# Synthesis of Some Novel Pyrido[2,3-*d*]pyrimidine and Pyrido[3,2-*e*][1,3,4]triazolo and Tetrazolo[1,5-*c*]pyrimidine Derivatives as Potential Antimicrobial and Anticancer Agents

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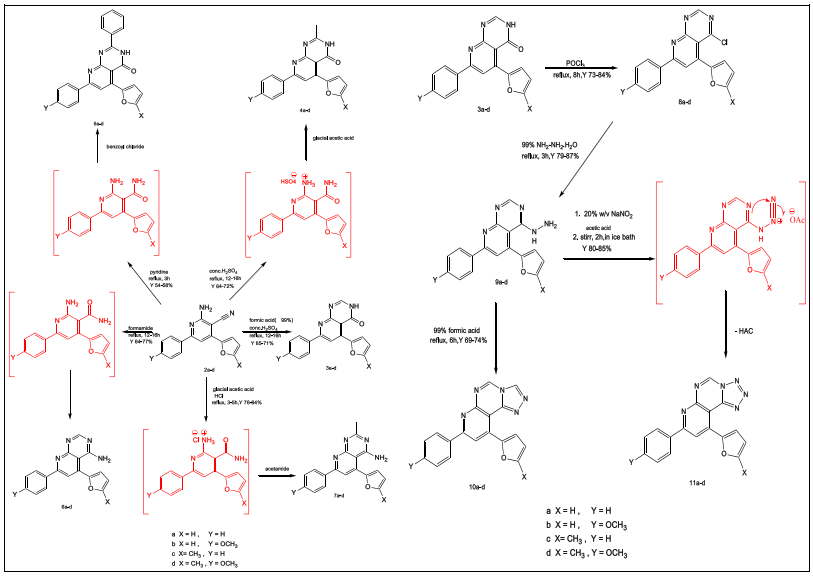



A novel series of pyrido[2,3-*d*]pyrimidines **3a**–**d**, **4a**–**d**, **5a**–**d**, **6a**–**d**, and **7a**–**d**; pyrido[3,2-*e*][1,3,4]triazolo; and tetrazolo[1,5-*c*]pyrimidines **10a**–**d** and **11a**–**d** was synthesized through different chemical reactions starting from 2-amino-3-cyano-4,6-diarylpyridines. The newly synthesized heterocycles were characterized by elemental analysis, IR, $^{1}$H-NMR, $^{13}$C-NMR, and mass spectral data. Compounds have been screened for their antibacterial and antifungal activities. The data showed that the presence of electron-donating group such as p-methoxyphenyl increases the antimicrobial activity. Also, the compounds have shown anticancer activity for colon and liver cancer cells.



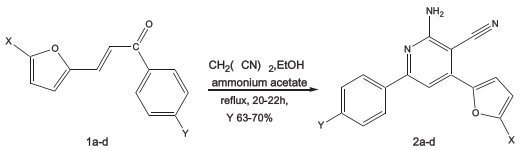
## INTRODUCTION

Pyrido[2,3-*d*]pyrimidines derivatives comprise a diverse and interesting group of drugs [1,2]. Pyrido[2,3-*d*] pyrimidines in general are extremely important for their biological activities. For example, some are antitumour [3], antibacterial [4], anticonvulsant [5], antipyretic [6], analgesic [7], and CNS depressant activity [8], Specifically, pyrido[2,3-*d*]pyrimidines known to inhibit *Pneumocystis carinii* (pc), *Toxoplasma gondii* (tg) of tumor cell lines in culture [9], and the activity is attributed to inhibition of dihydrofolate reductase [10,11].

## RESULTS AND DISCUSSION

**Chemistry.** In our work on pyrido[2,3-*d*]pyrimidines, we became interested to incorporate a triazol or tetrazol group in pyrimidine ring. The reason for this is that triazolo or tetrazolo derivatives are gaining importance because of their different and significant biological activities [12–14]. The synthesis of pyridino[2,3-*d*] pyrimidines is mainly by two ways, that is, annulations of pyrimidine ring over pyridine or vice versa [15]. The therapeutic importance of this nucleus is enthused us to develop selective procedures for synthesis in which substituents could be arranged in a pharmacophoric pattern to display high-order pharmacological activities.

The known starting materials 2-amino-3-cyano-4-(5-substituted furan-2-yl)-6-(4-substituted phenyl)pyridines **2a**–**d** according to Equation 1 [16].

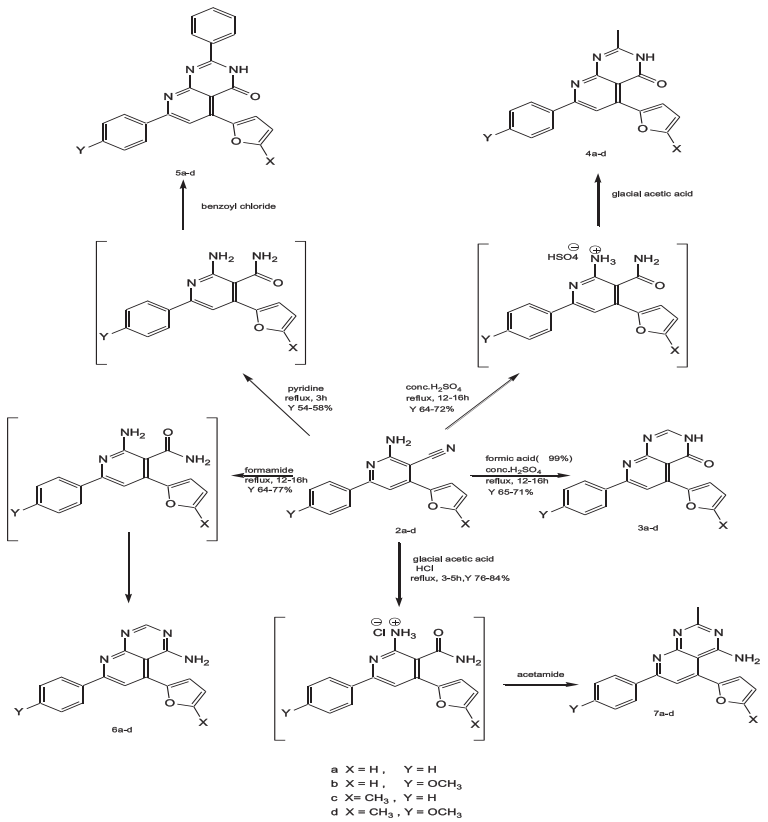



a X = H , Y = H
b X = H , Y = $OCH_3$
c X= $CH_3$ , Y = H
d X = $CH_3$ , Y = $OCH_3$

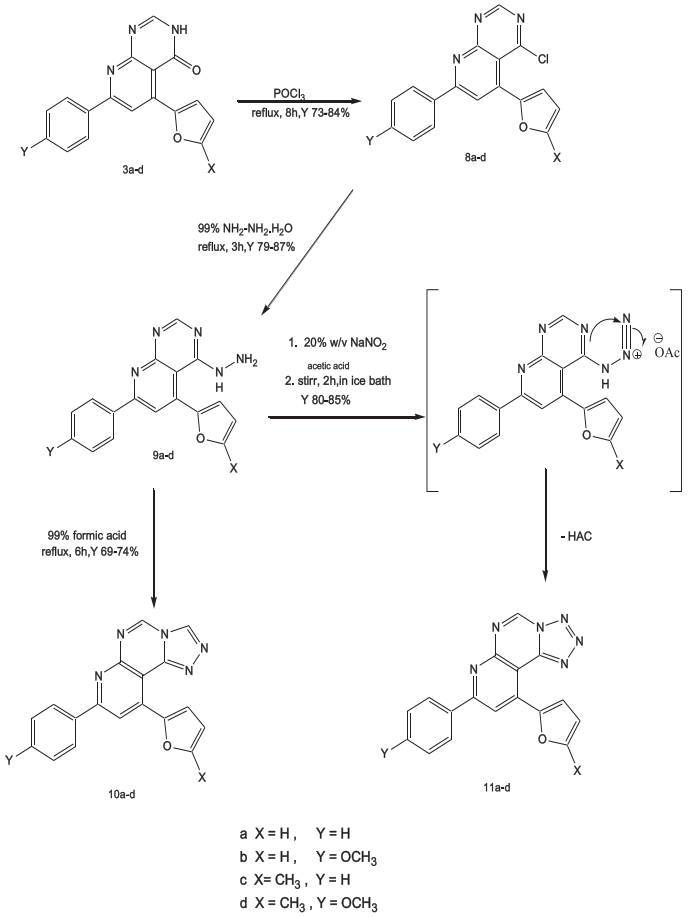
The synthetic pathways adopted for the preparation of the desired new compounds are illustrated in Schemes 1 and 2. The starting materials 2-amino-3-cyano-4,6-diphenyl pyridines **2a**–**d** were subjected to react with formic acid (99%), acetic acid in the presence of a few drops of concentrated sulfuric acid and benzoyl chloride in the presence of pyridine to give **3a**–**d**, **4a**–**d**, and **5a**–**d**, respectively, in high yield percent (Scheme 1). The structures of **3a**–**d**, **4a**–**d**, and **5a**–**d** were confirmed on the basis of their elemental and spectral data, the absence of cyano and amino groups of **2a**–**d**, and the appearance of IR absorption bands at 3184–3256 $cm^{-1}$ for NH groups and at 1663–1689 $cm^{-1}$ for C=O groups of the products. Furthermore, $^1$H-NMR spectrum also showed an exchangeable singlet signals at 10.13–13.37 ppm corresponding to NH groups, beside a singlet at δ 1.97–2.23 ppm for $CH_3$ of **4a**–**d**. Moreover, the mass spectrum showed molecular ion peaks at *m*/*z* 289, 303, and 365 corresponding to **3a**, **4a**, and **5a**, respectively. On the other hand, compounds **2a**–**d** were reacted with formamide and acetamide to give **6a**–**d** and **7a**–**d**, respectively. The IR spectrum of **6a**–**d** and **7a**–**d** revealed the absence of cyano group and the appearance of absorption bands at 3114–3449 $cm^{-1}$ for $NH_2$, and $^1$H-NMR spectrum showed an exchangeable singlet signal at 6.95–7.39 ppm for $NH_2$ and at 1.89–2.32 ppm for $CH_3$ of **7a**–**d**. The mass spectrum presented the molecular ion peaks at *m*/*z* 288 and 302 corresponding to **6a** and **7a**, respectively.

