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Parallel synthesis of condensed pyrimidine-thiones and their antitumor activities

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Abstract

Herein, we studied the formation of thiones via C=O group conversion into the C=S functional group-based tricyclic pyrimidinone systems using Lawesson's reagent and phosphorus pentasulfide as thionation agents. Naturally occurring alkaloids deoxyvasicinone and mackinazolinone were selected as templates for the modification of furo[2,3-*d*]pyrimidinone and pyrrolo[2,3-*d*]pyrimidinone scaffold. Research work was performed under the combinatorial and parallel synthesis of pyrimidine-based small molecules, along with a one-pot reaction strategy. All synthesized 54 novel pyrimidine-thiones were elucidated by ^{1}H-NMR, ^{13}C-NMR, and HRMS analysis. In addition, both series of thiones were evaluated for their antitumor activity against three types of the human cancer cell: cervical HeLa, breast MCF-7, and colon HT-29 lines. Compound with azepine fragment **13aa** (1-methyl-2-(4-(trifluoromethyl) phenyl)-1,6,7,8,9,10-hexahydro-4*H*-pyrrolo[2',3':4,5]pyrimido[1,2-*a*]azepine-4-thione) was most active derivative ($IC_{50} = 2.09 \pm 0.22$ μM) against the HT-29 cell line.

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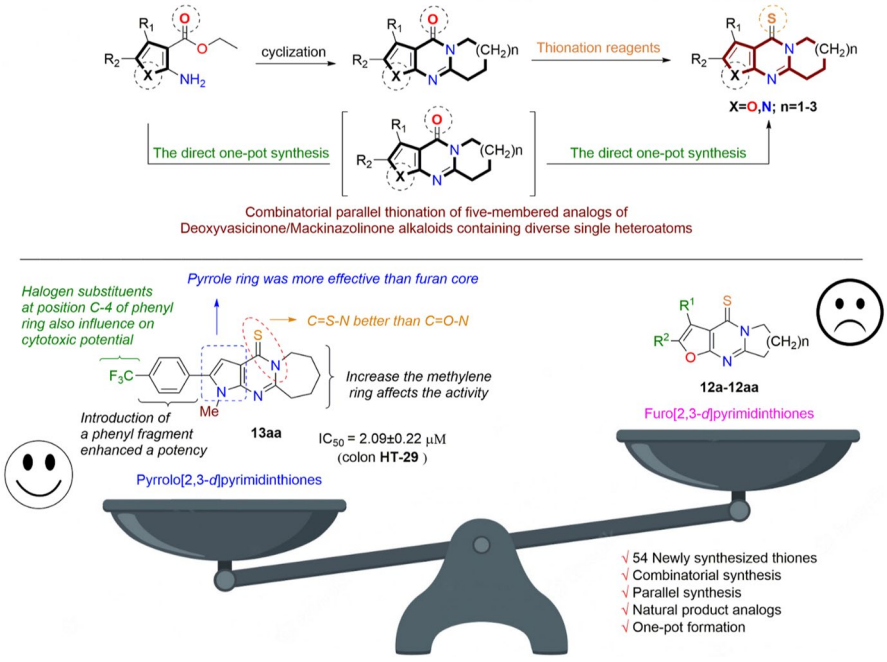
Graphical abstract



Keywords Thiones; parallel synthesis · furo[2,3-*d*]pyrimidinthione; pyrrolo[2,3-*d*] pyrimidinthione; antitumor activity

Introduction

Clinical trials show that most medical drugs which are used in cancer chemotherapy fail to produce satisfactory outcomes because of poor selectivity, efficiency, or various side effects [1–3]. Therefore, there is still a need to improve the drug development process [4, 5]. Pyrimidine-based small molecules [6] are a group of chemotherapeutic agents [7], which include 5-FU, capecitabine [8], decitabine [9], gemcitabine [10], raltitrexed [11], floxuridine [12], tegafur [13], and others [14]. These anticancer agents induce apoptosis while progressing via the cell cycle by inhibiting appropriate targets. In addition, pyrimidine-based small molecules are also included in system nitrogen, oxygen, and sulfur heteroatoms.

Organosulfur compounds are components or fragments in pyrimidine-based small molecules [15]. Herein, among the organosulfur derivatives (both natural and synthetic), thio-analogs of ketones are important scaffolds from chemical, medical [16], and industrial [17] points of view. Literature reports show that most organosulfur derivatives are found as potential inhibitors of various major enzymes, indicating the importance of these class compounds in medicinal chemistry [18–20].

Naturally occurring alkaloids deoxyvasicinone [21, 22] and mackinazolinone [23] are isolated from the plants *Adhatoda vasica* and *Mackilaya subulata* Philipson, respectively. These pyrimidine-based derivatives have demonstrated potential biological properties [24–26]. The tricyclic deoxyvasicinone system is part of other biologically important alkaloids including isaindigotone [27], tryptanthrin [28], and luotonin A [29], while mackinazolinone is part of rutaecarpine [30] and evodiamine [31], respectively (Fig. 1). Our research group performed large studies on the synthetic analogs of deoxyvasicinone and mackinazolinone, which containing carbonyl (C=O) group in the pyrimidine ring [32–38]. Results revealed that several modified analogs of these alkaloids exhibited satisfactory higher antiproliferative activity against a panel of human cancer cell lines [32, 39].

Spurred by this preliminary success, we proceeded to examine the combinatorial and parallel transformation of furo- and pyrrolopyrimidinones under thionation agents. An important field of research in medicinal and organic chemistry involves heterocyclic thiones forming the backbone of more complex organic compounds, especially those using other sites for their attachment to specific types of rapidly forming molecules [40, 41]. Therefore, herein, we studied the formation of thiones via C=O group conversion into the C=S functional group-based tricyclic pyrimidinone system using Lawesson's reagent [42–44] and phosphorus pentasulfide [45] as thionation agents. In addition, all synthesized thio-compounds were evaluated for their antitumor activity on human cancer cell cervical HeLa, breast MCF-7, and colon HT-29 lines.

Results and discussion

Chemistry

The thionation of bicyclic pyrimidines or related compounds with Lawesson's reagent has been presented earlier [46, 47]. Other pathways have also been reported in

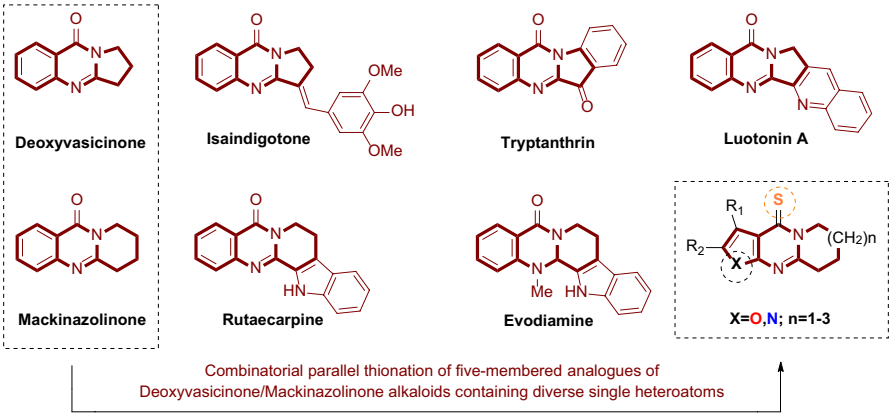
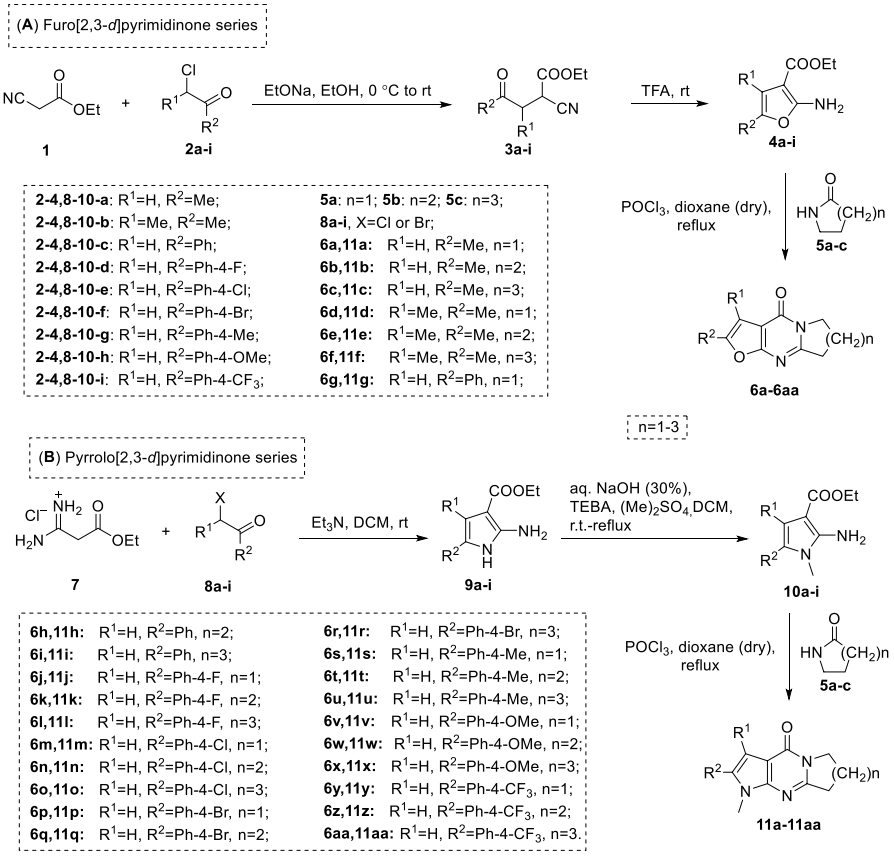


