# Synthesis of Novel Trifluoromethyl Group Containing Pyrido Furo/Thieno Pyrimidinone Derivatives and Their Anticancer Activity

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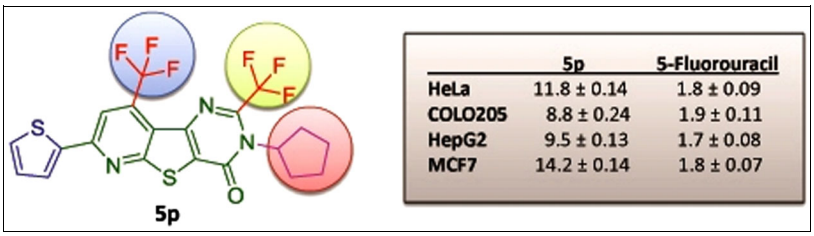


A series of novel trifluoromethyl group containing pyridofuro/thieno pyrimidinone derivatives **5a–p** were prepared starting from 2-oxo/thioxo-6-phenyl/thien-2-yl-4-(trifluoromethyl)-1,2-dihydropyridine-3-carbonitrile **1** compound on reaction with bromoethylacetate and further different primary aliphatic amines, under their refluxing conditions to afford amide tagged furo/thieno pyridine derivatives **4**. Compound **4** on reaction with trifluoroacetic acid and obtained novel trifluoromethyl group containing pyridofuro/thieno pyrimidinone derivatives **5a–p**. All the synthesized compounds **5a–p** were tested for anticancer activity on four cancer cell lines such as HeLa cervical cancer (CCL-2), COLO 205 colon cancer (CCL-222), HepG2 liver cancer (HB-8065), MCF7 breast cancer (HTB-22), and one normal cell line (HEK 293); compounds **5m**, **5n**, and **5p** are found to be more promising anticancer activity at micromolar concentration and found to be nontoxic on normal cell line.

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## INTRODUCTION

As per the reports of the World Health Organization, around 8.8 million people died due to cancer disease among all over the world in 2015 [1]. Among all over the drugs, anticancer drugs that were synthesized from molecules play a central and lead position in medicinal chemistry [2–4] and are essential part of the chemical and life sciences. Heterocyclic compounds are powerful scaffolds for many biological systems [5] and play an important role to design and discovery of new physiologically/pharmacologically active molecules [6]. Five-membered and six-membered fused heterocycles that consist nitrogen and oxygen or sulfur atoms have played an important role and reported to exhibit a broad spectrum of biological activities [7] like antimalarial [8], antifolate [9–13], antiviral [14], and potential radiation protection agents [15–19]. Based on the importance of fused heterocyclic compounds and we encouraged by previous reports, we are encouraged by them and focused our attention on the synthesis of tricyclic and bicyclic hetero cyclic ring systems and their biological activity to find promising anticancer agents. Moreover, literature says that the fluorine [20] or trifluoromethyl [21,22] groups at an appropriate position of an organic molecule effectually change the properties of molecule in terms of lipid solubility and oxidative thermal stability and after that enhance the transport mechanism and bio-efficacy. In the year of 2018, Santhosh Kumar *et al*. was reported the synthesis of pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives, which molecules doesn't contain trifluoro methyl group at 2nd position [23].

We are encouraged by them, and based on the importance [24–26], we drafted and synthesized a series of novel trifluoromethyl group containing pyrido furo/thieno pyrimidinone derivatives and submitted for anticancer activity against four cancer cell lines such as HeLa cervical cancer, COLO 205 colon cancer, HepG2 liver cancer, MCF7 breast cancer, and one (healthy) normal cell line (HEK 293). Among all the compounds, **5m**, **5n**, and **5p** compounds showed promising anticancer activity at micromolar concentration and found to be nontoxic to normal cell line.

