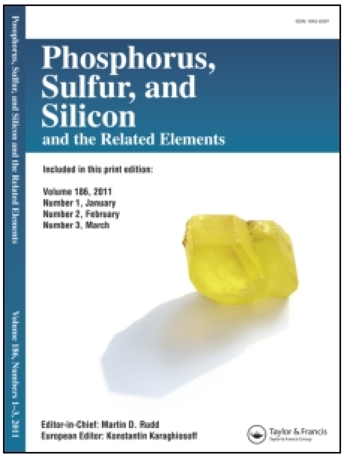

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Synthesis and Inhibitory Activity Against Epstein-Barr Virus of Some New 1,2,3,4-Tetrahydropyrimidine-2-thiones

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Synthesis and Inhibitory Activity Against Epstein-Barr Virus of Some New 1,2,3,4-Tetrahydropyrimidine-2-thiones

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1,2,3,4-Tetrahydropyrimidine-2-thiones ***4a–n*** *were synthesized through the reaction of aromatic aldehydes* ***1a–n****, ethyl acetoacetate* ***(2)*** *and thiourea* ***(3)****. The structures of all newly synthesized heterocyclic compounds elucidated by the use of IR, ^{1}H NMR, mass spectra, and elemental analyses. The inhibitory activity against the Epstein-Barr Virus early antigen (EBA-VA) of all newly synthesized heterocyclic compounds were evaluated.*

Keywords 1,2,3,4-tetrahydropyrimidinethione; antigen; ethylaceto-acetate; Epstein-Barr Virus; inhibitory activity; and thiourea

INTRODUCTION

The reported biological activities of polysubstituted pyrimidine for the treatment of hypoxemia,[1] neuronosis,[2] neuropathy,[3] as well as their antitumor effect,[4] and their physiological activity[5] stimulated our interest to synthesize several derivatives of these ring systems, and to develop derivatives to investigate in our medicinal chemistry program. In the last two decades, uracil, and oxopyrimidine derivatives have been investigated extensively in relation to their antiviral and antitumoral properties[6]; but the pyrimidinethiones have less attention in

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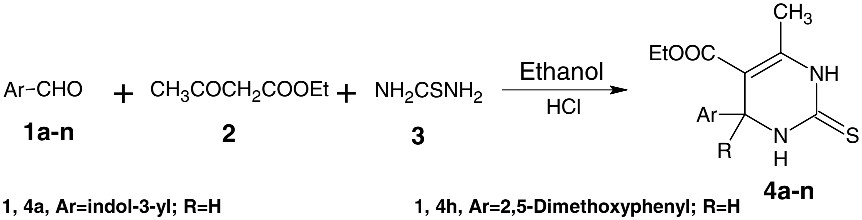
spite of their synthetic potentiality,[7–11] so we report here a method to prepare a new derivatives of 1,2,3,4-tetrahydrpyrimidinethiones and evaluate their inhibitory activity against the Epstein-Barr Virus early antigen (EBA-VA).

