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Synthesis and Evaluation of The Antiviral Activity of Novel 2-Thiopyrimidine-5-Carbonitrile Derivatives

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Abstract

2-Thiouracil-5-carbonitriles are promising compounds that have been prepared in the last twenty years and have proven to have a diversity of biological activity. In line with this approach, our new compounds were prepared starting from Biginilli condensation of thiourea, ethyl cyanoacetate and 2,4-dichlorobenzaldehyde in basic medium to give a thiouracil derivative **(1)** which was subjected to chlorination at4th position by $POCl_3/PCl_5$ mixture producing chloropyrimidine **(2)** which in turns was reacted with *m*-aminoacetophenone in pyridine yielding acetyl derivative **(3)** which was condensed with a series of four aromatic aldehydes in alcoholic sodium hydroxide producing chalcones **(4a-d)** which were used as starting materials for the preparation of many heterocyclic nuclei by their reaction with hydrazine, phenyl hydrazine, hydroxylamine HCl, thiourea, ethyl cyanoacetate /ammonium acetate, malononitrile, ethyl cyanoacetate/acetic acid/sodium acetate to give pyrazoline, phenyl pyrazoline, isoxazoline, thiopyrimidine, pyridinone, aminopyridine and aminopyran derivatives **(5a-d)** to **(11a-d)** respectively . Some of the newly synthesized compounds were investigated as antiviral agents. Compounds **(2)**, **(7a)**, and **(7c)** show promising antiviral activity against Bovine Viral Diarrhea Virus (BVDV). The structures of the newly synthesized compounds were confirmed by elemental analysis in addition to spectral data and physical methods.

Key words: Antiviral, 2-Thiouracil-5- carbonitriles, synthesis, and evaluation.

1. Introduction

There is no doubt that the war on viruses is the talk of hour because of what we are suffering now from the complications of viral diseases and the most dangerous Covid-19.

Bovine viral virus (BVDV) is an individual of the genus Pestivirus, belonging to the family Flaviviridae. It is a small spherical positive sense single-stranded enveloped RNA virus of 40 to 60 nm in diameter [1] . It infects cattle of all ages and is distributed worldwide, although some countries have recently eradicated it [2]. BVDV may be subclinical or can become a severe fatal disease [3]. Efforts of scientists have united to produce and prepare effective antiviral agents. Some studies indicated that 2-thiouracil has an inhibitory effect on the growth of some culture viruses[4,5], but this inhibitory effect is very limited against animal viruses such as inhibiting the growth of vaccinia virus in tissue culture[6] as well as its ability to disable poliovirus[7]. In this context, we have focused in this research on thiouracil compounds due to diversity of their biological activity as antibacterial [8–11], antifungal [12–14], anticancer [15–18], antioxidant [19,20], antiviral [21–23], antithyroid [24,25]…etc. agents. Here we developed a program aimed to synthesize substituted thiouracils in an attempt to improve the antiviral activity of 2-thiouracil itself and we hope that in the future we will continue to test these compounds as anti-covid-19, either through our research or in cooperation with the relevant authorities. Substituted thiouracils were classically prepared from the three-component condensation of aromatic aldehydes, thiourea, and acetoacetic esters, this reaction was discovered by the Italian chemist P.Beginilli in 1893 [26]. Biginilli reaction was not commonly used but in the last 20-30 years this class of heterocyclic compounds received considerable attention as a type of promising heterocyclic scaffolds with a significant pharmacological potential. As antiviral agents some substituted thiouracil showed potent activity, For example some 5-substituted methyl-2-thiouracil had exhibited large activity against Herpes Simplex virus type-1 [27]. A

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huge number of 2-thiouracil-5-carbonitriles were prepared in the last twenty years and had screened for their biological activity [28,29]. Their incorporation with other heterocyclic nuclei such as isoxazoline, pyrazoline, pyridine, pyrimidine, thiazoline….etc. had enriched their potential activity [30].

Materials and Methods:

Instrumentation

All melting points are uncorrected and were measured using an Electro thermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA). ^{1}HNMR spectra (300MHz) were recorded in dimethyl sulfoxide (DMSO) by employing tetra methyl silane (TMS) as an internal standard on Varian Mercury 300 MHz NMR Spectrometer (Varian, UK) and the chemical shifts (δ) were expressed as ppm against TMS as internal standard. Mass spectra were recorded on a 70 eV EI MS-QP 1000 (Shimadzu, Japan). Microanalyses were operated using vario, Elemental apparatus (Shimadzu). The progress of all the reactions was monitored by TLC on silica gel 60 for TLC (Merck) using chloroform-methanol (3:1) as mobile phase and spots were visualized by iodine vapors or by irradiation with UV-light (254nm). The target compounds were synthesized as outlined in scheme I, and II.

Experimental:

6-(2,4-dichlorophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile:(1). Prepared as in literature [31].

4-chloro-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (2).

A mixture of (1) (0.01mol), and phosphorus pentachloride (0.01mol) in phosphorus oxychloride (20 ml) was heated on a steam bath for 3h. The reaction mixture was poured onto crushed ice. The precipitate was filtered off, dried, and then recrystallized from DMF/water.

Yield: 85%: mp: 175-177ºC: IR (KBr cm^{-1}): 3355 (NH), 3150 (CH, aromatic), 2217 (C≡N), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.60-8.00(m, 3H, Ar-H), 11.30 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 315 ([M]$^{+\cdot}$,11%), 317 ([M+2]$^{+\cdot}$, 3.6%), 319 ([M+4]$^{+\cdot}$, 1.2%); Anal. Calcd. For $C_{11}H_4Cl_3N_3S$ (316): C, 41.73; H, 1.27; N, 13.27%. Found: C, 41.81; H, 1.15; N, 13.32%.

4-[(3-acetylphenyl)amino]-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (3).

A mixture of (2) (0.01mol), and *m*-amino acetophenone (0.01mol) was heated under reflux in 25 ml absolute ethanol, and few drops of pyridine for 10h. The reaction mixture was cooled and poured into acidified ice/water. The produced solid was filtered off, dried, and recrystallized from DMF/water.

Yield: 83%: mp: 188-190ºC: IR (KBr cm^{-1}): 3332 (NH), 3100 (CH, aromatic), 2980 (CH, aliphatic), 2217 (C≡N), 1740 (C=O), 1275 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 2.10 (s, 3H, $COCH_3$), 7.60-8.10(m, 7H, Ar-H), 10.20 (s, 1H, NH, D_2O exchangeable), 11.30 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 414 ([M]$^{+\cdot}$, 25%), 416 ([M+2]$^{+\cdot}$, 8.3%), 418 ([M+4]$^{+\cdot}$, 2.7%) ; Anal. Calcd. for $C_{19}H_{12}Cl_2N_4OS$ (415): C, 54.95; H, 2.91; N, 13.49%. found: C, 54.84; H, 2.89; N, 13.52%.

4-(3-((E)-3-(4-aryl)acryloyl)phenylamino)-6-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile: (4a-d).

A mixture of (3) (0.01mol), and aromatic aldehyde (0.01mol) in 50ml ethanolic sodium hydroxide solution (10%) was stirred at room temperature for 24h, and then was refluxed for 1h. the reaction mixture was cooled, poured in ice-cold water, and neutralized with HCl. the precipitate was filtered off and recrystallized from DMF/water.

(E)-6-(2,4-dichlorophenyl)-4-((3-(3-(4-methoxyphenyl)acryloyl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (4a).