## CHEMISTRY

The 2(1*H*) pyridine **1** was treated with 2-bromoethyl acetate in basic conditions ($K_2CO_3$) to produce selectively 2-*O*-ethylacetoxy-3-cyano-4-trifluoromethyl-6-substituted pyridine derivatives **2**. For cyclization of compounds **2**, treated with DMF using potassium carbonate as base to produce furo[2,3-*b*]pyridine derivatives **3**, the reaction sequence involves selective O-alkylation and abstraction of proton by base from an active methylene followed by cyclization onto nitrile

carbon. This type of cyclization is also known as Thorpe–Ziegler cyclization. Compound **3** was further treated with various types of primary aliphatic amines under neat conditions and reflux at their own boiling points to afford amide derivatives **4**. Amide derivatives **4** on reaction with trifluoroacetic acid in the presence of HCl, $H_2O$ reflux condition and obtained 2-trifluoromethyl pyridofuro/thieno pyrimidinone derivatives **5a–p**. All the synthesized compounds **5a–p** were screened for anticancer activity against four cancer cell lines such as HeLa cervical cancer (CCL-2), COLO 205 colon cancer (CCL-222), HepG2 liver cancer (HB-8065), MCF7 breast cancer (HTB-22), and one (healthy) normal cell line (HEK 293). Compounds **5m**, **5n**, and **5p** showed promising anticancer activity at micromolar concentration. The synthetic sequence is drawn in Scheme 1, and products are tabulated in Table 1.

Compounds were tested for *in vitro* against four cancer cell lines such as HeLa cervical cancer (CCL-2), COLO 205 colon cancer (CCL-222), HepG2 liver cancer (HB-8065), MCF7 breast cancer (HTB-22), and HEK 293 human embryonic kidney cells (CRL-1573) using MTT

**Scheme 1.** Synthesis of novel trifluoromethyl group containing pyrido furo/thieno pyrimidinone derivatives **5a–p**.

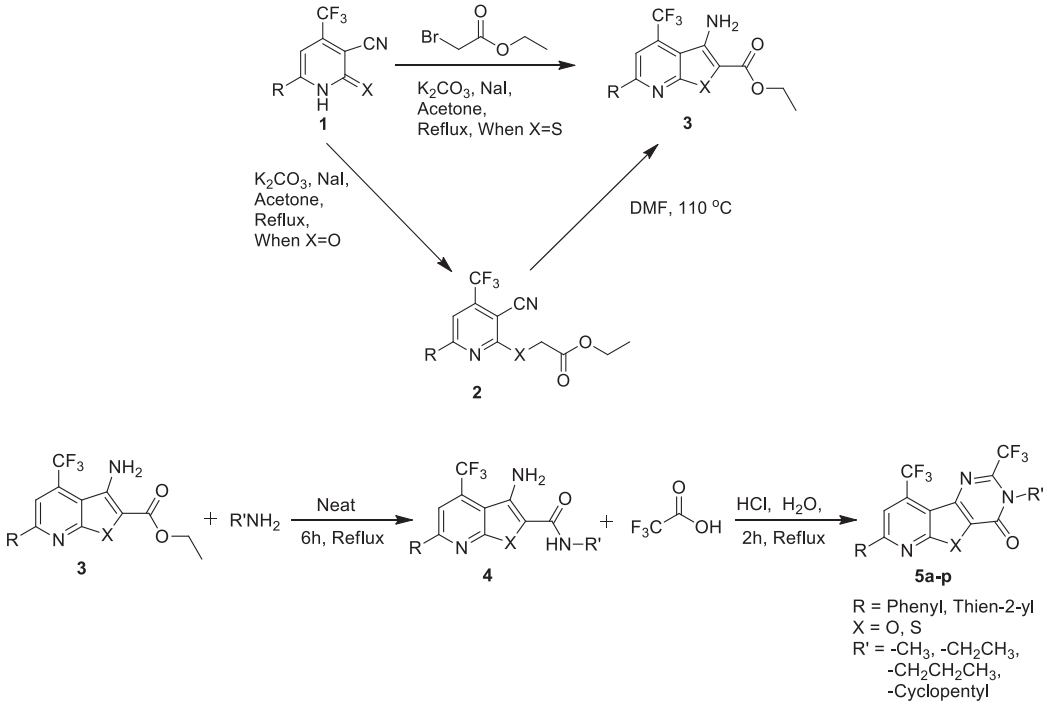


**Table 1**

Preparation of novel trifluoromethyl group containing pyrido furo/thieno pyrimidinone derivatives **5a–p**.