RESULTS AND DISCUSSION

Chemistry

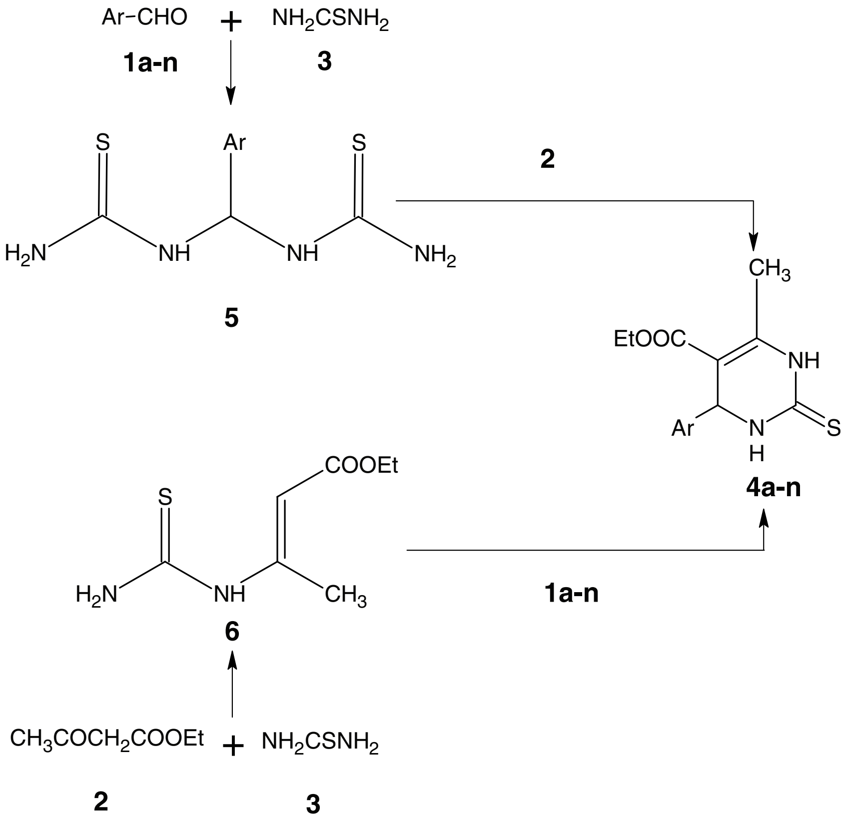
Although several publications[12–15] have been appeared concerning the synthesis of tetrahydropyrimidinethiones by direct *Biginelli* condensation using ethyl acetoacetate as β-ketoester component of classical *Biginelli* reaction[12] with aromatic aldehydes and thiourea, no approach using heterocyclic aldehydes has been reported. Thus, it has been found that a ternary mixture of heterocyclic aldehydes **1a–e**, ethyl acetoacetate **2** and thiourea **3** in absolute ethanol containing a catalytic amount of concentrated hydrochloric acid gave reaction products **4a–e** whose structures were elucidated by considering the data of elemental analyses, IR, ^{1}H NMR, as well as mass spectra (cf. Experimental section and Scheme 1).

The formation of **4a–e** seemed to proceed via either reaction of N,N′-(2-arylmethylene)bis(thiourea) **5**, *formed through the reaction of thiourea (3) and heterocyclic aldehydes 1a–e*, with ethyl acetoacetate **(2)** or ethyl 3-(thiocarbamoylamino)but-2-enoate **6**, *formed through the*



1, 4a, Ar=indol-3-yl; R=H
b, Ar=Pyridin-3-yl; R=H
c, Ar=1,3-Benzodioxol-5-yl; R=H
d, Ar=5-Methyl-2-furyl; R=H
e, Ar=2-Thienyl; R=H
f, Ar=5-Methyl-2-thienyl; R=CH_3
g, Ar=2,4-Dimethoxyphenyl; R=H
1, 4h, Ar=2,5-Dimethoxyphenyl; R=H
1, 4i, Ar=4-Florophenyl; R=H
1, 4j, Ar=4-Methylphenyl; R=H
1, 4k, Ar=3-Nitrophenyl; R=H
1, 4l, Ar=2-Florophenyl; R=H
1, 4m, Ar=2-Hydroxyphenyl; R=H
1, 4n, Ar=4-Hydroxyphenyl; R=H

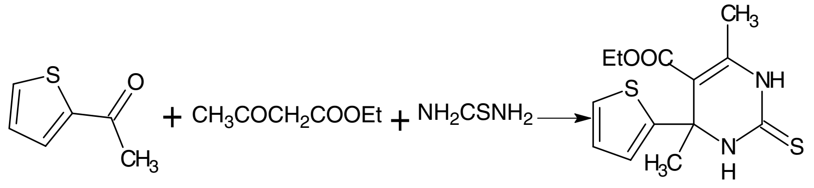
SCHEME 1



SCHEME 2

reaction of thiourea ***(3)*** *and ethyl acetoacetate (2)*, with aldehydes **1a–e** (cf. Scheme 2).[11–15]