Yield: 80%: mp: 153-155ºC: IR (KBr cm^{-1}): 3362 (NH), 3170 (CH, aromatic), 2986 (CH, aliphatic), 2217 (C≡N), 1746 (C=O), 1272 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.70(s, 3H, OCH_3), 6.90 (d, 1H, CH=CH), 7.20 (d, 1H, CH=CH), 7.04-8.06 (m, 11H, Ar-H), 10.00 (s, 1H, NH, D_2O exchangeable), 11.00 (s, 1H, NH, D_2O exchangeable), 11.30 (s, 1H, NH, D2O exchangeable); MS (EI) *m/z*: 532([M]$^{+\cdot}$, 65.3%), 534 ([M+2]$^{+\cdot}$, 21.7%), 536 ([M+4]$^{+\cdot}$,7.2%); Anal. Calcd. for $C_{27}H_{18}Cl_2N_4O_2S$ (533): C, 60.79; H, 3.40; N, 10.50%. found: C, 60.65; H, 3.52; N, 10.47%.

4-((3-cinnamoylphenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (4b).

Yield: 77%: mp: 180-182ºC: IR (KBr cm^{-1}): 3340 (NH), 3183 (CH, aromatic), 2204 (C≡N), 1735 (C=O), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 6.90 (d, 1H, CH=CH), 7.08 (d, 1H, CH=CH), 7.43-8.63 (m, 12H, Ar-H), 10.50 (s, 1H, NH, D_2O exchangeable), 11.18 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 502([M]$^{+\cdot}$, 45.2 %), 504 ([M+2]$^{+\cdot}$, 15%), 506 ([M+4]$^{+\cdot}$, 5%); Anal. Calcd. for $C_{26}H_{16}Cl_2N_4OS$ (503): C, 62.03; H, 3.20; N, 11.13%. found: C, 62.38; H, 2.96; N, 11.42%.

(E)-6-(2,4-dichlorophenyl)-4-((3-(3-(4-nitrophenyl)acryloyl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (4c).

Yield: 83%: mp: 198-200ºC: IR (KBr cm^{-1}): 3338 (NH), 3187 (CH, aromatic), 2222 (C≡N), 1745

(C=O), 1550 (N=O), 1300 (N-O), 1273 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 6.80(d, 1H, CH=CH), 7.10 (d, 1H, CH=CH), 7.30-8.50 (m, 11H, Ar-H), 10.30 (s, 1H, NH, D_2O exchangeable), 11.20 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 547([M]$^{+\cdot}$, 40 %), 549 ([M+2]$^{+\cdot}$,13.3%), 551 ([M+4]$^{+\cdot}$,4.4%); Anal. Calcd. for $C_{26}H_{15}Cl_2N_5O_3S$ (548): C, 56.95; H, 2.76; N, 12.77%. found: C, 56.67; H, 2.53; N, 12.85%.

(E)-4-((3-(3-(4-bromophenyl)acryloyl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (4d).

Yield: 78%: mp: 132-134ºC: IR (KBr cm^{-1}): 3350 (NH), 3193 (CH, aromatic), 2249 (C≡N), 1742 (C=O), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.00 (d, 1H, CH=CH), 7.20 (d, 1H, CH=CH), 7.40-8.70 (m, 11H, Ar-H), 10.40 (s, 1H, NH, D_2O exchangeable), 11.30 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 580([M]$^{+\cdot}$, 35.6 %), 582 ([M+2]$^{+\cdot}$,35.6%); Anal. Calcd. for $C_{26}H_{15}BrCl_2N_4OS$ (582): C, 53.63; H, 2.60; N, 9.62%. found: C, 53.82; H, 2.48; N, 9.75%.

(±)4-(3-(4,5-dihydro-5-(4-aryl)-1H-pyrazol-3-yl)phenylamino)-6-(2,4dichlorophenyl)-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile: (5a-d).

A mixture of (4a-d) (0.001mol) and hydrazine hydrate 98% (0.5 ml) was refluxed in absolute ethanol for 8h. The reaction mixture was allowed to cool and poured in ice-water. The precipitate was filtered off, dried, and recrystallized from DMF/ water.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (5a).

Yield: 82%: mp: 190-192ºC: IR (KBr cm^{-1}): 3340 (NH), 3197 (CH, aromatic), 2980(CH, aliphatic), 2205 (C≡N), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.10 (s, 1H, pyrazoline CH_2), 4.00 (s, 1H, pyrazoline CH_2), 3.80 (s, 3H, OCH_3), 5.60 (s, 1H, pyrazoline CH), 7.20-8.30 (m, 11H, Ar-H), 10.10 (s, 1H, NH, D_2O exchangeable), 10.30 (s, 1H, NH, D_2O exchangeable), 10.90 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 546([M]$^{+\cdot}$, 42 %), 548 ([M+2]$^{+\cdot}$, 14%), 550 ([M+4]$^{+\cdot}$, 4.6%); Anal. Calcd. for $C_{27}H_{20}Cl_2N_6OS$ (547): C, 59.24; H, 3.68; N, 15.35%. found: C, 59.53; H, 3.35; N, 15.61%.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (5b).

Yield: 74%: mp: 220-222ºC: IR (KBr cm^{-1}): 3332 (NH), 3179 (CH, aromatic), 2217 (C≡N), 1273 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.30(s, 1H, pyrazoline CH_2), 4.20 (s, 1H, pyrazoline CH_2), 5.50 (s, 1H, pyrazoline CH), 7.30-8.50 (m, 12H, Ar-H), 10.00 (s, 1H, NH, D_2O exchangeable), 10.40 (s, 1H, NH, D_2O exchangeable), 11.00 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 516 ([M]$^{+\cdot}$, 54.2 %), 518 ([M+2]$^{+\cdot}$, 18.1%), 520 ([M+4]$^{+\cdot}$, 6%); Anal. Calcd. for $C_{26}H_{18}Cl_2N_6S$ (517): C, 60.35; H, 3.51; N, 16.24%. found: C, 60.51; H, 3.80; N, 16.07%.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (5c).

Yield: 80%: mp: 268-270ºC: IR (KBr cm^{-1}): 3372 (NH), 3165 (CH, aromatic), 2221 (C≡N), 1500 (N=O), 1350 (N-O), 1276 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.00 (s, 1H, pyrazoline CH_2), 4.10 (s, 1H, pyrazoline CH_2), 5.30 (s, 1H, pyrazoline CH), 7.10-8.20 (m, 11H, Ar-H), 10.20 (s, 1H, NH, D_2O exchangeable), 10.50 (s, 1H, NH, D_2O exchangeable), 11.20 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 561 ([M]$^{+\cdot}$, 36.4 %), 563 ([M+2]$^{+\cdot}$, 12.1%), 565 ([M+4]$^{+\cdot}$, 4%); Anal. Calcd. for $C_{26}H_{17}Cl_2N_7O_2S$ (562): C, 55.52; H, 3.05; N, 17.43%. found: C, 55.28; H, 3.24; N, 17.65%.

(±)4-((3-(5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (5d).

Yield: 76%: mp: 213-215ºC: IR (KBr cm^{-1}): 3384 (NH), 3177 (CH, aromatic), 2296 (C≡N), 1271 (C=S); ^{1}HNMR (DMSO-d$_6$), δ(ppm): 3.40 (s, 1H, pyrazoline CH_2), 4.30 (s, 1H, pyrazoline CH_2), 5.40 (s, 1H, pyrazoline CH), 7.50-8.90 (m, 11H, Ar-H), 10.20 (s, 1H, NH, D_2O exchangeable), 10.60 (s, 1H, NH, D_2O exchangeable), 11.00 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 594 ([M]$^{+\cdot}$, 93 %), 596 ([M+2]$^{+\cdot}$, 93 %); Anal. Calcd. for $C_{26}H_{17}BrCl_2N_6S$ (596): C, 52.37; H, 2.87; N, 14.09%. found: C, 52.19; H, 2.64; N, 13.92%.