| Entry | Compound | R | X | R′ | Yield (%) |
|---|---|---|---|---|---|
| 1. | **5a** | Phenyl | O | $-CH_3$ | 89 |
| 2. | **5b** | Phenyl | O | $-CH_2CH_3$ | 90 |
| 3. | **5c** | Phenyl | O | $-CH_2CH_2CH_3$ | 91 |
| 4. | **5d** | Phenyl | O | −Cyclopentyl | 86 |
| 5. | **5e** | Phenyl | S | $-CH_3$ | 88 |
| 6. | **5f** | Phenyl | S | $-CH_2CH_3$ | 89 |
| 7. | **5g** | Phenyl | S | $-CH_2CH_2CH_3$ | 85 |
| 8. | **5h** | Phenyl | S | −Cyclopentyl | 81 |
| 9. | **5i** | Thien-2-yl | O | $-CH_3$ | 92 |
| 10. | **5j** | Thien-2-yl | O | $-CH_2CH_3$ | 88 |
| 11. | **5k** | Thien-2-yl | O | $-CH_2CH_2CH_3$ | 89 |
| 12. | **5l** | Thien-2-yl | O | −Cyclopentyl | 80 |
| 13. | **5m** | Thien-2-yl | S | $-CH_3$ | 91 |
| 14. | **5n** | Thien-2-yl | S | $-CH_2CH_3$ | 92 |
| 15. | **5o** | Thien-2-yl | S | $-CH_2CH_2CH_3$ | 89 |
| 16. | **5p** | Thien-2-yl | S | −Cyclopentyl | 82 |

**Table 2**

*In vitro* cytotoxicity of compounds **5a–p**.

| Compound | $IC_{50}$ values (in μM) | | | | |
|---|---|---|---|---|---|
| | HeLa | COLO 205 | HepG2 | MCF7 | HEK 293 |
| **5a** | 31.1 ± 0.23 | 22.2 ± 0.13 | 26.1 ± 0.61 | 29.5 ± 0.21 | 68 ± 0.18 |
| **5b** | — | — | 52.2 ± 0.41 | 49.1 ± 0.45 | 102 ± 0.24 |
| **5c** | 27.6 ± 0.13 | 34.6 ± 0.31 | 41.4 ± 0.26 | 25.2 ± 0.22 | 78 ± 0.16 |
| **5d** | — | — | — | 112.5 ± 0.16 | 75 ± 0.46 |
| **5e** | 21.1 ± 0.21 | 31.5 ± 0.34 | 61.2 ± 0.41 | — | 59 ± 0.29 |
| **5f** | 54.2 ± 0.52 | — | 52.6 ± 0.61 | 63.7 ± 0.12 | — |
| **5g** | 30.1 ± 0.11 | 41.8 ± 0.18 | 64.7 ± 0.30 | 52.8 ± 0.28 | 83 ± 0.12 |
| **5h** | 48.5 ± 0.23 | 27.7 ± 0.24 | 21.5 ± 0.51 | 36.8 ± 0.54 | 87 ± 0.59 |
| **5i** | 65.6 ± 0.11 | — | 79.6 ± 0.31 | — | 61 ± 0.25 |
| **5j** | — | — | — | — | 96 ± 0.47 |
| **5k** | — | — | — | — | 78 ± 0.28 |
| **5l** | 55.3 ± 0.29 | 58.9 ± 0.33 | 50.9 ± 0.39 | 68.4 ± 0.54 | 109 ± 0.22 |
| **5m** | 10.1 ± 0.22 | 13.5 ± 0.46 | 11.8 ± 0.53 | 12.6 ± 0.28 | — |
| **5n** | 12.2 ± 0.18 | 16.1 ± 0.11 | 18.3 ± 0.28 | 12.8 ± 0.32 | — |
| **5o** | 21.4 ± 0.28 | 22.8 ± 0.28 | 21.8 ± 0.52 | 18.5 ± 0.42 | 56 ± 0.37 |
| **5p** | 11.8 ± 0.14 | 8.8 ± 0.24 | 9.5 ± 0.13 | 14.2 ± 0.14 | — |
| **5-Fluorouracil** (standard control) | 1.8 ± 0.09 | 1.9 ± 0.11 | 1.7 ± 0.08 | 1.8 ± 0.07 | 19.6 ± 0.18 |

