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Synthesis and Biological Evaluation of Novel Pyrimidine Derivatives as Potential Anticancer agents

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A B S T R A C T

A new series of novel pyrimidine derivatives (2-30)wereobtained from propylthiouracil (1) and evaluated for antitumour activity. The newly synthesized compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR, MS and elemental analysis. Eight of the synthesized compounds were selected and tested by National Cancer Institute (NCI), USA, for anticancer activity against 60 different human tumour cell lines. Among the compounds tested, 2-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-propyl-3,4-dihydropyrimidin-4-one (26)(NSC 771835)was found to be the most active candidate of the series at fivedose level screening with no selectivity towards any cell panels.

Keywords: Antitumour agents, Pyrimidine derivative, Propylthiouracil, NCI-USA

A R T I C L E I N F O

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1. Introduction

A recent World Health Organization (WHO) report states that by the year 2030 the incidence of cancer worldwide will grow by approximately 75%, doubling in some of the developing countries [1]. The term cancer encompasses a wide range such as CNS cancer, ovarian cancer, renal cancer, breast cancer and colon cancer. Malignant cancers are very common and are the second largest cause of death in the West after cardiovascular disease. Treating such cancers is one of the major challenges of this century and is a concern for medical communities all over the world. The diversity of tumour types and their great similarity to normal cells are the main obstacles that prevent the discovery of a cure [2-7]. Chemotherapy is one of the medical options for cancer management. Antineoplastic drugs in medical use can elicit their cytotoxic activity by impairing cellular mitosis via numerous possible mechanisms of actions being either antimetabolites, alkylating agents and topoisomerase inhibitors or, most recently, signal transduction inhibitors [8,9].

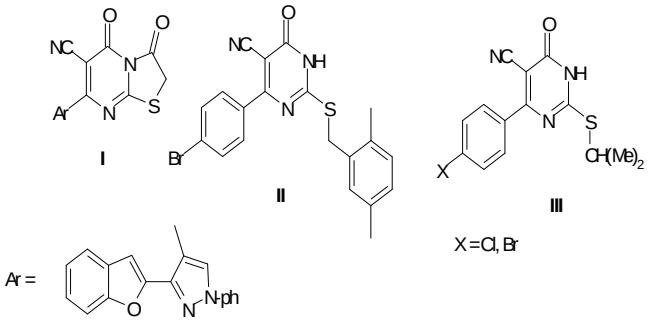


Figure. 1. Structures of some potent 5-cyano-2-thiouracils.

Many pyrimidine- and fused pyrimidine- based vascular endothelial growth factor receptor (VEGFR) and cellular-Src (c-Src) inhibitors were approved by the Food and Drug Administration (FDA) as first and second line cancer therapy agents against breast cancer, bone cancer, prostate cancer, acute lymphocytic leukemia and other types of cancer [10,11]. Among the numerous known examples are the pyrimidine (imatinib, dasatinib, nilotinib and pazopanib) and pyrrole (sunitinib) tyrosine kinase inhibitors (TKIs),which are in clinical use as anticancer agents due to their high activity towards several families of receptor and non-receptor tyrosine[12,13].

Pyrimidineplays a vital role in metabolic functions serving as a moiety of biomolecules, e.g., nucleic acids, as well as key building blocks for pharmaceuticals such asantiviral and antitumour [14-30].Similarly, the related thiouracil derivatives are potential therapeutics as antiviral, antioxidant and anticancer [31-34]. Moreover, a literature survey revealed that the thiouracil carbonitrile ring system has occupieda marked position in the design and synthesis of novel chemotherapeutic agents with remarkable antitumour**I**, hepatitis C virus (HCV) inhibitors **II** and antimicrobial activities **III** (figure. 1) [35-37]. Furthermore, 4-hydrazinothiopyrimidine-5-carbonitriles were synthesized from 4-chloro derivatives [38,39]. These hydrazine derivatives exerted promising antibacterial, antifungal and anticancer activities [40-42].In addition, the reactions of hydrazinopyrimidines with formic acid, triethylortho formate (TEOF) and CS_2(one carbon donor moieties) afforded the corresponding triazolo pyrimidines [43], which are known to exhibit interesting pharmaceutical activities. Depending upon the previously mentioned facts, the synthesis and *invitro* antitumour activity of new series of novel pyrimidine derivatives were selected as the subject of this research work.

2. Materials and Methods

2.1. Chemistry

All melting points were measured on a Gallenkamp melting point apparatus (Weiss- Gallenkamp, London, UK). IR spectra were recorded on KBr disks on a pye Unicam SP 3300 and Shimadzu FT IR 8101 PC Infrared Fourier Transform Spectrometer (pye Unicam Ltd. Cambridge, England and Shimizu, Tokyo, Japan, respectively). ^{1}HNMR and ^{13}CNMR spectra were recorded on Gemini 300 MHz,Chemical shifts were recorded in ppm (δ) from an internal tetramethyl-silane standard in deuterochloroform or deuterodimethyl sulfoxide as specified below. Elemental analysis (C, H and N) was performed by a VarioIIICHN analyzer (Germany) on Micro-analytical Centre of Cairo University, Giza, Egypt. All compounds were within ± 0.4% of the theoretical values. Mass spectra were recorded on a DI analysis Shimizu QP-2010 plus mass spectrometer. TLC experiments were performed on 0.2 mm Merck pre-coated Silica gel 60 F254 aluminium sheets and the spots were visualized under a UV lamp. Propylthiouracil (**1**) was purchased from Sigma-Aldrich. The chemical reagents used in synthesis were purchased from Fluka, Merck and Sigma-Aldrich.

Synthesis of 2-(benzylsulfanyl)-6-propyl-3,4-dihydro pyrimidin-4-one (2)

A mixture of propylthiouracil (**1**) (2.38 g, 14 mmol) and NaH (0.33 g, 14 mmol) in dry DMF (20 mL) was stirred. The colour of the mixture changed and hydrogen gas was evolved for 30 min.Benzyl bromide (3.59 g, 21 mmol) was then added. The mixture was heated under reflux for 5 h, at 90 ºC, cooled to room temperature and poured into icewater. The precipitate formed was filtered, washed, dried and crystallized from ethylacetate/petroleum ether mixture.Yield (2.47 g, 68%); mp: 118-119 ºC; IR (KBr) γ /cm^{-1}: 3438 (NH), 2960 (C-HAr), 1663 (C=O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ⁄ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.52-1.66 (m, 2H, CH_2), 2.40 (t, 2H, CH_2, J=7.43 Hz), 3.35 (s, 1H, NH, D_2O exchangeable), 4.38 (s, 2H, SCH_2), 5.37 (s, 1H, CH Pyrimidine), 7.32-7.43 (m, 5H, Ar-H); ^{13}CNMR (DMSO- d_6) δ⁄ppm:13.44 (CH_3), 20.36 (CH_2), 33.49 (SCH_2), 35.59 (CH_2), 106.80 (C5 Pyrimidine), 126.72-128.99 (Ar-C), 135.23, 161.29, 165.17 (N=C-NH), 171.48 (C=O) ppm; Anal. Calc. for $C_{14}H_{16}N_2OS$ (260.35): C, 64.58%; H, 6.19%; N, 10.76%. Found: C, 64.50%; H,

6.43%; N, 10.67%; MS m/z: 260.12 (M^+, 36.90), 227.00 (21.30), 149.00 (17.20), 91.00 (100.00).

Synthesis of 2-hydrazinyl-6-propyl-3, 4-dihydro pyrimidin-4-one (3).

A solution of propylthiouracil (**1**) or 2-(benzylsulfanyl)-6-propyl-3,4-dihydropyrimidin-4-one (**2**) (1.70 g or 2.60 g, 10mmol) in methanol (30 mL) and hydrazine hydrate (10 mL) was heated under reflux for 3 h.On cooling, the solid product was filtered off, dried and crystallized from methanol.

Yield (1.22 g, 73%); mp: 203-204 ºC; IR (KBr) γ /cm^{-1}: 3347 (NH), 3200 (NH_2), 1665 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.85 (t, 3H, CH_3,J=6.63 Hz), 1.50-1.62 (m, 2H, CH_2),1.97 (s, 2H, NH_2,D_2O exchangeable), 2.21 (t, 2H, CH_2, J=7.43 Hz), 3.24 (s, 1H, NH, D_2O exchangeable),5.23 (s, 1H, NH,D_2O exchangeable), 5.36 (s, 1H, CH Pyrimidine); ^{13}CNMR (DMSO-d_6) δ / ppm: 13.46 (CH_3), 20.69 (CH_2), 39.51 (CH_2), 99.32 (C5 Pyrimidine), 157.16 (N=C-NH), 162.52 (C=O) ppm, Anal. Calc. for $C_7H_{12}N_4O$ (168.19): C, 49.99%; H, 7.19%; N, 33.31%. Found: C, 49.60%; H, 7.43%; N, 33.67; MS m/z: 168.80 (M^+, 5.20), 123.00 (38.10), 110.00 (100.00).

