International Research Journal of Natural and Applied Sciences
ISSN: (2394-4077)
Impact Factor- 5.46, Volume 6, Issue 9, September 2019
Website- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

One-pot Multicomponent Synthesis of Some Pyridine-3-carbonitrile Derivatives and Their Biological Evaluation as Cytotoxic Agent

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Abstract: A series of 2-amino-3-cyano-4,6-disubistituted pyridines supported with some functionalities reported to contribute to significant chemotherapeutic potential were synthesized via One-pot Multicomponent reaction and evaluated for their cytotoxic activity. Ten compounds exhibited cytotoxic potential against a panel of three human tumor cell lines. Compounds **29**, **35**, and **36** proved to be the most active agents with a broad spectrum of cytotoxic activity. Analog **35** was considered as the most active cytotoxic agent, being about two times more active than doxorubicin against the colon HT29 carcinoma cell line.

Keywords: Synthesis, 3-Cyanopyridines, Pyrido-pyrimidines, Cytotoxic

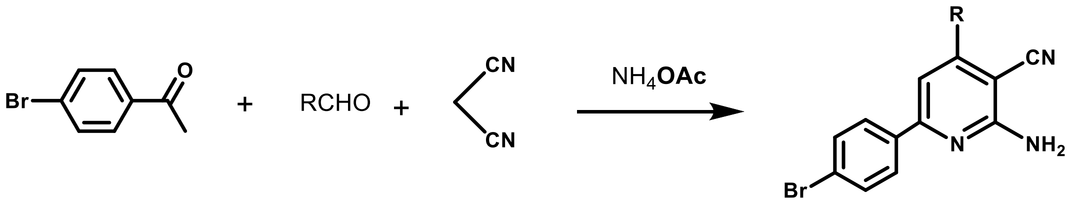
1. Introduction

Cancer is a growing public problem whose estimated worldwide new incidence is about 6 million cases per year. It is the second major cause of death after cardiovascular diseases and is characterized by unregulated proliferation of cells. Therefore, such rapid spread of cancer has stimulated an unprecedented level of medicinal chemistry research activity directed towards the search for new structure leads that may be of use in designing novel antitumor drugs. In this view, much interest has been focussed on pyridines and pyridine fused-ring systems since they are proved to be biological versatile compounds possessing variety of activities. Among these, wide range of chemotherapeutic activities have been ascribed to pyridine derivatives including the antimicrobial[1-3], antitubercular [4,5], antiamoebic[6], antiparasitic[7,8], antiviral [9.10]. Moreover, particular interest has been focussed on cyanopyridine derivatives owing to their well documented anticancer[11-15] activities. Motivated by these facts, it was thought worthwhile to synthesize and investigate the anticancer and antimicrobial activities of some new hydroxyl- and amino-cyanopyridine derivatives. Furthermore, some structure hybrids comprising both the pyridine and some biologically active rings such as aryl or theinyl moieties, in one and the same structure entity. This combination is suggested in an attempt to investigate the influence of such hybridization on the anticipated anticancer and/or antimicrobial activity, hoping to discover a new structure lead that would have a remarkable biological significance. The target compounds were rationalized so as to comprise the pharmacophores and functionalities that are believed to be responsible for the biological significance of some relevant anticancer agents. The substitution pattern of such derivatives was selected so as to confer different electronic environment to the molecules that would affect their pharmacokinetics.

2. Results and discussion

2.1 Chemistry

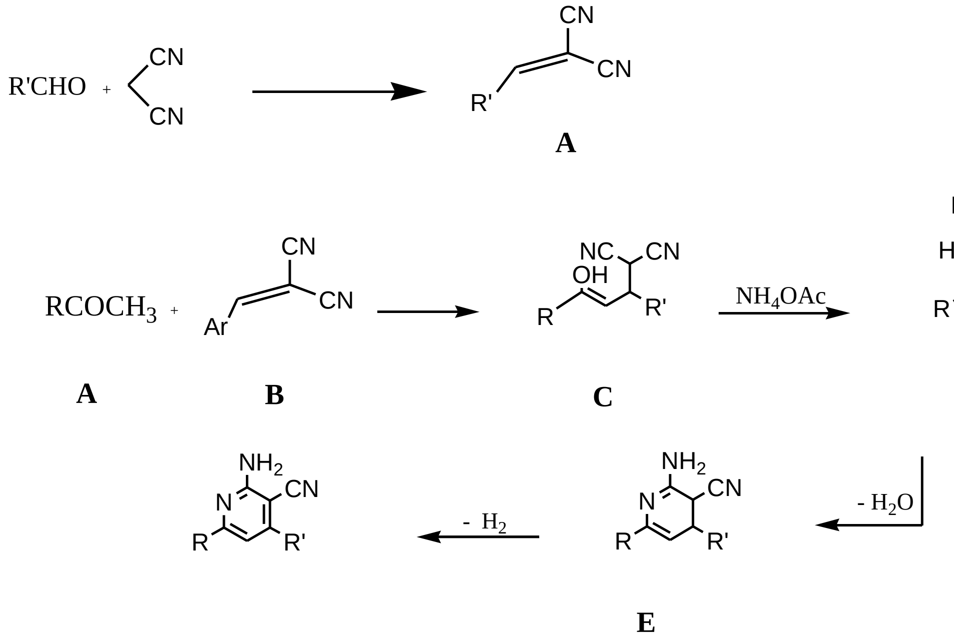
The 2-Amino-3-cyano-4,6-disubistituted pyridines **1-6** were be directly prepared via one-pot multicomponent reaction (MCR) of the appropriate aromatic aldehydes, 4-bromoacetophenone, malononitrile and excess of ammonium acetate in boiling ethanol (Scheme 1).



1-6

Scheme 1

Such type of reactions has received considerable interest since it is easier to perform, gives higher yields and less time consuming. The formation of the above 2-aminopyridines may be explained according to the following mechanism: The reaction seemed to be started by first addition of active hydrogen of compound **A** to the ethylenic double bond of compound **B** to give **C**. Ammonia was added to the nitrile group in **C** to give **D** which loss a molecule of water to yield **E**, which in turn was converted to the final product by auto-oxidation (Scheme 2).