However, treatment of the pyrido[2,3-*d*]pyrimidin-4-ones derivatives **3a**–**d** with phosphorous oxychloride gave the expected 4-chloropyrido[2,3-*d*]pyrimidin-4-ones derivatives **8a**–**d** (Scheme 2). The proposed structures of **8a**–**d** were supported by their elemental and spectral data. The spectrum of **8a**–**d** showed the absence the NH and carbonyl group of **3a**–**d**, and the mass spectrum of **8a** presented the molecular ion peaks (*m*/*z*): 307 ($M^+$, 90.45), 309 ($M^{+2}$, 29.65), which agree with chlorine atom isotopes ratio. The hydrazinolysis of **8a**–**d** using (99%) hydrazine hydrate yielded the corresponding pyrido[2,3-*d*]pyrimidin-4-yl]hydrazine derivatives **9a**–**d**, which were cyclized with formic acid or sodium nitrite in the presence of acetic acid resulted in the expected pyrido[3,2-*e*][1,3,4]triazolo[1,5-*c*]pyrimidine **10a**–**d** and pyrido[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine **11a**–**d** derivatives, respectively (Scheme 2). The $^1$H-NMR of **9a**–**d** showed the presence of the amino group signals at 4.14–4.73 ppm and NH signals at 8.41–8.65 ppm. Additionally, IR spectrum of **9a**–**d** confirmed the obtained structure by the presence of absorption bands at 3193–

**Scheme 1.** Formation of Pyrido[2,3-d]pyrimidines derivatives from 2-amino-3-cyano-4,6diaryl pyridines.



**Scheme 2.** Formation of pyrido[3,2-e][1,3,4]triazolo and tetrazolo [1,5-c] pyrimidines from Pyrido[2,3-d]pyrimidines derivatives.




3421 $cm^{-1}$ for (NH and $NH_2$ stretching), while the absence of the amino and NH groups signals in both IR and $^1$H-NMR spectrum of **10a**–**d** and **11a**–**d**, and their elemental analysis confirmed their structures.

## BIOLOGICAL ACTIVITIES

**Antimicrobial activity.** The antimicrobial screenings for the synthesized compounds were undertaken using agar well-diffusion assay [17,18]. Table 1 listed the screening results of the tested compounds against the Gram-negative bacteria (*Escherichia coli* ATCC873 and *Pseudomonas* sp. ATCC9027), Gram-positive bacteria (*Bacillus subtilis* ATCC6051, *Streptococcus pneumonia* ATCC6303, and *Staphylococcus aureus* ATCC6538P), and three fungal strains (*Aspergillusniger* ATCC6275, *Penicillium* sp. ATCC11709, and *Candida albicans* ATCC2091). The obtained data revealed that most of the compounds showed moderate-to-excellent activities against the microorganisms used at a dose of 1 μg/mL. Compounds showing inhibition of at least 15 mm were considered active and were further evaluated for their minimal inhibitory concentration (MIC) (Table 2) by means of the agar well-diffusion method in DMSO. Streptomycin (10 μg/disc) was used as a standard antibacterial, while Ketoconazole (5 μg/disc) was used as standard antifungal. DMSO was used as a blank, which exhibited no activity against any of the used organisms.

It is well noticed that compounds **2a**, **2c**, **3a**, **3c**, **4a**, **4c**, **5a**, **5c**, **6a**, **6c**, **7a**, **7c**, **8a**, **8c**, **9a**, **9c**, **10a**, **10c**, **11a**, and **11c** showed lower potency against all bacteria and fungi compared with the reference standard. These compounds **2b**,**2d**, **3d**, **4b**, **5b**, **6b**, **6d**,**7b**, **7d**, and **10b** were equipotent similar to reference standard. Furthermore, compounds **3b**, **4d**, **5d**, **8b**, **8d**, **9b**, **9d**, **10d**, **11b**, and **11d** were found to be more active than reference standard. In this work, we have synthesized compounds, which were screened for their antimicrobial activities. The tested compounds **3b**, **4d**, **5d**, **8b**, **8d**, **9b**, **9d**, **10d**, **11b**, and **11d** demonstrated excellent antimicrobial activity. The data showed that the presence of electron-donating group such as p-methoxyphenyl increase. Hence, this study has widened the scope for evolving the new and promising antimicrobial drugs.

**Table 1**

Inhibition zones (IZ) in mm of the synthesized compounds at 1 µg/mL.

| | Gram-positive bacteria | | | Gram-negative bacteria | | Antifungal | | |
|---|---|---|---|---|---|---|---|---|
| Entry | *Bacillus subtilis* | *Streptococcus pneumonia* | *Staphylococcus aureas* | *Escherichia coli* | *Pseudomonas* sp. | *Aspergillusniger* | *Penicillium* sp. | *Candida albicans* |
| **2a** | 2 | 3 | 5 | 6 | 2 | 1 | 7 | 9 |
| **2b** | 5 | 10 | 11 | 13 | 8 | 7 | 10 | 7 |
| **2c** | 3 | 1 | 3 | 5 | 3 | 2 | 8 | 9 |
| **2d** | 15 | 16 | 18 | 20 | 22 | 15 | 18 | 19 |
| **3a** | 4 | 6 | 7 | 8 | 3 | 1 | 5 | 6 |
| **3b** | 19 | 20 | 22 | 24 | 29 | 20 | 23 | 25 |
| **3c** | 2 | 3 | 9 | 13 | 14 | 8 | 12 | 10 |
| **3d** | 16 | 15 | 18 | 19 | 23 | 16 | 18 | 20 |
| **4a** | 1 | 3 | 6 | 10 | 2 | 10 | 13 | 10 |
| **4b** | 10 | 13 | 18 | 19 | 23 | 13 | 17 | 19 |
| **4c** | 5 | 1 | 7 | 8 | 6 | 1 | — | — |
| **4d** | 20 | 18 | 23 | 25 | 29 | 16 | 17 | 19 |
| **5a** | — | — | 9 | 11 | 7 | 4 | 9 | 11 |
| **5b** | 9 | 12 | 14 | 14 | 9 | 8 | 14 | 13 |
| **5c** | — | 7 | 10 | 8 | 11 | — | 11 | 10 |
| **5d** | 19 | 18 | 21 | 23 | 29 | 20 | 23 | 25 |
| **6a** | 5 | 1 | 7 | 8 | 1 | 2 | 4 | 1 |
| **6b** | 11 | 13 | 16 | 19 | 23 | 15 | 19 | 20 |
| **6c** | 3 | 1 | 8 | 10 | 12 | 1 | 5 | 7 |
| **6d** | 12 | 11 | 15 | 17 | 21 | 13 | 18 | 19 |
| **7a** | — | 2 | 11 | 10 | 14 | 10 | 9 | 13 |
| **7b** | 11 | 13 | 12 | 14 | 8 | 14 | 16 | 11 |
| **7c** | 5 | — | 11 | 13 | — | 12 | 10 | 8 |
| **7d** | 11 | 14 | 16 | 15 | 17 | 12 | 16 | 15 |
| **8a** | 17 | 15 | 19 | 20 | 25 | 16 | 19 | 20 |
| **8b** | 19 | 20 | 22 | 24 | 29 | 19 | 22 | 24 |
| **8c** | 15 | 11 | 13 | 16 | 19 | 12 | 18 | 19 |
| **8d** | 20 | 21 | 25 | 24 | 28 | 19 | 24 | 25 |
| **9a** | 11 | 10 | 16 | 18 | 22 | 14 | 18 | 19 |
| **9b** | 18 | 19 | 22 | 23 | 28 | 18 | 22 | 23 |
| **9c** | 15 | 10 | 12 | 14 | 19 | 11 | 17 | 19 |
| **9d** | 19 | 21 | 23 | 22 | 27 | 19 | 23 | 26 |
| **10a** | 1 | 3 | — | 5 | 1 | — | — | — |
| **10b** | 15 | 14 | 17 | 19 | 22 | 14 | 17 | 16 |
| **10c** | 3 | 9 | 1 | 10 | 3 | 2 | 1 | 1 |
| **10d** | 19 | 18 | 22 | 25 | 29 | 19 | 24 | 21 |
| **11a** | 2 | 3 | — | 6 | 1 | — | — | — |
| **11b** | 20 | 17 | 24 | 25 | 31 | 20 | 25 | 27 |
| **11c** | 2 | 8 | 1 | 9 | 3 | 3 | 1 | 2 |
| **11d** | 18 | 21 | 23 | 23 | 30 | 22 | 26 | 24 |
| Streptomycin | 18 | 17 | 20 | 22 | 27 | — | — | — |
| Ketoconazole | — | — | — | — | — | 18 | 21 | 20 |