Fig. 1 Main approach of present work and structurally diverse condensed pyrimidinones

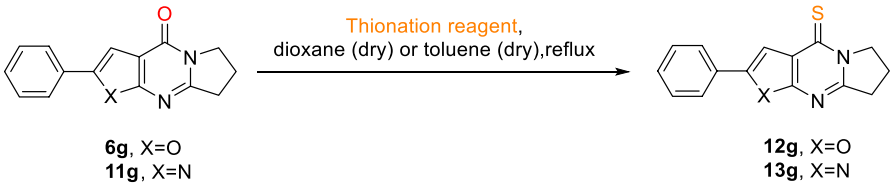


Scheme 1 Synthesis of furo[2,3-*d*]pyrimidinone **6a-6aa** and pyrrolo[2,3-*d*]pyrimidinone **11a-11aa** intermediates

the literature for the C=O group conversion into C=S [48, 49]. However, we turned our attention toward a milder route in order to perform systematic combinatorial and parallel thionation of the diverse molecular systems, along with their evaluation of the antitumor activity.

The formation of furo[2,3-*d*]pyrimidinone **6a-6aa** and pyrrolo[2,3-*d*]pyrimidinone **11a-11aa** intermediates were described in our recently reported research (Scheme 1) [50]. Proceeding from this research, we have studied a parallel thionation process of the obtained pyrimidinones **6a-6aa** and **11a-11aa**. First, in an example of compounds **6** and **11 g**, thionation conditions were investigated. Subjecting pyrimidinones **6** and **11 g** to reflux in the presence of Lawesson's reagent in toluene or dioxane results in conversion of the oxygen of the carbonyl group convers to sulfur; under these conditions, the products **12** and **13 g** are obtained in 56 and 88% yields, respectively (Table 1). In our research, we also used other thionation agents in order to convert C=O to C=S (for example with P_2S_5). All reactions using this reagent were performed in dioxane or toluene. Unfortunately, all efforts to introduce "thion" functionality into the tricyclic

Table 1 Optimization of the conditions for thionation of **6 g** and **11 g**



6g, X=O
11g, X=N

12g, X=O
13g, X=N

Entry	Thionation reagent	Intermediate	Temperature	Time (h)	Product	Yield (%)
1	Lawesson's Reagent	**6 g**	reflux	12	**12 g**	56
2	Phosphorus pentasulfide ($P_2S_{5)}$	**6 g**	reflux	4	**12 g**	74
3	Lawesson's Reagent	**11 g**	reflux	3	**13 g**	88
4	Phosphorus pentasulfide ($P_2S_{5)}$	**11 g**	reflux	6	**13 g**	49

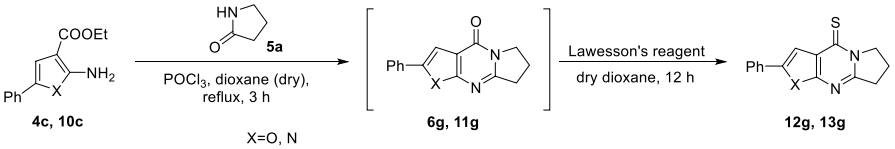
annulated system were not similar in both series of pyrimidinones. Herein, Lawesson's reagent was more suitable for the thionation of pyrrolo[2,3-*d*]pyrimidinone (for compound **11 g**), while using the P_2S_5 afforded a final thion-product (**12 g**) of furo[2,3-*d*] pyrimidinone derivatives yielded in quit good yields (74%, Table 1).

The direct one-pot formation of target thiones (**12** and **13 g**) from five-membered 2-amino esters (**4c** and **10c**) was also investigated (Scheme 2). When 2-amino esters are subjected to condensation in the presence of lactam (**5a**) and $POCl_3$ (dioxane used as reaction solvent) leads to intermediates (**6** and **11 g**) after 3–4 h, the addition of Lawesson's reagent into the reaction mixture followed by a reaction time of 4 h gives thiones **12** and **13 g**.

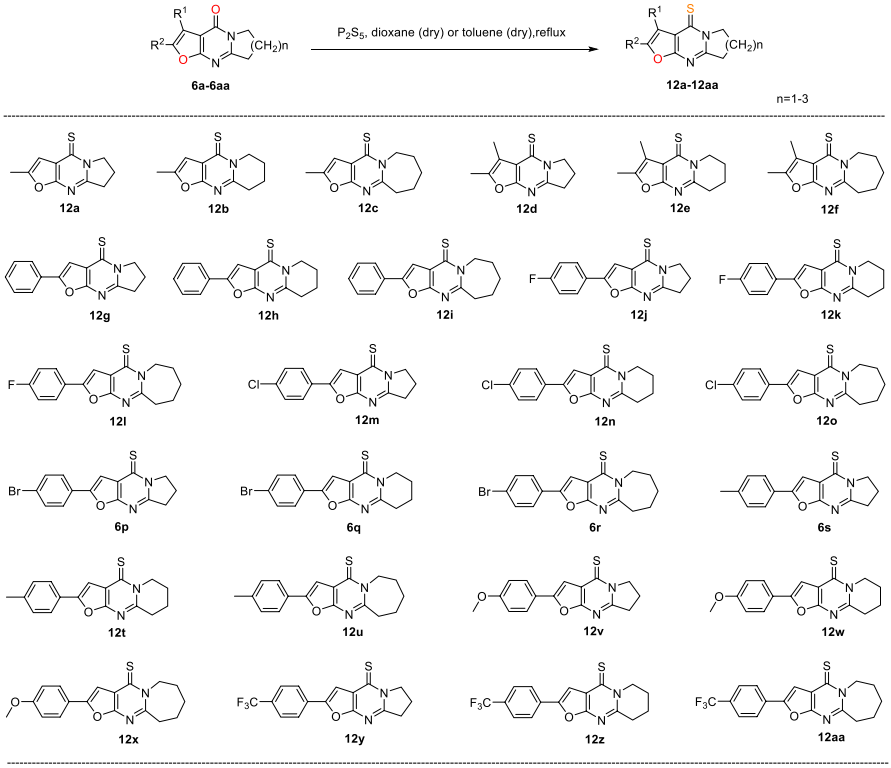
As discussed above, pyrimidine-thiones were produced in two cases, where dioxane or toluene and only dioxane (in the one-pot reaction, Scheme 2) were used as a solvent. Although we performed direct one-pot synthesis (from mono-cyclic esters), increased yields were observed in the synthetic pathways from the furo[2,3-*d*]pyrimidinone **6 g** and pyrrolo[2,3-*d*]pyrimidinone **11 g** intermediates. The direct one-pot synthesis from ester's desired thiones was obtained at a low yield, as well as workup procedures were a bit difficult, because of the formed side products. However, further development of this method may serve to obtain pyrimidine-thiones via a simple pathway.

Thus, the combinatorial and parallel thionation of pyrimidinones was achieved using Lawesson's reagent toward the pyrrolo[2,3-*d*]pyrimidinthiones (Scheme 3) and P_2S_5 in the furo[2,3-*d*]pyrimidinthiones (Scheme 4), respectively.