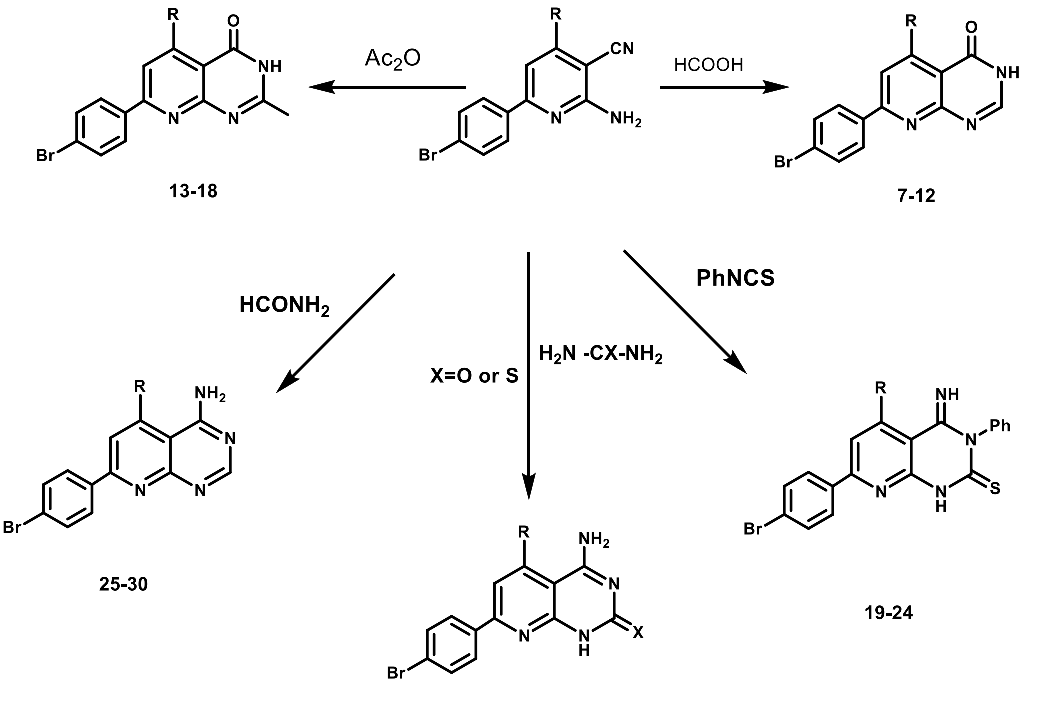
R=4-BrC$_6$H$_4$

Scheme 2

The IR spectra of these compounds revealed absorption bands at 3249-3287 cm^{-1} characteristic for the NH$_2$ and at 2232-2378 cm^{-1} attributed to the CN group. Their structure was further confirmed from their ^{1}HNMR which exhibited beside the aromatic protons an exchangeable singlet of two proton intensity at δ 7.74 – 8.10 due to the NH$_2$ group, The structures were further supported from their ^{13}C NMR spectral data which showed the expected number of aliphatic and aromatic carbon. Heating the 3-cyanopyridinone derivatives **1-6** with formic acid resulted in the formation of the targeted 7-(4-bromophenyl)-5-substituted-3H-pyrido[2,3-d]pyrimidin-4-ones **7-10** (Scheme 2). Their IR spectra were characterized by the absence of the CN group absorption and the appearance of new sharp absorption bands at 1648-1656 cm^{-1} due to the new C=O groups as well as an NH absorption bands at 3285-3314 cm^{-1}. Their ^{1}H NMR which showed beside the aromatic protons an exchangeable singlet of one proton intensity at δ 7.83-8.15 for the NH group. The structures were further supported by ^{13}C NMR spectral data which showed the expected number of carbons signals (see experimental section). On the other hand, treatment the 2-Aminopyridine derivatives **1-6** with acetic anhydride in presence of few drops of concentrated sulphuric acid gave the corresponding 2-methyl-5,7-disubstituted-3H-pyrido[2,3-d]pyrimidin-4-ones **13-18**. Their IR spectra of lacked the CN bands exists in the starting pyridines and exhibited a carbonyl absorption bands at 1650-1658 cm^{-1}. The ^{1}H-NMR spectra showed beside the aromatic protons an exchangeable singlet of one proton intensity at δ 8.14-8.22 due to the NH group as well as a singlet of three proton intensity at δ2.22-2.42 ppm due to the new CH$_3$

group introduced in position-2. Moreover, their ^{13}C NMR spectral data exhibited beside the expected number of aliphatic and aromatic carbons, a new singlet at δ 20.19-21.40 ppm due to the CH_3 group as well as a CO signal at δ168.8-173.2.

Condensation of the original compounds **1-6** with phenyl isothiocyanate in alkaline medium afforded the corresponding N-phenylthiocarbamoyl analogs **19-24**. The IR spectra of these compounds showed C=S absorption at 1217-1236 cm^{-1} as well as an NH absorptions in the regions 3300-3379 cm^{-1}. The structures were further supported from their ^{1}H NMR which showed the aromatic protons an exchangeable singlet of one proton intensity at δ6.65-6.81 . Further confirmation for the structure arises from their ^{13}C NMR spectral data which exhibited the expected number of aliphatic and aromatic carbons as well as a thio carbony signal at δ188.84-197.20. Furthermore heating the key inter mediates **1-6** with formamide, afforded the corresponding 7-(4-Bromophenyl)-4-Amino-5-substituted-pyrido[2,3-d]pyrimidines **25-30** in good yields. The IR spectra of these compounds exhibited NH_2 absorbtion bands in the region at 3249-3387cm^{-1} attributed to the NH_2 group. Their ^{13}C NMR spectral data exhibited the expected number of aliphatic and aromatic carbons. Finally, It is worth to mentioning that, direct condensation of **1-6** either with thiourea or urea was utilized as fruitful way for a one-step synthesis of the target pyrimidine-2-thiones **31-36** and pyrimidine-2-ones **37-42** respectively. The IR spectra of these derivatives **31-42** were characterized by the disappearance of the absorptions of the cyano group and the appearance of broad absorption bands in the regions 3275-3462 cm^{-1} due to the NH_2 and NH groups as well as a thiocarbonyl absorbtion at 1266-1279 cm^{-1} for compounds **31-36** and a carbonyl bands in the regions 1660-1667 for compounds **37-42**. Their ^{1}H-NMR spectra showed beside the aromatic protons a broad singlet of two proton intensity at δ6.65-6.80 for the NH_2 group. Further confirmation for the structure arises from their ^{13}C NMR spectral data (see experimental section) which exhibited beside the expected number of aliphatic and aromatic carbons, a signals at δ 180.22-183.53 or δ166.24-168.34 for the CS(in case of compounds **31-36** or C (in case of compounds **37-42**



31-42

2.2 In Vitr oMTT Cytotoxicity Assay

Twenty analogs **4,5,6,10,11,12,17,18,21,22,23,24,29,30,35,36,39,40,41** and **42** were evaluated for their *in vitro* cytotoxic effect via the standard MTT [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] method[16,17] against a panel of three human tumor cell lines, namely, colon carcinoma HT29, hepatocellular carcinoma HePG2, and Caucasian breast adenocarcinoma MCF7. The results are presented in Table 1 as LC50 (μM) which is the lethal concentration of the compound that causes death of 50% of the cells in 24 h. The obtained data revealed that the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards ten of the tested compounds, namely,**5,6,11,12,17,18,29,30,35** and **36** whereas the rest compounds were either marginally active or even totally inactive. Regarding the activity against the human colon carcinoma HT29, this cell line proved to be very sensitive to all the ten active compounds. In particular, it revealed distinctive sensitivity towards compounds **29**, **35** and **36** (LC50 28.2, 24.4, and 25.5 μM, resp.) even higher than doxorubicin (LC50 40.0 μM), the reference standard cytotoxic agent utilized in this assay. Meanwhile, compounds **17** and **30** (LC50 48.6 and 44.3 μM, resp.) were nearly equipotent to doxorubicin (LC50 40.0 μM), whereas compounds **11,12** and **18** (LC50 60.2,72.6 and 71.2 μM, resp.) showed moderate cytotoxic potential against the same cell line. Shifting to the hepatocellular carcinoma HepG2, this cell line showed mild to weak sensitivity towards seven of the tested analogs with LC50 range 56.6-112.3 μM, when compared to doxorubicin (LC50 3.0 μM). Among these, the highest activity was displayed by compounds **29, 35** and