Synthesis of 6-propyl-1, 2, 3, 4-tetrahydropyrimidin-2, 4-dithione (4).

A mixture of propyl thiouracil (**1**)(1.70 g, 10mmol) and P_2S_5 (4.44 g, 20mmol) in dry pyridine (20 mL) was heated under reflux for 2 h, allowed to cool and poured into icewater.The precipitate formed was filtered, dried and then crystallized from DMF.Yield (1.39 g, 75%); mp: 215-216 ºC; IR (KBr) γ /cm^{-1}: 3438 (NH), 1561 (C=S); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.85 (t, 3H, CH_3,J=6.63 Hz), 1.50-1.62 (m, 2H, CH_2), 2.21 (t, 2H, CH_2, J=7.43 Hz), 5.61 (s, 1H, CH Pyrimidine), 8.88 (s, 1H, NH,D_2O exchangeable), 9.20 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_7H_{10}N_2S_2$ (186.29): C, 45.13%; H, 5.41%; N, 15.04%. Found: C, 44.80%; H, 5.43%; N, 15.37%; MS m/z: 186.00 (M^+, 50.40), 125.21(31.13), 110.24(100.00).

Synthesis of 2-(benzylsulfanyl)-6-propyl-3,4-dihydro pyrimidin-4-thione (5)

A mixture of 2-(benzylsulfanyl)-6-propyl-3,4-dihydropyrimidin-4-one (**2**) (2.60 g, 10 mmol) and P_2S_5 (4.44 g, 20mmol) in dry pyridine (20 mL) was heated under reflux for 2 h, allowed to cool and poured into icewater. The precipitate formed was filtered, dried and crystallized from DMF.

Yield (1.87 g, 68%); mp: 112-113 ºC; IR (KBr) γ /cm^{-1}: 3438 (NH), 1561 (C= S); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.52-1.71 (m, 2H, CH_2), 2.40 (t, 2H, CH_2, J=7.43 Hz), 3.25 (s, 1H, NH, D_2O exchangeable), 4.38 (s, 2H, SCH_2), 5.37 (s, 1H, CH Pyrimidine), 7.16-7.43 (m, 5H, Ar-H);Anal. Calc. for $C_{14}H_{16}N_2S_2$ (276.42): C, 60.83%; H, 5.83%; N, 10.13%. Found: C, 60.50%; H, 5.53%; N, 10.37%; MS m/z: 276.14 (M^+, 36.91), 227.43(21.31), 149.53(17.25), 91.00(100.00).

Synthesis of 7-propyl-2*H*, 3*H*, 5*H*-[1,3] thiazolo[3,2-*a*] pyrimidine-3,5-dione (6)

A mixture of propylthiouracil (**1**) (1.70 g, 10mmol) and chloroacetyl acetate (1.84 g, 15mmol) in absolute ethanol (20 mL) with a few drops of triethylamine was heated under reflux for 3 h. The solvent was removed under vacuum.The residual solid was filtered, dried and crystallized from ethanol. Yield (2.73 g, 65%); mp: 230-231 ºC; IR (KBr) γ /cm^{-1}: 1649 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.92 (t, 3H, CH_3,J=6.63 Hz), 1.69-1.96 (m, 2H, CH_2), 2.66 (t, 2H, CH_2, J=7.43 Hz), 4.13 (s, 2H, CH_2), 5.74 (s, 1H, CH Pyrimidine); Anal. Calc. for $C_9H_{10}N_2O_2S$ (210.26): C, 51.40%; H, 4.76%; N, 13.30%. Found: C, 51.50%; H, 5.02%; N, 13.67%; MS m/z: 210.50 (M^+, 15.00), 182.70 (100.00).

General procedure for the synthesis of 7-9

To a solution of the propylthiouracil (**1**) (1.70 g, 10mmol) in aqueous potassium hydroxide [0.36 g, 10mmole in distilled water (16 mL)] was added a solution of aryl bromide (10 mmol) in methanol (20 mL). The mixture was stirred at room temperature until reaction was judged complete by TLC.The mixture was poured into icewater, and the precipitate formed was filtered, washed, dried and crystallized from methanol.

2-[(2-Oxo-2-phenylethyl)sulfanyl]-6-propyl-3,4-dihydropyrimidin-4-one (7)

Yield (2.30 g, 80%); mp: 180-181 ºC; IR (KBr) γ /cm^{-1}: 3434 (NH), 1644 (C=O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm : 0.68 (t, 3H, CH_3,J=6.63 Hz), 1.59-1.63 (m, 2H, CH_2), 2.08 (t, 2H, CH_2, J=7.43 Hz), 3.25 (s, 1H, NH,D_2O exchangeable), 4.71 (s, 2H, SCH_2), 5.91 (s, 1H, CH Pyrimidine), 7.48-7.76 (m, 5H, Ar-H); ^{13}CNMR (DMSO-d_6) δ / ppm: 13.22 (CH_3), 20.31 (CH_2), 37.24 (-CH_2-), 40.35 (SCH_2), 124.36 (CH Pyrimidine), 128.13 - 128.60 (Ar-C), 133.29 (N=C-NH), 136.14 (C=O), 193.46 (SCH_2 C=O) ppm;Anal. Calc. for $C_{15}H_{16}N_2O_2S$ (288.36): C, 62.48%; H, 5.59%; N, 9.71%. Found: C, 62.50%; H, 5.43%; N, 9.67%; MS m/z: 290.00 (M^++2, 1.30), 289.10 (M^++1, 0.90), 287.80 (M^+, 5.30), 255.80 (2.10), 105.00 (100.00).

2-[{2-(4-Methylphenyl)2-oxo-ethyl}sulfanyl]-6-propyl-3,4-dihydropyrimidin-4-one (8)

Yield (2.47 g, 82%); mp: 170-171 ºC; IR (KBr) γ /cm^{-1}: 3434 (NH), 1645 (C=O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.68 (t, 3H, CH_3,J=6.63 Hz), 1.59-1.63 (m, 2H, CH_2), 2.33 (s, 3H, CH_3), 2.49 (t, 2H, CH_2, J=7.43 Hz),3.27 (s, 1H, NH,D_2O exchangeable), 4.73 (s, 2H, SCH_2), 5.91 (s, 1H, CH Pyrimidine), 7.48 -7.76 (m, 4H, Ar-H);Anal. Calc. for $C_{16}H_{18}N_2O_2S$ (302.39): C, 63.55%; H, 6.00%; N, 9.26%. Found: C, 63.50%; H, 5.83%; N, 9.47%; MS m/z: 302.12 (M^+, 3.80), 284.20 (3.10), 269.80 (5.00), 118.80 (100.00).

2-[{2-(4-Bromophenyl)2-oxo-ethyl}sulfanyl]-6-propyl-3,4-dihydropyrimidin-4-one (9)

Yield (2.93 g, 80%); mp: 190-191 ºC; IR (KBr) γ /cm^{-1}: 3434 (NH), 1644 (C=O), 1596-1484(C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.68 (t, 3H, CH_3,J=6.63 Hz), 1.59-1.63 (m, 2H, CH_2), 2.08 (t, 2H, CH_2, J=7.43 Hz), 3.23 (s, 1H, NH,D_2O exchangeable), 4.74 (s, 2H, SCH_2), 5.91 (s, 1H, CH Pyrimidine), 7.48-7.76 (m, 5H, Ar-H);Anal. Calc. for $C_{15}H_{15}N_2O_2SBr$ (367.26): C, 49.06%; H, 4.12%; N,

7.63%. Found: C, 49.20%; H, 4.43%; N, 7.67%; MS m/z: 367.70 (M^+, 10.90), 335.80 (7.90), 183 (100.00).

Synthesis of 5-propyl-3*H*,7*H*-[1,2,3,4]tetrazolo[1,5-*a*]pyrimidin-7-one (10)

A mixture of compound **3** (1.68 g, 10mmol) in dilute HCl (10 mL) andsolution ofsodium nitrite(0.67 g, 10mmol) in water (3 mL) was stirred for 1 h. in an ice bath. The formed precipitate was filtered off and recrystallized from methanol.Yield (1.30 g, 73%); mp: 165-166 ºC; IR (KBr) γ /cm^{-1}: 3400 (NH), 1698 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.86 (t, 3H, CH_3,J=6.63 Hz), 1.57-1.69 (m, 2H, CH_2), 2.42 (t, 2H, CH_2, J=7.43 Hz),3.36 (s,1H, NH,D_2O exchangeable), 5.67 (s, 1H, CH Pyrimidine); Anal. Calc. for $C_7H_9N_5O$ (179.17): C, 46.92%; H, 5.06%; N, 39.09%. Found: C, 46.60%; H, 5.23%; N, 39.17%; MS m/z: 179.70 (M^+, 35.00), 151.40 (100.00).

Synthesis of 5-propy-3*H*, 7*H*-[1,2,4]triazolo[1,5-*a*] pyrimidin-7-one (11).