**Table 2**

The MIC of the compounds tested against organisms.

| Compound (mg/mL) | Gram-positive bacteria | | | Gram-negative bacteria | | Antifungal | | |
|---|---|---|---|---|---|---|---|---|
| | *Bacillus subtilis* | *Streptococcus pneumonia* | *Staphylococcus aureas* | *Escherichia coli* | *Pseudomonas* sp. | *Aspergillusniger* | *Penicillium* sp. | *Candida albicans* |
| **2b** | — | 50 | — | 50 | 50 | 50 | 50 | 25 |
| **3b** | 100 | 100 | 100 | 100 | 50 | 100 | 50 | 100 |
| **4d** | — | 25 | 50 | 100 | 50 | 50 | 50 | 50 |
| **5d** | — | — | 25 | 50 | — | — | 50 | — |
| **6d** | — | 50 | — | 100 | 100 | 50 | — | — |
| **7d** | 50 | — | 50 | 50 | 25 | 50 | — | — |
| **8b** | 100 | 50 | 100 | 50 | 100 | 25 | 100 | 100 |
| **8d** | 100 | 25 | 100 | 100 | 100 | 100 | 100 | 100 |
| **9b** | 25 | 50 | 50 | 100 | 100 | 100 | 100 | 100 |
| **9d** | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 100 |
| **10b** | 50 | 100 | 100 | 50 | 100 | 100 | 100 | 100 |
| **10d** | 100 | 50 | 100 | 50 | 100 | 100 | 100 | 25 |
| **11b** | 75 | 50 | 100 | 100 | 50 | 50 | 100 | 100 |
| **11d** | 50 | 75 | 100 | 100 | 100 | 100 | 25 | 100 |
| Streptomycin | 50 | 50 | 50 | 75 | 50 | — | — | — |
| Ketoconazole | — | — | — | — | — | 50 | 75 | 75 |

**Table 3**

Evaluation of cytotoxicity of pyrido-pyrimidine derivatives against HCT-116.

| | Samples concd (μg) | | | | | | |
|---|---|---|---|---|---|---|---|
| Cell viability (%) | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.56 | 0.00 |
| **2d** | 8.03 | 10.25 | 12.08 | 21.04 | 37.28 | 44.88 | 100.00 |
| **3d** | 23.44 | 27.45 | 30.01 | 50.58 | 59.89 | 67.99 | 100.00 |
| **4d** | 28.15 | 29.14 | 39.85 | 58.12 | 60.01 | 73.12 | 100.00 |
| **5d** | 33.50 | 36.98 | 41.98 | 52.87 | 68.21 | 78.54 | 100.00 |
| **6d** | 3.55 | 5.99 | 10.02 | 15.00 | 21.82 | 32.40 | 100.00 |
| **7d** | 7.84 | 11.13 | 12.17 | 25.09 | 34.89 | 44.15 | 100.00 |
| **8d** | 9.01 | 10.22 | 11.55 | 21.41 | 29.12 | 40.03 | 100.00 |
| **9d** | 6.78 | 8.35 | 11.29 | 18.46 | 26.37 | 36.28 | 100.00 |
| **10d** | 19.12 | 25.28 | 34.24 | 63.92 | 74.27 | 89.87 | 100.00 |
| **11d** | 20.55 | 29.99 | 45.81 | 61.87 | 79.82 | 90.69 | 100.00 |
| Vinblastine standard | 12.16 | 15.54 | 18.92 | 39.86 | 47.30 | 58.11 | 100.00 |

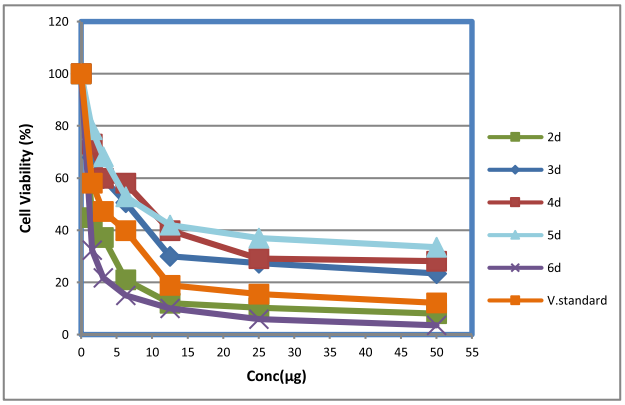



**Figure 1.** The inhibitory activities against HCT-116 cell lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

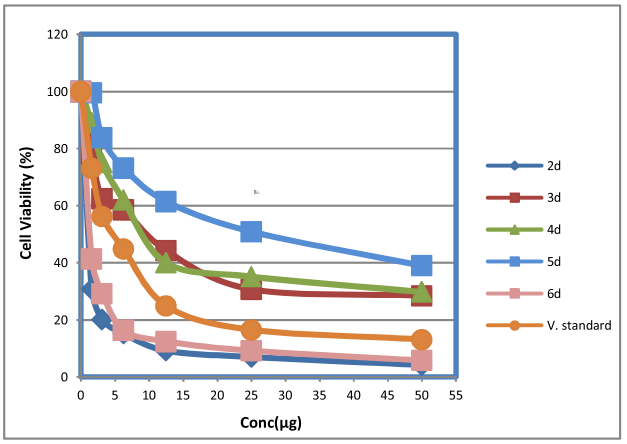



**Figure 3.** The inhibitory activities against HepG-2 cell lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

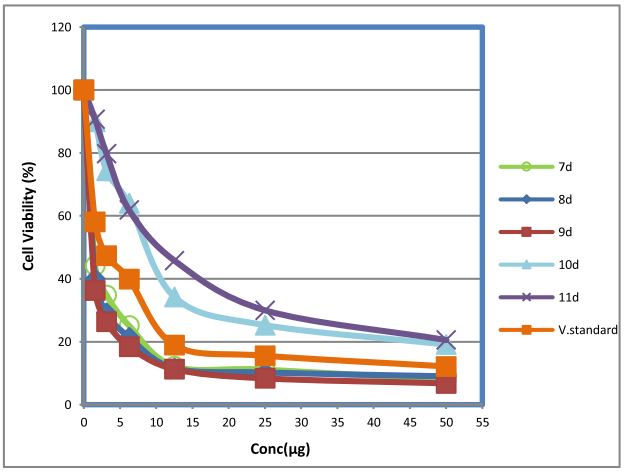



**Figure 2.** The inhibitory activities against HCT-116 cell lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

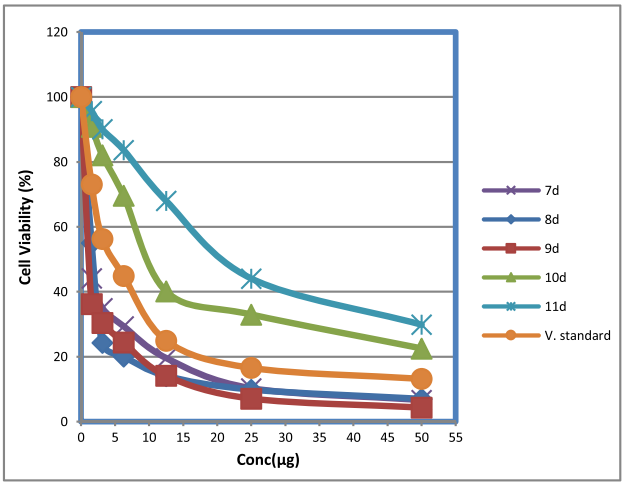



**Figure 4.** The inhibitory activities against HepG-2 cell lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Table 4**

Evaluation of cytotoxicity of pyrido-pyrimidine derivatives against HepG-2.