Surprisingly, 1-(thiophen-2-yl)ethanone **(1f)** reacted with thiourea **(3)** and ethyl acetoacetate **(2)** under the same experimental conditions to afford a reaction product **4f**. The ^{1}H NMR spectrum of this reaction product revealed the signals of thiophene, CH_3 at 5-position of thiophene in addition to that of $COO\underline{CH_2CH_3}$ protons (cf. Experimental section). Moreover, its mass spectrum gave parent peak at m/z = 296 that corresponded to the molecular weight of the molecular formula $C_{13}H_{16}N_2O_2S_2$of the assigned structure **4f** and peak at m/z = 281, which corresponded to the fragment at M-15 (loss of CH_3 at 6-position) and this confirm that the reaction proceeded by the same above-mentioned mechanism (cf. Scheme 2). Considering



1f **2** **3** **4f**

SCHEME 3 ethyl 4,6-dimethyl-4-(2-thienyl)-22thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate

the above-mentioned data in addition to that of elemental analyses **4f** could be formulated as ethyl 4,6-dimethyl-4-(2-thienyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (cf. Scheme 3).

Similarly, aromatic aldehydes **1g–n** reacted with ethyl acetoacetate **(2)** and thiourea **(3)** as a ternary mixture in ethanolic hydrochloric acid to give the corresponding tetrahydropyridinethione derivatives **4g–n,** respectively. The structures of these reaction products were elucidated by considering the data of elemental analyses, IR, ^{1}H NMR, and mass spectra (cf. Experimental section). It is important to refer that the structures **4g–n** formed through the same mechanistic pathway (cf. Scheme 2).

Pharmacological Activity

The present study aimed to synthesize a new series of tetrahydropyrimidinethione having heterocyclic or substituted benzene ring directly linked to the 4-position of the pyrimidine moiety. We have studied the inhibitory activity of the newly synthesized heterocyclic compounds against the Epstein-Barr Virus early antigen (EBA-VA) activation introduced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and a structure activity relationship was deduced.

Compounds **4c**, **4d**, **4g**, and **4h** exhibit promising activity at concentration (mol. Ratio/TPA) 1000 (cf. Figures 1–4).

CONCLUSIONS

Structure activity correlation of the obtained results revealed that the addition of both benzene ring (containing methoxy group, dimethoxy groups, dioxal group) and 5-methyl-2-furyl to the 4-position of pyrimidine moiety increases the activity (% of TPA Reduction) more than the addition of fluoro, nitro, hydroxy groups over the pyrimidine moiety. The

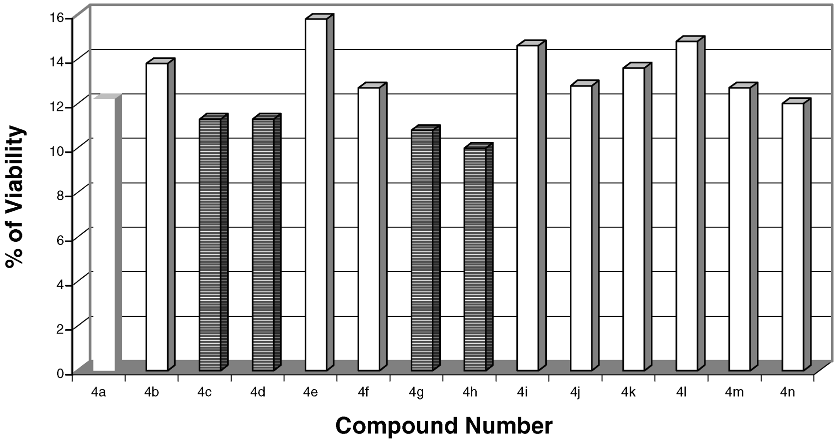


Figure 1 Inhibitory activity of the newly synthesized heterocyclic compounds at concentration 1000 of TPA.

overall results coincided with the previous conclusion that O-purenyl structure or group was revealed to possess remarkable Inhibitory activity against TPA induced EBV-EA potency.