It should be noted that using a P_2S_5 in the furo[2,3-*d*]pyrimidinthiones formation, we observed the ability of substituents in the yield of final products. The methyl



Scheme 2 One-pot formation of pyrimidine-thiones **12** and **13 g**



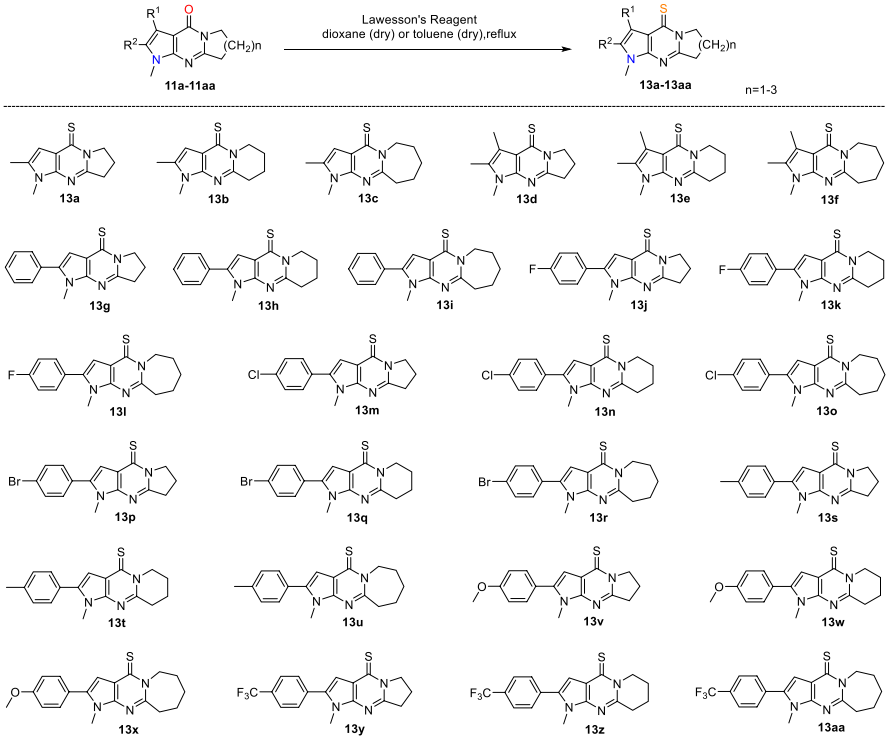
Scheme 3 A library of the synthesized tricyclic furo[2,3-*d*]pyrimidinthiones (**12a-12aa**)

group at C-2 or C-2/C-3 positions of the furan ring influence to form of thiones in lower yield (18–44%), followed to increase compounds yield (up to 74%) by introducing a phenyl substituent at position C-2. Introduction of the 4-substituted phenyl group at C-2 gave desired thiones up to 99%. Therefore, phosphorus pentasulfide was found as a convenient thionation agent on furo[2,3-*d*]pyrimidinthiones **12j-12aa**. Furthermore, using a P_2S_5 in the furo[2,3-*d*]pyrimidinthiones, only products were formed during checking at TLC (thin-layer chromatography), while with Lawesson's reagent was formed several bi-products. In the case of pyrrolo[2,3-*d*] pyrimidinthiones, final products were yielded up to 99% using Lawesson's reagent as a thionation agent, and no bi-products were observed.

All synthesized furo[2,3-*d*]pyrimidinthiones (**12a-12aa**) were elucidated by ^{1}H-NMR, ^{13}C-NMR, and HRMS as described in the experimental part, as well as X-ray analysis for derivative **12c** (Fig. 2).

Antitumor activity

All the synthesized condensed pyrimidine-thiones **12a-12aa** and **13a-13aa** were tested for their cytotoxic activity against three types of human cancer cell: cervical



Scheme 4 A library of the synthesized tricyclic pyrrolo[2,3-*d*]pyrimidinthiones (**13a-13aa**)

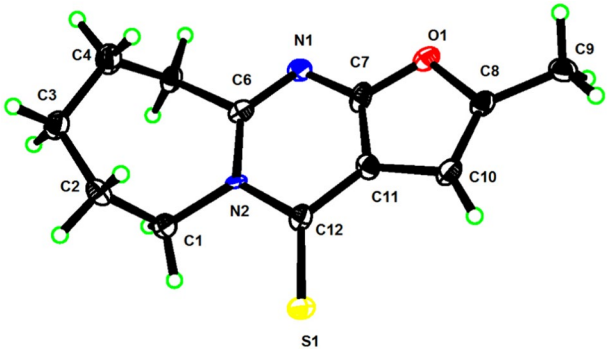


Fig. 2 Crystal structure of compound **12c** (CCDC: 2,169,324)

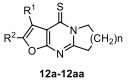
HeLa, breast MCF-7, and colon HT-29 lines using the MTT method (Table 2). Doxorubicin (DOX) is used as a reference drug. As shown in Table 2, furo[2,3-*d*] pyrimidinthiones were inactive and showed IC_{50} values ≥ 50 μM, except of derivatives **12e** (40.45 ± 2.03) and **12 g** (17.68 ± 0.83) on HT-29 cell lines. In general,

among the furopyrimidinthione series, there was no real trend between the various side-rings and substituents on the pyrimidinone system concerning the activity. However, several pyrrolo[2,3-*d*]pyrimidinthiones demonstrated weak to high cytotoxic activity against selected human cancer cell lines. Among the twenty-seven synthesized pyrrolo[2,3-*d*]pyrimidinthione derivatives, fourteen samples were quite active with IC_{50} values ≤ 50. In addition, results revealed that among the human cancer cell lines evaluated, the HT-29 cancer cell line was more sensitive. Derivatives **13j**, **13 l**, **13o**, **13r**, **13t**, **13z**, and **13aa** have exhibited higher activity with IC_{50} values ranging from 2.09 ± 0.22 to 9.84 ± 0.88 μM. All these compounds contain phenyl or 4-substituted phenyl group at position C-2. It was observed that the increase of methylene groups effect cytotoxic activity (Fig. 3). For example, a compound with azepine fragment **13aa** (1-methyl-2-(4-(trifluoromethyl)phenyl)-1,6,7,8,9,10-hexahydro-4*H*-pyrrolo[2',3':4,5]pyrimido[1,2-*a*]azepine-4-thione) was most active derivative ($IC_{50} = 2.09 \pm 0.22$ μM) against the HT-29 cell line (Fig. 3). Therefore, a further detailed study is needed on this way, where obtained leading compounds may serve a good candidate as anticancer agents.

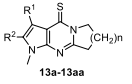
Conclusion

We have designed and synthesized novel 2-substituted furo[2,3-*d*]pyrimidinthione and pyrrolo[2,3-*d*]pyrimidinthione derivatives under combinatorial and parallel synthesis strategy. This heterocyclic system is furan/pyrrole analogs of the naturally occurring deoxyvasicinone mackinazolinone alkaloids. In general, two thionation agents were used to study systematic thionation. Results revealed that Lawesson's reagent was more suitable for the thionation of pyrrolo[2,3-*d*]pyrimidinones, while using P_2S_5 afforded a final thion-products of furo[2,3-*d*]pyrimidinones in a quit good yield. All of the newly designed thiones were evaluated against the human cancer cell lines Hela, MCF-7, and HT-29. The furo[2,3-*d*]pyrimidinthione series were not demonstrated antitumor activity against selected cell lines. However, several pyrrolo[2,3-*d*]pyrimidinthiones showed weak to high cytotoxic activity. Among the twenty-seven synthesized pyrrolo[2,3-*d*]pyrimidinthione derivatives, fourteen samples were quite active with IC_{50} values ≤ 50. Azepine containing derivative **13aa** (1-methyl-2-(4-(trifluoromethyl)phenyl)-1,6,7,8,9,10-hexahydro-4*H*-pyrrolo[2',3':4,5]pyrimido[1,2-*a*]azepine-4-thione) displayed a high ($IC_{50} = 2.09 \pm 0.22$ μM) antitumor activity against the HT-29 cell line, which further modification could be serve as leading compound in anticancer drug design investigations.