| | Samples concd (µg) | | | | | | |
|---|---|---|---|---|---|---|---|
| Cell viability (%) | 50 | 25 | 12.5 | 6.25 | 3.125 | 1.56 | 0.00 |
| **2d** | 4.19 | 7.01 | 9.20 | 15.19 | 20.11 | 30.88 | 100.00 |
| **3d** | 28.50 | 30.73 | 44.29 | 58.66 | 62.55 | 77.99 | 100.00 |
| **4d** | 29.87 | 35.22 | 40.11 | 62.03 | 75.18 | 89.12 | 100.00 |
| **5d** | 38.99 | 50.91 | 61.49 | 73.20 | 83.84 | 99.54 | 100.00 |
| **6d** | 5.79 | 9.21 | 12.47 | 16.38 | 29.18 | 41.40 | 100.00 |
| **7d** | 6.67 | 10.28 | 19.58 | 29.38 | 35.01 | 44.15 | 100.00 |
| **8d** | 7.00 | 9.87 | 14.10 | 20.08 | 24.28 | 55.03 | 100.00 |
| **9d** | 4.33 | 7.09 | 14.11 | 24.39 | 30.37 | 36.28 | 100.00 |
| **10d** | 22.54 | 32.98 | 40.09 | 69.57 | 82.07 | 90.87 | 100.00 |
| **11d** | 29.85 | 44.07 | 67.99 | 83.67 | 90.04 | 95.69 | 100.00 |
| Vinblastine standard | 13.16 | 16.54 | 24.92 | 44.86 | 56.30 | 73.11 | 100.00 |

**Anticancer activity.** In this study, the anticancer activity of the 10 synthesized pyrido-pyrimidine derivatives has been evaluated on human cancer cell lines, representing colon and liver cancer. The inhibitory activities against colon carcinoma cells (HCT-116) and hepatocellular carcinoma cells (HepG-2) was tested using different concentrations of the samples (50, 25, 12.5, 6.25, 3.125, and 1.56 µg), and the cell viability (%) was determined by colorimetric method.

The 50% inhibitory concentration (IC50) of the HCT-116 cell line was calculated from Table 3 and Figures 1 and 2.

The 50% inhibitory concentration (IC50) of the HepG-2 cell line was calculated from Table 4 and Figures 3 and 4.

The results of 50% inhibitory concentration (IC50) data are summarized in Table 5.

In comparison with standard antitumor drug vinblastine, compounds **2b**, **6b**, **7b**, **8b**, and **9b** were found to be active against HCT-116 and HepG-2 cell lines, while another compounds **3b**, **4b**, **5b**, **10b**, and **11b** were observed to be weak active against HCT-116 and HepG-2.

## IN VITRO STUDIES

**Cell lines.** Human colon carcinoma (HCT-116) cells and human hepatocellular carcinoma (HepG-2) cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% $CO_2$ and were subcultured two to three times a week.

**Cytotoxic assay of pyrido-pyrimidine derivatives.** The cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24 h at 37°C in a humidified incubator with 5% $CO_2$. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2), and simultaneously, the cells were treated with 100 µL from different dilutions of the test sample in fresh maintenance medium and incubated at 37°C. A control of untreated cells was made in the absence of the test sample. Six wells were used for each concentration of the test sample. Every 24 h, the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet [19,20] followed by cell lysis using 33% glacial acetic acid and read the absorbance at 490 nm using ELISA reader (SunRise, TECAN, Inc., Morrisville, NC) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation.

The number of viable cells was determined using ELISA reader as previously mentioned before, and the percentage of viability was calculated as [1-(ODt/ODc × 100%], where ODt is the mean optical density of wells treated with the test sample and ODc is the mean optical density of untreated cells.

The 50% inhibitory concentration (IC50), which is the concentration required to cause toxic effect in 50% of inactivated cells, was estimated from graphic plots.

**Table 5**

IC50 (µg) values of pyrido-pyrimidine derivatives after 24 h continuous exposure of tumor cell lines.

| | Tumor type/cell line | |
|---|---|---|
| Compound no. | HCT-116 | HepG-2 |
| **2d** | 3.40 | 7.12 |
| **3d** | 35.12 | 42.15 |
| **4d** | 22.97 | 28.45 |
| **5d** | 51.38 | 60.29 |
| **6d** | 4.12 | 6.83 |
| **7d** | 6.81 | 5.99 |
| **8d** | 3.55 | 8.29 |
| **9d** | 4.82 | 10.12 |
| **10d** | 43.58 | 62.10 |
| **11d** | 34.01 | 49.82 |
| Vinblastine standard | 2.78 | 5.11 |

**IC50 value** is the concentration that induces 50% growth inhibition compared with untreated control cells. **HCT-116** means human colon carcinoma cell lines. **HepG-2** means human hepatocellular carcinoma cell lines.

## CONCLUSIONS

A new series of pyrido-pyrimidine derivatives were prepared in good yield. The structures of these compounds were confirmed by IR, $^{1}$H-NMR, $^{13}$C-NMR, MS, and elemental analysis. Antitumor activities of synthesized compounds were evaluated on human colon and liver cancer cell lines. As a result of the cell culture studies, all of the compounds have shown anticancer activity for colon and liver cancer cells. In conclusion, novel pyrido-pyrimidine derivatives might be potentially useful in the field of cancer treatment. Finally, compounds **2b**, **6b**, **7b**, **8b**, and **9b** can be suggested as potent candidates for colon and liver cancer drug.

## EXPERIMENTAL

**Chemistry.** Melting points were determined on MEL_TEMP II apparatus and are uncorrected. IR spectra (KBr) were measured on Perkin-Elmer FTIR spectrophotometer, $^{1}$H NMR, and $^{13}$C NMR spectra were recorded on JEOL (500 MHz), in DMSO-$d_6$ as solvent, using tetramethylsilane (TMS) as internal

reference standard. The chemical shifts values are expressed in ppm. The NMR spectra were performed at Faculty of Science, Alexandria University. Elemental microanalyses were performed at the Micro Analytical Center, Faculty of Science, Cairo University. All compounds were within ±0.4% of the theoretical values. Mass spectra were run on DI analysis Shimadzu QP-2010 plus mass spectrometer at the Micro Analytical Unit of Cairo University. The progress of the reaction and the purity of the compounds were monitored by TLC analytical silica gel plates 60 F254. The chemical reagents used in synthesis were purchased from Fluka, Sigma, and Aldrich.

**General method for the preparation of 5-(5-substituted furan-2-yl)-7-(4-substituted phenyl)-3*H*-pyrido[2,3-d]pyrimidin-4-one (3a–d).** A mixture of **2a–d** (0.01 mol) in formic acid (99%, 30 mL) and catalytic amount of concd $H_2SO_4$ was heated under reflux for 16 h. Then the reaction mixture was cooled and poured into ice cold water, and the separated solid was filtered, washed with water, dried, and recrystallized from the proper solvent, ethanol, or DMF.

***5-Furan-2-yl-7-phenyl-3H-pyrido[2,3-d]pyrimidin-4-one 3a.*** White crystals, yield: 71%, mp: 289–291°C. IR (KBr) $cm^{-1}$: 3201 (NH), 3077 (C–H aromatic), 1676 (C=O amide) and 1623, 1589 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 6.82–8.27 (m, 10H, Ar–H), 10.86 (1H, s, NH amide, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, CDCl3): δ = 105.0, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.7, 163.0, 170.0. Mass (*m*/*z*): 289 ($M^+$, 91.23), 261 (M-CO, 73.23), 235 (M-$C_2NO$, 63.11), 77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{17}H_{11}N_3O_2$ (289): C, 70.59; H, 3.81; N, 14.53. Found: C, 70.88; H, 3.72; N, 14.41.

***5-Furan-2-yl-7-(4-methoxyphenyl)-3H-pyrido[2,3-d]pyrimidin-4-one 3b.*** White crystals, yield: 66%, mp: 276–278°C. IR (KBr) $cm^{-1}$: 3188 (NH), 3062 (C–H aromatic), 1668 (C=O amide) and 1611, 1578 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.65 (s, 3H, $OCH_3$), 6.95–8.27 (m, 9H, Ar–H), 10.13 ((1H, s, NH amide, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 56.0, 105.0, 111.5, 114.6 (2 C), 118.5, 120.7, 128.1(2 C), 129.0, 142.0, 147.5, 154.0, 157.7, 160.6, 170.0. *Anal*. Calcd for $C_{18}H_{13}N_3O_3$ (319): C, 67.71; H, 4.08; N, 13.17. Found: C, 67.58; H, 3.92; N, 13.31.

***5-(5-Methylfuran-2-yl)-7-phenyl-3H-pyrido[2,3-d]pyrimidin-4-one 3c.*** White crystals, yield: 70%, mp: 272–274°C. IR (KBr) $cm^{-1}$: 3223 (NH), 3087 (C–H aromatic), 1686 (C=O amide) and 1621, 1583 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.02 (s, 3H, $CH_3$), 7.07–8.31 (m, 9H, Ar–H), 11.27 ((1H, s, NH amide, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 15.0, 105.0, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.7, 163.0, 170.0. *Anal*. Calcd for $C_{18}H_{13}N_3O_2$ (303): C, 71.29; H, 4.29; N, 13.86. Found: C, 70.98; H, 4.22; N, 13.51.