(±)4-(3-(4,5-dihydro-5-(4-aryl)-1-phenyl-1H-pyrazol-3-yl)phenylamino)-6-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile: (6a-d).

A mixture of (4a-d) (0.001mol) and phenyl hydrazine (0.001mol) in 20ml absolute ethanol was refluxed for 5-8h. After completion of the reaction, the reaction mixture was cooled, poured in ice-water, and the solid separated was filtered off, dried, and recrystallized from DMF/water.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (6a).

Yield: 80%: mp: 220-222ºC: IR (KBr cm^{-1}): 3216 (NH), 2217 (CN), 3186 (CH, aromatic), 2971(CH, aliphatic), 2217 (C≡N), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.10 (s, 1H, pyrazoline CH_2), 3.90 (s, 1H, pyrazoline CH_2), 3.70 (s, 3H, OCH_3), 5.70 (s, 1H, pyrazoline CH), 7.50-8.30 (m, 16H, Ar-H), 10.00 (s, 1H, NH, D_2O exchangeable), 11.30 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 622([M]$^{+\cdot}$, 21.3%), 624 ([M+2]$^{+\cdot}$, 7.1%), 626 ([M+4]$^{+\cdot}$, 2.4%) ; Anal. Calcd. for $C_{33}H_{24}Cl_2N_6OS$

(623): C, 63.56; H, 3.88; N, 13.48%. found: C, 63.44; H, 3.65; N, 13.37%.

(±)6-(2,4-dichlorophenyl)-4-((3-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (6b).

Yield: 68%: mp: >300ºC: IR (KBr cm^{-1}): 3339 (NH), 3183 (CH, aromatic), 2233 (C≡N), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.30 (s, 1H, pyrazoline CH_2), 4.00 (s, 1H, pyrazoline CH_2), 5.90 (s, 1H, pyrazoline CH), 7.20-8.50 (m, 17H, Ar-H), 10.20 (s, 1H, NH, D_2O exchangeable), 11.10 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 592 ([M]$^{+\cdot}$, 25.2 %), 594 ([M+2]$^{+\cdot}$, 8.4%), 596 ([M+4]$^{+\cdot}$, 2.8%); Anal. Calcd. for $C_{32}H_{22}Cl_2N_6S$ (593): C, 64.76; H, 3.74; N, 14.16%. found: C, 64.49; H, 3.86; N, 14.33%.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (6c).

Yield: 75%: mp: 275-277ºC: IR (KBr cm^{-1}): 3347 (NH), 3187 (CH, aromatic), 2220 (C≡N), 1530 (N=O), 1400 (N-O), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.20 (s, 1H, pyrazoline CH_2), 4.10 (s, 1H, pyrazoline CH_2), 5.60 (s, 1H, pyrazoline CH), 7.40-8.60 (m, 16H, Ar-H), 10.40 (s, 1H, NH, D_2O exchangeable), 11.40 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 637 ([M]$^{+\cdot}$, 23.5 %), 639 ([M+2]$^{+\cdot}$, 7.8%), 641 ([M+4]$^{+\cdot}$, 2.6%); Anal. Calcd. for $C_{32}H_{21}Cl_2N_7O_2S$ (638): C, 60.19; H, 3.32; N, 15.36%. found: C, 60.35; H, 3.18; N, 15.60%.

(±)4-((3-(5-(4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (6d).

Yield: 70%: mp: 263-265ºC: IR (KBr cm^{-1}): 3216 (NH), 3191 (CH, aromatic), 2217 (CN), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.00 (s, 1H, pyrazoline CH_2), 3.80 (s, 1H, pyrazoline CH_2), 6.00 (s, 1H, pyrazoline CH), 7.30-8.60 (m, 16H, Ar-H), 10.30 (s, 1H, NH, D_2O exchangeable), 11.30 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 670 ([M]$^{+\cdot}$, 27.6 %), 672 ([M+2]$^{+\cdot}$, 27.6%); Anal. Calcd. for $C_{32}H_{21}BrCl_2N_6S$ (672): C, 57.16; H, 3.15; N, 12.50%. found: C, 57.42; H, 2.98; N, 12.26%.

(±) 4-(3-(4,5-dihydro-5-(4-aryl) isoxazol-3-yl) phenylamino)-6-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile: (7a-d).

A mixture of (4a-d) (0.001mol), hydroxylamine hydrochloride (0.001mol) and sodium hydroxide (0.1 g) in 80% ethanol (15ml) was refluxed for 4h. The reaction mixture was allowed to cool at room temperature and then kept overnight in the freezer. The precipitate was filtered off, dried, and recrystallized from DMF/water.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-(4-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (7a).

Yield: 70%: mp: 212-214ºC: IR (KBr cm^{-1}): 3315 (NH), 3183 (CH, aromatic), 2983 (CH, aliphatic), 2204 (C≡N), 1271 (C=S); ^{1}H-NMR (DMSO-d_6), δ (ppm): 3.20 (s, 1H, isoxazoline CH_2), 4.00 (s, 1H, isoxazoline CH_2), 3.70 (s, 3H, OCH_3), 5.50 (s, 1H, isoxazoline CH), 7.20-8.05 (m, 11H, Ar-H), 10.00 (s, 1H, NH, D_2O exchangeable), 10.80 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 547 ([M]$^{+\cdot}$, 19.3 %), 549 ([M+2]$^{+\cdot}$, 6.4%), 551 ([M+4]$^{+\cdot}$, 2.1%) ; Anal. Calcd. for $C_{27}H_{19}Cl_2N_5O_2S$ (548): C, 59.13; H, 3.49; N, 12.77%. found: C, 60.11; H, 3.52; N, 12.86%.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-phenyl-4,5-dihydroisoxazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (7b).

Yield: 62%: mp: 233-235ºC: IR (KBr cm^{-1}): 3300 (NH), 3186 (CH, aromatic), 2227(C≡N), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.30 (s, 1H, isoxazoline CH_2), 4.10 (s, 1H, isoxazoline CH_2), 5.70 (s, 1H, isoxazoline CH), 7.10-8.20 (m, 12H, Ar-H), 10.40 (s, 1H, NH, D_2O exchangeable), 11.10 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 517([M]$^{+\cdot}$, 15.6 %), 519 ([M+2]$^{+\cdot}$, 5.2%), 521 ([M+4]$^{+\cdot}$, 1.7%) ; Anal. Calcd. for $C_{26}H_{17}Cl_2N_5OS$ (518): C, 60.24; H, 3.31; N, 13.51%. found: C, 60.51; H, 3.14; N, 13.68%.

(±)6-(2,4-dichlorophenyl)-4-((3-(5-(4-nitrophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (7c).