36 (LC50 60.3,56.6 and 70.4 μM, resp.). On the other hand, the human breast cancer MCF 7 emerged as the least sensitive among the cell lines tested as its growth was affected by the presence of only six test compounds. However, a remarkable growth inhibition potential was shown by analogs **29**, **35**, and **36** as evidenced from their LC50 values (LC50 10.5, 7.8 and 8.5 μM, resp.),which represents about 40–60%of the activity of doxorubicin (LC50 4.0 μM). Further interpretation of the results revealed that compounds **29**,**30,35** and **36** showed considerable broad spectrum cytotoxic activity against the three tested human tumor cell lines. In particular, compounds **29,35** and **36** proved to be the most active members in this study with special effectiveness against both the colon carcinoma HT29 (almost twice as active as doxorubicin; LC50 28.2,24.4 and 25.5 versus 40 μM, resp.) and human breast cancer MCF 7 (about 40– 60% of the activity of doxorubicin; LC50 10.5, 7.8, and 8.5 versus 4.0 μM, resp.).

A close examination of the structures of the active compounds showed that the nature of substituent (R), together with ring entity (mono- or bicyclic), seemed to influence the cytotoxic activity. In this context, compounds substituted with the benzo[*d*][1,3]dioxol-5-yl counterpart (**5, 11, 17, 29** and **35**) were in favor of better cytotoxic activity, when compared with their 2-thienyl congeners (**6,12,18,30** and **36**), as revealed from their LC50 values in Table 1. Moreover, the bicyclic pyrido[2,3-*d*]pyrimidines proved to be more active than the monocyclic nicotinonitriles. In this view, although the starting nicotinonitriles **1**–**6** lacked cytotoxic efficacy, yet the bicyclic pyrido[2,3-d]pyrimidine derivatives **7-18** showed overall mild to moderate activity, among which analog **17** (R = 3,4-$(OCH_2O)C_6H_3$)was relatively the most active regarding both potency and spectrum. Cyclization of the nicotinonitriles **1**–**6** with phenyl isothiocyanate or urea yielded the substituted bicyclic pyrido[2,3-*d*]pyrimidines **19-24** and **37-42** which were all inactive against the three tested cell lines. However, isosteric replacement of 2-one functionality in pyrido[2,3-*d*]pyrimidine-2(1*H*)-ones **37-42**, with a 2-thione group, yielded two remarkable active analogs, namely, **35** (R = 3,4-$OCH_2O)C_6H_3$) and **36** (R = 2-thienyl).

Table 1. Cytotoxic effects LC_{50} (μM) [a] of the active compounds on some human tumor cell lines using the MTT assay.

Compd no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
5	135.4	110.2	-[b]
6	140.5	-	-
11	60.2	105.5	-
12	72.6	-	-
17	48.6	95.3	88.2
18	71.2	112.3	-
29	28.2	60.3	10.5
30	44.3	-	21.9
35	24.4	56.6	7.8
36	25.5	70.4	8.5
Doxorubicin [c]	40.0	3.0	4.0

[a]LC50: Lethal concentration of the compound which causes death of 50% of cells in 24h (μM).

[b]Totally inactive against this cell line.

[c] positive control cytotoxic agent.

3. Experimental

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. 1H and 13C NMR spectra were recorded on a Bruker WM-600 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-*d6* as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

2-Amino-7-(4-Bromophenyl)-3-cyano-5-substituted pyridines (1-6)

A mixture of the aromatic aldehyde (10 mmol), 4-bromoacetophenone (1.99g, mmol), malononitrile (0.66 g, 10 mmol) and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (50 mL) was refluxed for 4-5 h. The reaction mixture was cooled and the formed precipitate was filtered, washed with water, dried and recrystallized from the appropriate solvent.

1 (R = 4-$CH_3OC_6H_4$): Recrystallized from ethanol as needles. (1.9g 78%) m.p. 104-106°C.$\nu_{max.}$(cm^{-1}, KBr): 3275,3378(NH_2), 2218(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,3H,CH_3O), 8.12 (s,2H,NH_2), 7.05-7.78 (m,9H,ArH+H-5).^{13}CNMR (δ/ppm,DMSO-d_6): 55.8 (CH_3O), 113.7 (CN), 85.6, 113.7, 114.7, 114.8, 114.8 121.7, 128.4, 128.4 129.6, 129.6, 132.1, 131.3, 138.0, 154.4, 156.1, 161.1, 161.9 (ArC). Anal.% Calcd for C19H14BrN3O: C, 60.02; H, 3.71; N, 11.05. Found: C, 59.96; H, 3.74; N, 11.02.

2 (R = 3,4-$(CH_3O)_2C_6H_3$): Recrystallized from ethanol as needles. (2.5g 82%) m.p. 240-242°C.$\nu_{max.}$(cm^{-1}, KBr): 3254,3374 (NH_2), 2225(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,6H,2CH_3O), 7.98 (s,2H,NH_2), 7.04 – 7.76 (m,8H,ArH+H-5).^{13}CNMR (δ/ppm,DMSO-d_6): 55.8, 56.1,(CH_3O), 113.7 (CN), 85.6, 98.8, 107.1, 111.1, 119.4, 121.7, 128.4, 129.4, 132.1, 138.0, 156.1, 156.6, 161.8, 162.1 (ArC). Anal.% Calcd for $C_{20}H_{16}BrN_3O_2$: C, 58.55; H, 3.93; N, 10.24. Found: C, 58.59; H, 3.90; N, 10.27.

3 (R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from ethanol as needles. (1.9g 80%) m.p. 236-238°C.$\nu_{max.}$(cm^{-1}, KBr): 3232,3367 (NH_2), 2228 (CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.80 (s,3H,CH_3O), 5.35 (s,1H,OH), 7.74 (s,2H,NH_2), 7.18-7.65 (m,8H,ArH+H-5).^{13}CNMR (δ/ppm,DMSO-d_6): 56.1 (CH_3O), 113.7 (CN), 85.6, 114.7, 115.8, 121.7, 122.3, 128.4, 128.4, 132.1, 132.1, 148.0, 149.2, 154.4, 156.1, 161.9 (ArC). Anal.% Calcd for $C_{19}H_{14}BrN_3O_2$: C, 57.53; H, 3.01; N, 10.60. Found: C, 57.82; H, 3.03; N, 10.61.

4 (R = 4-NO_2-C_6H_4): Recrystallized from ethanol as needles. (2.02g, 76%) m.p. 233-235°C. $\nu_{max.}$ (cm^{-1}, KBr): 3353, 3353 (NH_2), 2219(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 7.87 (s,2H,NH_2), 6.98-7.76 (m,9H-ArH+H-5). ^{13}CNMR (δ/ppm,DMSO-d_6): 113.7 (CN), 85.6, 114.7, 121.7, 124.4, 124.4, 128.3, 128.3, 128.4, 128.4, 132.1, 138.0, 145.1, 148.4, 154.4, 156.1, 161.9 (ArC). Anal.% Calcd for C18H11BrN4O2: C, 54.70; H, 2.81; N, 14.18. Found: C, 54.76; H, 2.79; N, 14.24.