***5-(5-Methylfuran-2-yl)-7-(4-methoxyphenyl)-3H-pyrido[2,3-d]pyrimidin-4-one 3d.*** White crystals, yield: 65%, mp: 281–283°C. IR (KBr) $cm^{-1}$: 3205 (NH), 3076 (C–H aromatic), 1683 (C=O amide) and 1632, 1576 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.13 (s, 3H, $CH_3$), 3.76 (s, 3H, $OCH_3$), 7.02–8.39 (m, 8H, Ar–H), 11.08 (1H, s, NH amide, $D_2O$ exchangeable). *Anal*. Calcd for $C_{19}H_{15}N_3O_3$ (333): C, 68.47; H, 4.50; N, 12.61. Found: C, 68.51; H, 4.44; N, 12.67.

**General procedure for synthesis of 5-(5-substituted furan-2-yl)-2-methyl-7-(4-substituted phenyl)-3*H*-pyrido[2,3-d]pyrimidin-4-one (4a–d).** A mixture of **2a–d** (0.01 mol) in acetic acid (30 mL) and catalytic amount of concd $H_2SO_4$ was heated under reflux for 16 h. The reaction mixture was cooled and poured into ice cold water, and the separated solid was filtered, washed with water, dried, and recrystallized from DMF.

***5-(Furan-2-yl)-2-methyl-7-phenyl-3H-pyrido[2,3-d]pyrimidin-4-one 4a.*** White crystals, yield: 72%, mp: 312–314°C IR (KBr) $cm^{-1}$: 3143 (NH), 3022 (C–H aromatic), 2976 (C–H aliphatic), 1672 (C=O), 1612, 1574 (C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.23 (s, 3H, $CH_3$), 6.91–8.34 (m, 9H, Ar–H), 12.23 (s, 1H, NH, $D_2O$ exchangeable). (*m*/*z*): 303 (M+, 83.72), 275 (M-CO, 65.27), 77 ($C_6H_5$, 100). $^{13}$C NMR (100 MHz, CDCl3): δ = 19.5, 105.0, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.7, 163.0, 170.0. *Anal*. Calcd for $C_{18}H_{13}N_3O_2$ (303): C, 71.29; H, 4.29; N, 13.86. Found: C, 71.11; H, 4.17; N, 13.77.

***5-(Furan-2-yl)-2-methyl-7-(4-methoxyphenyl)-3H-pyrido[2,3-d]pyrimidin-4-one 4b.*** White crystals, yield: 69%, mp: 304–306°C IR (KBr) $cm^{-1}$: 3221 (NH), 3043 (C–H aromatic), 2985 (C–H aliphatic), 1683 (C=O), 1617, 1579 (C=C, C=N); $^1$H-NMR (DMSO-$d_6$) 2.18 (s, 3H, $CH_3$), δ ppm: 3.91 (s, 3H, $OCH_3$), 6.87–8.23 (m, 8H, Ar–H), 11.87 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 19.5, 56.0, 105.0, 111.5, 114.6 (2 C), 118.5, 120.7, 128.1(2 C), 129.0, 142.0, 147.5, 154.0, 157.7, 160.6, 170.0. *Anal*. Calcd for $C_{19}H_{15}N_3O_3$ (333): C, 68.47; H, 4.50; N, 12.61. Found: C, 68.34; H, 4.53; N, 12.58.

***5-(5-Methylfuran-2-yl)-2-methyl-7-phenyl-3H-pyrido[2,3-d]pyrimidin-4-one 4c.*** White crystals, yield: 69%, mp: 317–319°C IR (KBr) $cm^{-1}$: 3198 (NH), 3009 (C–H aromatic), 2956 (C–H aliphatic), 1663 (C=O), 1607, 1575 (C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.09 (s, 3H, CH3), 2.27 (s, 3H, CH3), 6.73–8.29 (m, 8H, Ar–H), 11.39 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 15.0, 19.5, 105.0, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.7, 163.0, 170.0 (*Anal*. Calcd for $C_{19}H_{15}N_3O_2$ (317): C, 71.92; H, 4.73; N, 13.25. Found: C, 71.74; H, 4.63; N, 13.34.

***5-(5-Methylfuran-2-yl)-2-methyl-7-(4-methoxyphenyl)-3H-pyrido[2,3-d]pyrimidin-4-one 4d***. White crystals, yield: 64%, mp: 326–328°C IR (KBr) $cm^{-1}$: 3202 (NH), 3014 (C–H aromatic), 2978 (C–H aliphatic), 1676 (C=O), 1613, 1578 (C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 1.97(s, 3H, $CH_3$), 2.23 (s, 3H, $CH_3$), 3.85 (s, 3H, $OCH_3$), 6.54–8.21 (m, 7H, Ar–H), 11.39 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 15.0, 19.5, 56.0, 105.0, 111.5, 114.6 (2 C), 118.5, 120.7, 128.1(2 C), 129.0, 142.0, 147.5, 154.0, 157.7, 160.6, 170.0 (*Anal*. Calcd for $C_{20}H_{17}N_3O_3$ (347): C, 69.16; H, 4.90; N, 12.10. Found: C, 69.03; H, 4.82; N, 12.21.

**General method for the preparation of 5-(5-substituted furan-2-yl)-7-(4-substituted phenyl)-2-phenyl-3*H*-pyrido[2,3-d]pyrimidin-4-one (5a–d)**. A mixture of **2a–d** (0.01 mol) and benzoyl chloride (20 mL) was heated for 3 h. The reaction mixture was allowed to cool; the formed solid was washed with ethanol and recrystallized from DMF.

***5-(Furan-2-yl)-2,7-diphenyl-3H-pyrido[2,3-d]pyrimidin-4-one 5a***. White crystals, yield: 56%, mp: 324–326°C IR (KBr) $cm^{-1}$: 3209 (NH), 3022 (C–H aromatic), 2955 (C–H aliphatic), 1686 (C=O), 1601, 1535(C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 6.95–8.34 (m, 14H, Ar–H), 12.89 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 105.0, 111.5, 118.5, 120.7, 125.9, (2C), 127.1(3 C), 128.6 (2C), 129.0 (2 C), 129.9, 132.9, 139.7, 142.0, 147.5, 154.0, 157.7, 164.0, 170.0 (*m*/*z*): 365 (M+, 90.34), 337(M-CO, 77.23), 77 (C6H5, 100). *Anal*. Calcd for $C_{23}H_{15}N_3O_2$ (365): C, 75.62; H, 4.11; N, 11.51. Found: C, 75.43; H, 4.19; N, 11.32.

***5-(Furan-2-yl)-7-(4-methoxyphenyl)-2-phenyl-3H-pyrido[2,3-d]pyrimidin-4-one 5b***. Yellowish white crystals, yield: 52%, mp: 305–307°C IR (KBr) $cm^{-1}$: 3233 (NH), 3035 (C–H aromatic), 2942 (C–H aliphatic), 1676 (C=O), 1613, 1562(C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.43 (s, 3H, $OCH_3$), 6.78–8.46 (m, 13H, Ar–H), 12.79 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 56.0, 105.0, 111.5, 114.6 (2 C), 118.5, 120.7, 125.9 (2C), 128.1(2 C), 128.6 (2C), 129.0, 129.9, 132.9, 142.0, 147.5, 154.0, 157.7, 160.6, 170.0. *Anal*. Calcd for $C_{24}H_{17}N_3O_3$ (395): C, 72.91; H, 4.30; N, 10.63. Found: C, 72.87; H, 4.33; N, 10.45.

***5-(5-Methylfuran-2-yl)-2,7-diphenyl-3H-pyrido[2,3-d]pyrimidin-4-one 5c***. White crystals, yield: 58%, mp: 313–315°C IR (KBr) $cm^{-1}$: 3233 (NH), 3035 (C–H aromatic), 2942 (C–H aliphatic), 1676 (C=O), 1613, 1562 (C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.24 (s, 3H, $CH_3$), 6.93–8.37(m, 13H, Ar–H), 13.04 (s, 1H, NH, $D_2O$ exchangeable). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 15.0, 105.0, 111.5, 118.5, 120.7, 125.9(2C), 127.1(3 C), 128.6(2C), 129.0 (2 C), 129.9, 132.9, 139.7, 142.0, 147.5, 154.0, 157.7, 163.0, 170.0. *Anal*. Calcd for C24H17N3O2(379): C, 75.99; H, 4.49; N, 11.08. Found: C, 75.82; H, 4.51; N, 10.97.