EXPERIMENTAL

Melting points of the newly synthesized heterocyclic compounds were determined by capillary method and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 529 in nujol mull. ^{1}H NMR spectra of a few selected compounds were recorded on a 300 MHZ Bruker WH-200 pulsed FT.NMR spectrometer using DMSO-d_6 as solvent and tetramethylsilane as an internal standard. All chemical shift values are expressed in δ scale, mass spectra of some of the selected compounds were recorded on a JEOL JMS-D-300mass spectrometer operating at 70 V.

Synthesis of 2-Thioxo-1,2,3,4-tetrahydropyrimidinethiones 4a–n—General Procedure

To a solution of thiourea (3.8 g, 0.05 mol), appropriate aromatic or heterocyclic aldehydes (0.05 mol), ethyl acetoacetate (0.05 mol), in absolute ethanol (20 mL) a few drops (8 drops) of 37% HCl was added. The reaction mixture was refluxed for 7–10 h, and then allowed to stand for 24 h for crystallization. The product was filtrated and washed with ethanol, dried, and recrystallized from the proper solvent.

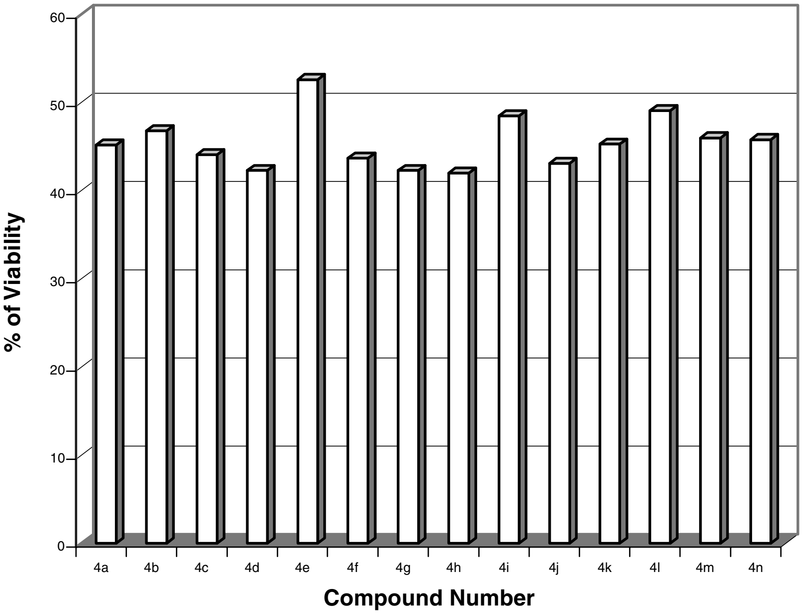


Figure 2 Inhibitory activity of the newly synthesized heterocyclic compounds at concentration 500 of TPA.

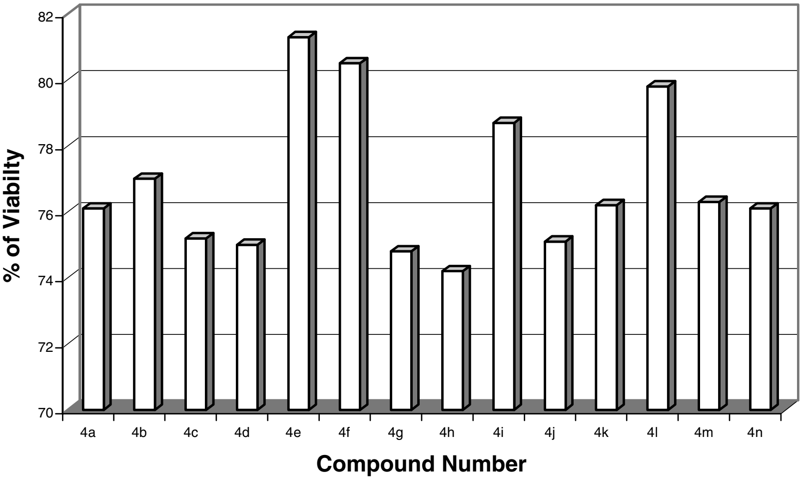


Figure 3 Inhibitory activity of the newly synthesized heterocyclic compounds at concentration 100 of TPA.