Yield: 73%: mp: 251-253ºC: IR (KBr cm^{-1}): 3330 (NH), 3172 (CH, aromatic), 2295 (C≡N), 1500 (N=O), 1375 (N-O), 1272 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 2.90 (s, 1H, isoxazoline CH_2), 3.80 (s, 1H, isoxazoline CH_2), 5.30 (s, 1H, isoxazoline CH), 7.00-8.30 (m, 11H, Ar-H), 10.20 (s, 1H, NH, D_2O exchangeable), 10.90 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 562 ([M]$^{+\cdot}$, 26.5 %), 564 ([M+2]$^{+\cdot}$, 8.8%), 566 ([M+4]$^{+\cdot}$, 2.9%) ; Anal. Calcd. for $C_{26}H_{16}Cl_2N_6O_3S$ (563): C, 55.43; H, 2.86; N, 14.92%. found: C, 55.26; H, 3.02; N, 14.73%.

(±)4-((3-(5-(4-bromophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (7d).

Yield: 65%: mp: 225-227ºC: IR (KBr cm^{-1}): 3305 (NH), 3181 (CH, aromatic), 2300 (C≡N), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.20 (s, 1H, isoxazoline CH_2), 4.30 (s, 1H, isoxazoline CH_2), 5.10 (s, 1H, isoxazoline CH), 7.20-8.50 (m, 11H, Ar-H), 10.30 (s, 1H, NH, D_2O exchangeable), 11.20 (s, 1H, NH, D_2O exchangeable); MS (EI) *m/z*: 595([M]$^{+\cdot}$, 18.3 %), 597 ([M+2]$^{+\cdot}$, 18.3%); Anal. Calcd. for $C_{26}H_{16}BrCl_2N_5OS$ (597): C, 52.28; H, 2.70; N, 11.73%. found: C, 52.54; H, 2.48; N, 11.91%.

4-(3-(1,2-dihydro-6-(4-aryl)-2-thioxopyrimidin-4-yl)phenylamino)-6-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile: (8a-d).

A mixture of (4a-d) (0.001mol), thiourea (0.001mol) and sodium hydroxide (0.1g) in 80% ethanol (15ml) was refluxed for 6h. The reaction mixture was cooled, poured onto ice-water and the precipitate was filtered off, dried, and recrystallized from DMF/water.

6-(2,4-dichlorophenyl)-4-((3-(6-(4-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (8a).

Yield: 72%: mp: >300ºC: IR (KBr cm^{-1}): 3308 (NH), 3180 (CH, aromatic), 2973 (CH, aliphatic) 2216 (C≡N), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.90 (s, 3H, OCH_3), 7.30-8.20 (m, 11H, Ar-H), 8.30 (s, 1H, thiopyrimidine H), 10.00 (s,1H, NH, D_2O exchangeable), 10.40 (s,1H, NH, D_2O exchangeable), 10.80 (s,1H, NH, D2O exchangeable); MS (EI) *m/z*: 588 ($[M]^{+\cdot}$, 24.6%), 590 ($[M+2]^{+\cdot}$, 8.2%), 592 ($[M+4]^{+\cdot}$, 2.7%); Anal. Calcd. for $C_{28}H_{18}Cl_2N_6OS_2$ (589): C, 57.05; H, 3.08; N, 14.26%. found: C, 56.86; H, 3.21; N, 14.45%.

6-(2,4-dichlorophenyl)-4-((3-(6-phenyl-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (8b).

Yield: 70%: mp: 286-288ºC: IR (KBr cm^{-1}): 3340 (NH), 3192 (CH, aromatic), 2236(C≡N), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.10-8.30 (m, 12H, Ar-H), 8.50 (s, 1H, thiopyrimidine H), 10.20 (s,1H, NH, D_2O exchangeable), 10.50 (s,1H, NH, D_2O exchangeable), 11.00 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 558 ($[M]^{+\cdot}$, 30.3%), 560 ($[M+2]^{+\cdot}$, 10.1%), 562 ($[M+4]^{+\cdot}$, 3.4%); Anal. Calcd. for $C_{27}H_{16}Cl_2N_6S_2$ (559): C, 57.96; H, 2.88; N, 15.02%. found: C, 58.02; H, 2.64; N, 15.31%.

6-(2,4-dichlorophenyl)-4-((3-(6-(4-nitrophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (8c).

Yield: 80%: mp: 240-242ºC: IR (KBr cm^{-1}): 3328 (NH), 3176 (CH, aromatic), 2273 (C≡N), 1550 (N=O), 1300 (N-O), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.20-8.10 (m, 11H, Ar-H), 8.40 (s, 1H, thiopyrimidine H), 10.40 (s,1H, NH, D_2O exchangeable), 10.60 (s,1H, NH, D_2O exchangeable), 10.90 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 603 ($[M]^{+\cdot}$, 18.6 %), 605 ($[M+2]^{+\cdot}$, 6.2%), 607 ($[M+4]^{+\cdot}$, 2.1%); Anal. Calcd. for $C_{27}H_{15}Cl_2N_7O_2S_2$ (604): C, 53.65; H, 2.50; N, 16.22%. found: C, 53.44; H, 2.81; N, 16.39%.

4-((3-(6-(4-bromophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (8d).

Yield: 77%: mp: 288-290ºC: IR (KBr cm^{-1}): 3316 (NH), 3184 (CH, aromatic), 2265(C≡N), 1272 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.00-8.40 (m, 11H, Ar-H), 8.60 (s, 1H, thiopyrimidine H), 10.20 (s,1H, NH, D2O exchangeable), 10.60 (s,1H, NH, D_2O exchangeable), 11.20 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 636 ($[M]^{+\cdot}$, 13.3 %), 638 ($[M+2]^{+\cdot}$, 13.3%); Anal. Calcd. for $C_{27}H_{15}BrCl_2N_6S_2$ (638): C, 50.80; H, 2.37; N, 13.16%. found: C, 50.55; H, 2.48; N, 13.37%.

4-(3-(5-cyano-1,6-dihydro-4-(4-aryl)-6-oxopyridin-2-yl)phenylamino)-6-(2,4-dichlorophenyl)-1,2-dihydro-2-thioxopyrimidine-5-carbonitrile: (9a-d).

An ethanolic mixture of (4a-d) (0.001mol) and ethyl cyano acetate (0.001mol) in the presence of ammonium acetate (0.01mol) and acetic acid (10 ml) was refluxed for 8h. The reaction mixture was allowed to cool, and the precipitate was filtered off, dried, and recrystallized from DMF/water.

Another procedure:

A mixture of acetyl derivative (3) (0.001mol), the appropriate aldehyde (0.001mol), ethyl cyano acetate (0.001mol) and excess ammonium acetate (0.02mol) in 30 ml absolute ethanol was refluxed for 6-10h. The reaction mixture was concentrated, filtered off and the filtrate was poured into ice/water and the produced precipitate was filtered off, dried and crystallized from DMF/water.

4-((3-(5-cyano-4-(4-methoxyphenyl)-6-oxo-1,6-dihydropyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (9a).

Yield: 78%: mp: 230-232ºC: IR (KBr cm^{-1}): 3306 (NH), 3190 (CH, aromatic), 2963 (CH, aliphatic), 2212(C≡N), 1675 (C=O), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.70 (s, 3H, OCH_3), 7.10-8.05 (m, 11H, Ar-H), 10.30 (s,1H, NH, D_2O exchangeable), 10.80 (s,1H, NH, D_2O exchangeable), 12.00 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 596 ($[M]^{+\cdot}$, 12.6 %), 598 ($[M+2]^{+\cdot}$, 4.2%), 600 ($[M+4]^{+\cdot}$, 1.4%); Anal. Calcd. for $C_{30}H_{18}Cl_2N_6O_2S$ (597): C, 60.31; H, 3.04; N, 14.07%. found: C, 60.64; H, 3.31; N, 13.95%.