A mixture of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (2.52 g, 15 mmol) and formic acid (85%) (20mL) was heated under reflux for 6-8 h.The reaction mixture was cooled and the separated solid was filtered, washed with ethanol, dried and crystallized from ethanol.

Yield (1.73 g, 65%); mp: 148-149 ºC; IR (KBr) γ /cm^{-1}: 3400 (NH), 1712 (C= O); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.86 (t, 3H, CH_3,J=6.63 Hz), 1.57-1.69 (m, 2H, CH_2), 2.42 (t, 2H, CH_2, J=7.43 Hz),3.32 (s,1H, NH,D_2O exchangeable), 5.57 (s, 1H, CH Pyrimidine), 6.50 (s,1H, CH);Anal. Calc. for $C_8H_{10}N_4O$ (178.19): C, 53.92%; H, 5.66%; N, 31.44%. Found: C, 53.70%; H, 5.43%; N, 31.67%; MS m/z: 178.00 (M^+, 41.80), 149.80 (100.00).

Synthesis of 3-methyl-7-propyl-3*H*, 5*H*-[1,2,4] triazolo [4,3-*a*]pyrimidin-5-one (12)

A mixture of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (1.68 g, 10 mmol), acetic acid (20 mL) and acetic anhydride (10 mL) was heated under reflux for 3-5 h.The reaction mixture was cooled and the separated solid was filtered, washed with ethanol, dried and crystallized from ethanol.Yield (1.05 g, 55%); mp: 198-199 ºC; IR (KBr) γ /cm^{-1}: 3400 (NH), 1715 (C= O); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.86 (t, 3H, CH_3,J=6.63 Hz), 1.57-1.69 (m, 2H, CH_2), 2.42 (t, 2H, CH_2, J=7.43 Hz), 2.68 (s, 3H, CH_3),3.38 (s,1H, NH,D_2O exchangeable), 5.51 (s, 1H, CH Pyrimidine); ^{13}CNMR (DMSO-d_6) δ / ppm: 13.22 (CH_3), 13.29 (CH_3), 20.98 (CH_2), 36.84 (-CH_2-), 95.96 (C5 Pyrimidine), 143.17, 150.35, 157.56 (N=C-NH), 164.47 (C=O) ppm;Anal. Calc. for $C_9H_{12}N_4O$ (192.21): C, 56.24%; H, 6.29%; N, 29.15%. Found: C, 56.50%; H, 6.43%; N, 29.47%; MS m/z: 192.00 (M^+, 4.30), 149.00 (5.60), 104.00 (100.00).

Synthesis of 7-propyl-3-sulfanylidene-3*H*,5*H*-[1,2,4] triazolo [4,3-*a*]pyrimidin-5-one (13)

A solution of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (3.36 g, 20mmol) in ethanol (50 mL), was added to a solution of potassium hydroxide [(0.72 g, 20mmol) dissolved in water (2 mL)] and carbon disulfide (5 mL) was heated under reflux for 15 h.The solvent was evaporated and the residue was dissolved in water, filtered off and acidified with dilute HCl. The formed precipitate was filtered off, washed with water and crystallized from ethanol.Yield (2.43 g, 58%); mp: 236-237 ºC; IR (KBr) γ /cm^{-1}: 1672 (C= O), 1394 (C=S); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.93 (t, 3H, CH_3,J=6.63 Hz), 1.57-1.69 (m, 2H, CH_2), 2.42 (t, 2H, CH_2, J=7.43 Hz), 5.79 (s, 1H, CH Pyrimidine);Anal. Calc. for $C_8H_8N_4OS$ (208.24): C, 46.14%; H, 3.87%; N, 26.9%. Found: C, 46.50%; H, 3.53%; N, 26.67%; MS m/z: 210.00 (M^++2, 94.30), 209.00 (M^++1, 42.70), 208.00 (M^+, 18.50), 177.00 (100.00).

General procedure for the synthesis of compounds 14-18

Equimolar amount of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) and the appropriate aldehyde (10mmol) in methanol (50 mL) in the presence of a catalytic amount of glacial acetic acid was heated under reflux for 3 h.The reaction mixture was cooled,and the separated solid was filtered, washed with methanol, dried and crystallized from methanol.

2-[-2-(Phenylmethylidene)hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (14)

Yield (1.66 g, 65%); mp: 120-121 ºC; IR (KBr) γ /cm^{-1}: 3346 (NH), 1667 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.54-1.64 (m, 2H, CH_2), 2.26 (t, 2H, CH_2, J=7.43 Hz),3.43 (s, 1H, NH,D_2O exchangeable),5.49 (s, 1H, CH Pyrimidine), 6.76-7.59 (m, 5H, Ar-H), 8.30 (s, 1H, N=CH), 9.55 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{14}H_{16}N_4O$ (256.30): C, 65.61%; H, 6.29%; N, 21.86%. Found: C, 65.50%; H, 6.43%; N, 21.67%; MS m/z: 258.00 (M^+ +2, 1.60),257.00 (M^+ +1, 12.60), 256.00 (M^+, 79.00), 228.00 (22.00), 179.00 (72.40), 125.00 (100.00).

2-{2-[(4-Methoxyphenyl)methylidene]hydrazin-1-yl}-6-propyl-3,4-dihydropyrimidin-4-one (15)

Yield (1.85 g, 65%); mp: 160-161 ºC; IR (KBr) γ /cm^{-1}: 3434 (NH), 1675 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.54-1.66 (m, 2H, CH_2), 2.26 (t, 2H, CH_2, J=7.43 Hz),3.41 (s, 1H, NH,D_2O exchangeable),3.87 (s, 3H, OCH_3), 5.43 (s, 1H, CH Pyrimidine), 6.76-7.59 (m, 4H, Ar-H), 7.98 (s, 1H, N=CH), 9.67 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{15}H_{18}N_4O_2$ (286.32): C, 62.92%; H, 6.34%; N, 19.57%. Found: C, 62.70%; H, 6.43%; N, 19.67%; MS m/z (%): 288.15 (M^+ +2, 1.22), 286.50 (M^+, 13.84), 152.50 (13.28), 133.65 (52.57), 90.55 (100.00).

2-{2-[(4-Hydroxyphenyl) methylidene] hydrazin-1-yl}-6-propyl-3,4-dihydropyrimidin-4-one (16)

Yield (1.76 g, 65%); mp: 158-159 ºC; IR (KBr) γ /cm^{-1}: 3425 (NH), 1663 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.54-1.66 (m, 2H, CH_2), 2.26 (t, 2H, CH_2, J=7.43 Hz),3.41 (s, 1H, NH,D_2O exchangeable),5.43 (s, 1H, CH Pyrimidine), 6.76-7.59 (m, 4H, Ar-H), 7.91 (s, 1H, N=CH), 9.79 (s, 1H, NH,D_2O exchangeable), 11.27 (s, 1H, OH,D_2O exchangeable);Anal. Calc. for $C_{14}H_{16}N_4O_2$ (272.30): C, 61.75%; H, 5.92%; N, 20.58%. Found: C, 61.50%; H, 5.63%; N, 20.67%; MS m/z: 272.35 (M^+, 94.79), 255.75 (38.14), 137.65 (35.49), 77.10 (100.00).

2-{2-[(4-Hydroxy-3-methoxyphenyl) methylidene] hydrazine-1-yl}-6-propyl-3,4-dihydropyrimidin-4-on(17)

Yield (1.96 g, 65%); mp: 108-109 ºC; IR (KBr) γ /cm^{-1}: 3419 (NH), 1641 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz),

1.54-1.66 (m, 2H, CH_2), 2.26 (t, 2H, CH_2, J=7.43 Hz),3.43 (s, 1H, NH,D_2O exchangeable),3.84 (s, 3H, OCH_3), 5.47 (s, 1H, CH Pyrimidine), 6.76-7.59 (m, 3H, Ar-H), 7.94 (s, 1H, N=CH), 9.73 (s, 1H, NH,D_2O exchangeable), 11.27 (s, 1H, OH,D_2O exchangeable);^{13}CNMR (DMSO-d_6) δ / ppm: 13.46 (CH_3), 20.60 (CH_2), 38.14 (CH_2), 55.91 (OCH_3), 100.65 (C5Pyrimidine), 110.05-125.81 (Ar-C), 144.77, 147.94, 148.57, 152.45(N=C-NH), 171.86 (C=O) ppm;Anal. Calc. for $C_{15}H_{18}N_4O_3$ (302.32): C, 59.59%; H, 6.00%; N, 18.53%.Found: C, 59.50%; H, 5.80%; N, 18.67%; MS m/z: 302.40 (M^+, 10.01), 165.10 (15, 01), 149.45 (34.38), 51.45 (100.00).