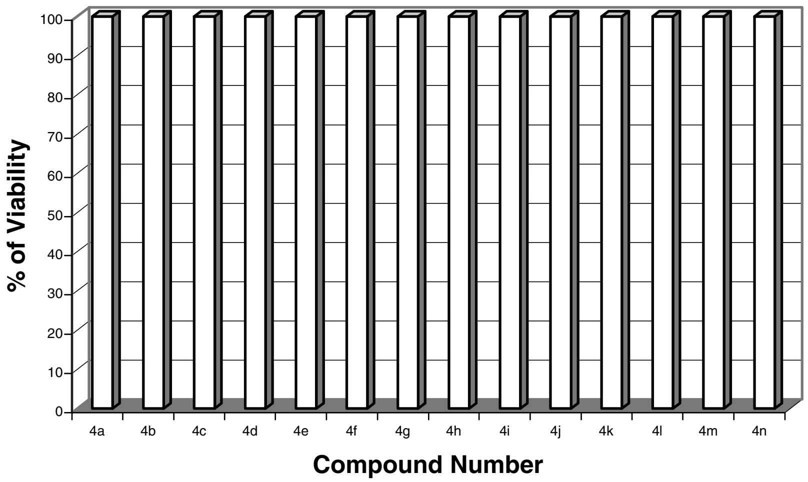


Figure 4 Inhibitory activity of the newly synthesized heterocyclic compounds at concentration 10 of TPA.

Ethyl 1,2,3,4-Tetrahydro-4-(1H-indol-3-yl)-6-methyl-2-thioxopyrimi-dine-5-carboxylate (4a)

Recrystallized from methanol m.p. 245−250°C as dark brown solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.22(t, $J = 5.5$ Hz, 3H, CH_3), 1.57(s, 3H, CH_3), 4.19(q, $J = 5.7$ Hz, 2H, CH_2), 5.20(d, 1H, d, $J = 3.75$ Hz,H-4), 7.18(m, 4H aromatic indol), 10.22(s, 1H, NH); IR (cm^{-1}): 3325(NH), 1705(C=O), 1583 (C=S). MS m/z(%): 315(90), 299(33), 286(12), 273(76); anal calcd. for $C_{16}H_{17}N_3O_2S$: C,60.93; H,5.43; N,13.32; S,10.17; found C, 60.98; H, 5.10; N, 14.01; S, 10.22.

Ethyl 1,2,3,4-Tetrahydro-6-methyl-4-(pyridine-3-yl)-2-thioxopyrimi-dine-5-carboxylate (4b)

Recrystallized from methanol m.p. 75−79°C as yellow solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.30 (t, $J = 5.9$ Hz, 3H, CH_3), 1.71(s, 3H, CH_3), 4.22(q, $J = 6.1$ Hz, 2H, CH_2), 5.13(d, 1H, d, $J = 3.45$ Hz, H-4), 7.62, 8.65(m, 5H, pyridine); IR (cm^{-1}): 3385(NH), 1720(C=O), 1580 (C=S). MS m/z(%): 262(100), 247(13), 231(72); anal. calcd. for $C_{13}H_{15}N_3O_2S$: C, 56.31, H, 5.41; N, 15.15; S, 11.55; found: C, 56.27; H, 5.38; N, 15.19; S, 11.22.

Ethyl 4-(1,3-Benzodioxol-5-yl)-6-methyl-2- thioxo-1,2,3,4-tetrahydro- pyrimidine-5-carboxylate (4c)

Recrystallized from methanol m.p. 172−175°C as white solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.22(t, $J = 5.9$ Hz, 3H, CH_3), 1.81(s, 3H,

CH_3), 4.15(q, $J=$ 6.5 Hz, 2H, CH_2), 5.34(d, 1H, d, $J=$ 3.80 Hz, H-4), 5.91(s, 2H, CH_2), 6.46–6.54 (m, 3H, ArH's); IR (cm^{-1}): 3405(NH), 1729(C=O), 1570(C=S). MS m/z (%): 320(100), 305(77) 288(53), 275(22); anal. calcd. for $C_{15}H_{16}N_2O_4S$: C, 56.24; H, 5.03; N, 8.74; S, 10.01; found C, 56.98; H,5.52; N, 8.44; S, 10.11.