5 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from ethanol as needles. (1.9g 68%) m.p. 198-200°C.$\nu_{max.}$(cm^{-1}, KBr): 3175,3358(NH_2), 2223(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.03 (s,2H,CH_2), 8.10 (s,2H,NH_2), 7.68-7.87 (m,8H,ArH+H-5).^{13}CNMR (δ/ppm,DMSO-d_6):

91.2(CH_2), 113.6, 115.4,117.8 (CN), 121.6,125.5,126.3, 127.0, 128.2,129.2,139.4, 142.2,148.0,150.5, 162.5, 164.9(ArC). Anal.% Calcd for $C_{19}H_{12}Br N_3O_2$: C, 57.89; H, 3.07; N, 10.66. Found: C, 57.82; H, 3.03; N, 10.61.

6 (R=2-Theinyl): Rrecrystallized from ethanol as needles. (2.02g, 73%) m.p. 200-202°C. $\nu_{max.}$ (cm^{-1}, KBr): 3416, 3198 (NH_2), 2220(CN). ^{1}HNMR (δ/ppm, DMSO-d_6): 7.75 (s,2H,NH_2), 7.10-7.65 (m,8H,ArH+H-5). ^{13}CNMR (δ/ppm,DMSO-d_6): 91.4,110.9, 118.0 (CN), 122.2, 125.3, 126.4, 127.2,128.4,129.3,139.8,142.7,151.2, 162.8, 165.5(ArC). Anal.% Calcd for $C_{16}H_{10}BrN_3S$: C, 53.94; H, 2.83; N, 11.80. Found: C, 53.88; H, 2.79; N, 11.85.

7-(4-Bromophenyl)-5-substituted-3H-pyrido[2,3-d]pyrimidin-4-ones (7-12)

A mixture of the appropriate 2-aminopyridine (10 mmol) and formic acid (5 ml) was heated in a boiling water bath for 30 min. After being cooled to room temperature, the reaction mixture was poured onto ice-cold water, the precipitated solid product was filtered, washed with water, dried and recrystallized from the appropriate solvent.

7 (R = 4-$CH_3OC_6H_4$): Recrystallized from ethanol as needles.(2.5g, 70%) m.p.120-122 °C.ν_{max} (cm^{-1}, KBr): 3314(NH), 1648 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 3.81(s,3H,CH_3O) 7.96(s,1H,CH); 7.05-7.78 (m,9H,Ar H); 8.00 (s, 1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 55.8 (CH_3O), 114.8, 114.8, 117.9, 120.9, 121.7, 128.4, 128.4, 129.6, 129.6, 132.1, 132.1, 133.2, 138.0, 145.7, 152.4, 153.9, 154.8, 161.1 (Ar C), 161.0 (CO). Anal.% Calcd for $C_{20}H_{14}BrN_3O_2$: C, 58.84; H, 3.46; N, 10.29. Found: C, 58.79; H, 3.43; N, 10.33.

8 (R = 3,4-$(CH_3O)_2C_6H_3$): Recrystallized from ethanol as needles.(2.3g, 72%) m.p.194-196 °C.ν_{max} (cm^{-1}, KBr): 3285(NH), 1652 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83(s,6H,2CH_3O)7.90(s,1H,CH); 6.89-7.70 (m,8H,Ar H); 7.83 (s, 1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 55.8, 56.1, (CH_3O), 98.8, 107.1, 117.9, 119.4, 120.9, 121.7, 128.4, 128.4, 129.4, 132.1, 132.1, 138.0, 145.7, 152.4, 153.9, 154.8, 161.8 (Ar C), 161.0 (CO). Anal.% Calcd for $C_{21}H_{16}BrN_3O_3$: C, 57.55; H, 3.68; N, 9.59. Found: C, 57.62 ; H, 3.65 ; N, 9.56.

9 (R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from ethanol as needles.(2.0g, 69%) m.p.158-160 °C.ν_{max} (cm^{-1}, KBr): 3300(NH), 1656 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83(s,3H,CH_3O) 5.35(s,1H,OH); 6.89-7.82(m,8H,Ar H); 7.96(s,1H,CH) 8.12 (s,1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 56.1 (CH_3O), 113.9, 115.8, 117.9, 120.9, 121.7, 122.3, 128.4, 128.4, 132.1, 132.1, 138.0, 138.3, 145.7, 148.0, 149.2, 152.4, 153.9 (Ar C), 161.0 (CO). Anal.% Calcd for $C_{20}H_{14}BrN_3O_3$: C, 56.62; H, 3.33; Br, N, 9.90. Found: C, 56.74 ; H, 3.36 ; N, 9.86.

10 (R = 4-NO_2-C_6H_4): Recrystallized from ethanol as needles.(2.0g, 72%) m.p.239-240 °C.ν $_{max}$ (cm^{-1}, KBr): 3299 (NH), 1653 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 7.96(s,1H,CH); 7.12-8.70(m,9H,Ar H); 7.92 (s, 1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 117.9, 120.9, 121.7, 124.4, 124.4, 128.3, 128.3, 128.4, 128.4, 132.1, 138.0, 148.4, 145.7, 152.4, 153.9, 154.8, (Ar C), 161.0 (CO). Anal.% Calcd for $C_{19}H_{11}BrN_4O_3$: C, 53.92; H, 2.62; N, 13.24. Found: C, 58.89; H, 2.59; N, 13.27.

11 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from ethanol as needles.(2.3g, 72%) m.p.110-112 °C.ν $_{max}$ (cm^{-1}, KBr): 3312 (NH), 1652 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 6.11(s,2H,CH_2); 6.80-7.98(m,9H,Ar H); 8.08 (s, 1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 91.5 (CH_2), 113.2, 116.7,119.3,120.1, 121.7,122.8,125.5,127.2, 131.6,142.3,144.0,148.2,151.7,157.3,164.0, 173.6 (Ar C), 170.2(CO). Anal.% Calcd for $C_{20}H_{12}BrN_3O_3$: C, 56.89; H, 2.86 ; N, 9.95.Found: C, 56.82 ; H, 2.79 ; N, 9.91.

12 (R=2-Theinyl): Rrecrystallized from methanol as needles. (2.0g,70%) m.p.128-130 °C. ν $_{max}$ (cm^{-1}, KBr): 3298 (NH), 1650 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.72-7,69(m,9H,ArH);8.15(s,1H,NH).^{13}CNMR(δ/ppm,DMSO-d_6):118.5,120.6,121.7, 122.8, 125.3, 127.5,129.4, 132.3,138.4,142.3,147.3,157.8, 162.5, 173.1 (Ar C), 170.3 (CO). Anal.% Calcd for $C_{17}H_{10}BrN_3OS$: C, 53.14; H, 2.62 ; N, 10.94. Found: C, 53.03 ; H, 2.60 ; N, 11.03.