2-[2-(Glucosemethylidene)hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (18): Yield (2.14 g, 65%); mp: 173-174 ºC; IR (KBr) γ /cm^{-1}: 4440 (OH), 3419 (NH), 1641 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ/ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.54-1.66 (m, 2H, CH_2), 2.26 (t, 2H, CH_2, J=7.43 Hz),3.43 (s, 1H, NH,D_2O exchangeable),3.60 (m, 1H, CH), 3.69 (d, 1H, CH, J=5.43 Hz), 3.72 (d, 2H, CH_2, J=6.43 Hz), 4.15 (d, 1H, CH, J=5.43 Hz), 4.22 (s, 1H, OH,D_2O exchangeable), 4.49 (s, 1H, OH,D_2O exchangeable), 4.51 (s, 1H, OH,D_2O exchangeable), 5.04 (s, 1H, OH,D_2O exchangeable), 5.08 (s, 1H, OH,D_2O exchangeable), 5.44 (t, 1H, CH, J=4.53 Hz), 5.49 (s, 1H, CH Pyrimidine), 7.90 (d, 1H, N=CH, J=5.53 Hz), 9.17 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{13}H_{22}N_4O_6$(330.01): C, 47.27%; H, 6.60%; N, 16.96%. Found: C, 47.50%; H, 6.80%; N, 16.67%; MS m/z: 329.95 (M^+, 0.02), 137.30 (14.20), 124.25 (33.81), 52.70 (100.00).

General procedure for the synthesis of compounds 19-24

Equimolar amount of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**), and the appropriate ketone (10mmol) in methanol (50 mL) in the presence of a catalytic amount of glacial acetic acid were heated under reflux for 3 h.The reaction mixture was cooled, and the separated solid was filtered, dried and crystallized from methanol.

2-[2-(1-Phenylethylidene) hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (19).

Yield (1.75 g, 65%); mp: 135-136 ºC; IR (KBr) γ /cm^{-1}: 3433 (NH), 1661 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ/ppm: 0.91 (t, 3H, CH_3,J=6.63 Hz), 1.55-1.67 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.33 (t, 2H, CH_2, J=7.43 Hz), 5.45 (s, 1H, CH Pyrimidine), 7.37-8.01 (m, 5H, Ar-H), 9.81 (s, 1H, NH,D_2O exchangeable), 11.28 (s, 1H, NH,D_2O exchangeable);^{13}CNMR (DMSO-d_6) δ / ppm: 13.38 (CH_3), 14.09 (N=CCH_3), 20.94 (CH_2), 36.88 (CH_2), 126.46 (C5Pyrimidine), 128.02-128.79 (Ar-C), 143.17, 150.35, 157.56 (N=C-NH), 164.47 (C=O) ppm;Anal.Calc.for$C_{15}H_{18}N_4O$ (270.32): C, 66.64%; H, 6.71%; N, 20.73%. Found: C, 66.50%; H, 6.43%; N, 20.67%; MS m/z: 270.90 (M^+, 1.67), 132.55 (14.62), 117.75 (17.13), 103.15 (86.15), 77.00 (100.00).

2-[2-(1(4-Methylphenyl)ethylidene)hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (20).

Yield (1.84 g, 65%); mp: 196-197 ºC; IR (KBr) γ /cm^{-1}: 3445 (NH), 1663 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ/ppm: 0.91 (t, 3H, CH_3,J=6.63 Hz), 1.55-1.67 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.34 (t, 2H, CH_2, J=7.43 Hz), 5.43 (s, 1H, CH Pyrimidine), 7.37-8.01 (m, 4H, Ar-H), 9.55 (s, 1H, NH,D_2O exchangeable), 9.86 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{16}H_{20}N_4O$ (284.35): C, 67.58%; H, 7.09%; N, 19.70%. Found: C, 67.50%; H, 7.43%; N, 19.67%; MS m/z: 286.55 (M^++2, 0.13), 284.85 (M^+, 0.13), 147.55 (10.16), 117.25 (90.18), 51.00 (100.00).

2-[2-(1(4-Hydroxyphenyl)ethylidene)hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (21)

Yield (1.85 g, 65%); mp: 246-247 ºC; IR (KBr) γ /cm^{-1}: 3500 (OH), 3445 (NH), 1663 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.91 (t, 3H, CH_3,J=6.63 Hz), 1.55-1.67 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.33 (t, 2H, CH_2, J=7.43 Hz), 5.45 (s, 1H, CH Pyrimidine), 7.37-8.01 (m, 4H, Ar-H), 8.31 (s, 1H, OH,D_2O exchangeable), 9.78 (s, 1H, NH,D_2O exchangeable), 9.92 (s, 1H, NH,D_2O exchangeable); Anal. Calc. for $C_{15}H_{18}N_4O_2$ (286.32): C, 62.92%; H, 6.34%; N, 19.57%. Found: C, 62.80%; H, 6.43%; N, 19.67%; MS m/z: 286.65 (M^+, 95.71), 271.65 (66.97), 146.10 (79.88), 58.75 (100.00).

2-[2-(1(4-Bromophenyl) ethylidene) hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (22)

Yield (2.26 g, 65%); mp: 203-204 ºC ; IR (KBr) γ /cm^{-1}: 3419 (NH), 1649 (C= O), 1596-148(C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ/ppm: 0.91 (t, 3H, CH_3,J=6.63 Hz), 1.55-1.67 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.35 (t, 2H, CH_2, J=7.43 Hz), 5.45 (s, 1H, CH Pyrimidine), 7.37-8.01 (m, 4H, Ar-H), 9.65 (s, 1H, NH,D_2O exchangeable), 9.91 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{15}H_{17}N_4OBr$ (349.22): C, 51.59%; H, 4.91%; N, 16.04%. Found: C, 51.50%; H, 4.63%; N, 16.17%; MS m/z: 352.15 (M^++2, 1.30), 349.55 (M^+, 22.88), 181.90 (19.97), 102.50 (74.05), 66.90 (100.00).

2-[2-(1(4-Aminophenyl)ethylidene)hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (23)

Yield (1.85 g, 65%); mp: 217-218 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 3333 (NH_2), 1661 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.91 (t, 3H, CH_3,J=6.63 Hz), 1.55-1.67 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.35 (t, 2H, CH_2, J=7.43 Hz), 4.54 (s, 2H, NH_2,D_2O exchangeable), 5.41 (s, 1H, CH Pyrimidine), 7.37-8.01 (m, 4H, Ar-H), 9.60 (s, 1H, NH,D_2O exchangeable), 10.38 (s, 1H, NH,D_2O exchangeable); Anal. Calc. for $C_{15}H_{19}N_5O$ (285.34): C, 63.14%; H, 6.71%; N, 24.54%. Found: C, 63.30%; H, 6.43%; N, 24.67%; MS m/z: 285.05 (M^+, 0.68), 117.90 (100.00).

2-[2-(Butan-2-ylidene) hydrazin-1-yl]-6-propyl-3,4-dihydropyrimidin-4-one (24).

Yield (1.44 g, 65%); mp: 160-161 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 1659 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ/ppm: 0.91 (t, 3H, CH_3,J=6.63 Hz), 1.55-1.67 (m, 2H, CH_2), 1.80 (t, 3H, CH_3, J=7.00 Hz), 2.08 (s, 3H, CH_3), 2.20-3.32 (m, 2H, CH_2), 2.37 (t, 2H, CH_2, J=7.43 Hz), 5.48 (s, 1H, CH Pyrimidine), 9.62 (s, 1H, NH,D_2O exchangeable), 9.96 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{11}H_{18}N_4O$ (222.28): C, 59.44%; H, 8.16%; N, 25.20%. Found: C, 59.50%; H, 8.43%; N, 25.67%; MS m/z: 222.45 (M^+, 2.01), 67.35 (83.62), 54.55 (100.00).

Synthesis of 2-(5-amino-3-oxo-2,3-dihydro-1*H*-pyrazol-1-yl)-6-propyl-3,4-dihydropyrimidin-4-one (25).

A mixture of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (1.68 g, 10 mmol) and ethyl cyanoacetate (1.13 g, 10mmol)in dioxane (20 mL) and a few drops of triethylamine was heated under reflux for 4 h. The reaction mixture was then concentrated, cooled to room temperature, and the formed precipitate was filtered off and crystallized from ethanol.Yield (1.52 g, 65%); mp: 145-146 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 3347 (NH_2), 1652 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.50-1.62 (m, 2H, CH_2), 2.25 (t, 2H, CH_2, J=7.43 Hz),3.29 (s, 1H, H Pyrazol), 3.57 (s, 2H, NH_2,D_2O exchangeable), 5.36 (s, 1H, CH Pyrimidine), 8.32 (s, 1H, NH,D_2O exchangeable), 8.64 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{10}H_{13}N_5O_2$ (235.24): C, 51.06%; H, 5.57%; N, 29.77%. Found: C, 51.20%; H, 5.43%; N, 29.67%; MS m/z (%): 235.05 (M^+, 5.38), 179.75 (10.09), 76.15 (87.50), 49.75 (100.00).

Synthesis of 2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-propyl-3,4-dihydropyrimidin-4-one (26) .