***5-(5-Methylfuran-2-yl)-7-(4-methoxyphenyl)-2-phenyl-3H-pyrido[2,3-d]pyrimidin-4-one 5d***. Yellow crystals, yield: 54%, mp: 309–311°C IR (KBr) $cm^{-1}$: 3210 (NH), 3027 (C–H aromatic), 2953 (C–H aliphatic), 1680 (C=O), 1606, 1542 (C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.09 (s, 3H, $CH_3$), 3.61 (s, 3H, $OCH_3$), 7.06–8.52 (m, 12H, Ar–H), 13.37 (s, 1H, NH, $D_2O$ exchangeable). *Anal*. Calcd for $C_{25}H_{19}N_3O_3$ (409): C, 73.35; H, 4.65; N, 10.27. Found: C, 73.41; H, 4.56; N, 10.38.

**General procedure for synthesis of 5-(5-substituted furan-2-yl)-7-(4-substituted phenyl)-pyrido[2,3-d]pyrimidin-4-ylamine (6a–d)**. A mixture of **2a–d** (0.01 mol) and formamide (20 mL) was refluxed on an oil bath for 16 h. The reaction mixture was allowed to cool and poured into ice cold water, and the separated solid was filtered, washed with water, dried, and recrystallized from ethanol.

***5-(Furan-2-yl)-7-phenylpyrido[2,3-d]pyrimidin-4-ylamine 6a***. White crystals, yield: 77%, mp: 314–316°C IR (KBr) $cm^{-1}$: 3287, 3412 ($NH_2$ stretching), 3066 (C–H aromatic), 1623, 1586 (C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 6.95 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.13–8.43 (m, 8H, Ar–H), 7.68 (s, 1H, Ar–H of pyridine), 8.43 (s, 1H, Ar–H of pyrimidine). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 105.0, 105.2, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.1, 157.9, 159.2, 167.7 (*m*/*z*): 288 (M+, 93.65), 77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{17}H_{12}N_4O$ (288): C, 70.83; H, 4.17; N, 19.44. Found: C, 70.63; H, 4.23; N, 19.23.

***5-(Furan-2-yl)-7-(4-methoxyphenyl)pyrido[2,3-d]pyrimidin-4-ylamine 6b***. White crystals, yield: 68%, mp: 299–301°C IR (KBr) $cm^{-1}$: 3276, 3433 ($NH_2$ stretching), 3076 (C–H aromatic), 1613, 1575 (C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.62 (s, 3H, $OCH_3$),7.05 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.24–8.37 (m, 7H, Ar–H), 7.51 (s, 1H, Ar–H of pyridine), 8.56 (s, 1H, Ar–H of pyrimidine). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 56.0, 105.0, 105.2, 111.5, 114.6 (2 C), 118.5, 120.7, 128.1(2 C), 129.0, 142.0, 147.5, 154.0, 157.1, 157.9, 159.2, 167.7. *Anal*. Calcd for $C_{18}H_{14}N_4O_2$ (318): C, 67.72; H, 4.40; N, 17.61. Found: C, 67.65; H, 4.34; N, 17.68.

***5-(5-Methylfuran-2-yl)-7-phenylpyrido[2,3-d]pyrimidin-4-ylamine 6c***. White crystals, yield: 73%, mp: 308–310°C IR (KBr) $cm^{-1}$: 3244, 3421 ($NH_2$ stretching), 3053 (C–H aromatic), 1604, 1562 (C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.14 (s, 3H, $CH_3$),7.12 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.18–8.28 (m, 7H, Ar–H), 7.87 (s, 1H, Ar–H of pyridine), 8.49 (s, 1H, Ar–H of pyrimidine). $^{13}$C NMR (100 MHz, CDCl3): δ = 15.0, 105.0, 105.2, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.1, 157.9, 159.2, 167.7. *Anal*. Calcd for $C_{18}H_{14}N_4O$ (302): C, 71.52; H, 4.64; N, 18.54. Found: C, 71.42; H, 4.60; N, 18.45.

***5-(5-Methylfuran-2-yl)-7-(4-methoxyphenyl)pyrido[2,3-d]pyrimidin-4-ylamine 6d***. White crystals, yield: 64%, mp: 302–304°C IR (KBr) $cm^{-1}$: 3276, 3449 ($NH_2$ stretching),

3032 (C–H aromatic), 1596, 1562 (C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.05 (s, 3H, $CH_3$), 3.71 (s, 3H, $OCH_3$),7.03 (s, 2H, $NH_2$, $D_2O$ exchangeable), 7.21–8.13 (m, 6H, Ar–H), 7.52 (s, 1H, Ar–H of pyridine), 8.31 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{19}H_{16}N_4O_2$ (332): C, 68.67; H, 4.82; N, 16.87. Found: C, 68.45; H, 4.73; N, 16.63.

**General method for the preparation of 5-(5-substituted furan-2-yl)-2-methyl-7-(4-substituted phenyl)pyrido[2,3-d] pyrimidin-4-ylamine (7a–d)**. A mixture of **2a–d** (0.01 mol) and acetamide (0.01 mol) was refluxed in mixture of glacial acetic acid and hydrochloric acid (3:1) for 5 h. The reaction mixture was allowed to cool and poured into ice cold water, and the separated solid was filtered, washed with water, dried, and recrystallized from DMF.

***5-(Furan-2-yl)-2-methyl-7-phenylpyrido[2,3-d]pyrimidin-4-ylamine 7a***. White crystals, yield: 84%, mp: 286–288°C IR (KBr) cm$^{-1}$: 3114, 3323 ($NH_2$ stretching), 3052 (C–H aromatic),1563, 1544(C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.46 (s, 3H, $CH_3$),7.23 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.89–8.34 (m, 9H, Ar–H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 20.9, 102.2, 105.2, 111.5, 118.5, 120.7, 127.1(3 C), 129.0 (2 C), 139.7, 142.0, 144.9, 154.0, 157.9, 158.8, 165.9, 167.9 (*m*/*z*): 302 (M+, 89.77), 77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{18}H_{14}N_4O$ (302): C, 71.52; H, 4.64; N, 18.54. Found: C, 71.67; H, 4.45; N, 18.39.

***5-(Furan-2-yl)-2-methyl-7-(4-methoxyphenyl)-2-methylpyrido [2,3-d]pyrimidin-4-ylamine 7b***. Yellow crystals, yield: 79%, mp: 277–279°C IR (KBr) cm$^{-1}$: 3176, 3384 ($NH_2$ stretching), 3025 (C–H aromatic),1573, 1565 (C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.19 (s, 3H, $CH_3$), 3.93 (s, 3H, $OCH_3$),7.34 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.84–8.47 (m, 8H, Ar–H).$^{13}$C NMR (100 MHz, $CDCl_3$): δ = 20.9, 56.0, 102.2, 105.2, 111.5, 114.6 (2 C), 118.5, 120.7, 128.1(2 C), 129.0, 142.0, 144.9, 154.0, 157.9, 158.8, 165.9, 167.9 Calcd for $C_{19}H_{16}N_4O_2$ (332): C, 68.67; H, 4.82; N, 16.87. Found: C, 68.48; H, 4.64; N, 16.90.

***5-(5-Methylfuran-2-yl)-2-methyl-7-phenylpyrido[2,3-d] pyrimidin-4-ylamine 7c***. White crystals, yield: 76%, mp: 265–267°C IR (KBr) cm$^{-1}$: 3201, 3425 ($NH_2$ stretching), 3078 (C–H aromatic),1602, 1575(C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.09 (s, 3H, $CH_3$), 2.24 (s, 3H, $CH_3$),7.11 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.84–8.47 (m, 8H, Ar–H). 13C NMR (100 MHz, $CDCl_3$): δ = 15.0, 20.9, 102.2, 105.2, 111.5, 118.5, 120.7, 127.1 (3 C), 129.0 (2 C), 139.7, 142.0, 147.5, 154.0, 157.9, 158.8, 165.9, 167.9 Calcd for $C_{19}H_{16}N_4O$ (316): C, 72.15; H, 5.06; N, 17.72. Found: C, 72.18; H, 4.89; N, 17.65.

***5-(5-Methylfuran-2-yl)-2-methyl-7-(4-methoxyphenyl)pyrido[2,3-d]pyrimidin-4-ylamine 7d***. White crystals, yield: 81%, mp: 275–277°C IR (KBr) cm$^{-1}$: 3233, 3432 ($NH_2$ stretching), 3061 (C–H aromatic),1607, 1572(C=N, C=C); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.09 (s, 3H, $CH_3$), 2.24 (s, 3H, $CH_3$), 3.29 (s, 3H, $OCH_3$),7.02 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.78–8.56 (m, 7H, Ar–H). $^{13}$C NMR (100 MHz, CDCl3): δ = 15.1, 20.9. 56.0, 105.0, 111.5, 114.6 (2 C), 118.5, 120.7, 125.9 (2C), 128.1(2 C), 128.6 (2C), 129.0, 129.9, 132.9, 142.0, 147.5, 154.0, 157.7, 158.8, 165.9, 167.9 Calcd for $C_{20}H_{18}N_4O_2$ (346): C, 69.36; H, 5.20; N, 16.19. Found: C, 69.41; H, 5.02; N, 16.21.