Table 2 In vitro cytotoxic activity of the synthesized pyrimidinthiones (**12a-12aa** and **13a-13aa**) against selected cell lines



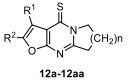
12a-12aa



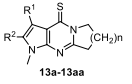
13a-13aa

Compd	R_1	R_2	n	Cell lines (IC_{50}, μM)			Compd	R_1	R_2	n	Cell lines (IC_{50}, μM)		
				HeLa	MCF-7	HT-29					HeLa	MCF-7	HT-29
12a	H	Me	1	≥ 50	≥ 50	≥ 50	**13a**	H	Me	1	≥ 50	≥ 50	≥ 50
12b	H	Me	2	≥ 50	≥ 50	≥ 50	**13b**	H	Me	2	≥ 50	≥ 50	≥ 50
12c	H	Me	3	≥ 50	≥ 50	≥ 50	**13c**	H	Me	3	≥ 50	≥ 50	≥ 50
12d	Me	Me	1	≥ 50	≥ 50	≥ 50	**13d**	Me	Me	1	≥ 50	≥ 50	≥ 50
12e	Me	Me	2	≥ 50	≥ 50	40.45 ± 2.03	**13e**	Me	Me	2	≥ 50	≥ 50	≥ 50
12f	Me	Me	3	≥ 50	≥ 50	≥ 50	**13f**	Me	Me	3	≥ 50	≥ 50	≥ 50
12 g	H	Ph	1	≥ 50	≥ 50	17.68 ± 0.83	**13 g**	H	Ph	1	≥ 50	≥ 50	≥ 50
12 h	H	Ph	2	≥ 50	≥ 50	≥ 50	**13 h**	H	Ph	2	≥ 50	≥ 50	≥ 50
12i	H	Ph	3	≥ 50	≥ 50	≥ 50	**13i**	H	Ph	3	4.61 ± 0.21	≥ 50	7.96 ± 0.90
12j	H	4-F-Ph	1	≥ 50	≥ 50	≥ 50	**13j**	H	4-F-Ph	1	≥ 50	≥ 50	≥ 50
12 k	H	4-F-Ph	2	≥ 50	≥ 50	≥ 50	**13 k**	H	4-F-Ph	2	≥ 50	≥ 50	≥ 50
12 l	H	4-F-Ph	3	≥ 50	≥ 50	≥ 50	**13 l**	H	4-F-Ph	3	≥ 50	≥ 50	9.84 ± 0.88
12 m	H	4-Cl-Ph	1	≥ 50	≥ 50	≥ 50	**13 m**	H	4-Cl-Ph	1	≥ 50	≥ 50	≥ 50
12n	H	4-Cl-Ph	2	≥ 50	≥ 50	≥ 50	**13n**	H	4-Cl-Ph	2	≥ 50	≥ 50	17.87 ± 1.71
12o	H	4-Cl- Ph	3	≥ 50	≥ 50	≥ 50	**13o**	H	4-Cl- Ph	3	≥ 50	≥ 50	4.00 ± 0.23
12p	H	4-Br-Ph	1	≥ 50	≥ 50	≥ 50	**13p**	H	4-Br-Ph	1	24.98 ± 0.82	40.82 ± 1.63	≥ 50
12q	H	4-Br-Ph	2	≥ 50	≥ 50	≥ 50	**13q**	H	4-Br-Ph	2	≥ 50	≥ 50	≥ 50
12r	H	4-Br-Ph	3	≥ 50	≥ 50	≥ 50	**13r**	H	4-Br-Ph	3	≥ 50	≥ 50	2.78 ± 0.21
12 s	H	4-Me-Ph	1	≥ 50	≥ 50	≥ 50	**13 s**	H	4-Me-Ph	1	35.74 ± 1.03	≥ 50	≥ 50

Table 2 (continued)



12a-12aa



13a-13aa

Compd	R_1	R_2	n	Cell lines (IC_{50}, μM)			Compd	R_1	R_2	n	Cell lines (IC_{50}, μM)		
				HeLa	MCF-7	HT-29					HeLa	MCF-7	HT-29
12t	H	4-Me-Ph	2	≥ 50	≥ 50	≥ 50	**13t**	H	4-Me-Ph	2	≥ 50	≥ 50	3.52 ± 0.31
12u	H	4-Me-Ph	3	≥ 50	≥ 50	≥ 50	**13u**	H	4-Me-Ph	3	≥ 50	26.99 ± 0.57	10.32 ± 0.63
12v	H	4-OMe-Ph	1	≥ 50	≥ 50	≥ 50	**13v**	H	4-OMe-Ph	1	≥ 50	≥ 50	≥ 50
12w	H	4-OMe-Ph	2	≥ 50	≥ 50	≥ 50	**13w**	H	4-OMe-Ph	2	≥ 50	≥ 50	19.47 ± 1.39
12x	H	4-OMe-Ph	3	≥ 50	≥ 50	≥ 50	**13x**	H	4-OMe-Ph	3	≥ 50	≥ 50	22.56 ± 1.60
12y	H	4-CF_3-Ph	1	≥ 50	≥ 50	≥ 50	**13y**	H	4-CF_3-Ph	1	9.07 ± 0.18	≥ 50	≥ 50
12z	H	4-CF_3-Ph	2	≥ 50	≥ 50	≥ 50	**13z**	H	4-CF_3-Ph	2	7.01 ± 0.15	94.29 ± 2.55	2.66 ± 0.17
12aa	H	4-CF_3-Ph	3	≥ 50	≥ 50	≥ 50	**13aa**	H	4-CF_3-Ph	3	20.25 ± 0.77	≥ 50	2.09 ± 0.22
DOX	–	–	–	0.57 ± 0.016	0.18 ± 0.011	0.82 ± 0.029	**DOX**	–	–	–	0.57 ± 0.016	0.18 ± 0.011	0.82 ± 0.029

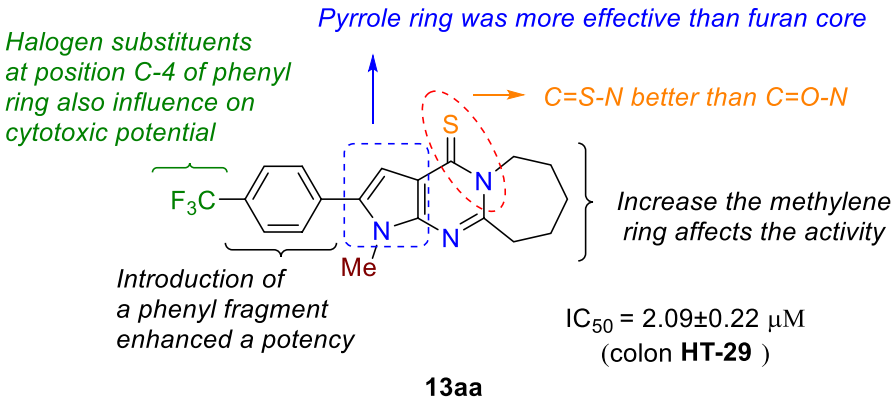


Fig. 3 Structure–activity relationship for active derivative **13aa**

Experimental section

Chemistry

Materials and methods

All reagents and solvents were purchased from Sigma and used without further purification. Thin-layer chromatography (TLC) was performed on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254) and visualized by UV light (254 nm). The products were purified by column chromatography over silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh). Melting points were determined on a Buchi B-540 apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian 400 MHz NMR spectrometer in $CDCl_3$, using TMS as an internal standard. High-resolution mass spectra (HRMS) were recorded on an AB SCIEX QSTAR Elite quadrupole time-of-flight mass spectrometry.

General procedure for synthesis of compounds 12a-12aa A solution of 1-mmol furo[2,3-*d*]pyrimidinones (**6a-6aa**) in 10 mL of dried toluene or dioxane was stirred under reflux with 1.1 mmol (222 mg) of phosphorus pentasulfide (P_2S_5). After 4 h, the reaction mixture was cooled to 20–25 °C, the solvent was removed under reduced pressure and the residue column chromatographed using petroleum ether: EtOAc (1:1 − 3:1) as an eluent to give solid thiones **12a-12aa**.

2-Methyl-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12a) Yield 25%, yellow solid, m.p.159–160 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62 (s, 1H), 4.52 (t, J = 8.0 Hz, 2H), 3.32 (t, J = 8.1 Hz, 2H), 2.42 (s, 3H), 2.41 – 2.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.57, 159.91, 159.64, 154.09, 120.54, 104.06, 52.50, 33.02, 19.12, 14.09; HRMS (ESI): calcd for $C_{10}H_{10}N_2OS$ $[M+H]^+$: 207.0592, found 207.0592.

2-Methyl-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12b) Yield 18%, yellow solid, m.p.139–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.61 (s, 1H), 4.57 (t, J = 6.2 Hz, 2H), 3.07 (t, J = 6.8 Hz, 2H), 2.39 (s, 3H), 2.09–2.00 (m, 2H), 1.99–1.90 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.58, 157.43, 156.87, 153.88, 121.18, 104.70, 48.83, 32.63, 22.37, 18.72, 13.98; HRMS (ESI): calcd for $C_{11}H_{12}N_2OS$ $[M+H]^+$: 221.0749, found 221.0749.