4-((3-(5-cyano-6-oxo-4-phenyl-1,6-dihydropyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (9b).

Yield: 64%: mp: 244-246ºC: IR (KBr cm^{-1}): 3324 (NH), 3195 (CH, aromatic), 2229 (C≡N), 1680 (C=O), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.30-8.40 (m, 12H, Ar-H), 10.20 (s,1H, NH, D_2O exchangeable), 11.00 (s,1H, NH, D_2O exchangeable), 11.90 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 566 ($[M]^{+\cdot}$, 30.3%), 568 ($[M+2]^{+\cdot}$, 10.1%), 570 ($[M+2]^{+\cdot}$, 3.4%); Anal. Calcd. for $C_{29}H_{16}Cl_2N_6OS$ (567): C, 61.38; H, 2.84; N, 14.81%. found: C, 61.15; H, 2.96; N, 14.63%.

4-((3-(5-cyano-4-(4-nitrophenyl)-6-oxo-1,6-dihydropyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (9c).

Yield: 75%: mp: 223-225ºC: IR (KBr cm^{-1}): 3327 (NH), 3192 (CH, aromatic), 2200 (C≡N), 1670 (C=O), 1550 (N=O), 1365 (N-O), 1271 (C=S);

^{1}HNMR (DMSO-d_6), δ(ppm): 7.20-8.50 (m, 11H, Ar-H), 10.40 (s,1H, NH, D_2O exchangeable), 10.90 (s,1H, NH, D_2O exchangeable), 11.80 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 611 ([M]$^{+\cdot}$, 15.3 %), 613 ([M+2]$^{+\cdot}$, 5.1%), 615 ([M+4]$^{+\cdot}$, 1.7%); Anal. Calcd. for $C_{29}H_{15}Cl_2N_7O_3S$ (612): C, 56.87; H, 2.47; N, 16.01%. found: C, 56.72; H, 2.63; N, 15.89%.

4-((3-(4-(4-bromophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (9d).

Yield: 60%: mp: 262-264ºC: IR (KBr cm^{-1}): 3280 (NH), 3197 (CH, aromatic), 2237 (C≡N), 1687 (C=O), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 7.10-8.20 (m, 11H, Ar-H), 10.10 (s,1H, NH, D_2O exchangeable), 10.70 (s,1H, NH, D_2O exchangeable), 12.00 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 644 ([M]$^{+\cdot}$, 21%), 646 ([M+2]$^{+\cdot}$, 21%); Anal. Calcd. for $C_{29}H_{15}BrCl_2N_6OS$ (646): C, 53.89; H, 2.34; N, 13.00%. found: C, 53.58; H, 2.47; N, 12.86%.

4-((3-(6-amino-5-cyano-4-(4-aryl)pyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (10a-d).

A mixture of (4a-d) (0.001mol), Malonitrile (0.001mol), and ammonium acetate (0.03mol) as catalyst was refluxed in acetic acid (10 ml) for 8h. The reaction mixture was cooled and poured onto ice-water. The solid obtained was filtered off, dried, and recrystallized from DMF/water.

4-((3-(6-amino-5-cyano-4-(4-methoxyphenyl)pyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (10a).

Yield: 68%: mp: 253-255ºC: IR (KBr cm^{-1}): 3430 (NH_2), 3243 (NH), 3190 (CH, aromatic), 2982 (CH, aliphatic), 2214 (C≡N), 1272 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 3.70 (s, 3H, OCH_3), 5.50 (s, 2H, NH_2, D_2O exchangeable), 7.06-8.05 (m, 12H, Ar-H), 10.40 (s,1H, NH, D_2O exchangeable), 11.20 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 595 ([M]$^{+\cdot}$, 33.6 %), 597 ([M+2]$^{+\cdot}$, 11.2%), 599 ([M+4]$^{+\cdot}$, 3.7%); Anal. Calcd. for $C_{30}H_{19}Cl_2N_7OS$ (596): C, 60.41; H, 3.21; N, 16.44%. found: C, 60.72; H, 3.37; N, 16.83%.

4-((3-(6-amino-5-cyano-4-phenylpyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (10b).

Yield: 70%: mp: 268-270ºC: IR (KBr cm-1): 3420 (NH_2), 3267 (NH), 3187 (CH, aromatic), 2228 (C≡N), 1273 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 5.30 (s, 2H, NH_2, D_2O exchangeable), 7.20-8.30 (m, 13H, Ar-H), 10.50 (s,1H, NH, D_2O exchangeable), 11.40 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 565 ([M]$^{+\cdot}$, 24.3%), 567 ([M+2]$^{+\cdot}$, 8.1%), 569 ([M+4]$^{+\cdot}$, 2.7%); Anal. Calcd. for $C_{29}H_{17}Cl_2N_7S$ (566): C, 61.49; H, 3.03; N, 17.31%. found: C, 61.73; H, 2.92; N, 17.56%.

4-((3-(6-amino-5-cyano-4-(4-nitrophenyl)pyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (10c).

Yield: 74%: mp: 240-242ºC: IR (KBr cm^{-1}): 3380 (NH_2), 3227 (NH), 3175 (CH, aromatic), 2250 (C≡N), 1500 (N=O), 1360 (N-O), 1275 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 5.60 (s, 2H, NH_2, D_2O exchangeable), 7.10-8.20 (m, 12H, Ar-H), 10.20 (s,1H, NH, D_2O exchangeable), 11.00 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 610 ([M]$^{+\cdot}$, 10.5 %), 612 ([M+2]$^{+\cdot}$, 3.5%), 614 ([M+4]$^{+\cdot}$, 1.2%); Anal. Calcd. for $C_{29}H_{16}Cl_2N_8O_2S$ (611): C, 56.97; H, 2.64; N, 18.33%. found: C, 57.05; H, 2.48; N, 18.21%.

4-((3-(6-amino-4-(4-bromophenyl)-5-cyanopyridin-2-yl)phenyl)amino)-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile: (10d).

Yield: 72%: mp: 282-284ºC: IR (KBr cm^{-1}): 3396 (NH_2), 3236 (NH), 3187 (CH, aromatic), 2245 (C≡N), 1273 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 5.70 (s, 2H, NH_2, D_2O exchangeable), 7.40-8.50 (m, 12H, Ar-H), 10.50 (s,1H, NH, D_2O exchangeable), 11.40 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 643 ([M]$^{+\cdot}$, 15.2%), 645 ([M+2]$^{+\cdot}$, 15.2%); Anal. Calcd. for $C_{29}H_{16}BrCl_2N_7S$ (645): C, 53.97; H, 2.50; N, 15.19%. found: C, 53.76; H, 2.64; N, 15.35%.

(±)Ethyl-6-amino-2-(3-((5-cyano-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)phenyl)-4-(4-aryl)-2H-pyran-5-carboxylate: (11a-d).

A mixture of (4a-d) (0.001mol), ethyl cyano acetate (0.001mol) and sodium acetate (0.03mol) was refluxed in acetic acid (10 ml) for 8h, then cooled and poured into ice/HCl solution. The produced solid was collected, filtered off, dried, and recrystallized from DMF/water.

(±)Ethyl-6-amino-2-(3-((5-cyano-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)phenyl)-4-(4-methoxyphenyl)-2H-pyran-5-carboxylate: (11a).

