn

Table(1): The effect of some newly synthesized compounds against human cultured liver carcinoma cell line (HEPG2), human breast mammary gland (MCF7), prostate cancer(PC3).

Compound	LC_{50} (µMol)		
	HepG2	PC3	MCF7
1a	**195.9**	**150**	**51.4**
1b	-	-	-
2b	-	-	-
3a	-	-	**48.5**
3b	-	-	-
4a	-	-	-
5b	-	-	-
6a	-	-	-
6b	**46.3**	-	-
6c	-	-	-
6d	-	**61.1**	**28.35**
Doxorubicin	**69.55**	**54**	**47.8**

LC_{50} : The concentration which gives 50% cell lethality
- : Inactive

CONCLUSION

This work deals with synthesis of new thiopyrimidine derivatives. 11 compounds were selected as representatives to evaluate their cytotoxic potency. Based on the above data it is worthwhile to mention that compounds **3a, 6b, 6d** show high potency against different human cancer cell lines in comparison with the reference drug Doxorubicin.

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