7-(4-Bromophenyl)-2-methyl-5-substituted-3H-pyrido[2,3-d]pyrimidin-4-ones (13-18)

A mixture of the starting 2-aminopyridine (10 mmol), acetic anhydride (5 ml) and conc. H_2SO_4 (0.5 ml) was heated in a boiling water bath for 10 min, then cooled, poured onto ice-cold water, treated with 20% NaOH solution till alkaline (pH 11), the crude solid product was filtered , dried and recrystallized from the appropriate solvent.

13 (R = 4-$CH_3OC_6H_4$): Recrystallized from ethanol as needles. (2.1g, 72%) m.p. 84-86 °C. ν $_{max}$ (cm^{-1}, KBr): 3308 (NH), 1650 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,3H,CH_3O) 2.42 (s,3H,CH_3), 7.00-7.70(m,9H,Ar H); 8.14 (s,1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 21.40 (CH_3), 55.8 (CH_3O), 114.8, 114.8, 117.9, 120.9, 121.7, 128.4, 128.4, 129.6, 129.6, 132.1, 132.1, 133.2, 152.4, 153.9, 154.3, 154.8, 161.1 (Ar C), 161.0 (CO). Anal.% Calcd for $C_{21}H_{16}BrN_3O_2$: C, 59.73; H, 3.82; N, 9.95. Found: C, 59.68; H, 3.80; N, 10.02.

14 (R = 3,4-(CH_3O)$_2C_6H_3$): Recrystallized from ethanol as needles. (2.6g, 74%) m.p. 140-142°C. ν $_{max}$ (cm^{-1}, KBr): 3325 (NH), 1658 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,6H,2-CH_3O) 2.22 (s,3H,CH_3), 6.79-7.65 (m,8H,Ar H); 8.20 (s,1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 20.19 (CH_3), 55.8, 56.1 (CH_3O), 98.9, 107.1, 117.9, 119.4, 120.9, 121.7, 128.4, 128.4, 129.4, 132.1, 138.0, 153.9, 154.3, 154.8, 161.8, 162.1 (Ar C), 161.0 (CO). Anal.% Calcd for $C_{22}H_{18}BrN_3O_3$: C, 59.45; H, 3.88; N, 10.27. Found: C, 59.39; H, 3.90; N, 10.24.

15 (R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from ethanol as needles. (2.1g, 73%) m.p. 104-106 °C. ν $_{max}$ (cm^{-1}, KBr): 3322 (NH), 1656 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 3.80 (s,3H,CH_3O) 2.34 (s,3H,CH_3), 5.35(s,1H,OH); 6.89-7.75(m,8H,Ar H); 8.16(s,1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 21.4 (CH_3), 56.1 (CH_3O), 113.9, 115.8, 117.9, 120.9, 121.7, 122.3, 128.4, 128.4, 132.1, 132.1, 138.0, 138.3, 148.0, 149.2, 152.4, 153.9, 154.3, 154.8, (Ar C), 161.0 (CO). Anal.% Calcd for $C_{21}H_{16}BrN_3O_3$: C, 57.55; H, 3.68; N, 9.59. Found: C, 57.59; H, 3.70; N, 10.02.

16 (R = 4-NO_2-C_6H_4): Recrystallized from ethanol as needles. (2.0g, 70%) m.p. 150-152 °C. ν $_{max}$ (cm^{-1}, KBr): 3298 (NH), 1654(C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 2.38 (s,3H,CH_3), 7.69-8.4.5 (m,9H,Ar H); 8.20 (s,1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 21.40 (CH_3), 117.9, 120.9, 121.7, 124.4, 124.4, 128.3, 128.4, 132.1, 132.1, 138.0, 147.0, 148.4, 152.4, 153.9, 154.3, 154.8, (Ar C), 161.0 (CO). Anal.% Calcd for C20H13BrN4O3: C, 54.94; H, 3.00; N, 12.81. Found: C, 54.99; H, 2.97; N, 12.86.

17 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from ethanol as needles. (1.7g, 52%) m.p. 220-222°C. ν $_{max}$ (cm^{-1}, KBr): 3265 (NH), 1651 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): ^{1}HNMR (δ/ppm, DMSO-d_6): 2.32 (s,3H,CH_3), 6.16(s,2H,CH_2); 6.82-7.96(m,8H,Ar H); 8.23 (s,1H,NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 21.22 (CH_3), 91.3 (CH_2), 113.5, 116.3,118.2,119.9,120.0,122.1,125.4,127.6,131.4,142.6,144.2, 152.8, 148.1,157.9, 163.4, 171.7 (Ar C), 173.2(CO). Anal.% Calcd for $C_{21}H_{14}BrN_3O_3$: C, 57.82; H, 3.23; N, 9.63. Found: C, 57.91; H, 3.17; N, 9.75.

18 (R=2-Theinyl): Rrecrystallized from ethanol as needles. (2.0g, 63%) m.p. 248-250°C. ν $_{max}$ (cm^{-1}, KBr): 3270(NH), 1652 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 2.35 (s,3H,CH_3), 6.68-7.77(m,8H,ArH);8.25(s,1H,NH).^{13}CNMR(δ/ppm,DMSO-d_6): 21.08 (CH_3), 118.8,120.5,121.8, 122.9, 125.2, 127.4,129.3, 132.2,138.3,142.2,147.1,157.7, 163.1, 172.9 (Ar C), 170.2 (CO). Anal.% Calcd for $C_{18}H_{12}BrN_3OS$: C, 54.24; H, 3.04; N, 10.55. Found: C, 54.17; H, 3.00; N, 10.61.

4-Imino-3-phenyl-7-(4-Bromophenyl)-5-substituted-3,4-dihydro-1H-pyrido[2,3-d]-pyrimidine-2-thiones (19-24)

A mixture of the appropriate 2-aminopyridine (10 mmol), phenyl isothiocyanate (0.15 g, 15 mmol) in pyridine (15 ml) was refluxed for 4 h. After cooling, the solid product was filtered off, washed thoroughly with water, dried and recrystallized from the appropriate solvent.

19 (R = 4-$CH_3OC_6H_4$): Recrystallized from ethanol as needles. (2.8g, 70%) m.p. 198-200°C. ν $_{max}$ (cm^{-1}, KBr): 3379 (NH), 1628 (C=N), 1217 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,3H,CH_3O), 6.68 (s,1H,C=NH);6.82-7.56(m,15H, ArH+ NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.8 (CH_3O), 108.1, 110.6, 114.8, 114.8, 121.7, 124.8, 128.4, 128.4, 128.7, 128.7, 129.0, 129.0, 129.6, 132.1, 133.2, 133.2, 135.7, 138.0, 148.1, 149.5, 153.3, 161.1 (ArC), 156.2 (C=NH), 188.84 (CS). Anal.% Calcd for C26H19BrN4OS: C, 60.59; H, 3.72; N, 10.87. Found: C, 60.46; H, 3.70; N, 10.84.