Yield: 70%: mp: 290-292ºC: IR (KBr cm^{-1}): 3410 (NH_2), 3242 (NH), 3182 (CH, aromatic), 2968 (CH, aliphatic), 2220 (C≡N), 1785 (C=O), 1271 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 1.40 (t, 3H, OCH_2CH_3), 3.90 (s, 3H, OCH_3), 4.20 (q, 2H, OCH_2CH_3), 6.60 (s, 2H, NH_2, D_2O exchangeable), 7.30 (s, 1H, chiral H), 7.40-8.30 (m, 12H, Ar-H), 10.10 (s,1H, NH, D_2O exchangeable), 10.60 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 645 ([M]$^{+\cdot}$, 13.3 %), 647 ([M+2]$^{+\cdot}$, 4.4%), 649 ([M+4]$^{+\cdot}$, 1.5%); Anal. Calcd. for $C_{32}H_{25}Cl_2N_5O_4S$ (646): C, 59.45; H, 3.90; N, 10.83%. found: C, 59.52; H, 3.74; N, 10.67%.

(±)Ethyl-6-amino-2-(3-((5-cyano-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)phenyl)-4-phenyl-2H-pyran-5-carboxylate: (11b).

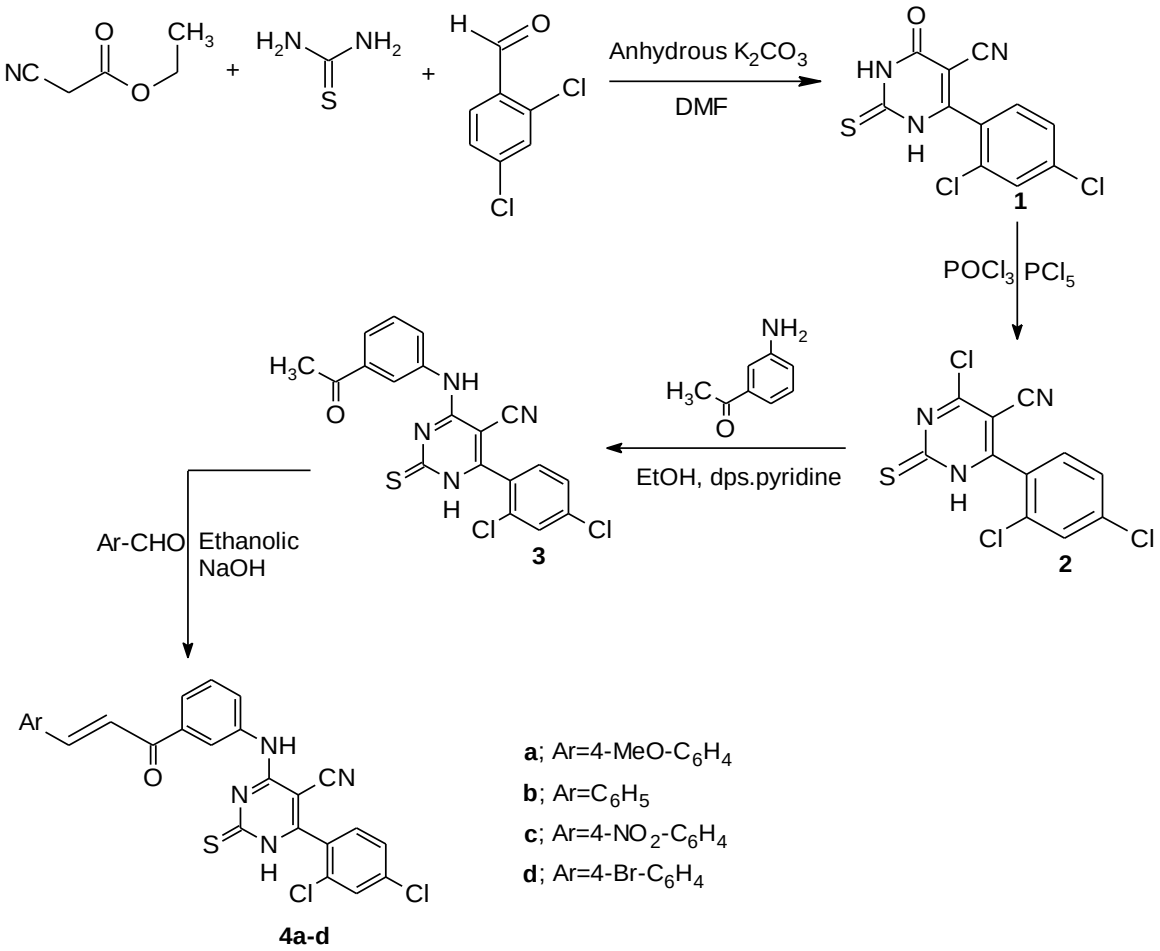
Yield: 65%: mp: 243--245ºC: IR (KBr cm^{-1}): 3425 (NH_2), 3258 (NH), 3158 (CH, aromatic), 2976 (CH, aliphatic), 2270 (C≡N), 1778 (C=O), 1272 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 1.40 (t, 3H, OCH_2CH_3), 4.00 (q, 2H, OCH_2CH_3), 6.40 (s, 2H, NH_2, D_2O exchangeable), 7.10 (s, 1H, chiral H), 7.30-8.40 (m, 13H, Ar-H), 10.20 (s,1H, NH, D_2O exchangeable), 10.50 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 615 ([M]$^{+\cdot}$, 19.6 %), 617 ([M+2]$^{+\cdot}$, 6.5%), 619 ([M+4]$^{+\cdot}$, 2.2%); Anal. Calcd. for $C_{31}H_{23}Cl_2N_5O_3S$ (616): C, 60.39; H, 3.76; N, 11.36%. found: C, 60.16; H, 3.92; N, 11.31%.

(±)Ethyl-6-amino-2-(3-((5-cyano-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)phenyl)-4-(4-nitrophenyl)-2H-pyran-5-carboxylate: (11c).