2-Methyl-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12c) Yield 40%, yellow solid, m.p.119–120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.61 (s, 1H), 5.09 (t, J = 5.0 Hz, 2H), 3.20 (t, J = 6.8 Hz, 2H), 2.41 (s, 3H), 1.93–1.81 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.11, 161.19, 157.26, 154.23, 121.06, 105.24, 49.83, 38.07, 29.14, 26.15, 24.95, 14.02. HRMS (ESI): calcd for $C_{12}H_{14}N_2OS$ $[M+H]^+$: 235.0905, found 235.0904.

2,3-Dimethyl-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12d) Yield 26%, yellow solid, m.p.223–224 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50 (t, J = 7.5 Hz, 2H), 3.29 (t, J = 8.1 Hz, 2H), 2.42 (s, 3H), 2.38–2.29 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.32, 159.64, 159.43, 148.96, 118.90, 113.60, 52.15, 32.99, 18.96, 11.48, 9.74; HRMS (ESI): calcd for $C_{11}H_{12}N_2OS$ $[M+H]^+$: 221.0749, found 221.0749.

2,3-Dimethyl-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12e) Yield 44%, yellow solid, m.p. 179–180 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (t, J = 6.2 Hz, 2H), 3.06 (t, J = 6.8 Hz, 2H), 2.45 (s, 3H), 2.32 (s, 3H), 2.10–2.01 (m, 2H), 1.99–1.90 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.49, 157.39, 156.71, 148.78, 119.64, 113.92, 48.02, 32.69, 22.48, 18.73, 11.55, 10.17; HRMS (ESI): calcd for $C_{12}H_{14}N_2OS$ $[M+H]^+$: 235.0905, found 235.0906.

2,3-Dimethyl-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12f) Yield 38%, yellow solid, m.p.114–115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.08 (t, 2H), 3.17 (t, J = 6.8 Hz, 2H), 2.44 (s, 3H), 2.31 (t, J = 5.0 Hz, 3H), 1.92–1.79 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.90, 160.93, 157.20, 149.01, 119.40, 114.13, 48.66, 38.06, 29.06, 26.05, 24.93, 11.52, 10.06; HRMS (ESI): calcd for $C_{13}H_{16}N_2OS$ $[M+H]^+$: 249.1062, found 249.1062.

2-Phenyl-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12 g) Yield 74%, yellow solid, m.p. 234.5–235.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 7.2 Hz, 2H), 7.44 (t, J = 7.4 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.25 (s, 1H), 4.55 (t, J = 7.4 Hz, 2H), 3.36 (t, J = 8.1 Hz, 2H), 2.39 (dt, J = 15.7, 7.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.09, 160.62, 159.95, 154.38, 129.14, 128.99, 128.97, 124.66, 121.17, 102.38, 52.57, 33.16, 19.10; HRMS (ESI): calcd for $C_{15}H_{13}N_2OS$ $[M+H]^+$: 269.0749, found 269.0750.

2-Phenyl-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12 h) Yield 73%, yellow solid, m.p. 199.4–200.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 7.4 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.36 (t, J = 7.3 Hz, 1H), 7.25 (s, 1H), 4.60 (t, J = 6.1 Hz, 2H), 3.13 (t, J = 6.8 Hz, 2H), 2.13–2.05 (m, 2H), 2.03–1.94 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.06, 157.66, 154.05, 129.06, 128.92, 128.89, 124.64, 121.67, 103.03, 48.99, 32.77, 22.37, 18.71, 18.39; HRMS (ESI): calcd for $C_{16}H_{15}N_2OS$ $[M+H]^+$: 283.0905, found 283.0908.

2-Phenyl-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12i) Yield 23%, yellow solid, m.p. 190.6–191.8 °C. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.79 (d, J = 7.3 Hz, 2H), 7.44 (t, J = 7.4 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.23 (s, 1H), 5.11 (t, J = 4.1 Hz, 2H), 3.24 (t, J = 6.4 Hz, 2H), 1.95–1.85 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.61, 161.93, 157.51, 154.44, 129.15, 128.96, 124.96, 124.71, 121.59, 103.62, 49.90, 38.21, 29.14, 26.13, 24.93; HRMS (ESI): calcd for $C_{16}H_{15}N_2OS$ $[M+H]^+$: 297.1062, found 297.1064.

2-(4-Fluorophenyl)-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12j) Yield 99%, yellow solid, m.p255.5–257.4.°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (dd, J = 8.6, 5.3 Hz, 2H), 7.11 (t, J = 8.6 Hz, 2H), 7.01 (s, 1H), 4.21 (t, J = 7.3 Hz, 2H), 3.21 (t, J = 8.0 Hz, 2H), 2.40 –2.27 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.22, 164.02, 161.54, 160.89, 157.74, 151.40, 126.18, 126.10, 116.09, 115.87, 107.30, 99.89, 46.84, 32.39, 19.62. HRMS (ESI): calcd for $C_{15}H_{12}FN_2OS$ $[M+H]^+$: 287.0654, found 287.0647.

2-(4-Fluorophenyl)-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12 k) Yield 99%, yellow solid, m.p. 220.9–222.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, J = 8.1, 5.6 Hz, 2H), 7.15 (s, 1H), 7.11 (t, J = 8.5 Hz, 2H), 4.59 (t, J = 6.1 Hz, 2H), 3.12 (t, J = 6.7 Hz, 2H), 2.14–1.94 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.07, 164.32, 161.83, 157.73, 153.11, 126.61, 126.61, 125.27, 121.65, 116.20, 115.98, 102.72, 49.04, 32.80, 22.38, 18.71. HRMS (ESI): calcd for $C_{16}H_{14}FN_2OS$ $[M+H]^+$: 301.0811, found 301.0810.

2-(4-Fluorophenyl)-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12 l) Yield 99%, yellow solid, m.p. 201.6–202.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, J = 8.7, 5.3 Hz, 2H), 7.16 (s, 1H), 7.13 (t, J = 8.8 Hz, 2H), 5.15–5.06 (m, 2H), 3.23 (t, J = 5.0 Hz, 2H), 1.96–1.83 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.57, 164.37, 161.99, 157.46, 153.48, 126.64, 125.30, 121.53, 116.25, 116.03, 103.29, 49.89, 38.17, 29.10, 26.08, 24.88; HRMS (ESI): calcd for $C_{17}H_{16}FN_2OS$ $[M+H]^+$: 315.0967, found 315.0961.

2-(4-Chlorophenyl)-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12 m) Yield 44%, yellow solid, m.p. 243.3–244.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H), 7.06 (s, 1H), 4.22 (t, J = 7.3 Hz, 2H), 3.21 (t, J = 8.0 Hz, 2H), 2.39 – 2.28 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.31, 161.14, 157.69, 151.16, 134.32, 129.10, 127.92, 125.47, 107.30, 100.70, 46.86, 32.42, 19.61. HRMS (ESI): calcd for $C_{15}H_{12}ClN_2OS$ $[M+H]^+$: 303.0359, found 303.0351.

2-(4-Chlorophenyl)-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12n) Yield 99%, yellow solid, m.p. 220.6–222.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.5 Hz, 2H), 7.24 (s, 1H), 4.60 (t, J = 6.2 Hz, 2H), 3.14 (t, J = 6.8 Hz, 2H), 2.10 (p, J = 6.2 Hz, 2H), 1.99 (p, J = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.24, 157.94, 157.75, 152.91, 134.95, 129.21, 127.44, 125.84, 121.62, 103.54, 49.05, 32.82, 22.38, 18.71. HRMS (ESI): calcd for $C_{16}H_{14}ClN_2OS$ $[M+H]^+$: 317.0515, found 317.0508.

2-(4-Chlorophenyl)-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12o) Yield 99%, yellow solid, m.p. 207.3–208.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 7.06 (s, 1H), 4.46–4.36 (m, 2H), 3.14–3.05 (m, 2H), 1.94–1.73 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.12, 161.75, 158.81, 151.12, 134.35, 129.12, 127.93, 125.52, 106.70,

101.05, 42.87, 37.64, 29.54, 27.49, 24.95. HRMS (ESI): calcd for $C_{17}H_{16}ClN_2OS$ $[M+H]^+$: 331.0672, found 331.0665.

2-(4-Bromophenyl)-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12p) Yield 99%, yellow solid, m.p. 270.8–272.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, $J=8.4$ Hz, 2H), 7.54 (d, $J=8.6$ Hz, 2H), 7.08 (s, 1H), 4.22 (t, $J=7.4$ Hz, 2H), 3.21 (t, $J=8.0$ Hz, 2H), 2.40 – 2.27 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.33, 161.19, 157.68, 151.18, 132.04, 128.34, 125.70, 122.50, 107.31, 100.82, 46.87, 32.43, 19.60. HRMS (ESI): calcd for $C_{15}H_{12}BrN_2OS$ $[M+H]^+$: 346.9854, found 346.9841.