"—" indicates $IC_{50}$ value >112.5 μg/mL. Cell lines used are as follows: HeLa cervical cancer (CCL-2), COLO 205 colon cancer (CCL-222), HepG2 liver cancer (HB-8065), MCF7 breast cancer (HTB-22), and HEK 293 human embryonic kidney cells (CRL-1573).

assay [27]. $IC_{50}$ values of the test compounds for 24 h on each cell line were calculated and presented in Table 2.

## RESULTS AND DISCUSSIONS

**Anticancer activity.** All the compounds, except **5d**, **5j**, and **5k**, showed activity against four cancer cell lines at micromolar concentration. Among all the compounds, **5m**, **5n**, and **5p** showed promising activity, while the remaining compounds showed moderate activity. Among the derivatives, compound **5p** exhibited significant activity on COLO 205 and HepG2 cells at $IC_{50} < 9.5$ μg/mL, and other derivatives **5m**, **5n**, and **5o** also showed promising activity on all cell lines at $IC_{50} < 22.8$ μg/mL. Compound **5m** was considered as the more potent towards all the cancer cell lines. The structure–activity relationship studies revealed that the thieno-2-yl group at the sixth position shows more activity compared with phenyl group at the sixth position and also ($-CF_3$) trifluoromethyl groups at a strategic position. The presence of $CF_3$ group increases the properties of lipid solubility and after that enhances the transport mechanism and bio-efficacy. Thieno fused pyridine is an additional advantage in promoting cytotoxicity compared with furo fused pyridine ring.

## CONCLUSION

In conclusion, a series of novel trifluoromethyl group containing pyridofuro/thieno pyrimidinone derivatives **5a–p** were prepared and submitted for anticancer activity against four human cancer cell lines and one normal cell line. Among all the compounds tested, the compounds **5m**, **5n**, and **5p** showed significant anticancer activity against all the cell lines at micromolar concentration and found to be nontoxic on normal cell line.

## EXPERIMENTAL

**Cytotoxicity assay.** The cytotoxicity was assessed using the MTT assay [27].

**General procedure for the preparation of 2,3-dimethyl-7-substituted-9-(trifluoromethyl) pyrido[3′,2′:4,5]furo[3,2-*d*]pyrimidin-4(3*H*)-one derivatives (5a–p).** 3-Amino-*N*-methyl-6-substituted-4-(trifluoromethyl)furo[2,3-*b*]pyridine-2-carboxamide **4** (4 mmol) and trifluoroacetic acid (4 mmol) were taken in an RB. To this solution, added 5 ml HCl and 5 ml $H_2O$. The mixture was refluxed at 100°C for 2 h, and after completion of the reaction, reaction mixture was poured on crushed ice, and solid was formed and washed with excess water and dried.