**General method for the preparation of 4-chloro-5-(5-substituted furan-2-yl)-7-(4-substituted phenyl)pyrido[2,3-d] pyrimidine (8a–d)**. A mixture of **3a–d** (0.01 mol) in phosphorous oxychloride (20 mL) was refluxed for 8 h. The reaction mixture was allowed to cool and poured into ice cold water, stirred well, filtered, washed with water, dried, and recrystallized from ethanol.

***4-Chloro-5-(furan-2-yl)-7-phenylpyrido[2,3-d]pyrimidine 8a***. White crystals yield: 83%, mp: 192–194°C. IR (KBr) cm$^{-1}$: 3089 (C–H aromatic) 1589, 1577 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 6.67–8.19 (m, 8H, Ar–H), 7.88 (s, 1H, Ar–H of pyridine), 8.43 (s, 1H, Ar–H of pyrimidine). (*m*/*z*): 307 (M+, 90.45), 309 (M + 2, 29.65).77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{17}H_{10}N_3OCl$ (307): C, 66.45; H, 3.26; N, 13.68. Found: C, 66.29; H, 3.52; N, 13.52.

***4-Chloro-5-(furan-2-yl)-7-(4-methoxyphenyl)pyrido[2,3-d] pyrimidine 8b***. Yellow crystals, yield: 74%, mp: 183–185°C. IR (KBr) cm$^{-1}$: 3044 (C–H aromatic) 1597, 1566 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.52 (s, 3H, $OCH_3$), 6.56–8.31 (m, 7H, Ar–H), 7.32 (s, 1H, Ar–H of pyridine), 8.39 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{18}H_{12}N_3O_2Cl$ (337): C, 64.10; H, 3.56; N, 12.46. Found: C, 64.16; H, 3.46; N, 12.37.

***4-Chloro-5-(5-methylfuran-2-yl)-7-phenylpyrido[2,3-d] pyrimidine 8c***. White crystals, yield: 79%, mp: 188–190°C. IR (KBr) cm$^{-1}$: 3076 (C–H aromatic) 1572, 1553 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.17 (s, 3H, $CH_3$), 6.54–8.23 (m, 7H, Ar–H), 8.21 (s, 1H, Ar–H of pyridine), 8.55 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{18}H_{12}N_3OCl$ (321): C, 67.92; H, 3.74; N, 13.08. Found: C, 67.68; H, 3.81; N, 13.01.

***4-Chloro-5-(5-methylfuran-2-yl)-7-(4-methoxyphenyl)pyrido[2,3-d]pyrimidine 8d***. White crystals, yield: 75%, mp: 195–197°C. IR (KBr) cm$^{-1}$: 3035 (C–H aromatic) 1599, 1583 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 1.92 (s, 3H, $CH_3$), 3.40 (s, 3H, $OCH_3$), 6.92–8.46 (m, 6H, Ar–H), 8.02 (s, 1H, Ar–H of pyridine), 8.37 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{19}H_{14}N_3O_2Cl$ (351): C, 64.96; H, 3.99; N, 11.96. Found: C, 64.81; H, 3.84; N, 11.83.

**General procedure for synthesis of 5-(5-substituted furan-2-yl)-7-(4-substituted phenyl)-pyrido[2,3-d]pyrimidin-4-yl)hydrazine (9a–d)**. A mixture of **8a–d** (0.01 mol) in 99% hydrazine hydrate (15 mL) and ethanol (10 mL) was refluxed for 3 h. The reaction mixture was allowed to cool and poured into ice cold water, stirred well, filtered, washed with water, dried, and recrystallized from ethanol.

***5-(Furan-2-yl)-7-phenylpyrido[2,3-d]pyrimidin-4-yl)hydrazine 9a***. White crystals, yield: 87%, mp: 265–267°C. IR (KBr) $cm^{-1}$: 3207, 3232 and 3409 (NH and $NH_2$ stretching), 3064 (C–H aromatic) 1579, 1557 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 4.14 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.83–8.31 (m, 8H, Ar–H), 7.92 (s, 1H, Ar–H of pyridine), 8.34 (s, 1H, Ar–H of pyrimidine), 8.54 (s, 1H, NH, $D_2O$ exchangeable). (*m*/*z*): 303 (M+, 82.22), 77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{17}H_{13}N_5O$ (303): C, 67.33; H, 4.29; N, 23.10. Found: C, 67.41; H, 4.31; N, 23.01.

***5-(Furan-2-yl)-7-(4-methoxyphenyl)pyrido[2,3-d]pyrimidin-4-yl)hydrazine 9b***. White crystals, yield: 83%, mp: 248–250°C. IR (KBr) $cm^{-1}$: 3193, 3201 and 3386 (NH and $NH_2$ stretching), 3079 (C–H aromatic) 1603, 1578 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.73 (s, 3H, $OCH_3$), 4.38 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.64–8.22 (m, 8H, Ar–H and H of pyridine), 8.29 (s, 1H, Ar–H of pyrimidine), 8.41 (s, 1H, NH, $D_2O$ exchangeable). *Anal*. Calcd for $C_{18}H_{15}N_5O_2$ (333): C, 64.86; H, 4.50; N, 21.02. Found: C, 64.66; H, 4.34; N, 20.87.

***5-(5-Methylfuran-2-yl)-7-phenypyrido[2,3-d]pyrimidin-4-yl) hydrazine 9c***. White crystals, yield: 84%, mp: 254–256°C; IR (KBr) $cm^{-1}$: 3267, 3288 and 3421 (NH and $NH_2$ stretching), 3087 (C–H aromatic) 1609, 1574 (conjugated C=C, C=N); $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.02 (s, 3H, $CH_3$), 4.67 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.49–8.18 (m, 8H, Ar–H and H of pyridine), 8.31 (s, 1H, Ar–H of pyrimidine), 8.65 (s, 1H, NH, $D_2O$ exchangeable). *Anal*. Calcd for $C_{18}H_{15}N_5O$ (317): C, 68.14; H, 4.73; N, 22.08. Found: C, 68.01; H, 4.75; N, 21.89.

***5-(5-Methylfuran-2-yl)-7-(4-methoxyphenyl)pyrido[2,3-d] pyrimidin-4-yl)hydrazine 9d***. Yellow crystals, yield: 79%, mp: 243–245°C. IR (KBr) $cm^{-1}$: 3235, 3263 and 3413 (NH and $NH_2$ stretching), 3065 (C–H aromatic) 1589, 1562(conjugated C=C, C=N).$^1$H-NMR (DMSO-$d_6$) δ ppm: 2.23 (s, 3H, $CH_3$), 3.92 (s, 3H, $OCH_3$), 4.73 (s, 2H, $NH_2$, $D_2O$ exchangeable), 6.79–8.23 (m, 7H, Ar–H and H of pyridine), 8.36 (s, 1H, Ar–H of pyrimidine), 8.57 (s, 1H, NH, $D_2O$ exchangeable). *Anal*. Calcd for $C_{19}H_{17}N_5O_2$ (347): C, 65.71; H, 4.99; N, 20.17. Found: C, 65.48; H, 4.82; N, 20.03.

**General procedure for synthesis of 10-(5-substituted furan-2-yl)-8-(4-substituted phenyl)pyrido[3,2-e][1,3,4] triazolo[1,5-*c*]pyrimidine (10a–d)**. A mixture of **9a–d** (0.01 mol) in formic acid (99%, 30 mL) was refluxed for 6 h. The reaction mixture was allowed to cool and poured into ice cold water, stirred well, filtered, washed with water, dried, and recrystallized from ethanol.

***10-(Furan-2-yl)-8-phenylpyrido[3,2-e][1,3,4]triazolo[1,5-c] pyrimidine 10a***. Yellowish white crystals, yield: 74%, mp: 235–237°C. IR (KBr) $cm^{-1}$: 3032 (C–H aromatic) and 1601, 1543 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 6.72–8.05 (m, 9H, Ar–H), 8.64 and 9.03 (s, 1H, Ar–H of pyrimidine and s, 1H, Ar–H of triazol). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 105.0, 111.6, 119.9, 120.9, 127.1 (3C), 129.0(2C), 139.7, 142.0, 144.9, 147.9 (2C), 154.0, 156.9, 157.9, 158.0 (*m*/*z*): 313 (M+, 89.23), 77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{18}H_{11}N_5O$ (313): C, 69.01; H, 3.51; N, 22.36. Found: C, 69.23; H, 3.41; N, 22.43.