A mixture of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (1.68 g, 10mmol) and acetylacetone(1.00 g, 10mmol) in dioxane (20 mL) and a few drops of triethylamine was heated under reflux for 4 h. The reaction mixture was then concentrated and cooled to room temperature, and the formed precipitate was filtered off and crystallized from ethanol.Yield (1.50 g, 65%); mp: 123-124 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 1652 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.50-1.62 (m, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.55 (s, 3H, CH_3) 2.57 (t, 2H, CH_2, J=7.43 Hz),3.29 (s, 1H, H Pyrazol), 5.36 (s, 1H, CH Pyrimidine), 8.64 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{12}H_{16}N_4O$ (232.28): C, 62.05%; H, 6.94%; N, 24.12%. Found: C, 62.30%; H, 6.73%; N, 24.27%; MS m/z (%): 232.80 (M^+, 47.10), 204.00 (100.00).

Synthesis of 2-(3,5-diamino-1*H*-pyrazol-1-yl)-6-propyl-3,4-dihydropyrimidin-4-one (27).

A mixture of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (1.68 g, 10mmol) and malononitrile(0.66 g, 10mmol) in dioxane (20 mL) and a few drops of triethylamine was heated under reflux for 4 h. The reaction mixture was then concentrated and cooled to room temperature, and the formed precipitate was filtered off and crystallized from ethanol.Yield (1.52 g, 65%); mp: 155-156 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 3347 (NH_2), 1653 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.50-1.62 (m, 2H, CH_2), 2.25 (t, 2H, CH_2, J=7.43 Hz),3.29 (s, 1H, H Pyrazol), 3.57 (s, 2H, NH_2,D_2O exchangeable),5.36 (s, 1H, CH Pyrimidine), 5.43 (s, 2H, NH_2,D_2O exchangeable),8.64 (s, 1H, NH,D_2O exchangeable); ^{13}CNMR (DMSO-d_6) δ / ppm: 13.48 (CH_3), 20.69 (CH_2), 38.67 (CH_2), 99.27 (=CH), 125.81 (C5 Pyrimidine), 144.77 (N=C-NH), 152.45 (=C-NH), 157.16 (NH_2-C=N), 169.47 (C=O) ppm; Anal. Calc. for $C_{10}H_{14}N_6O$ (234.25): C, 51.27%; H, 6.02%; N, 35.88%. Found: C, 51.30%; H, 6.02%; N, 35.67%; MS m/z (%): 234.00 (M^+, 0.13), 230.65(0.13), 140.20 (20.15), 95.25 (15.49), 51.65 (100.00).

Synthesis of3-[2-(4-oxo-6-propyl-3,4-dihydropyrimidin-2-yl)hydrazine-1-yldene] propanenitrile (28).

A mixture of triethylamine (10 mmol), acrylonitrile (1.59 g, 30 mmol) and 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (1.68 g, 10mmol) in absolute ethanol (20 mL) was refluxed for 3 h. The solvent was removed under vacuum, and the solid residue was crystallized from ethanol.

Yield (1.42 g, 65%); mp: 186-187 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 2360 (CN), 1647 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.50-1.62 (m, 2H, CH_2), 2.25 (t, 2H, CH_2, J=7.43 Hz), 3.55(d, 2H, CH_2, J=6.72 Hz), 5.52 (s, 1H, CH Pyrimidine), 7.18 (t, 1H, CH, J=6.73 Hz), 9.79 (s, 1H, NH,D_2O exchangeable), 10.29 (s, 1H, NH,D_2O exchangeable); Anal. Calc. for $C_{10}H_{13}N_5O$ (219.24): C, 54.78%; H, 5.98%; N, 31.94%. Found: C, 54.50%; H, 6.02%; N, 31.67%; MS m/z (%): 219.80 (M^+, 5.80), 140.70 (29.60), 135.00 (78.40), 76.80 (100.00).

Synthesis of 3-[(4-oxo-6-propyl-3,4-dihydropyrimidin-2-yl)amino]-1-phenyl-thiourea (29).

A mixture of triethylamine (10 mmol), phenyl isothiocyanate (3.97 g, 30mmol) and 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3)** (3.36 g, 20mmol) in absolute ethanol (20 mL) was refluxed for 3 h. The solvent was removed under vacuum, and the solid residue was crystallized from ethanol.Yield (3.93 g, 65%); mp: 202-203 ºC; IR (KBr) γ /cm^{-1}: 3449 (NH), 1649 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.89 (t, 3H, CH_3,J=6.63 Hz), 1.72-1.84 (m, 2H, CH_2), 2.58 (t, 2H, CH_2, J=7.43 Hz), 5.74 (s, 1H, CH Pyrimidine), 7.12-7.43 (m, 5H, Ar-H), 9.71 (s, 1H, NH,D_2O exchangeable), 10.41 (s, 1H, NH,D_2O exchangeable), 10.59 (s, 1H, NH,D_2O exchangeable), 10.63 (s, 1H, NH,D_2O exchangeable);Anal. Calc. for $C_{14}H_{17}N_5OS$ (303.38): C, 55.42%; H, 5.65%; N, 23.08%. Found: C, 55.50%; H, 5.42%; N, 23.17%; MS m/z: 303.40 (M^+, 20.34), 137.40 (15.26), 52.70 (100.00).

Synthesis of 4-phenyl-8-propyl-6*H*-pyrimido[2,1-*c*][1,2,4]triazin-6-one (30).

A mixture of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) (3.36 g, 20mmol) and phencyl bromide (1.99 g, 10mmol) was heated under reflux in dry ethanol in presence of a catalytic amount of triethylamine for 3 h. The excess of solvent was distilled off and the solid hydrbromide that separated was collected by filtration, suspended in water and neutralized by aqueous sodium carbonate solution.It was filtered, washed with water, dried and recrystallized from ethanol.

Yield (3.45 g, 65%); mp: 235-236 ºC; IR (KBr) γ /cm^{-1}: 1649 (C= O), 1596-1484 (C=C, C=N ring); ^{1}HNMR (DMSO-d_6) δ / ppm: 0.87 (t, 3H, CH_3,J=6.63 Hz), 1.54-1.66 (m, 2H, CH_2), 2.26 (t, 2H, CH_2, J=7.43 Hz), 4.71 (s, 1H, CHPyrimidine),5.97 (s, 1H, H Triazine), 7.48- 7.76(m, 5H, Ar-H); ^{13}CNMR (DMSO-d_6) δ / ppm:13.43(CH_3), 20.69 (CH_2), 38.66 (CH_2), 62.67 (=CH), 65.22 (C3Pyrimidine), 126.47-129.80 (Ar-C), 143.17 (C-Triazine), 157.56 (=C-N), 164.47 (C=O) ppm;Anal. Calc. for $C_{15}H_{14}N_4O$ (266.29): C, 67.65%; H, 5.30%; N, 21.04%. Found: C, 67.50%; H, 5.02%; N, 21.27%; MS m/z (%): 266.00 (M^+, 17.30),

238.60 (9.60), 210.60 (5.80), 137.80 (25.00), 124.90 (65.40), 102.00 (100.00).

2.2. Pharmacology

***In- vitro* cancer screen at NCI-USA**

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds that exhibit significant growth inhibition are evaluated against the 60 cell panel at five dose levels. The human tumour cell lines of the cancer-screening panel are grown in RPMI 1640 medium containing 5% foetal bovine serum and 2mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well micro titre plates in 100 µL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the micro titre plates are incubated at 37 ºC, 5% CO_2, 95% air and 100% relative humidity for 24h prior to addition of experiential drugs. After 24h, two plates of each cell line are fixed in situ with TCAto represent a measurement of the cell population for each cell line the time of drug addiction (Tz).

Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing50µg/ml gentamicin. Additional four-fold, 10-fold or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate micro titre wells already containing 100 µl of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48h at 37 ºC, 5% CO_2, 95% air and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 ºC.

The supernatant is discarded. Bound stain is subsequently solubilized with 10mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentration levels. Percentage growth inhibition is calculated as:

$[(Ti - Tz)/(C- Tz)] \times 100$ for concentrations for which $Ti >/= Tz$

$[(Ti – Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI_{50}) is calculated from $[(Ti - Tz)/(C- Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment compared with that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/(C- Tz)] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested [44-46].