***3-Methyl-7-phenyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5] furo[3,2-d]pyrimidin-4(3H)-one (5a).*** Whitish solid; mp 191–193°C; IR (KBr, $cm^{-1}$): 1692 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 3.74 (s, 3H, $-CH_3$), 7.53–7.59 (m, 3H, Ar—H), 8.17 (s, 1H, Ar—H), 8.18–8.21 (m, 2H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 35.6, 119.5, 124.0, 125.2, 126.3, 127.1, 128.2, 130.0, 132.0, 134.7, 137.4, 139.6, 141.9, 143.3, 145.6, 157.0; MS (ESI): *m/z* [$(M + H)^+$]: 414. *Anal.* Calcd for $C_{18}H_9F_6N_3O_2$: C,

52.31; H, 2.20; N, 10.17%. Found: C, 52.32; H, 2.19; N, 10.19%.

***3-Ethyl-7-phenyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5b).*** Whitish solid; mp 209–211°C; IR (KBr, $cm^{-1}$): 1682 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.48 (t, $J$ = 6.79, 3H, —$CH_3$), 4.22 (q, $J$ = 6.79, 2H, —$CH_2$), 7.52–7.59 (m, 3H, Ar—H), 8.16–8.24 (m, 2H, Ar—H), 8.28 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 12.6, 38.0, 120.7, 123.6, 124.0, 125.3, 126.3, 127.6, 128.0, 130.9, 133.7, 136.1, 138.1, 139.7, 141.9, 143.6, 158.5; MS (ESI): $m/z$ [(M + H)$^+$]: 428. *Anal*. Calcd for $C_{19}H_{11}F_6N_3O_2$: C, 53.41; H, 2.59; N, 9.83%. Found: C, 53.40; H, 2.61; N, 9.84%.

***7-Phenyl-3-propyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5c).*** Whitish solid; mp 198–200°C; IR (KBr, $cm^{-1}$): 1678 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.03 (t, $J$ = 6.31, 3H, —$CH_3$), 1.60–1.69 (m, 2H, —$CH_2$), 4.38 (t, $J$ = 7.32, 2H, —$CH_2$), 7.52–7.58 (m, 3H, Ar—H), 8.11–8.18 (m, 2H, Ar—H), 8.23 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 11.8, 21.7, 46.3, 120.7, 123.5, 125.5, 126.6, 128.2, 129.0, 129.5, 133.4, 133.9, 135.1, 139.4, 141.8, 144.4, 146.3, 158.6; MS (ESI): $m/z$ [(M + H)$^+$]: 442. *Anal*. Calcd for $C_{20}H_{13}F_6N_3O_2$: C, 54.43; H, 2.97; N, 9.52%. Found: C, 54.44; H, 2.98; N, 9.53%.

***3-Cyclopentyl-7-phenyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5d).*** Whitish solid; mp 204–206°C; IR (KBr, $cm^{-1}$): 1678 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.57–1.66 (m, 4H, —$CH_2$—), 1.68–1.76 (m, 2H, —$CH_2$—), 1.94–1.99 (m, 2H, —$CH_2$—), 5.32–5.39 (m, 1H, —CH—), 7.95–8.03 (m, 3H, Ar—H), 8.12–8.18 (m, 2H, Ar—H), 8.35 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 23.8, 32.5, 48.8, 120.4, 122.2, 122.9, 123.5, 124.7, 125.8, 127.2, 130.6, 133.3, 134.0, 139.4, 142.5, 143.4, 147.2, 158.5; MS (ESI): $m/z$ [(M + H)$^+$]: 400. *Anal*. Calcd for $C_{22}H_{15}F_6N_3O_2$: C, 56.54; H, 3.23; N, 8.99%. Found: C, 56.55; H, 3.24; N, 9.01%.

***3-Methyl-7-phenyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5e).*** Whitish solid; mp 188–190°C; IR (KBr, $cm^{-1}$): 1678 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 3.53 (s, 3H, —$CH_3$), 7.52–7.58 (m, 3H, Ar—H), 7.94 (s, 1H, Ar—H), 8.10–8.17 (m, 2H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 34.0, 119.4, 124.0, 125.4, 126.3, 128.1, 130.0, 130.6, 132.0, 134.5, 137.3, 139.7, 143.2, 145.3, 146.9, 161.5; MS (ESI): $m/z$ [(M + H)$^+$]: 430. *Anal*. Calcd for $C_{18}H_9F_6N_3OS$: C, 50.35; H, 2.11; N, 9.79%. Found: C, 50.36; H, 2.12; N, 9.78%.