Ethyl 1,2,3,4-Tetrahydro-6-methyl-4-(5-methyl-2-furyl)-2-thioxopyrimidine-5-carboxylate (4d)

Recrystallized from methanol m.p. >350°C as colorless solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.19(t, $J=$ 4.9 Hz, 3H, CH_3), 1.41(s, 3H, CH_3), 2.22(s, 3H, CH_3), 4.55(q, $J=$ 5.8 Hz, 2H, CH_2), 5.13(d, 1H, d, $J=$ 3.28 Hz, H-4), 5.82(dd, $J=$ 6.1 Hz, 1H, CH), 5.92(dd, $J=6.3$ Hz, 1H, CH); IR (cm^{-1}): 3407(NH), 1708(C=O), 1587(C=S); MS m/z(%): 280(100), 251(60) 207(87); anal. calcd. for $C_{13}H_{16}N_2O_3S$: C, 55.69; H, 5.75; N, 9.99; S, 11.44; found C, 55.52; H, 6.01; N, 10.01; S, 11.43.

Ethyl 1,2,3,4-Tetrahydro-6-methyl-4-(2-thienyl)-2-thioxopyrimidine-5-carboxylate (4e)

Recrystallized from methanol m.p. 166 −171°C as colorless solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.09(t, $J=6.9$ Hz, 3H, CH_3), 1.91(s, 3H, CH_3), 2.42(s, 3H, CH_3), 4.35(q, $J=$ 7.3 Hz, 2H, CH_2), 5.32(d, 1H, d, $J=3.67$ Hz, H-4), 6.42–6.95 (m, 3H, thiophene); IR (cm^{-1}): 3397(NH), 1700(C=O), 1574(C=S); MS m/z(%): 282(100), 267(20); anal. calcd. for $C_{12}H_{14}N_2O_2S_2$: C, 51.04; H, 5.00; N, 9.92; S, 22.71; found C, 51.01; H, 5.91; N, 10.31; S, 23.03.

Ethyl 1,2,3,4-Tetrahydro-4,6-dimethyl-4-(thiophene-2-yl)-2-thioxopyrim-idine-5-carboxylate (4f)

Recrystallized from methanol m.p. 195−198°C as a yellow solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.09(t, J = 6.1 Hz, 3H, CH_3), 1.61(s, 3H, CH_3), 1.92(s, 3H, CH_3), 4.35(q, J = 6.3 Hz, 2H, CH_2), 5.22(d, 1H, d, J = 3.55 Hz, H-4), 6.52-6.85 (m, 3H, thiophene); IR (cm^{-1}): 3347(NH), 1702(C=O), 1584(C=S); MS m/z(%): 296(100), 281(10) 265(74); anal. calcd. for. $C_{13}H_{16}N_2O_2S_2$: C, 52.58; H, 5.44; N, 9.45; S, 21.64; found C, 51.92; H, 5.21; N, 10.01; S, 21.03.

Ethyl 1,2,3,4-Tetrahydro-4-(2,4-dimethoxyphenyl)-6-methyl-2-thioxo-pyrimidine-5-carboxylate (4g)

Recrystallized from ethanol m.p. 120−125°C as a brown solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.11(t, $J = 5.01$ Hz, 3H, CH_3), 1.27(s, 3H, CH_3), 3.81(s, 6H, 2-OCH_3), 4.22(q, $J = 5.7$ Hz, 2H, CH_2); 5.17(d, 1H, d, $J = 3.54$ Hz, H-4), 6.16–6.84(m, 3H, ArH's). IR (cm^{-1}): 3330(NH), 1717(C=O), 1572(C=S). MS m/z (%): 336(100), 321(37) 305(13), 261(41); anal. calcd. for $C_{16}H_2ON_2O_4S$: C, 57.09; H, 6.01; N, 8.31; S, 9.51; found: C, 57.11; H, 6.12; N, 8.33; S, 9.49.