20 (R = 3,4-$(CH_3O)_2C_6H_3$): Recrystallized from ethanol as needles. (3.1g, 76%) m.p. 144-146 °C. ν $_{max}$ (cm^{-1}, KBr): 3321 (NH), 1669 (C=N), 1220 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,3H,CH_3O), 6.69 (s,1H,C=NH) ; 6.68-7.69 (m,14H,ArH+NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 55.8, 56.1, (CH_3O), 98.8, 107.1, 108.0, 108.1, 110.6, 119.4, 121.7, 128.4, 128.4, 128.4, 128.7, 128.7, 129.0, 129.0, 129.4, 132.1, 132.1, 148.1, 149.5, 153.3, 161.8, 162.1 (ArC), 156.2 (C=NH), 188.84 (CS).Anal.% Calcd for $C_{26}H_{17}BrN_4O_2S$: C, 59.45; H, 3.88; N, 10.27 Found: C, 59.51; H, 3.90; N, 10.31.

21 (R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from ethanol as needles. (3.0g, 74%) m.p. 192-194 °C. ν $_{max}$ (cm^{-1}, KBr): 3300 (NH), 1669 (C=N), 1236 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.82 (s,3H,CH_3O) 5.36 (s,1H,OH), 6.65 (s,1H, C=NH);6.25-8.29(m,14H,ArH+NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 56.1 (CH_3O), 108.1,110.6, 113.9, 115.8, 121.7, 122.4, 128.4, 128.4, 128.4, 128.7, 128.7, 129.0, 129.0, 132.1, 132.1, 138.0, 135.7, 138.3, 148.0, 148.1, 149.2, 149.5, 153.3 (ArC), 156.2 (C=NH), 197.20 (CS).Anal.% Calcd for C26H19BrN4O2S: C, 58.76; H, 3.60; N, 10.54. Found: C, 58.69; H, 3.63; N, 10.57.

22 (R = 4-NO_2-C_6H_4): Recrystallized from ethanol as needles. (2.9g, 72%) m.p. 180-182°C. ν $_{max}$ (cm^{-1}, KBr): 3298 (NH), 1618 (C=N), 1224 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.70 (s,1H,C=NH);7.03-7.98(m,15H,ArH+NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 108.1, 110.6, 114.8, 114.8, 124.4, 128.3, 124.8, 128.4, 128.4, 129.0, 129.0,132.1, 147.0, 148.1, 148.4, 149.5, 153.3, 156.2, (ArC), 156.2 (C=NH), 190.14 (CS). Anal.% Calcd for C25H16BrN5O2S: C, 56.61; H, 3.04; N, 13.20. Found: C, 56.57; H, 3.01; N, 13.17S.

23 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from ethanol as needles. (3.3g, 78%) m.p. 198-200°C. ν $_{max}$ (cm^{-1}, KBr): 3321 (NH), 1637 (C=N), 1223 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 5.98 (s,2H,CH_2),6.78 (s,1H,C=NH);6.88-8.12(m,14H,ArH+NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 91.3 (CH_2), 108.6, 109.8, 113.8, 116.1, 120.4, 124.8, 125.5, 127.1, 127.4, 128.8, 129.3, 131.6, 139.5, 140.0, 148.1, 149.2,158.2 160.8 (ArC), 164.4 (C=NH), 181.2 (CS).Anal.% Calcd for $C_{26}H_{17}BrN_4O_2S$: C, 58.99; H, 3.24; N, 10.58. Found: C, 58.91; H, 3.26; N, 10.45.

24(R=2-Theinyl): Rrecrystallized fromethanol as needles. (3.4g, 76%) m.p. 180-182°C. ν $_{max}$ (cm^{-1}, KBr): 3284(NH), 1642 (C=N), 1228 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.81 (s,1H,C=NH); 7.34-7.82(m,14H,ArH+NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 108.4, 110.9, 121.6,122.8,124.5, 125.5, 126.8, 127.2, 127.4, 128.9, 132.4,139.8, 142.5, 144.4, 149.0, 158.3, 160.0 (ArC), 164.6 (C=NH), 189.23 (C=S). Anal.% Calcd for $C_{23}H_{15}BrN_4S_2$: C, 56.21; H, 3.08; N,14.40. Found: C, 56.12; H, 3.06; N, 14.51.

4-Amino -7-(4-Bromophenyl)-5-substituted-pyrido[2,3-d]pyrimidines (25-30)

A mixture of the appropriate 2-aminopyridine (10 mmol) and formamide (10 ml) was heated under reflux for 2-3 h. The reaction mixture was cooled and the precipitated solid product was collected, washed with cold ethanol and recrystallized from the appropriate solvent.

25 (R = 4-$CH_3OC_6H_4$): Recrystallized from DMF/H_2O as needles. (2.8g, 70%) m.p. 100-102°C. ν $_{max}$ (cm^{-1}, KBr): 3249, 3368 (NH_2). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,3H,CH_3O), 6.72 (s,2H,NH_2), 7.08-7.72 (m,9H,ArH), 8.27 (s, 1H, H-2). ^{13}CNMR(δ/ppm,DMSO-d_6): 55.8 (CH_3O), 106.7, 114.8, 114.8, 119.3, 121.7, 128.4, 128.4, 129.6, 129.6, 132.1 135.8, 138.0, 150.2, 155.0, 157.3, 157.4, 161.1 (ArC). Anal.% Calcd for C20H15BrN4O: C, 58.98; H, 3.71; N, 13.76. Found: C, 58.96; H, 3.69; N, 13.73.

26 (R = 3,4-$(CH_3O)_2C_6H_3$): Recrystallized from DMF/H_2O as needles. (3.2g, 75%) m.p. 178-180 °C. ν $_{max}$ (cm^{-1}, KBr): 3232, 3379 (NH_2). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.82 (s,6H,2-CH_3O), 6.74 (s,2H,NH_2),6.89-8.29 (m,9H,ArH), 8.27 (s, 1H, H-2). ^{13}CNMR(δ/ppm,DMSO-d_6): 55.8, 56.1 (CH_3O) , 98.8, 106.7, 107.1, 119.3, 119.4, 121.7, 128.4, 128.4, 129.4, 132.1, 132.1, 138.0, 150.2, 151.2, 155.0, 157.3, 157.4, 161.8, 162.1 (ArC). Anal.% Calcd for C21H17BrN4O2: C, 57.68; H, 3.92; N, 12.81. Found: C, 57.66; H, 3.90; N, 12.79.

27 (R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from DMF/H_2O as needles. (3.0g, 73%) m.p. 191-194 °C. ν $_{max}$ (cm^{-1}, KBr): 3289, 3367 (NH_2). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.80 (s,3H,CH_3O), 5.35 (s,1H,OH),6.89-7.71 (m,8H,ArH), 8.20 (s, 1H, H-2). ^{13}CNMR(δ/ppm,DMSO-d_6): 56.1 (CH_3O), 106.7, 113.9, 115.8, 119.3, 121.7, 122.3, 128.4, 128.4, 131.6, 132.1, 132.1, 138.0, 148.0, 150.2, 157.3, 157.4 (ArC). Anal.% Calcd for C20H15BrN4O2: C, C, 56.75; H, 3.57; Br, N, 13.24. Found: C, 56.73; H, 3.55; N, 13.26.