3. Results and Discussion

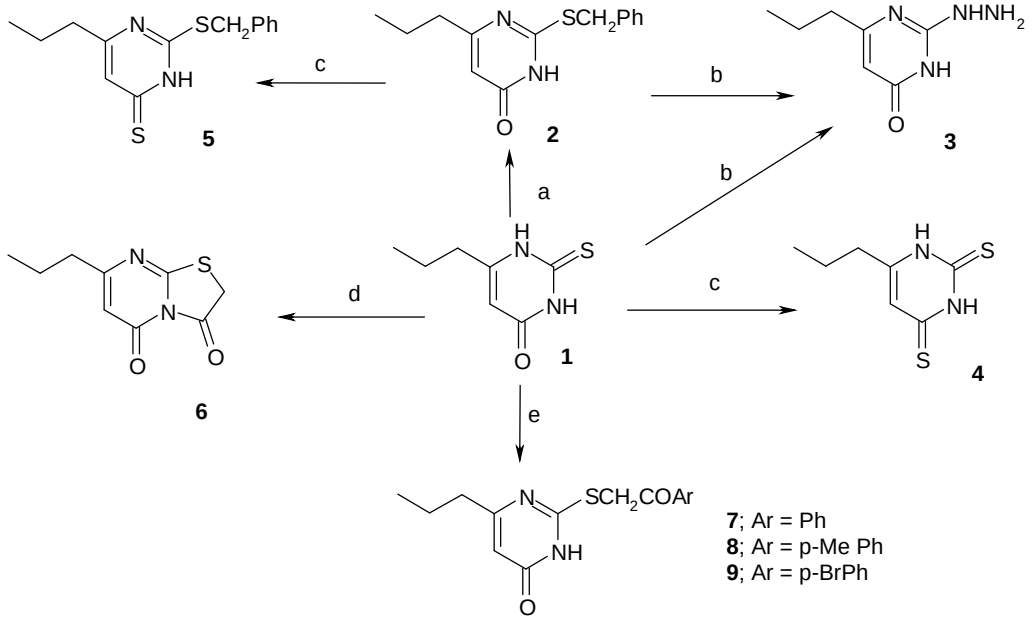
3.1. Chemistry

The synthetic pathways adopted for the synthesis of the novel pyrimidine derivatives are illustrated in schemes 1-3. Propylthiouracil (**1**)was found to be excellent building blocks for the synthesis of several heterocyclic ring systems.When**1** was treated with benzyl bromide, it gave *S*-substituted, thiouracil 2-(benzylsulfanyl)-6-propyl-3,4-dihydropyrimidin-4-one (**2**). Reacting propylthiouracil (**1)** with hydrazine hydrate afforded 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**). The structure of **3** was further confirmed by its alternate synthesis from the reaction of **2** with hydrazine hydrate.Refluxing propylthiouracil (**1**)and 2-(benzylsulfanyl)-6-propyl-3,4-dihydropyrimidin-4-one (**2**) with P_2S_5 gave **4** and **5,** respectively. Moreover, treatment of propylthiouracil (**1**)with chloroacetyl acetate, afforded 7-propyl-2*H*,3*H*,5*H*-[1,3]thiazolo[3,2-*a*]pyrimidine-3,5-dione (**6**). The 2-[(2-oxo-2-arylethyl) sulfanyl]-6-propyl-3,4-dihydropyrimidin-4-one (**7-9**) were obtained through reacting propylthiouracil (**1**) with phenacyl bromide derivatives (Scheme 1).

2-Hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**) was used as key compound for synthesis of other fused heterocyclic. In this investigation, the reaction of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin -4-one (**3**) with aqueous solution ofsodium nitrite resulted in 5-propyl-3*H*,7*H*-[1,2,3,4] tetrazolo [1,5-*a*]pyrimidin-7-one (**10**). Cyclization of **3** with formic acid (85%) or acetic anhydride/acetic acid, afforded triazolopyrimidines (**11**and **12**), respectively. Treatment of 2-hydrazinyl-6-propyl-3,4-dihydropyrimidin-4-one (**3**)with carbon disulphide, afforded 7-propyl-3-sulfanylidene-3*H*,5*H*-[1,2,4] triazolo [4,3-*a*] pyrimidin-5-one (**13**).

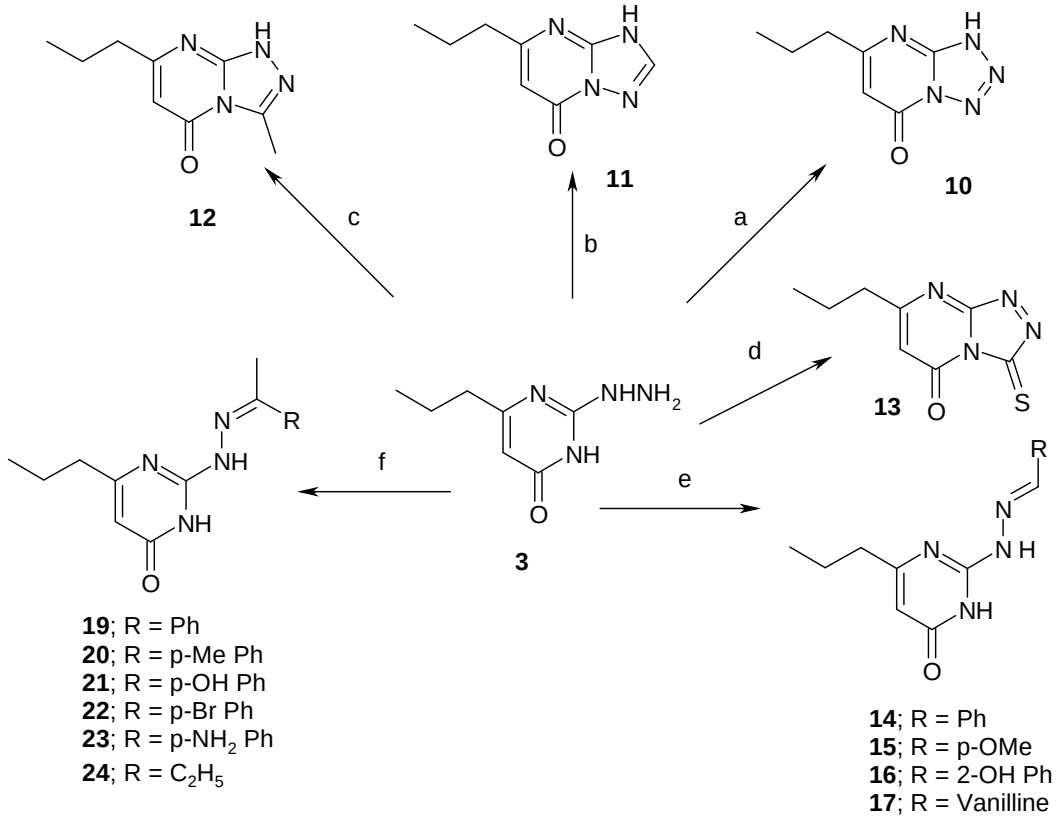
Schiff's bases (**14-24**) were obtained by the reaction of **3** with aldehyde and ketone derivatives (Scheme 2). Furthermore, reaction of **3** with ethyl cyanoacetate, acetylacetone and malononitrile, gave **25-27**, respectively. On the other hand, the reaction of **3** with acrylonitrile, yielded 3-[2-(4-oxo-6-propyl-3,4-dihydropyrimidin-2-yl) hydrazine-1-yldene] propanenitrile **(28).** Treatment of **3** with phenyl isothiocyanate, resulted 3-[(4-oxo-6-propyl-3,4-dihydro pyrimidin-2-yl)amino-1-phenyl-thiourea (**29**).

Finally, the reaction of **3** with phenacyl bromide, afforded 4-phenyl-8-propyl-6*H*-pyrimido [2,1-*c*] [1,2,4]triazin-6-one (**30**). The structures of new compounds were confirmed by MS, IR, ^{1}HNMR, ^{13}CNMR and elemental analysis.



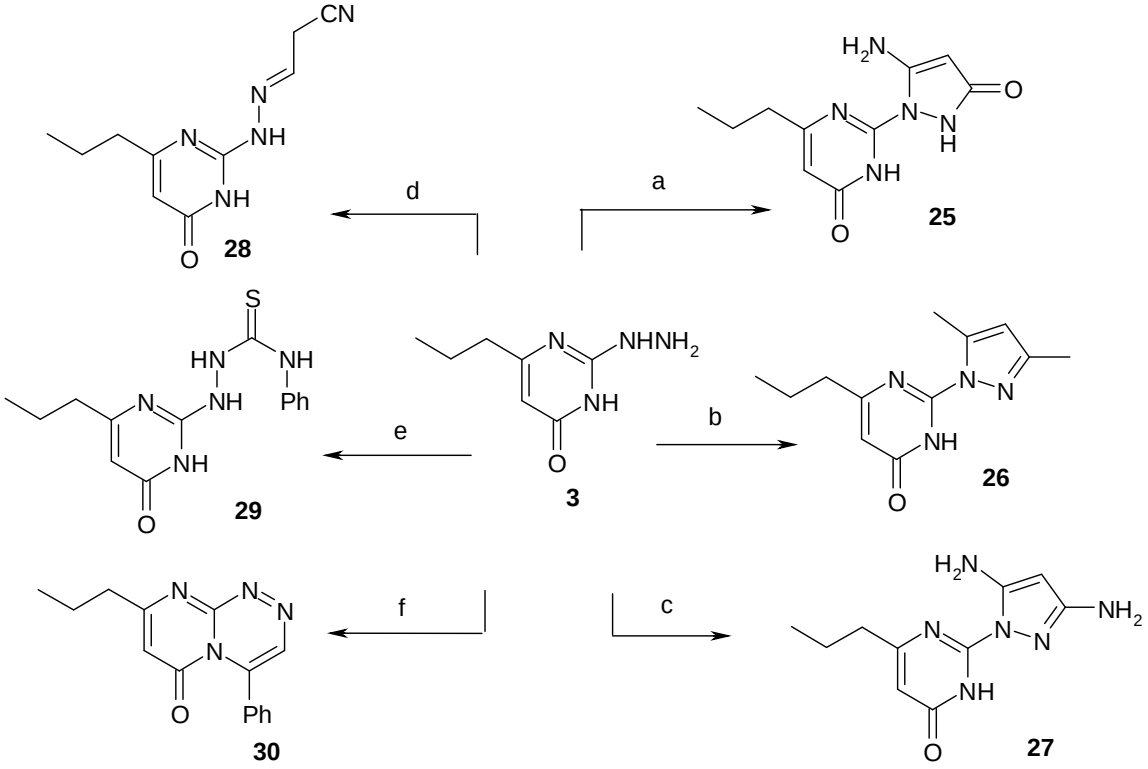
Reagents : (a) $PhCH_2Br$, NaH ; (b) $NH_2NH_2.H_2O$; (c) P_2S_5 ; (d) $ClCH_2CO_2Et$; (e) $ArCOCH_2Br$, KOH