2-(4-Bromophenyl)-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12q) Yield 89%, yellow solid, m.p. 234.4–235.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, $J=8.4$ Hz, 2H), 7.54 (d, $J=8.6$ Hz, 2H), 7.08 (s, 1H), 4.08 (t, $J=6.0$ Hz, 2H), 3.02 (t, $J=6.6$ Hz, 2H), 2.07 – 1.90 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.41, 159.00, 157.02, 150.80, 132.03, 128.38, 125.72, 122.46, 106.66, 100.91, 42.57, 31.92, 22.02, 19.12. HRMS (ESI): calcd for $C_{16}H_{14}BrN_2OS$ $[M+H]^+$: 361.0010, found 361.0001.

2-(4-Bromophenyl)-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12r) Yield 99%, yellow solid, m.p. 213.5–214.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, $J=8.4$ Hz, 2H), 7.57 (d, $J=8.5$ Hz, 2H), 7.23 (s, 1H), 5.15–5.06 (m, 2H), 3.28–3.20 (m, 2H), 1.96–1.83 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.71, 162.22, 157.56, 153.27, 132.18, 127.85, 126.06, 123.24, 121.48, 104.19, 49.89, 38.20, 29.10, 26.08, 24.87. HRMS (ESI): calcd for $C_{17}H_{16}BrN_2OS$ $[M+H]^+$: 375.0167, found 375.0161.

2-(p-Tolyl)-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12 s) Yield 73%, yellow solid, m.p. 262.9–264.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, $J=8.1$ Hz, 2H), 7.22 (d, $J=8.0$ Hz, 2H), 7.02 (s, 1H), 4.21 (t, $J=7.4$ Hz, 2H), 3.20 (t, $J=8.0$ Hz, 2H), 2.37 (s, 3H), 2.36–2.26 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.08, 160.55, 157.82, 152.59, 138.65, 129.53, 126.71, 124.24, 107.34, 99.42, 46.81, 32.36, 21.33, 19.63. HRMS (ESI): calcd for $C_{16}H_{15}N_2OS$ $[M+H]^+$: 283.0905, found 283.0898.

2-(p-Tolyl)-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12t) Yield 99%, yellow solid, m.p. 202.0–203.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, $J=8.0$ Hz, 2H), 7.25 (d, $J=7.8$ Hz, 2H), 7.20 (s, 1H), 4.61 (t, $J=6.1$ Hz, 2H), 3.14 (t, $J=6.7$ Hz, 2H), 2.39 (s, 3H), 2.10 (p, $J=6.2$ Hz, 2H), 1.99 (p, $J=6.5$ Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.87, 157.56, 157.42, 154.42, 139.32, 129.62, 126.22, 124.64, 121.79, 102.23, 49.00, 32.77, 22.40, 21.40, 18.74. HRMS (ESI): calcd for $C_{17}H_{17}N_2OS$ $[M+H]^+$: 297.1062, found 283.0898.

2-(p-tolyl)-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12u) Yield 99%, yellow solid, m.p. 185.6–186.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, $J=8.0$ Hz, 2H), 7.25 (d, $J=8.2$ Hz, 2H), 7.18 (s, 1H), 5.16–5.07 (m, 2H), 3.27–3.20 (m, 2H), 2.39 (s, 3H), 1.95–1.83 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.36, 161.68, 157.35, 154.77, 139.38, 129.64, 126.21, 124.66, 121.66, 102.77, 49.87, 38.16, 29.12, 26.11, 24.91, 21.40. HRMS (ESI): calcd for $C_{18}H_{19}N_2OS$ $[M+H]^+$: 311.1218, found 311.1211.

2-(4-Methoxyphenyl)-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12v) Yield 99%, yellow solid, m.p. 219.2–221.0 °C. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.72 (d, J = 8.7 Hz, 2H), 7.11 (s, 1H), 6.97 (d, J = 8.7 Hz, 2H), 4.55 (t, J = 7.5 Hz, 2H), 3.86 (s, 3H), 3.35 (t, J = 8.0 Hz, 2H), 2.39 (p, J = 7.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.63, 160.42, 160.11, 159.69, 154.60, 126.23, 121.77, 121.36, 114.45, 100.56, 55.38, 52.54, 33.08, 19.09. HRMS (ESI): calcd for $C_{16}H_{15}N_2O_2S$ $[M+H]^+$: 299.0854, found 299.0847.

2-(4-Methoxyphenyl)-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12w) Yield 99%, yellow solid, m.p. 206.6–207.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 8.8 Hz, 2H), 7.13 (s, 1H), 6.97 (d, J = 8.8 Hz, 2H), 4.62 (t, J = 6.2 Hz, 2H), 3.86 (s, 3H), 3.14 (t, J = 6.8 Hz, 2H), 2.10 (p, J = 6.2 Hz, 2H), 1.99 (p, J = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.69, 160.43, 157.49, 157.17, 154.39, 126.28, 121.97, 121.78, 114.44, 101.26, 55.38, 49.00, 32.76, 22.42, 18.76. HRMS (ESI): calcd for $C_{17}H_{17}N_2O_2S$ $[M+H]^+$: 313.1011, found 313.1002.

2-(4-Methoxyphenyl)-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12x) Yield 99%, yellow solid, m.p. 191.8–193.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 8.8 Hz, 2H), 7.08 (s, 1H), 6.96 (d, J = 8.8 Hz, 2H), 5.15–5.07 (m, 2H), 3.85 (s, 3H), 3.27–3.19 (m, 2H), 1.94–1.83 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.10, 161.44, 160.42, 157.24, 154.66, 126.26, 121.77, 121.72, 114.43, 101.75, 55.37, 49.87, 38.14, 29.12, 26.10, 24.91. HRMS (ESI): calcd for $C_{18}H_{19}N_2O_2S$ $[M+H]^+$: 327.1167, found 327.1158.

2-(4-(Trifluoromethyl)phenyl)-7,8-dihydrofuro[2,3-d]pyrrolo[1,2-a]pyrimidine-4(6H)-thione (12y) Yield 99%, yellow solid, m.p. 237.7–238.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 7.35 (s, 1H), 4.54 (t, J = 7.4 Hz, 2H), 3.38 (t, J = 8.1 Hz, 2H), 2.41 (p, J = 8.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.43, 161.38, 160.24, 152.44, 132.11, 130.42, 125.94, 124.64, 122.49, 120.86, 104.35, 52.58, 33.19, 18.99. HRMS (ESI): calcd for $C_{16}H_{12}F_3N_2OS$ $[M+H]^+$: 337.0622, found 337.0613.

2-(4-(Trifluoromethyl)phenyl)-6,7,8,9-tetrahydro-4H-furo[2,3-d]pyrido[1,2-a]pyrimidine-4-thione (12z) Yield 99%, yellow solid, m.p. 224.8–226.4 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 7.37 (s, 1H), 4.60 (t, J = 6.1 Hz, 2H), 3.15 (t, J = 6.7 Hz, 2H), 2.11 (p, J = 6.1 Hz, 2H), 2.00 (p, J = 6.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.59, 158.44, 158.02, 152.24, 132.15, 130.42, 125.94, 124.70, 122.50, 121.46, 105.14, 49.08, 32.87, 22.36, 18.68. HRMS (ESI): calcd for $C_{17}H_{14}F_3N_2OS$ $[M+H]^+$: 351.0779, found 351.0770.

2-(4-(Trifluoromethyl)phenyl)-7,8,9,10-tetrahydrofuro[2',3':4,5]pyrimido[1,2-a]azepine-4(6H)-thione (12aa) Yield 99%, yellow solid, m.p. 178.6–180.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 7.19 (s, 1H), 4.48–4.37 (m, 2H), 3.15–3.06 (m, 2H), 1.94–1.74 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.44, 162.30, 158.77, 150.52, 132.62, 129.96, 125.88, 124.35, 122.57, 106.67, 102.68, 42.91, 37.68, 29.53, 27.47, 24.92. HRMS (ESI): calcd for $C_{18}H_{16}F_3N_2OS$ $[M+H]^+$: 365.0935, found 365.0929.

General procedure for synthesis of compounds 13a-13aa The solution of 1-mmol pyrrolo[2,3-*d*]pyrimidinones (**11a-11aa**) in 10 mL of dried toluene or dioxane was stirred under reflux with 0.6 mmol (243 mg) of Lawesson's reagent. After 4 h (in 8–12 h for dioxane), the reaction mixture was cooled to 20–25 °C, the solvent was

removed under reduced pressure and the residue column chromatographed using petroleum ether: EtOAc (1:1 − 3:1) as an eluent to give solid thiones **13a-13aa**.