***3-Ethyl-7-phenyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5f).*** Whitish solid; mp 205–207°C; IR (KBr, $cm^{-1}$): 1682 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.51 (t, $J$ = 6.79, 3H, —$CH_3$), 4.21 (q, $J$ = 6.79, 2H, —$CH_2$), 7.54–7.62 (m, 3H, Ar—H), 8.21 (s, 1H, Ar—H), 8.24–8.29 (m, 2H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 11.5, 33.7, 120.5, 123.9, 125.0, 126.6, 127.9, 129.2, 130.4, 130.9, 133.7, 136.2, 139.8, 143.3, 145.3, 147.9, 160.6; MS (ESI): $m/z$ [(M + H)$^+$]: 444. *Anal*. Calcd for $C_{19}H_{11}F_6N_3OS$: C, 51.47; H, 2.50; N, 9.48%. Found: C, 51.49; H, 2.52; N, 9.50%.

***7-Phenyl-3-propyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5g).*** Whitish solid; mp 201–203°C; IR (KBr, $cm^{-1}$): 1678 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.03 (t, $J$ = 6.35, 3H, —$CH_3$), 1.82–1.86 (m, 2H, —$CH_2$), 4.15 (t, $J$ = 7.34, 2H, —$CH_2$), 7.56–7.62 (m, 3H, Ar—H), 8.12 (s, 1H, Ar—H), 8.20–8.24 (m, 2H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 11.9, 21.3, 46.7, 120.8, 123.7, 124.3, 125.0, 126.4, 127.7, 129.5, 133.3, 133.9, 135.1, 139.2, 141.7, 144.3, 146.4, 161.1; MS (ESI): $m/z$ [(M + H)$^+$]: 458. *Anal*. Calcd for $C_{20}H_{13}F_6N_3OS$: C, 52.52; H, 2.86; N, 9.19%. Found: C, 52.53; H, 2.87; N, 9.20%.

***3-Cyclopentyl-7-phenyl-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5h).*** Whitish solid; mp 212–214°C; IR (KBr, $cm^{-1}$): 1648 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.32–1.50 (m, 4H, —$CH_2$—), 1.52–1.58 (m, 2H, —$CH_2$—), 1.78–1.84 (m, 2H, —$CH_2$—), 5.32–5.38 (m, 1H, —CH—), 7.99–8.05 (m, 3H, Ar—H), 8.15–8.21 (m, 2H, Ar—H), 8.35 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 23.4, 43.3, 46.2, 120.4, 122.2, 123.5, 124.7, 125.4, 125.9, 126.9, 130.0, 132.7, 133.5, 139.4, 142.6, 143.5, 146.9, 160.3; MS (ESI): $m/z$ [(M + H)$^+$]: 484. *Anal*. Calcd for $C_{22}H_{15}F_6N_3OS$: C, 54.66; H, 3.13; N, 8.69%. Found: C, 54.67; H, 3.14; N, 8.71%.

***3-Methyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5i).*** Whitish solid; mp 191–193°C; IR (KBr, $cm^{-1}$): 1685 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 3.73 (s, 3H, —$CH_3$), 7.19 (dd, $J$ = 4.91, 1H, Ar—H), 7.57 (dd, $J$ = 4.91, 1H, Ar—H), 7.89 (dd, $J$ = 3.77, 1H, Ar—H), 8.02 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 36.4, 119.5, 121.7, 123.4, 124.1, 125.1, 127.4, 128.4, 133.5, 134.7, 137.3, 139.9, 142.2, 143.3, 147.4, 161.5; MS (ESI): $m/z$ [(M + H)$^+$]: 420. *Anal*. Calcd for $C_{16}H_7F_6N_3O_2S$: C, 45.83; H, 1.68; N, 10.02%. Found: C, 45.84; H, 1.69; N, 10.03%.