Scheme 1. Synthetic route for the preparation of the target compounds **2-9**



Reagents: (a) $NaNO_2$, HCl ; (b) HCO_2H ; (c) $MeCO_2H$, AC_2O ; (d) CS_2, KOH ; (e) ArCHO ; (f) ArCOMe

Scheme 2. Synthetic route for the preparation of the target compounds **10-24**



Reagents: (a) $NCCH_2CO_2C_2H_5$; (b) $CH_2(COMe)_2$; (c) $CH_2(CN)_2$; (d) CH_2=CHCN ; (e) PhNCS ; (f) $PhCOCH_2Br$.

Scheme 3. Synthetic route for the preparation of the target compounds **25-30**

Pharmacology

***In-vitro* cancer screen at NCI-USA**

The structures of the final pyrazolopyrimidine-, triazolopyrimidine, thiazolopyrimidine- and propyl pyrimidine based products were submitted to the National Cancer Institute "NCI" (www.dtp.nci.nih.gov), Bethesda, Maryland, USA. Eight compounds were selected on the basis of degree of structure variation and computer modelling techniques for evaluation of their antineoplastic activity. The screening is a two-stage process, beginning with the evaluation of all compounds against 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five-dose levels.

The tumour growth inhibition properties of the eight compounds 6, 8, 11, 12, 25, 26, 27, and 30 with the NCI codes NSC 771838, NSC 771831, NSC 771832, NSC 771833, NSC 771834, NSC 771835, NSC 771836 and NSC 771837 selected among 2-**30** by the National Cancer Institute (NCI), USA, were screened on human tumour cell lines at 10^{-5} M at the NIH, Bethesda, Maryland, USA, under the drug discovery program of the NCI. Among the selected eight compounds, compound **26** (**NSC 771835**) was further screened for 5-log dose molar range as it has shown prominent cell growth inhibition at 10^{-5} M concentration against verity of cell lines.

Primary single high dose (10^{-5} M) full NCI 60 cell panels *in-vitro* assay

The selected compounds submitted to National Cancer Institute (NCI) for *invitro* anticancer assay were evaluated for their anticancer activity. Primary *in vitro* one dose anticancer assay was performed in full NCI60 cell panel representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48h. End point determinations were made with a protein binding dye, sulforhodamine B. Results for each compound were reported as a mean graph of the percent growth of the treated cells compared with the untreated control cells. Analysis of Historical Therapeutics Program (DTP) was performed, among the selected eight compounds, compound **26**(**NSC771835**), which satisfied predetermined threshold inhibition criteria, was further screened for 5-log dose molar range due to its prominent cell growth inhibition at 10^{-5} M concentration against a variety of cell lines. The tested 6-propylpyrimidin-4-one- based inhibitor demonstrated a remarkable and distinctive pattern of sensitivity against different NCI cell panel (Table 1). This compound **26**exhibited broad spectrum cell growth inhibition against leukemia cancer HL-60(TB)(cell growth promotion 12.8%, inhibition 87.2%), non -small cell lung cancer HOP-92(cell growth promotion 18.2%, inhibition

81.8%), Colon HCT-116 (cell growth promotion 29.5%, inhibition 70.5%),CNS cancer SF-295(cell growth promotion 17.8%, inhibition 82.2%), melanoma UACC-62 (cell growth promotion 33.7%, inhibition 65.9%), ovarian NCI/ADR-RES cancer cell line (cell growth promotion 22.8%, inhibition 77.2%), renal UO-31 cancer cell line (cell growth promotion 21.2%, inhibition 78.8%), prostate PC-3 cancer cell line (cell growth promotion 45.0%, inhibition 55.0%) and breast MCF7 cancer cell line (cell growth promotion 38.7%, inhibition 61.3%), at single dose assay concentration of 10^{-5} M.

***In-vitro* 5 dose full NCI 60 cell panel assay**

All 60 cell lines, representing nine tumour subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 and100 µM). The outcomes were used to create log concentration Vs% growth inhibition curves and three response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line, Table 2. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48h. Compound under investigation **26**(**NSC771835**)exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between 0.02–0.93 µM, Table 2.The highest activity achieved by compound **26** was against breast cancer, prostate cancer, renal cancer, colon cancer, ovarian cancer, melanoma cancer, non-small cell lung cancer, leukemia and CNS cancer, respectively. For example, CNS cancer: (SNB-75, GI_{50} 0.02µM), ovarian cancer: (OVCAR-5, GI_{50} 0.04µM), renal cancer: (RXF393, GI_{50} 0.05µM), breast cancer: (HS-578T, GI_{50} 0.07µM) and colon cancer: (HCC-2998, GI_{50} 0.07µM). As regards to the sensitivity, the criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel toward the test agent). Ratios between 3 and 6 refer to moderate selectivity; ratios greater than 6 indicate high selectivity towardsthe corresponding cell line, while compound not meeting either of these criteria were rated non-selective [44].

Structure-activity relationship

Structure-activity relationship, based on the number of cell lines that showed sensitivity towards each of the synthesized individual compounds, revealed that eight of the synthesized compounds were selected and tested for anticancer activity against 60 different human tumour cell lines. Among the compounds tested, compound **26**was found to be the most active candidate at five-dose level screening with no selectivity towards any cell panels. The GI_{50}, TGI, LC_{50} values revealed that compound **26** exhibited potential growth inhibition activity against most of the tested subpanel tumour cell lines. It showed promising activity toward several cell lines. Based on the results presented in table 2, we believed that the two methyl groups at C-3, C-5 position on pyrazole ring is essential for prompting the inhibitory activity. Compound **26** has the highest activity among the synthesized series. Its structure is characterized by pyrazole ring that is substituted with two lipophilic methyl groups. This structure is similar to that of **25** and **27** except that the pyrazole ring in these two compounds is substituted with polar groups. These polar groups seem to lower the antitumour activity of **25** and **26**. Despite increasing lipophilicity of **8**, this compound has worse activity. This suggests steric hindrance in this position of the propylthiouracil ring. Additionally, cyclization of **26** develops **11** and **12,** which greatly decrease the activity because the co-planar conformation of pyrazole's nitrogen significantly reduces the activity.

Table 1: Sixty human tumour cell line anticancer screening data at single dose assay (10^{-5} M concentration) as percent cell growth promotion of **6, 8, 11, 12, 25, 26, 27** and **30**

	6	***8***	***11***	***12***	***25***	***26***	***27***	***30***
Leukemia								
CCRF – CEM	97.71	89.41	94.83	98.49	93.75	21.45	94.57	89.53
HL – 60 (TB)	94.43	95.28	87.41	97.36	94.05	12.82	90.32	91.62
K - 562	96.94	85.26	81.12	96.76	91.76	18.51	97.46	91.24
MOLT - 4	91.60	91.47	85.42	97.13	87.64	25.35	91.02	93.11
RPMI - 8226	105.38	90.41	98.51	97.87	98.00	58.61	97.65	98.25
SR	83.92	81.69	78.64	95.49	92.33	14.36	85.54	83.13
Non-small cell Lung								
A549/ATCC	98.09	98.04	104.86	103.70	107.60	33.47	102.11	101.19
HOP-62	91.93	88.84	90.38	93.89	101.21	35.19	92.45	97.48
HOP-92	NT	72.41	NT	NT	95.82	18.26	87.72	NT
NCI-H226	93.72	112.71	108.11	96.40	104.16	74.76	98.42	103.99
NCI-H23	100.8	99.49	101.17	95.70	99.90	70.11	102.36	101.18
NCI-H322M	85.8	88.68	89.28	89.67	95.13	25.91	84.45	95.74
NCI-H460	109.4	108.90	110.69	112.39	107.14	26.80	109.58	109.95
NCI-H522	90.07	91.86	91.41	102.47	88.01	62.40	88.24	92.66
Colon cancer								