1,2-Dimethyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13a) Yield 77%, yellow solid, m.p. 272–273 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (s, 1H), 4.54 (t, J = 7.5 Hz, 2H), 3.62 (s, 3H), 3.26 (t, J = 8.0 Hz, 2H), 2.38 – 2.24 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.56, 156.52, 143.87, 135.43, 119.37, 102.26, 52.03, 32.87, 28.30, 19.46, 12.75; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 220.0908, found 220.0903.

1,2-Dimethyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13b) Yield 76%, yellow solid, m.p. 184–186 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 1H), 4.70 (t, J = 6.3 Hz, 2H), 3.62 (s, 4H), 3.04 (t, J = 6.8 Hz, 2H), 2.35 (s, 3H), 2.02 (p, J = 6.6, 6.1 Hz, 2H), 1.94 (p, J = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.32, 153.50, 141.87, 135.61, 120.14, 103.03, 47.84, 32.47, 28.04, 22.46, 18.92, 12.70; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 234.1065, found 234.1059.

1,2-Dimethyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13c) Yield 80%, yellow solid, m.p. 223–224 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.54 (s, 1H), 5.11 (s, 2H), 3.61 (s, 3H), 3.21 – 3.13 (m, 2H), 2.34 (s, 3H), 1.84 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.79, 157.73, 141.67, 135.82, 119.80, 103.60, 49.38, 38.09, 29.19, 28.03, 26.63, 25.44, 12.65; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 248.1221, found 248.1217.

1,2,3-Trimethyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13d) Yield 79%, yellow solid, m.p. 258–260 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (t, J = 7.2 Hz, 2H), 3.60 (s, 3H), 3.23 (t, J = 8.0 Hz, 2H), 2.57 (s, 3H), 2.34–2.21 (m, 5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.32, 156.12, 143.61, 131.00, 117.52, 111.84, 51.87, 32.89, 28.24, 19.32, 10.98, 9.84; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 234.1065, found 234.1060.

1,2,3-Trimethyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13e) Yield 82%, yellow solid, m.p. 179–180 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.04 (t, J = 4.2 Hz, 2H), 3.58 (s, 3H), 2.90 (t, J = 6.7 Hz, 2H), 2.36 (s, 3H), 2.21 (s, 3H), 1.94 (p, J = 6.1 Hz, 2H), 1.88 (p, J = 6.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.64, 152.30, 146.01, 127.29, 109.59, 104.28, 41.00, 31.71, 28.11, 22.38, 19.48, 9.74, 9.51; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 248.1221, found 248.1216.

1,2,3-Trimethyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13f) Yield 86%, yellow solid, m.p. 138–140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.10 (s, 2H), 3.59 (s, 3H), 3.14 (d, J = 7.2 Hz, 2H), 2.58 (s, 3H), 2.24 (s, 3H), 1.83 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.56, 157.39, 141.51, 131.30, 117.94, 112.38, 48.39, 38.16, 29.16, 28.01, 26.60, 25.50, 11.41, 9.91; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 262.1378, found 262.1372.

1-Methyl-2-phenyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13 g) Yield 88%, yellow solid, m.p. 195–196 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.37 (m, 5H), 6.90 (s, 1H), 4.58 (t, J = 7.5 Hz, 2H), 3.75 (s, 3H), 3.31 (t, J = 8.0 Hz, 2H), 2.34 (p, J = 7.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.75, 157.28, 144.85, 139.68, 131.41, 128.86, 128.72, 128.38, 119.77, 104.01,

52.09, 33.00, 30.28, 19.45; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 282.1065, found 282.1060.

1-Methyl-2-phenyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13 h) Yield 87%, yellow solid, m.p. 157–158 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.37 (m, 5H), 4.71 (t, $J=6.1$ Hz, 2H), 3.74 (s, 3H), 3.09 (t, $J=6.8$ Hz, 2H), 2.05 (p, $J=6.5$ Hz, 2H), 1.96 (p, $J=6.6$ Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.52, 154.30, 142.79, 139.80, 131.45, 128.84, 128.71, 128.36, 120.49, 104.82, 47.94, 32.59, 29.98, 22.47, 18.92; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 296.1221, found 296.1216.

1-Methyl-2-phenyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13i) Yield 90%, yellow solid, m.p. 140–141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.38 (m, 5H), 6.88 (s, 1H), 5.19–5.10 (m, 2H), 3.74 (s, 3H), 3.26–3.18 (m, 2H), 1.87 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.00, 158.51, 142.60, 140.02, 131.42, 128.84, 128.71, 128.38, 120.17, 105.41, 49.44, 38.18, 30.00, 29.20, 26.64, 25.44; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 310.1378, found 310.1373.

2-(4-Fluorophenyl)-1-methyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13j) Yield 94%, yellow solid, m.p. 230–232 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, $J=8.6$, 5.3 Hz, 2H), 7.16 (t, $J=8.6$ Hz, 2H), 6.86 (s, 1H), 4.57 (t, $J=7.5$ Hz, 2H), 3.71 (s, 3H), 3.31 (t, $J=8.0$ Hz, 2H), 2.34 (p, $J=7.9$ Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.83, 164.01, 161.54, 157.41, 144.76, 138.54, 130.72, 127.51, 119.69, 115.96, 115.75, 104.06, 52.09, 32.99, 30.16, 19.43; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 300.0971, found 300.0967.

2-(4-Fluorophenyl)-1-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13 k) Yield 94%, yellow solid, m.p. 179–180 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (dd, $J=8.4$, 5.4 Hz, 2H), 7.14 (t, $J=8.5$ Hz, 2H), 6.85 (s, 1H), 4.68 (t, $J=6.1$ Hz, 2H), 3.69 (s, 3H), 3.07 (t, $J=6.8$ Hz, 2H), 2.03 (p, $J=6.4$ Hz, 2H), 1.95 (p, $J=6.6$ Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.54, 163.97, 161.50, 154.43, 142.72, 138.64, 130.60, 127.54, 120.37, 115.94, 115.72, 104.80, 47.95, 32.59, 29.88, 22.44, 18.89; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 314.1127, found 314.1120.

2-(4-Fluorophenyl)-1-methyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13 l) Yield 99%, yellow solid, m.p. 123–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, $J=8.4$, 5.4 Hz, 2H), 7.16 (t, $J=8.5$ Hz, 2H), 6.85 (s, 1H), 5.15 (s, 2H), 3.71 (s, 3H), 3.22 (s, 2H), 1.88 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.09, 164.02, 161.55, 158.63, 142.56, 138.89, 130.71, 130.62, 127.53, 120.11, 115.96, 115.75, 105.46, 49.45, 38.20, 29.84, 29.20, 26.63, 25.43; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 328.1284, found 328.1279.

2-(4-Chlorophenyl)-1-methyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13 m) Yield 92%, yellow solid, m.p. 199–200 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.39 (m, 4H), 6.88 (s, 1H), 4.56 (t, $J=7.2$ Hz, 2H), 3.72 (s, 3H), 3.31 (t, $J=8.0$ Hz, 2H), 2.34 (p, $J=7.9$ Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.92, 157.55, 144.79, 138.30, 134.50, 130.01, 129.82, 129.01, 119.71, 104.34, 52.12, 32.99, 30.31, 19.40; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 316.0675, found 316.0672.

2-(4-Chlorophenyl)-1-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13n) Yield 98%, yellow solid, m.p. 151–153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 4H), 6.89 (s, 1H), 4.69 (t, J = 6.1 Hz, 2H), 3.72 (s, 3H), 3.08 (t, J = 6.7 Hz, 2H), 2.05 (p, J = 6.6 Hz, 2H), 1.96 (p, J = 6.5 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.72, 154.57, 142.80, 138.42, 134.47, 130.00, 129.88, 129.00, 120.43, 105.16, 47.99, 32.57, 30.05, 22.45, 18.88; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 330.0832, found 330.0826.

2-(4-Chlorophenyl)-1-methyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13o) Yield 98%, yellow solid, m.p. 164–166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 4H), 6.86 (s, 1H), 5.13 (s, 2H), 3.71 (s, 3H), 3.21 (d, J = 6.0 Hz, 2H), 1.86 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.18, 158.75, 142.69, 138.62, 134.48, 129.99, 129.86, 129.00, 120.10, 105.73, 49.43, 38.17, 29.99, 29.16, 26.61, 25.40; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 344.0988, found 344.0984.