***3-Ethyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl)pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5j).*** Whitish solid; mp 186–188°C; IR (KBr, $cm^{-1}$): 1685 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.38 (t, $J$ = 6.76, 3H, —$CH_3$), 4.39 (q, $J$ = 6.76, 2H, —$CH_2$), 7.19 (dd, $J$ = 4.88, 1H, Ar—H), 7.50 (dd, $J$ = 4.88, 1H, Ar—H), 7.78 (dd, $J$ = 3.76, 1H, Ar—H), 7.85 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 12.4, 42.5, 120.6, 123.5, 124.5, 126.9, 127.4, 128.8, 132.4, 134.6, 136.5, 137.1, 140.7, 143.3, 144.5, 146.9, 161.0; MS (ESI): $m/z$ [(M + H)$^+$]: 434. *Anal*. Calcd for $C_{17}H_9F_6N_3O_2S$: C, 47.12; H, 2.09; N, 9.70%. Found: C, 47.13; H, 2.10; N, 9.72%.

***3-Propyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl) pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5k).*** Whitish solid; mp 222–224°C; IR (KBr, $cm^{-1}$): 1682 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.04 (t, $J$ = 6.31, 3H, —$CH_3$), 1.62–1.67 (m, 2H, —$CH_2$—), 4.12 (t, $J$ = 7.31, 2H, —$CH_2$—), 7.18 (dd, $J$ = 4.90, 1H, Ar—H), 7.56 (dd, $J$ = 4.90, 1H, Ar—H), 7.83 (dd, $J$ = 3.77, 1H, Ar—H), 8.01 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 11.2, 22.7, 45.4, 120.6, 124.0, 124.5, 126.5, 127.9, 129.2, 130.9, 131.9, 133.6, 136.2, 139.7, 143.3, 146.4, 161.6; MS (ESI): *m/z* [(M + H)$^+$]: 448. *Anal*. Calcd for $C_{18}H_{11}F_6N_3O_2S$: C, 48.33; H, 2.48; N, 9.39%. Found: C, 43.34; H, 2.49; N, 9.40%.

***3-Cyclopentyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl) pyrido[3′,2′:4,5]furo[3,2-d]pyrimidin-4(3H)-one (5l).*** Whitish solid; mp 201–203°C; IR (KBr, $cm^{-1}$): 1678 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.60–1.69 (m, 2H, —$CH_2$—), 1.83–1.92 (m, 4H, —$CH_2$—), 2.29–2.38 (m, 2H, —$CH_2$—), 5.36–5.49 (m, 1H, —CH—), 7.20 (dd, $J$ = 4.91, 1H, Ar—H), 7.58 (dd, $J$ = 4.91, 1H, Ar—H), 7.87 (dd, 1H, Ar—H), 8.00 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 23.0, 32.3, 46.6, 119.3, 121.0, 123.7, 124.5, 127.8, 129.3, 130.5, 131.5, 132.1, 132.9, 139.4, 142.9, 143.8, 146.6, 160.9; MS (ESI): *m/z* [(M + H)$^+$]: 474. *Anal*. Calcd for $C_{20}H_{13}F_6N_3O_2S$: C, 50.74; H, 2.77; N, 8.88%. Found: C, 50.75; H, 2.78; N, 8.89%.