28 (R = 4-NO_2-C_6H_4): Recrystallized from DMF/H_2O as needles. (2.9g, 72%) m.p. 238-240°C. ν $_{max}$ (cm^{-1}, KBr): 3254, 3384 (NH_2). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.74 (s,2H,NH_2), 7.13-8.32 (m,9H,ArH), 8.22 (s, 1H, H-2). ^{13}CNMR(δ/ppm,DMSO-d_6): 106.7, 119.3, 121.7, 124.4, 124.4, 128.3, 128.3, 128.4, 128.4, 132.1, 132.1, 138.0, 148.4, 150.2, 155.0, 157.3, 157.4 (ArC). Anal.% Calcd for C19H12BrN5O2: C, 54.05; H, 2.86; N, 16.59. Found: C, 54.03; H, 2.84; N, 16.61.

29 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from DMF/H_2O as needles. (2.2g, 65%) m.p. 258-260°C. ν $_{max}$ (cm^{-1}, KBr): 3255, 3362 (NH_2). ^{1}HNMR (δ/ppm, DMSO-d_6): 5.96 (s,2H,CH_2), 6.63 (s,2H,NH_2),6.84-8.03(m,9H,ArH), 8.29 (s, 1H, H-2). ^{13}CNMR(δ/ppm,DMSO-d_6):92.1(CH_2),113.5, 115.4,120.1,124.6,126.4,127.1, 128.8, 129.1, 129.5,139.7, 142.7,148.0,151.0, 157.2, 160.5, 164.9(ArC). Anal.% Calcd for $C_{20}H_{13}BrN_4O_2$: C, 57.02; H, 3.11; N,13.30. Found: C, 56.90; H, 3.07; N, 13.21.

30 (R=2-Theinyl): Rrecrystallized from methanol as needles. (2.7g, 83%) m.p. 218-220°C. ν $_{max}$ (cm^{-1}, KBr): 3260, 3359 (NH_2). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.64 (s,2H,NH_2), 6.72-8.13 (m,9 H,ArH+ H-2).^{13}CNMR (δ/ppm,DMSO-d_6): 108.6, 110.7, 122.1, 125.5, 126.6, 127.0, 128.2,129.4,139.9,142.6,150.7, 157.3 158.1,162.5, 165.5 (ArC). Anal.% Calcd for $C_{17}H_{11}BrN_4S$: C, 53.27; H, 2.89; N,14.62. Found: C, 53.17; H, 2.83; N, 16.50.

General method for the preparation of 4-amino-7-(4-Bromophenyl)-5-substituted-1H-pyrido[2,3-d]pyrimidine-2-thiones (31-36) and 4-amino-7-(4-Bromophenyl)-5-substituted-1H-pyrido[2,3-d]pyrimidine-2-ones (37-42)

A mixture of the appropriate derivative 2-aminopyridine derivative (10 mmol) and thiourea (0.8 g, 10 mmol) or urea (0.6 g, 10 mmol) was fused at 260-300 ºC using sand bath for 1 h. The reaction mixture was allowed to attain room temperature, the crude solid product was treated with water, then rubbed with ethanol, filtered and recrystallized from the appropriate solvent.

31 (R = 4-$CH_3OC_6H_4$): Recrystallized from ethanol as needles. (2.6g, 72%) m.p. 266-269°C. ν $_{max}$ (cm^{-1}, KBr): 3275, 3372, 3452 (NH&NH_2), 1266 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.81 (s,3H,CH_3O), 6.65 (s,2H,NH_2), 7.01-8.01 (m,10H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 55.8 (CH_3O), 108.1, 110.6, 114.8, 121.7, 128.4, 128.4, 129.6, 129.6, 132.1, 132.1, 133.2, 138.0, 148.1, 149.5, 153.3, 156.8, 161.1 (ArC). 180.22 (CS). Anal.% Calcd for C20H15BrN4OS: C, 54.68; H, 3.44; N, 12.75; S, 7.30. Found: C, 54.61; H, 3.40; N,12.72; S, 7.27

32(R = 3,4-(CH_3O)$_2C_6H_3$): Recrystallized from ethanol as needles. (2.2g, 66%) m.p. 245-247°C. ν $_{max}$ (cm^{-1}, KBr): 3275, 3346, 3398 (NH&NH_2), 1279 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.83 (s,6H,2CH_3O), 6.75 (s,2H,NH_2), 6.79-8.11 (m,8H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 55.8, 56.1 (CH_3O) 98.8, 107.1, 108.1, 110.6, 119.4, 121.7, 128.4, 128.4, 129.4, 132.1, 132.1, 138.0, 148.1, 149.1, 153.3, 161.8, 162.1(ArC). 180.4 (CS). Anal.%

Calcd for C21H17BrN4O2S: C, 53.74; H, 3.65; Br, 17.02; N, 11.94; S, 6.83. Found: C, 53.69; H, 3.61; N,11.88; S, 6.79.

33(R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from ethanol as needles. (2.4g, 68%) m.p. >360°C. ν_{max} (cm^{-1}, KBr): 3273, 3364, 3462 (NH&NH_2), 1272(C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.80 (s,3H,CH_3O), 5.35 (s,1H,OH) 6.73 (s,2H,NH_2), 6.89-8.20 (m,8H,ArH+NH). ^{13}CNMR (δ/ppm,DMSO-d_6):56.1 (CH_3O), 108.1, 110.6, 113.9, 115.8, 121.7, 122.3, 128.4, 128.4, 132.1, 132.1, 138.0, 138.3, 148.0, 148.1, 149.2, 149.5, 153.3, 156.8 (ArC). 182.46 (CS). Anal.% Calcd for C21H17BrN4O2S: C, 53.74; H, 3.65; N, 11.94; S, 6.83. Found: C, 53.69; H, 3.61; N,11.88; S, 6.79.

34 (R = 4-NO_2-C_6H_4): Recrystallized from ethanol as needles. (2.2g, 67%) m.p. >360 °C. ν_{max} (cm^{-1}, KBr): 3287, 3327, 3436 (NH&NH_2) 1269 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.80 (s,2H,NH_2), 7.19-8.32 (m,9H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 108.1, 110.6, 121.7, 124.4, 124.4, 128.3, 128.3, 128.4, 128.4, 147.0, 148.1, 148.4, 149.5, 153.3, 156.8, (ArC). 183.53 (CS). Anal.% Calcd for C19H12BrN5O2S: C, 54.68; H, 3.44; Br, 18.19; N, 12.75; S, 7.30. Found: C, 54.61; H, 3.40; N,12.72; S, 7.27.

35 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from ethanol as needles. (2.6g, 73%) m.p. 230-232°C. ν_{max} (cm^{-1}, KBr): 3288, 3387, 3438 (NH&NH_2), 1273 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 5.96 (s,2H,CH_2), 6.69 (s,2H,NH_2), 6.88-8.03 (m,9H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6):92.1(CH_2),113.5,115.4,120.1, 124.6,126.4,127.1,128.8,129.1, 129.5,139.7, 142.7,148.0,151.0,159.3, 160.5, 164.9(ArC). 181.48 (CS). Anal.% Calcd for $C_{20}H_{13}BrN_4O_2S$: C, 52.99; H, 2.89; N,12.36; S, 7.07. Found: C, 52.93; H, 2.87; N,12.47; S, 7.00.