2-(4-Bromophenyl)-1-methyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13p) Yield 99%, yellow solid, m.p. 210–212 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 6.89 (s, 1H), 4.57 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 3.31 (t, J = 8.0 Hz, 2H), 2.35 (p, J = 7.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.97, 157.55, 144.91, 138.31, 131.96, 130.30, 130.27, 122.70, 119.73, 104.37, 52.10, 33.00, 30.20, 19.41; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 360.0170, found 360.0170.

2-(4-Bromophenyl)-1-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13q) Yield 99%, yellow solid, m.p. 196–197 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 6.90 (s, 1H), 4.69 (t, J = 6.3 Hz, 2H), 3.71 (s, 3H), 3.08 (t, J = 6.8 Hz, 2H), 2.05 (p, J = 7.0 Hz, 2H), 1.96 (p, J = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.74, 154.56, 142.94, 138.41, 131.93, 130.36, 130.25, 122.66, 120.42, 105.15, 47.97, 32.61, 30.01, 22.45, 18.89; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 374.0327, found 374.0300.

2-(4-Bromophenyl)-1-methyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13r) Yield 99%, yellow solid, m.p. 188–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 6.87 (s, 1H), 5.13 (s, 2H), 3.71 (s, 3H), 3.21 (d, J = 6.9 Hz, 2H), 1.87 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.21, 158.77, 142.75, 138.63, 131.95, 130.33, 130.25, 122.68, 120.12, 105.75, 49.43, 38.19, 29.98, 29.17, 26.62, 25.41; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 388.0483, found 388.0478.

1-Methyl-2-(p-tolyl)-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13 s) Yield 99%, yellow solid, m.p. 200–202 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 6.86 (s, 1H), 4.57 (t, J = 7.5 Hz, 2H), 3.73 (s, 3H), 3.31 (t, J = 8.0 Hz, 2H), 2.41 (s, 3H), 2.34 (p, J = 7.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.51, 157.15, 144.74, 139.81, 138.39, 129.43, 128.74, 128.48, 119.73, 103.57, 52.09, 32.99, 30.24, 21.32, 19.44; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 296.1221, found 296.1215.

1-Methyl-2-(p-tolyl)-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13t) Yield 95%, yellow solid, m.p. 159–161 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 7.9 Hz, 2H), 6.87 (s, 1H), 4.71

(t, J = 6.1 Hz, 2H), 3.72 (s, 3H), 3.08 (t, J = 6.7 Hz, 2H), 2.41 (s, 3H), 2.05 (p, J = 6.4 Hz, 2H), 1.96 (p, J = 6.5 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.24, 154.18, 142.71, 139.92, 138.36, 129.42, 128.71, 128.51, 120.44, 104.36, 47.92, 32.57, 30.00, 22.47, 21.33, 18.91; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 310.0378, found 310.1371.

1-Methyl-2-(p-tolyl)-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13u) Yield 99%, yellow solid, m.p. 149–151 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 7.9 Hz, 2H), 6.85 (s, 1H), 5.14 (s, 2H), 3.72 (s, 3H), 3.21 (s, 2H), 2.41 (s, 3H), 1.87 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.76, 158.36, 142.50, 140.14, 138.38, 128.72, 128.49, 120.15, 104.97, 49.41, 38.15, 29.96, 29.18, 26.64, 25.44, 21.32; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 324.1534, found 324.1528.

2-(4-Methoxyphenyl)-1-methyl-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13v) Yield 99%, yellow solid, m.p. 224–225 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 6.82 (s, 1H), 4.56 (t, J = 7.5 Hz, 2H), 3.85 (s, 3H), 3.71 (s, 3H), 3.30 (t, J = 8.0 Hz, 2H), 2.33 (p, J = 7.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.39, 159.76, 157.05, 144.53, 139.64, 130.20, 123.77, 119.77, 114.17, 103.27, 55.33, 52.11, 32.97, 30.12, 19.44; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 312.1171, found 312.1166.

2-(4-Methoxyphenyl)-1-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13w) Yield 95%, yellow solid, m.p. 178–179 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 8.7 Hz, 2H), 6.84 (s, 1H), 4.71 (t, J = 6.1 Hz, 2H), 3.86 (s, 3H), 3.71 (s, 3H), 3.08 (t, J = 6.8 Hz, 2H), 2.05 (p, J = 6.4 Hz, 2H), 1.96 (p, J = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.15, 159.74, 154.06, 142.61, 139.77, 130.19, 123.84, 120.51, 114.16, 104.06, 55.39, 47.93, 32.56, 29.91, 22.47, 18.93; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 326.1327, found 326.1321.

2-(4-Methoxyphenyl)-1-methyl-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13x) Yield 91%, yellow solid, m.p. 220–221 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.7 Hz, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.80 (s, 1H), 5.13 (s, 2H), 3.84 (s, 3H), 3.69 (s, 3H), 3.20 (d, J = 5.6 Hz, 2H), 1.86 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.60, 159.75, 158.27, 142.42, 139.96, 130.16, 123.79, 120.15, 114.16, 104.64, 55.33, 49.41, 38.16, 29.89, 29.19, 26.65, 25.44; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 340.1484, found 340.1480.

1-Methyl-2-(4-(trifluoromethyl)phenyl)-1,6,7,8-tetrahydro-4H-dipyrrolo[1,2-a:2',3'-d]pyrimidine-4-thione (13y) Yield 97%, yellow solid, m.p. 168–169 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 6.94 (s, 1H), 4.54 (t, J = 7.6, 7.2 Hz, 2H), 3.74 (s, 3H), 3.30 (t, J = 8.0 Hz, 2H), 2.34 (p, J = 7.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.21, 157.89, 145.22, 137.78, 134.92, 129.97, 128.89, 125.75, 122.60, 119.66, 105.13, 52.11, 33.01, 30.38, 19.37; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 350.0939, found 350.0933.

1-Methyl-2-(4-(trifluoromethyl)phenyl)-6,7,8,9-tetrahydropyrido[1,2-a]pyrrolo[2,3-d]pyrimidine-4(1H)-thione (13z) Yield 88%, yellow solid, m.p. 178–179 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.2 Hz, 2H), 7.61 (d, J = 8.1 Hz, 2H), 6.95 (s, 1H), 4.68 (t, J = 6.2 Hz, 2H), 3.74 (s, 3H), 3.08 (t, J = 6.8 Hz, 2H), 2.04 (p, J = 6.4 Hz, 2H), 1.95 (p, J = 6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$)

δ 179.04, 154.89, 143.17, 137.90, 135.00, 129.94, 128.87, 125.73, 122.62, 120.38, 105.98, 48.00, 32.63, 30.14, 22.43, 18.86; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 364.1095, found 364.1089.

1-Methyl-2-(4-(trifluoromethyl)phenyl)-1,6,7,8,9,10-hexahydro-4H-pyrrolo[2',3':4,5]pyrimido[1,2-a]azepine-4-thione (13aa) Yield 99%, yellow solid, m.p. 175–177 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.2 Hz, 2H), 7.60 (d, J = 8.2 Hz, 2H), 6.92 (s, 1H), 5.16–5.06 (m, 2H), 3.73 (s, 3H), 3.25–3.15 (m, 2H), 1.85 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.45, 159.08, 142.95, 138.10, 134.94, 129.93, 128.88, 125.69, 122.61, 120.05, 106.54, 49.42, 38.17, 30.09, 29.12, 26.58, 25.37; HRMS (ESI): calcd for $C_{19}H_{19}F_3N_3O$ $[M+H]^+$: 378.1252, found 378.1248.

Biology

Materials

Doxorubicin was purchased from BBI Inc. (Shanghai, China). The human cancer HeLa, MCF-7, and HT-29 cell lines were obtained from Chinese Type Culture Collection, CAS (Shanghai, China).

Cell cultures

Human HeLa, MCF-7, and HT-29 cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose and 0.37% sodium bicarbonate (Gibco, Rockville, MD, USA). All cell culture media contained with 10% FBS and antibiotic mix (1 × 100 μM penicillin A and 100 μM of streptomycin) and were grown at 37 °C in a humidified incubator (Binder, Germany) containing 95% air/5% CO_2 and have been fed every 3–4 days [51, 52].

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Authors' contributions B.S. performed the synthesis, biological experiments, and compounds analysis part. L.F. performed the design of synthesis, software, and compounds analysis part. K.B. designed a synthesis and wrote the main manuscript text. R.K. revised the manuscript. H.A.A. supervised the research and revised the main manuscript. J.Z. supervised the research and revised the main manuscript. All authors reviewed the manuscript.

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Declarations

Conflict of interests No potential conflict of interest was reported by the authors.

Ethical Approval Not applicable.

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