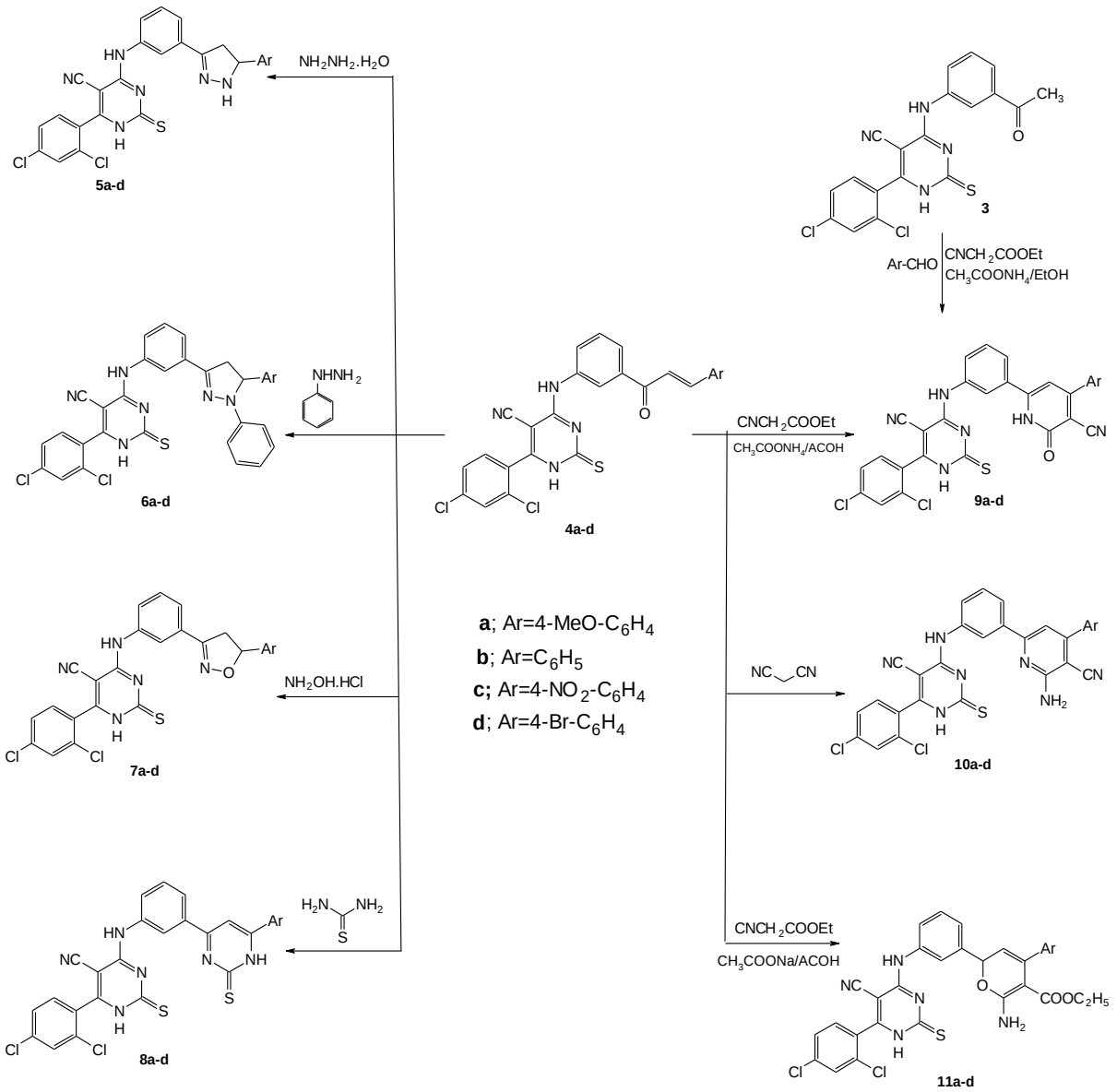
Yield: 72%: mp: 283--285ºC: IR (KBr cm^{-1}): 3388 (NH_2), 3274 (NH), 3172 (CH, aromatic), 2964 (CH, aliphatic), 2232 (C≡N), 1765 (C=O), 1500 (N=O), 1300 (N-O), 1274 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 1.30 (t, 3H, OCH_2CH_3), 4.30 (q, 2H, OCH_2CH_3), 6.20 (s, 2H, NH_2, D_2O exchangeable), 7.00 (s, 1H, chiral H), 7.10-8.30 (m, 12H, Ar-H), 10.40 (s,1H, NH, D_2O exchangeable), 10.80 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 660 ([M]$^{+\cdot}$, 24.3 %), 662 ([M+2]$^{+\cdot}$, 8.1%), 664 ([M+4]$^{+\cdot}$, 2.7%); Anal. Calcd. for $C_{31}H_{22}Cl_2N_6O_5S$ (661): C, 56.29; H, 3.35; N, 12.70%. found: C, 56.10; H, 3.57; N, 12.62%.

(±)Ethyl-6-amino-4-(4-bromophenyl)-2-(3-((5-cyano-6-(2,4-dichlorophenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)phenyl)-2H-pyran-5-carboxylate: (11d).

Yield: 66%: mp: >300ºC: IR (KBr cm^{-1}): 3435 (NH_2), 3281 (NH), 3184 (CH, aromatic), 2973 (CH, aliphatic), 2285 (C≡N), 1780 (C=O), 1270 (C=S); ^{1}HNMR (DMSO-d_6), δ(ppm): 1.40 (t, 3H, OCH_2CH_3), 4.40 (q, 2H, OCH_2CH_3), 6.50 (s, 2H, NH_2, D_2O exchangeable), 7.20 (s, 1H, chiral H), 7.40-8.50 (m, 12H, Ar-H), 10.00 (s,1H, NH, D_2O exchangeable), 10.70 (s,1H, NH, D_2O exchangeable); MS (EI) *m/z*: 693 ([M]$^{+\cdot}$, 12.5 %), 695 ([M+2]$^{+\cdot}$, 12.5%); Anal. Calcd. for $C_{31}H_{22}BrCl_2N_5O_3S$ (695): C, 53.54; H, 3.19; N, 10.07%. found: C, 53.28; H, 3.35; N, 10.19%.



Scheme I. Synthesis of compounds (4a-d).



Scheme II. Synthesis of compounds (5a-d) – (11a-d).

Antiviral activity

21 compounds of the newly synthesized 6-aryl-5-cyano-2-thiouracil derivatives were evaluated for their antiviral activity against bovine viral diarrhea virus (BVDV) by using serial dilution of the virus stock (NADL (National Animal Disease Laboratory) strain) on MDBK (Madin-Darby Bovine Kidney) cells. The experiment has been done using 10 fold serial dilutions from 10^{-3} to 10^{-7} of the virus material then100 µl of each dilution was transferred to 4 wells of the micro-titer plate and100 µl of cell suspension was added to each of the well containing virus dilution and also include 4 wells as cell control. the cytopathic effect in the wells inoculated with virus dilutions was observed after 3 to 4 days under microscope, virus cause rounding of normal cells. The virus titer is calculated by the method of Reed and Muench [32], the titer was $10^{-4.6}$ PFU per 0.1 ml. the antiviral activity of newly synthesized compounds was evaluated by BVDV plaque reduction assay [33].

BVDV plaque reduction assay

MDBK cells were seeded into 6 or 12-well cell culture plates and allowed to grow to confluence, then the cells were washed in phosphate buffered saline (PBS) and then infected with BVDV. After 2 h the virus inoculum was removed and replaced with 1.5 ml of DMEM (Dulbecco's Modified Eagle Medium) containing 0.5% Sea plaque agarose in addition, varying concentrations of test compound were added. After 3–4 days' incubation at 37°C and 5% CO_2, cells were fixed with 1.5 ml of 10%

formaldehyde solution for at least 2h at ambient temperature, then the agarose layer was gently removed in warm water and the fixed monolayers were stained with 0.3% methylene blue. Plaques were identified by microscopic examination and the percentage inhibition of plaque formation was estimated by comparing mean plaque numbers in test wells with the mean plaque number in the control wells containing no test compound.

Results and Discussion

The starting blocking unit 2-thiouracil derivative (1) was prepared[31,34] from the classic Biginelli condensation of ethyl cyanoacetate, 2,4-dichlorobenzaldehyde and thiourea in basic medium of anhydrous K_2CO_3.The IR-spectrum of this compound showed absorption at (2227 cm^{-1} and 1662 cm^{-1}) for C≡N and C=O respectively. ^{1}HNMR spectrum showed a singlet signal at δ 9.70 and δ 11.30 for 2 hydrogens of pyrimidine ring (2NH).

Compound (1) was then chlorinated by $POCl_3$ / PCl_5 mixture to afford chlorothiopyrimidine (2). Its IR-spectrum showed the disappearance of C=O absorption band.