36 (R=2-Theinyl): Rrecrystallized from ethanol as needles. (1.7g, 52%) m.p. 243-245°C. ν_{max} (cm^{-1}, KBr): 3279, 3329, 3460 (NH&NH_2), 1277 (C=S). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.73 (s,2H,NH_2), 6.67-8.11 (m,9H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 109.3, 110.7, 122.1, 125.5, 127.1, 127.6,127.9,129.4,139.6, 143.2,150.7,158.1,162.0, 165.5(ArC). 182.87 (CS). Anal.% Calcd for $C_{17}H_{11}BrN_4S_2$: C, 47.16; H, 2.67; N,13.49; S, 15.44. Found: C, 47.12; H, 2.64; N, 13.38; S, 15.35.

37 (R = 4-$CH_3OC_6H_4$): Recrystallized from DMF/H_2O as needles. (1.9g, 68%) m.p. 200-202°C. ν_{max} (cm^{-1}, KBr): 3245, 3347, 3428 (NH&NH_2), 1663 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.81 (s,3H,CH_3O), 6.69 (s,2H,NH_2), 7.05-8.20 (m,10H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 55.8(CH_3O), 108.1, 110.6, 114.8, 114.8, 121.7, 128.4, 128.4, 129.6, 129.6, 132.1, 132.1, 133.2, 138.0, 148.1, 149.5, 153.3, 156.8 (ArC). 166.24 (CO). Anal.% Calcd for C20H15BrN4O3: C, 56.75; H, 3.57; N, 13.24. Found: C, 56.74; H, 3.54; N;13.21.

38 (R = 3,4-$(CH_3O)_2C_6H_3$): Recrystallized from DMF/H_2O as needles. (2.0g, 70%) m.p. >360°C. ν $_{max}$ (cm^{-1}, KBr): 3292, 3374, 3402 (NH&NH_2), 1664 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.81 (s,6H,2CH_3O), 6,72 (s,2H,NH_2), 6.90-8.23 (m,9H, ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 55.8, 56.1(CH_3O), 98.8, 107.1, 108.1, 110.6, 119.4, 121.7, 128.4, 128.4, 129.4, 132.1, 132.1, 138.0, 148.1, 149.5, 156.8, (ArC). 168.34 (CO). Anal.% Calcd for C21H17BrN4O3: C, 55.64; H, 3.78; N, 12.36. Found: C, 55.58; H, 3.74; N;12.30.

39(R = 4-OH-3-$CH_3OC_6H_3$): Recrystallized from DMF/H_2O as needles. (2.1g, 72%) m.p. >360°C. ν $_{max}$ (cm^{-1}, KBr): 3286, 3356, 3449 (NH&NH_2), 1667 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 3.80 (s,3H,CH_3O), 5.35 (s,1H,OH),6.69 (s,2H,NH_2), 6.89-8.13 (m,9H,ArH+NH). ^{13}CNMR (δ/ppm,DMSO-d_6): 56.1 (CH_3O), 108.1, 110.6, 113.9, 115.8, 121.7,122.3, 128.4, 128.4, 128.4, 132.1, 132.1, 138.0, 138.3, 148.0, 148.1, 149.2, 149.5, 153.3, 156.8 (ArC). 167.19 (CO). Anal.% Calcd for C20H15BrN4O3: C, 54.69; H, 3.44; N, 12.75. Found: C, 54.66; H, 3.40; N;12.70.

40 (R = 4-NO_2-C_6H_4): Recrystallized from DMF/H_2O as needles. (1.8g, 66%) m.p. 260-262°C. ν $_{max}$ (cm^{-1}, KBr): 3277, 3357, 3448 (NH&NH_2), 1662 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.76 (s,2H,NH_2), 7.19-8.32 (m,10H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6): 108.1, 110.6, 114.8, 121.7,124.4, 124.4, 128.3, 128.3, 128.4, 128.4, 132.1, 132.1, 138.0, 147.0 148.1, 148.4, 149.5, 153.3, 156.8, (ArC). 166.97 (CO). Anal.% Calcd for C19H12BrN5O3: C, 52.07; H, 2.76; N, 15.98. Found: C, 52.01; H, 2.71; N;15.95.

41 (R= 4, 3-(OCH_2O)C_6H_3): Rrecrystallized from DMF/H_2O as needles. (2.2g, 75%) m.p. 260-262°C. ν $_{max}$ (cm^{-1}, KBr): 3279, 3326, 3460 (NH&NH_2), 1665 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.10 (s,2H,CH_2), 6.72 (s,2H,NH_2), 6.84-7.76 (m,9H,ArH+NH).^{13}CNMR (δ/ppm,DMSO-d_6):92.6(CH_2),113.5,115.6,122.1, 125.5,126.4,127.2,128.8,129.0, 129.4,139.6, 142.6,148.1,150.9,157.8, 160.2, 164.8(ArC). 166.45 (CO). Anal.% Calcd for $C_{20}H_{13}BrN_4O_3$: C, 54.94; H, 3.00; N,12.81. Found: C, 54.91; H, 2.97; N;12.89.

42 (R=2-Theinyl): Rrecrystallized from DMF/H_2O as needles. (2.1g, 73%) m.p. 250-252°C. ν $_{max}$ (cm^{-1}, KBr): 3284, 3346, 3455 (NH&NH_2), 1660 (C=O). ^{1}HNMR (δ/ppm, DMSO-d_6): 6.69 (s,2H, NH_2); 6.97-7.75 (m,8H,Ar H); 8.02 (s,1H, NH). ^{13}CNMR (δ/ppm, DMSO-d_6): 108.1, 110.3, 122.7, 125.2, 126.7, 127.3, 127.4, 129.0, 139.3, 142.6, 144.5, 158.4,159.3,166.1 (ArC), 166.52 (CO). Anal.% Calcd for $C_{17}H_{11}BrN_4OS$: C, 51.14; H, 2.78; N,14.03. Found: C, 51.05; H, 2.74; N, 14.07.

3.1. In Vitro MTT Cytotoxicity Assay.

The synthesized compounds were investigated for their *in vitro* cytotoxic effect via the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [16,17] against a panel of three human tumor cell lines, namely, Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2, and colon carcinoma HT29 and a normal non-transformed human foreskin fibroblast Hs27 cell line. The procedures were done in a sterile area using a laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch-cultured for 10 days and then seeded at concentration of 10 × 103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37∘C for 24 h under 5% CO2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium(without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of 100-50-25-12.5-6.25-3.125-1.56-0.78 μg/mL. DMSOwas employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL Penicillin Potassium, 10,000 μg/mL Streptomycin Sulphate, and 25 μg/mL Amphotericin B), and 1% L-Glutamine in

96-well flat bottom microplate at 37∘C under 5% CO2.After 24 h of incubation, the medium was aspirated and 40 μL of MTT salt (2.5μg/mL) was added to each well and

incubated for further 4 h at 37∘C under 5% CO2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37∘C. The absorbance was then measured using a microplate multiwall reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented in

Table 1 as LC50 (μM) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

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