***3-Methyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl) pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5m).*** Whitish solid; mp 208–209°C; IR (KBr, $cm^{-1}$): 1675 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 3.75 (s, 3H, —$CH_3$), 7.20 (dd, $J$ = 4.89, 1H, Ar—H), 7.59 (dd, $J$ = 4.89, 1H, Ar—H), 7.88 (dd, $J$ = 3.78, 1H, Ar—H), 8.01(s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 45.3, 120.5, 122.8, 124.3, 125.0, 126.7, 128.5, 129.7, 130.9, 133.0, 135.4, 140.5, 143.3, 146.0, 160.4; MS (ESI): *m/z* [(M + H)$^+$]: 366. *Anal*. Calcd for $C_{16}H_7F_6N_3OS_2$: C, 44.14; H, 1.62; N, 9.65%. Found: C, 44.15; H, 1.63; N, 9.66%.

***3-Ethyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl) pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5n).*** Whitish solid; mp 225–227°C; IR (KBr, $cm^{-1}$): 1672 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.41 (t, $J$ = 6.79, 3H, —$CH_3$), 4.41 (q, $J$ = 6.79, 2H, —$CH_2$), 7.19 (dd, $J$ = 4.90, 1H, Ar—H), 7.51 (dd, $J$ = 4.90, 1H, Ar—H), 7.78 (dd, $J$ = 3.78, 1H, Ar—H), 7.88 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 12.4, 43.8, 120.6, 124.0, 126.0, 126.5, 128.2, 129.2, 130.4, 130.9, 133.7, 136.2, 140.1, 143.0, 145.3, 147.2, 160.9; MS (ESI): *m/z* [(M + H)$^+$]: 450. *Anal*. Calcd for $C_{17}H_9F_6N_3OS_2$: C, 45.44; H, 2.02; N, 9.35%. Found: C, 45.45; H, 2.04; N, 9.36%.

***3-Propyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl) pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5o).*** Whitish solid; mp 212–214°C; IR (KBr, $cm^{-1}$): 1678 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.07 (t, $J$ = 6.32, 3H, —$CH_3$), 1.61–1.72 (m, 2H, —$CH_2$), 4.39 (t, $J$ = 7.32, 2H, —$CH_2$), 7.19 (dd, $J$ = 4.91, 1H, Ar—H), 7.52 (dd, $J$ = 4.91, 1H, Ar—H), 7.78 (dd, $J$ = 3.76, 1H, Ar—H), 7.87 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 12.5, 23.3, 45.4, 119.7, 122.8, 123.1, 124.0, 125.6, 126.7, 128.8, 132.0, 133.9, 135.0. 139.4, 142.0, 144.6, 146.7, 160.7; MS (ESI): *m/z* [(M + H)$^+$]: 464. *Anal*. Calcd for $C_{18}H_{11}F_6N_3OS_2$: C, 46.65; H, 2.39; N, 9.07%. Found: C, 46.66; H, 2.40; N, 9.08%.

***3-Cyclopentyl-7-(thiophen-2-yl)-2,9-bis(trifluoromethyl) pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidin-4(3H)-one (5p).*** Whitish solid; mp 218–220°C; IR (KBr, $cm^{-1}$): 1682 (=NCO—); $^1H$ NMR ($CDCl_3$, 300 MHz): δ 1.79–1.85 (m, 4H, —$CH_2$—), 1.89–1.95 (m, 2H, —$CH_2$—), 2.26–2.46 (m, 2H, —$CH_2$—), 5.29–5.38 (m, 1H, —CH—), 7.19 (dd, $J$ = 4.92, 1H, Ar—H), 7.59 (dd, $J$ = 4.92, 1H, Ar—H), 7.89 (dd, $J$ = 3.77, 1H, Ar—H), 8.02 (s, 1H, Ar—H); $^{13}C$ NMR ($CDCl_3$, 75 MHz): 24.0, 43.3, 45.4, 120.5, 122.4, 123.7, 125.2, 126.5, 127.1, 128.2, 130.0, 132.1, 134.5, 139.3, 142.1, 145.2, 160.7; MS (ESI): *m/z* [(M + H)$^+$]: 490. *Anal*. Calcd for $C_{20}H_{13}F_6N_3OS_2$: C, 49.09; H, 2.68; N, 8.59%. Found: C, 49.10; H, 2.69; N, 8.60%.

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