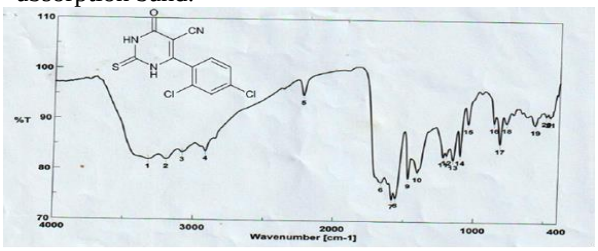


Fig (I) show chart of IR for compound (1)

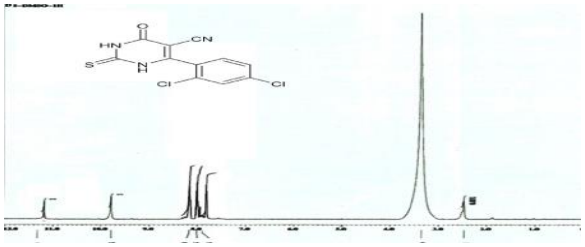


Fig.(II) show chart of ^{1}HNMR for compound (1)

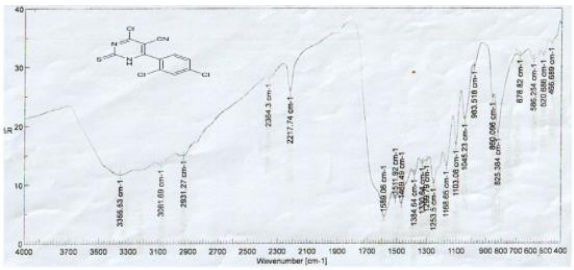


Fig (III) show chart of IR for compound (2).

This chloropyrimidine derivative (2) was condensed with *m*-amino-acetophenone yielding acetylphenyl aminothiopyrimidine derivative (3), its ^{1}HNMR spectrum showed the presence of a peak at δ 2.10 corresponding to 3H of CH_3CO group.

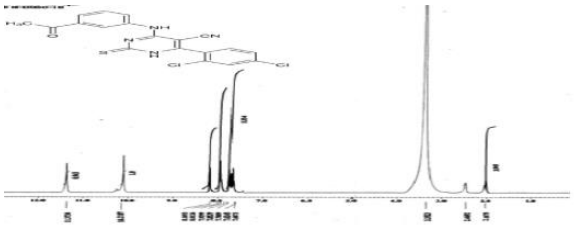


Fig.(IV) show chart of ^{1}HNMR for compound (3).

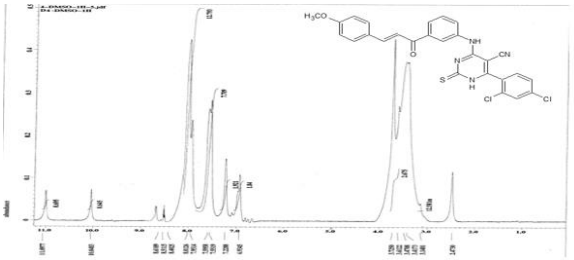


Fig.(V) show chart of ^{1}HNMR for compound (4b).

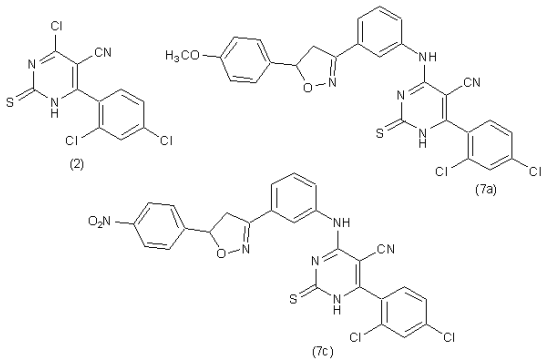


Fig. (VI): chemical structures of potent 2-Thiouracil-5-carbonitrile derivatives against BVDV

This acetyl derivative (3) was subjected to Claisen - Schmidt condensation with a series of aromatic aldehydes namely, *p*-anisaldehyde, benzaldehyde, *p*-nitrobenzaldehyde and *p*-bromobenzaldehyde to get chalcone derivatives (4a-d) respectively, where a characteristic ^{1}HNMR peak (dd, CH=CH) will appear in their ^{1}HNMR-charts.

Furthermore, these chalcone derivatives (4a-d) were cyclo-condensed with hydrazine, phenyl hydrazine, hydroxylamine HCl, thiourea, ethyl cyanoacetate/ ammonium acetate/acetic acid, malononitrile, ethyl cyanoacetate/acetic acid/sodium acetate to give pyrazoline, phenyl pyrazoline, isoxazoline, thiopyrimidine, pyridinone, aminopyridine and aminopyran derivatives (5a-d) to (11a-d) respectively. Some of the prepared compounds were screened for antiviral activity against Bovine Viral Diarrhea (BVDV) which is an economically significant disease of cattle. Compound (2), (7a) and (7c) showed promising antiviral activity.

Compound (2) showed good antiviral activity that has structural resemblance to Lamivudine which is a drug used to prevent and treat HIV/AIDS [35].
Compounds (7a) and (7c) are isoxazoline derivatives. Isoxazole ring have known antiviral activity as exemplified by Pleconaril drug which used for prevention of asthma exacerbation and common cold symptoms against picorna virus respiratory infections [36].

Antiviral assay results

21 compounds of the newly synthesized 6-aryl-5-cyano-2-thiouracil derivatives were evaluated for their antiviral activity against bovine viral diarrhea virus (BVDV) by measuring plaque forming unit (PFU) through BVDV plaque reduction assay. Among the prepared compounds (2), (7a), (7c) showed potent antiviral activity against BVDV compared with negative control (table 1).

Table 1: Antiviral results of the tested compounds

Comp. No.	10 µg/ml	notes
1	85 $*10^{-4.6}$ PFU	unreactive
2	2 $*10^{-4.6}$ PFU	potent
3	82 $*10^{-4.6}$ PFU	weak
4a	82$*10^{-4.6}$ PFU	weak
4b	85 $*10^{-4.6}$ PFU	unreactive
5a	85 $*10^{-4.6}$ PFU	unreactive
5c	85 $*10^{-4.6}$ PFU	unreactive
5d	60 $*10^{-4.6}$ PFU	weak
6a	82 $*10^{-4.6}$ PFU	weak
6c	85 $*10^{-4.6}$ PFU	unreactive
Positive control	85$*10^{-4.6}$ PFU	unreactive
7a	4 $*10^{-4.6}$ PFU	potent
7b	87 $*10^{-4.6}$ PFU	unreactive
7c	6 $*10^{-4.6}$ PFU	potent
8a	85 $*10^{-4.6}$ PFU	unreactive
8d	85 $*10^{-4.6}$ PFU	unreactive
9a	35 $*10^{-4.6}$ PFU	moderate
9b	85 $*10^{-4.6}$ PFU	unreactive
9d	85 $*10^{-4.6}$ PFU	unreactive
10a	83.4 $*10^{-4.6}$ PFU	weak
10c	85 $*10^{-4.6}$ PFU	unreactive
11b	85$*10^{-4.6}$ PFU	unreactive
Negative control	2 $*10^{-4.6}$ PFU	potent

CONCLUSION

In conclusion, as presented in this study, novel 2-thiouracil derivatives were synthesized and used them to prepare novel isoxazole, thiopyrimidine, pyridinones, aminopyridine, aminopyran derivatives. 21 of the synthesized compounds were investigated for their anti-viral activity against Bovine Viral Diarrhea Virus (BVDV); the biological results revealed that compound (2), (7a), and (7c) showed promising anti-viral activity against (BVDV). Thus, these compounds might be promising anti-viral candidates and there is a need for more studies.

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Conflict of interest

Authors would like to declare that there are no relationships or interests that could have direct or potential influence or impart bias on the work.

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