Ethyl 1,2,3,4-Tetrahydro-4-(2,5-dimethoxyphenyl)-6-methyl-2-thioxo-pyrimidine-5-carboxylate (4h)

Recrystallized from ethanol m.p. 214−217°C as a orange solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.27(t, $J = 4.6$ Hz, 3H, CH_3), 1.72(s, 3H, CH_3), 3.60(s, 6H, 2-OCH_3), 4.40(q, $J = 5.3$ Hz, 2H, CH_2), 5.12(d, 1H, d, $J = 3.65$ Hz, H-4), 6.49-6.67(m, 3H, ArH's); IR (cm^{-1}): 3327(NH), 1723(C=O), 1582(C=S); MS m/z(%): 336(89), 321(15) 305(60), 261(11); anal. calcd. for $C_{16}H_2ON_2O_4S$: C, 57.12; H, 5.99; N, 8.33; S, 9.53; found C, 57.35; H, 6.01; N, 8.28; S, 9.56.

Ethyl-4-(4-Fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrimi-dine-5-carboxylate (4i)

Recrystallized from ethanol m.p. 193−195°C as a white solid; ^{1}HNMR (300 MHz, DMSO-d_6): 1.18(t, $J = 5.1$ Hz, 3H, CH_3), 1.66(s, 3H, CH_3), 4.31(q, $J = 6.3$ Hz, 2H, CH_2), 5.46(d, 1H, d, $J = 3.75$ Hz, H-4), 6.88(m, 4H, ArH's); IR (cm^{-1}): 3390(NH), 1711(C=O), 1585(C=S); MS m/z(%) 294(100), 279(55) 265(20), 221(79); anal. calcd. for $C_{14}H_{15}FN_2O_2S$: C,57.10; H, 5.16; F, 6.43; N, 9.55; S, 10.91; found C, 57.22; H, 5.01; F, 6.39; N, 9.63; S, 10.90.

Ethyl-1,2,3,4-tetrahydro-6-methyl-2-thioxo-4-(4-methylphenyl)pyrimi-dine-5-carboxylate (4j)

Recrystallized from ethanol m.p. 185−188°C as a yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6): 1.28(t, $J = 6.2$ Hz, 3H, CH_3), 1.53(s, 3H, CH_3), 2.33(s, 3H, CH_3), 4.01(q, $J = 6.4$ Hz, 2H, CH_2), 5.49(d, 1H, d, $J = 3.87$ Hz, H-4), 6.94(m, 4H, ArH's); IR (cm^{-1}): 3310(NH), 1721(C=O), 1579(C=S). MS m/z(%): 290(100), 275(15) 261(12), 217(90); anal. calcd. for: $C_{15}H_{18}N_2O_2S$: C,62.04; H, 6.25; N, 9.65; S, 11.04; found C, 61.98; H, 5.89; N, 9.83; S, 11.13.

Ethyl 1,2,3,4-Tetrahydro-6-methyl-4-(3-nitrophyenyl)-2-thioxopyrim-idine-5-carboxylate (4k)

Recrystallized from ethanol m.p. 212−215°C as white powder solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.27(t, $J =$ 5.6 Hz, 3H,CH_3), 1.92(s, 3H, CH_3), 4.22(q, $J =$ 5.4 Hz, 2H, CH_2), 5.01(d, 1H, d, $J =$ 3.36 Hz, H-4), 7.76–8.01(m, 5H, ArH's); IR (cm^{-1}): 3319(NH), 1701(C=O), 1572(C=S). MS m/z(%): 321(14), 285(35) 275(100), 241(66); anal. calcd. For $C_{14}H_{15}N_3O_4S$: C, 53.01; H, 4.71; N, 13.09; S,10.01; found C, 53.01; H, 4.60; N, 12.92; S, 9.99.