COLO-205	102.38	117.15	111.81	107.48	116.23	73.11	113.34	114.03
HCC-2998	95.93	106.52	99.88	108.65	110.11	57.56	105.02	106.84
HCT-116	91.88	89.58	100.37	93.01	101.78	29.57	106.36	105.19
HCT-15	103.93	98.83	91.24	91.30	100.77	30.62	105.46	106.77
HT29	102.26	104.49	100.92	103.32	99.25	50.05	107.31	103.75
KM12	107.83	110.72	115.69	107.46	107.12	51.55	110.45	109.86
SW-620	105.66	108.13	108.21	105.70	100.04	47.78	105.74	110.75
CNS cancer								
SF-268	107.03	108.59	113.30	110.90	111.15	54.08	105.54	107.28
SF-V295	111.77	99.28	94.94	98.19	101.12	17.81	89.79	96.28
SF-539	111.21	104.00	94.47	98.00	97.02	49.47	94.17	101.15
SNB-19	107.16	108.61	107.36	102.74	107.45	49.74	106.66	111.49
SNB-75	81.67	92.86	102.72	97.99	95.14	76.98	97.24	93.22
U251	98.66	97.86	102.29	99.92	101.93	18.78	102.72	102.72
Melanoma cancer								
LOXIMVI	93.79	94.74	96.74	94.94	95.96	43.79	95.49	97.69
MALME-3M	97.27	98.21	96.91	87.46	78.76	66.00	81.67	96.31
M14	98.43	97.64	101.61	100.65	98.53	33.71	96.99	100.79
MDA-MB-435	97.94	102.47	105.50	101.99	99.16	47.16	91.70	96.69
SK-MEL-2	100.42	91.11	100.28	98.43	86.49	54.13	91.78	100.84
SK-MEL-28	106.94	106.03	112.74	104.19	115.53	61.00	109.57	110.60
SK-MEL-5	99.68	101.63	102.60	98.68	101.23	51.68	100.76	99.06
UACC-257	102.93	103.04	103.93	98.59	97.76	74.72	98.12	98.39
UACC-62	106.45	102.91	107.50	108.64	109.63	34.16	104.78	106.15
Ovarian cancer								
IGROV1	92.02	108.04	100.36	88.70	109.76	23.81	89.21	108.79
OVCAR-3	114.93	118.25	122.79	115.49	114.29	47.66	108.45	118.26
OVCAR-4	107.79	109.68	116.23	113.98	114.77	43.25	111.63	113.16
OVCAR-5	1O1.89	102.48	106.18	102.06	98.04	NT	103.49	101.95
OVCAR-8	102.86	97.90	103.34	106.12	105.47	27.52	97.88	100.59
NCI/ADR-RES	102.93	102.40	101.46	107.99	105.88	22.86	104.68	99.01
SK-OV-3	98.69	90.37	96.81	95.02	102.28	45.53	93.71	100.83
Renal cancer								
786-0	89.50	98.85	88.79	92.16	93.22	57.78	88.75	87.64
A-498	100.43	80.59	89.64	108.71	103.38	67.97	99.29	111.68
ACHN	105.55	94.05	104.99	98.69	100.95	35.86	105.22	98.80
CAKI-1	93.22	94.88	99.43	99.89	101.90	29.91	97.54	97.68
RXF393	109.79	125.45	106.70	115.61	113.79	87.28	114.62	104.65
SN12C	101.66	103.55	102.74	102.56	101.50	51.64	102.61	99.96
TK-10	94.00	98.32	99.99	104.01	94.83	47.59	98.70	95.82
UO-31	74.54	78.21	85.14	82.69	82.24	21.24	78.68	81.64
Prostate cancer								
PC-3	102.65	92.90	100.32	97.72	99.02	45.05	96.24	92.26
DU-145	110.81	122.52	125.10	117.88	114.86	66.65	108.87	111.42
Breast cancer								
MCF-7	100.56	103.65	100.13	92.63	99.51	38.73	97.11	94.20
MDA-MP-231/ATCC	119.49	106.67	118.29	108.77	115.08	48.24	114.56	112.88
HS-578T	112.52	103.84	133.28	116.88	109.90	79.98	98.60	112.69
BT-549	95.72	93.19	83.08	103.12	96.85	52.02	98.56	93.24
T-47D	83.70	97.14	87.81	79.95	101.36	50.31	101.14	115.82
MDA-MB-468	99.09	117.86	110.65	109.59	103.71	60.16	99.53	100.81

NT-Not Test

Table 2: NCI in vitro testing result of **26(NSC 771835)** at five dose level in µM

	GI_{50}	*Subpanel MID* [a]	*Selectivity ratio (MID [a] : MID [b])*	*TGI*	LC_{50}	IC_{50}
Leukemia		0.36	0.75			
CCRF – CEM	0.63			0.04	0.0!	0.35
HL – 60 (TB)	0.49			0.05	0.01	0.14
K - 562	0.30			0.01	0.01	0.23
MOLT - 4	0.29			0.01	0.01	0.14
RPMI - 8226	0.19			0.04	0.01	0.07
SR	0.29			0.01	0.01	0.18
Non-small cell Lung		0.34	0.79			
A549/ATCC	0.37			0.01	0.01	0.20
HOP-62	0.18			0.01	0.01	0.02
HOP-92	NT			NT	NT	NT
NCI-H226	0.17			0.02	0.01	0.02
NCI-H23	NT			NT	NT	NT
NCI-H322M	0.36			0.02	0.01	0.05
NCI-H460	0.79			0.01	0.01	0.56
NCI-H522	0.20			0.03	0.01	0.03
Colon cancer		0.23	1.17			
COLO-205	0.12			0.01	0.01	0.05
HCC-2998	0.07			0.01	0.01	0.01
HCT-116	0.30			0.01	0.01	0.21
HCT-15	0.58			0.01	0.01	0.33
HT29	0.15			0.03	0.01	0.09
KM12	0.19			0.01	0.01	0.11
SW-620	0.26			0.01	0.01	0.18
CNS cancer		0.40	0.67			
SF-268	0.32			0.01	0.01	0.08
SF-V295	0.93			0.11	0.01	0.28
SF-539	0.33			0.01	0.01	0.15
SNB-19	0.22			0.01	0.01	0.01
SNB-75	0.02			0.01	0.01	0.01
U251	0.59			0.01	0.01	0.24
Melanoma cancer		0.27	1.00			
LOXIMVI	0.35			0.01	0.01	0.22
MALME-3M	NT			NT	NT	NT
M14	0.35			0.01	0.01	0.18
MDA-MB-435	0.21			0.03	0.01	0.09
SK-MEL-2	0.08			0.02	0.01	0.01
SK-MEL-28	0.26			0.02	0.01	0.06
SK-MEL-5	0.32			0.02	0.01	0.14
UACC-257	0.16			0.03	0.01	0.02
UACC-62	0.44			0.03	0.01	0.19
Ovarian cancer		0.24	1.12			
IGROV1	0.44			0.01	0.01	0.14
OVCAR-3	0.13			0.01	0.01	0.03
OVCAR-4	0.21			0.01	0.01	0.01
OVCAR-5	0.04			0.01	0.01	0.01
OVCAR-8	0.42			0.01	0.01	0.18
NCI / ADR-RES	0.36			0.01	0.01	0.18
SK-OV-3	0.11			0.01	0.01	0.01
Renal cancer		0.22	1.22			
786-0	0.13			0.01	0.01	0.04
A-498	0.09			0.01	0.01	0.01
ACHN	0.25			0.01	0.01	0.13
CAKI-1	0.41			0.04	0.01	0.22

RXF393	0.05			0.01	0.01	0.01
SN12C	0.21			0.01	0.01	0.06
TK-10	NT			NT	NT	NT
UO-31	0.42			0.01	0.01	0.15
Prostate cancer		0.18	1.50			
PC-3	0.23			0.01	0.01	0.05
DU-145	0.13			0.01	0.01	0.04
Breast cancer		0.17	1.58			
MCF-7	0.23			0.01	0.01	0.15
MDA-MP-231 / ATCC	0.22			0.01	0.01	0.02
HS-578T	0.07			0.01	0.01	0.01
BT-549	0.14			0.01	0.01	0.01
T-47D	NT			NT	NT	NT
MDA-MB-468	0.21			0.01	0.01	0.01
MID [a]	0.27					

MID [a] = Average sensitivity of all cell line in µM; MID[b] = Average sensitivity of all cell line of a particular subpanel in µM, NT-Not Test

4. Conclusion

A new series of novel pyrimidine derivatives (**2**-**30**) were obtained from propylthiouracil (**1**)and evaluated for antitumour activity. Eight of the synthesized compounds were selected and tested by National Cancer Institute (NCI), USA, for anticancer activity against 60 different human tumour cell lines. Among the compounds tested, 2-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-6-propyl-3,4 dihydro pyrimidin-4-one (**26**) (**NSC 771835**)was found to be the most active candidate of the series at five-dose level screening with no selectivity towards any cell panels. Despite high toxicity of the test compounds, which limits their clinical application, these preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent and safer anticancer agent.

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