***10-(Furan-2-yl)-8-(4-methoxyphenyl)pyrido[3,2-e][1,3,4] triazolo[1,5-c]pyrimidine 10b***. Yellow crystals, yield: 69%, mp: 228–230°C. IR (KBr) $cm^{-1}$: 3052 (C–H aromatic) and 1612, 1562 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.77 (s, 3H, $OCH_3$), 6.56–8.17 (m, 8H, Ar–H), 8.58 and 8.93 (s, 1H, Ar–H of pyrimidine and s, 1H, Ar–H of triazol).$^{13}$C NMR (100 MHz, $CDCl_3$): δ = 56.0, 105.0, 111.6, 119.9, 120.9, 114.6 (2C), 128.1(2C), 132.0, 142.0, 144.9, 147.9(2C), 154.0, 156.9, 157.9, 158.0, 160.6. *Anal*. Calcd for $C_{19}H_{13}N_5O_2$ (343): C, 66.47; H, 3.79; N, 20.41. Found: C, 66.27; H, 3.52; N, 20.35.

***10-(5-Methylfuran-2-yl)-8-phenylpyrido[3,2-e][1,3,4]triazolo[1,5-c]pyrimidine 10c***. White crystals, yield: 73%, mp: 231–232°C. IR (KBr) $cm^{-1}$: 3033 (C–H aromatic) and 1623, 1539 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.31 (s, 3H, $CH_3$), 6.72–8.09 (m, 8H, Ar–H), 8.74 and 9.21 (s, 1H, Ar–H of pyrimidine and s, 1H, Ar–H of triazol). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 15.0, 105.0, 111.6, 119.9, 120.9, 127.1 (3C), 129.0(2C), 139.7, 142.0, 144.9, 147.9(2C), 154.0, 156.9, 157.9, 158.0. *Anal*. Calcd for $C_{19}H_{13}N_5O$ (327): C, 69.72; H, 3.98; N, 21.41. Found: C, 69.53; H, 3.84; N, 21.47.

***10-(5-Methylfuran-2-yl)-8-(4-methoxyphenyl)-[3,2-e][1,3,4] triazolo[1,5-c]pyrimidine 10d***. Yellow crystals, yield: 70%, mp: 237–239°C. IR (KBr) $cm^{-1}$: 3023 (C–H aromatic) and 1603, 1521 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.27 (s, 3H, $CH_3$), 3.86 (s, 3H, $OCH_3$), 6.88–8.23 (m, 7H, Ar–H), 8.94 and 9.30 (s, 1H, Ar–H of pyrimidine and s, 1H, Ar–H of triazol). $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 15.0, 56.0, 105.0, 111.6, 119.9, 120.9, 114.6 (2C), 128.1(2C), 132.0, 142.0, 144.9, 147.9(2C), 154.0, 156.9, 157.9, 158.0, 160.6. *Anal*. Calcd for $C_{20}H_{15}N_5O_2$ (357): C, 67.23; H, 4.20; N, 19.61. Found: C, 67.15; H, 4.04; N, 19.57.

**General procedure for synthesis of 10-(5-substituted furan-2-yl)-8-(4-substituted phenyl)-pyrido[3,2-*e*]tetrazolo[1,5-*c*]pyrimidine (11a–d)**. A total of 5 mL of an aqueous solution of (20% *w*/*v*) sodium nitrite was added slowly to **9a–d** (0.01 mol) in acetic acid (40 mL). The reaction mixture was stirred in ice bath for 2 h, then poured into water, stirred well, filtered, washed with water, dried, and recrystallized from ethanol.

***10-(Furan-2-yl)-8-phenylpyrido[3,2-e]tetrazolo[1,5-c] pyrimidine 11a***. White crystals, yield: 85%, mp: 248–250°C. IR (KBr) $cm^{-1}$: 3067 (C–H aromatic) and 1631, 1575 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 6.88–8.31 (m, 9H, Ar–H), 8.89 (s, 1H, Ar–H of pyrimidine). (*m*/*z*): 314 (M+, 87.98), 77 ($C_6H_5$, 100). *Anal*. Calcd for $C_{17}H_{10}N_6O$ (314): C, 64.97; H, 3.18; N, 26.75. Found: C, 65.04; H, 3.11; N, 26.56.

***10-(Furan-2-yl)-8-(4-methoxyphenyl)pyrido[3,2-e] tetrazolo[1,5-c]pyrimidine 11b***. Yellowish white crystals, yield: 81%, mp: 243–245°C. IR (KBr) $cm^{-1}$: 3072 (C–H aromatic) and 1641, 1583 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 3.33 (s, 3H, $OCH_3$), 6.89–8.34 (m, 8H, Ar–H), 9.24 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{18}H_{12}N_6O_2$ (344): C, 62.79; H, 3.49; N, 24.42. Found: C, 62.67; H, 3.33; N, 24.34.

***10-(5-Methylfuran-2-yl)-8-phenylpyrido[3,2-e]tetrazolo[1,5-c]pyrimidine 11c***. White crystals, yield: 80%, mp: 245–247°C. IR (KBr) $cm^{-1}$: 3102 (C–H aromatic) and 1634, 1565 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.13 (s, 3H, $CH_3$), 6.82–8.27 (m, 8H, Ar–H), 9.49 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{18}H_{12}N_6O$ (328): C, 65.85; H, 3.66; N, 25.61. Found: C, 65.71; H, 3.69; N, 25.53.

***10-(5-Methylfuran-2-yl-8-(4-methoxyphenyl))pyrido[3,2-e] tetrazolo[1,5-c]pyrimidine 11d***. Yellow crystals, yield: 83%, mp: 252–254°C. IR (KBr) $cm^{-1}$: 3083 (C–H aromatic) and 1622, 1551 (conjugated C=C, C=N). $^1$H-NMR (DMSO-$d_6$) δ ppm: 2.09 (s, 3H, $CH_3$), 3.67 (s, 3H, $OCH_3$), 6.56–8.01 (m, 7H, Ar–H), 9.57 (s, 1H, Ar–H of pyrimidine). *Anal*. Calcd for $C_{19}H_{14}N_6O_2$ (358): C, 63.69; H, 3.91; N, 23.46. Found: C, 63.55; H, 3.79; N, 23.37.

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## REFERENCES AND NOTES

[1] (a) Furth, P. S.; Reitman, M. S.; Gentles, R.; Cook, A. F. Tetrahedron Lett 1997, 38, 6643; (b) Furth, P. S.; Reitman, M. S.; Cook, A. F. Tetrahedron Lett 1997, 38, 5403.

[2] Zhang, F.; Zhao, Y.; Sun, L.; Ding, L.; Gu, Y.; Gong, P. Eur J Med Chem 2011, 46, 3149.

[3] Kurumurthy, C.; Sambasiva, P.; Veeraswamy, B.; Santhoshkumar, G.; Shanthan, P.; Narsaiah, B.; Velatooru, L. R.; Pamanji, R.; Venkateswara J Eur J Med Chem 2011, 46, 3462.

[4] (a) Masuda, S. JpnKokai TokkyoKohoJp 2000, 03, 106, 880. C.A.15: 183361d; (b) Tamura Jpn Kokai TokkyoKohoJp 2000, 61, 249, 983, C.A. 106: 213979.

[5] Ram, J.; VandenBerghe, D.; Vlietinck, A. J Heterocycl Chem 1988, 28, 217.

[6] Piper, J. R.; McCalab, G. S.; Montgomery, J. A.; Kishiuk, R. L.; Gamount, Y.; Sirotnak, F. M. J Med Chem 1986, 29, 1080.

[7] Robins, R. K.; Hitchings, G. H. J. Am Chem Soc 1958, 80, 3449.

[8] Hasan, M. F.; Madkour, A. M.; Saleem, I.; Rahman, J. M.; Mohammed, E. A. Heterocycles 1994, 38, 57.

[9] Lowe, J. A. Chem Abstr 112(1990), 21008 Austrian At. 1989, 388378, 378.

[10] Gangjee, A.; Adair, O. O.; Queener, S. F. J Med Chem 2003, 46, 5074.

[11] Murakami, H.; Koshimizu, S.; Matsabura, K. S. J Med Chem 1985, 28, 577.

[12] Iwamura, K. A.; El-Bayouk, M.; Basyouni, W. M.; Hosni, H. M.; El-Deen, A. S. JChemical Research (S) 1995, 314.

[13] El-Bayouk, K. A.; Basyouni, W. M.; Hosni, H. M. J Chemical Research (S) 1997, 452.

[14] Dave, C. G.; Shah, R. D. J Heterocyclic Chem 2000, 37, 757.

[15] Kataritzky, A.; Charles, R.; Rees, E. F.; Scriven, E. F. Comprehensive Heterocyclic Chemistry 1996, II 7, 591.

[16] NCCLS Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, which Grows Aerobically, fifth ed, Approved Standard M7- A5 ed.; NCCLS: Villanova, PA, 2000.

[17] Parakash, L.; Rashmischarma, S.; Goyal, R. D. Pharmazie 1993, 48, 221.

[18] Perez, C.; Paul, M.; Bazerque, P. Acta Bio Med Exp 1990, 15, 113.

[19] Mosmann, T. J Immunol Methods 1983, 55, 55.

[20] Gangadevi, V.; Muthumary, J. African, J Biotechnology 2007, 6, 1382.

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