Ethyl-4-(2-Fluorophenyl)-1,2,3,4-tetrahydro-6-methyl-2-thioxopyrim-idine-5-carboxylate (4l)

Recrystallized from mixture ethanol ethylacetate m.p. 87−90°C as a yellow solid; ^{1}H NMR (300MHz, DMSO-d_6): 1.12(t, $J =$ 6.1Hz, 3H, CH_3), 1.33(s, 3H, CH_3), 4.01(q, $J =$ 5.8 Hz, 2H, CH_2), 5.19(d, 1H, d, $J =$ 3.41 Hz, H-4), 6.82–7.10(m, 5H, ArH's); IR (cm^{-1}): 3298(NH), 1701(C=O), 1580(C=S); MS m/z(%): 294(44), 279(5) 265(20), 221(29); anal. calcd. for $C_{14}H_{15}FN_2O_2S$: C, 57.13; H, 5.14; F, 6.45; N, 9.52; S, 10.89; found C, 56.94; H, 5.34; F, 5.87; N, 10.01; S, 9.99.

Ethyl 1,2,3,4-Tetrahydro-4-(2-hydroxyphenyl)-6-methyl-2-thioxopyrim-idine-5-carboxylate (4m)

Recrystallized from methanol-water m.p. 199−203°C as a yellow solid; ^{1}H NMR (300MHz, DMSO-d_6): 1.37(t, $J =$ 6.8 Hz, 3H, CH_3), 1.90(s, 3H, CH_3), 4.67(q, $J =$ 6.9Hz, 2H, CH_2) 5.32(d, 1H, d, $J =$ 3.22 Hz, H-4), 6.65–6.95(m, 5H, ArH's); IR (cm^{-1}): 3366(NH), 1716(C=O), 1570(C=S); MS m/z(%): 292(100), 277(55) 261(30), 245(16); anal. calcd. for $C_{14}H_{16}N_2O_3S$: C, 57.52; H, 5.52; N, 9.58; S, 10.97; found C, 57.01; H, 5.33; N, 9.60; S, 10.96.

Ethyl 1,2,3,4-Tetrahydro-4-(4-hydroxyphenyl)-6-methyl-2-thioxopyrim-idine-5-carboxylate (4n)

Recrystallized from ethanol m.p. 87−90°C as a red solid; ^{1}HNMR (300MHz, DMSO-d_6): 1.12(t, J = 5.3 Hz, 3H, CH_3), 2.01(s, 3H, CH_3), 4.50(q, J = 4.4 Hz, 2H, CH_2), 5.16(d, 1H, d, J = 3.70 Hz, H-4), 6.62(dd, J = 7.7 Hz, 2H), 6.91(dd, J = 7.9 Hz, 2H); IR (cm^{-1}): 3156(NH), 1729(C=O), 1577(C=S); MS m/z(%): 292(100), 277(40); anal. calcd. for $C_{14}H_{16}N_2O_3S$: C, 57.50; H, 5.59; N, 9.61; S, 10.99; found: C, 57.52; H, 5.53; N, 9.58; S, 10.96.

Epstein-Barr Virus Activation Test

Materials and Methods

The newly synthesized heterocyclic compounds were tested for Epstein-Barr virus (EBV) genome carrying lymphoblastoid cells (Raji cells derived from Burkitt's lymphoma) were cultured in 10% fetal bovine serum (FBS) in RPMI-1640 medium (Sigma, MS, USA). Spontaneous activation of EBV-EA (early antigen) in our sub-line Raji cells less than 0.1%. The inhibition of EBV-EA activation was assayed using Raji cells (virus non producer type) as described previously.[16] The indicator cells (Raji cell, 1×10^6/ml) were incubated at 37°C for 48 hrs in 1 ml of medium containing n-butyric acid (4 mmol), TPA (32 pmol 20 ng in DMSO, 2 μl) as inducers and various amounts of test compounds in 5 μl DMSO. Smears were made from the cell suspension, and the activated cells that were stained by EBV-EA positive serum from nasopharyngeal carcinoma (NPC) patients were detected by an indirect immunofluoresence technique.[17] In each assay, at least 500 cells were counted, and the number of stained cells (positive cells) present was recorded. Triplicate assays were performed for each compound the average of EBV-EA induction of the test compounds was expressed as a relative ratio to the control experiment (100%), which was carried out only with n-butyric acid plus TPA. The viability of treated Raji cells was assayed by the trypan Blue staining method.

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