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Synthesis and antiproliferative evaluation of novel tetrahydrobenzo[4',5'] thieno [3',2':5,6]pyrido[4,3-*d*]pyrimidine derivatives

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A series of tetrahydrobenzo[4',5']thienophene[3',2':5,6]pyrido [4,3-*d*]pyrimidine-4-(3*H*)-one derivatives were synthesized and evaluated for their antiproliferative activities. Biological evaluation revealed that some compounds exhibited much stronger antiproliferative activitiy than the positive control Fluorouracil against KB and CEN2 cell lines. Compound 7a exhibited the highest antitumor activity against KB and CNE2 cell lines with IC_{50} values of 8.18 μM and 13.71 μM , respectively. The preliminary structure-activity relationships showed that introduction of tetrahydrobenzothieno as well as Schiff base units were favorable for the antiproliferative activity.

Cancer is a life-threatening disease, and about 8.0 million cancer deaths (around more than 20% of all deaths) occur annually in the world.[1] Although great progress has been made in the treatment cancer, unfortunately, no currently available anticancer drugs meet criterion of eradication of cancerous cells mainly due to undesirable side effects and a limited choice. Thus, there is always a constant and urgent need to develop alternative anticancer drugs with minimal side effects and higher efficacy.

In the process of developing anticancer drugs with novel chemical structure, many heteroaromatic compounds have displayed antiproliferative activities, and some of them became important anticancer drugs, such as Gefitinib and Tandutinib.[2-5] Recently, pyrido[4,3-*d*]pyrimidine scaffold was reported to exhibit interesting antitumor activity,[6-8] and pyrido[4,3-*d*]pyrimidine derivatives (such as **A**, **B**, Fig **1**) have been identified as a new class of cancer chemotherapeutic agents, potent inhibitors of epidermal growth factor receptor (EGFR).[9] On the other hand, tetrahydrobenzothieno group was a high efficient pharmacophore and widely used in the design of anticancer drugs. For example, (*E*)-9-Methyl-N-(2-styryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4yl)acridine-3,6-diamine (**C**, Fig **1**) was described to exert high anticancer activity.[10-12] In addition, in the course of finding anticancer drugs, Schiff base derivatives (such as **C**, **D**, Fig **1**) also have been identified as a new class of anticancer drugs.[13-15] Conjugation of two or three bioactive units is now accepted as an effective strategy for designing inhibitors, ligands and other drugs.[16] On the basis of antitumor activity of aforementioned pyrido[4,3-*d*]pyrimidine, tetrahydrobenzothieno as well as Schiff base, in order to find more potent and novel antitumor agents, we designed and synthesized a series of pyrido[4,3-*d*]pyrimidine derivatives, combined tetrahydrobenzothieno with Schiff base units, and evaluated the antiproliferative activity of these compounds.

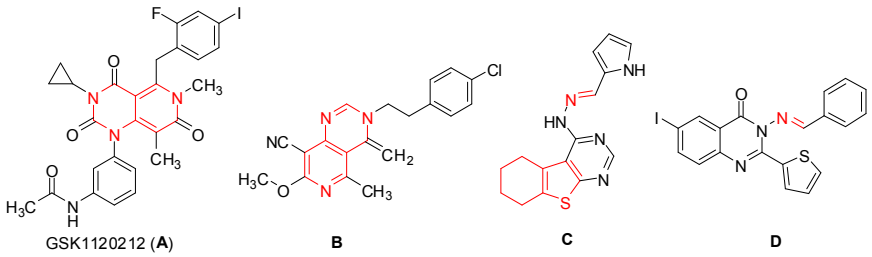
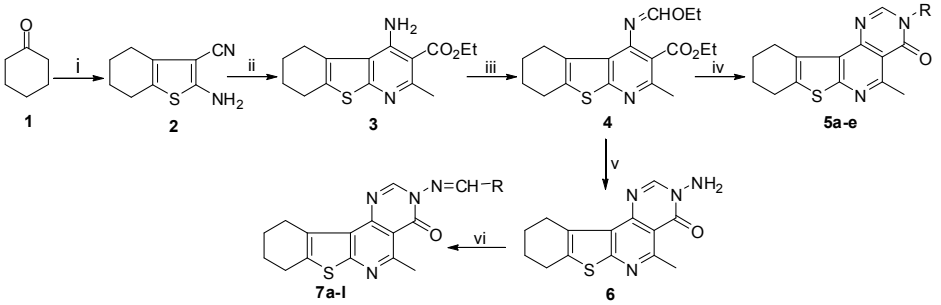


Fig 1. Representative anticancer pyrido[4,3-*d*]pyrimidines, tetrahydrobenzothieno and Schiff base.

Results and Discussion

Chemistry

General synthetic procedure for target compounds **5a-e**, **7a-l** was performed according to Scheme **1**. We used cyclohexanone as the starting material, and 2-amino-4,5,6,7-tetrahydro[1] benzothiophene-3-carbonitrile **2** was easily accessible via the Gewald reaction between cyclohexanone, elemental sulfur and propanedinitrile.[17] Treatment of **2** with tin tetrachloride at 130 °C gave **3**,[18] which was treated with triethyl orthoformate using acetic anhydride to produce the key intermediate **4**,[19] reddish brown oil. This oil was reacted directly without any purification with the corresponding amine to give pure target compounds **5a-e**. On the other hand, compound **4** was converted to **6** by refluxing with hydrazine hydrate in ethanol,[19] and then compound reacted with corresponding aromatic aldehydes to give Schiff base compounds **7a-l**.



Scheme 1 Reagents and solvents: (i) propanedinitrile, S_8, morpholine, 50 °C, 12 h; (ii) $CH_3COCH_2COOCH_2CH_3$, $SnCl_4$, 130 °C, 6 h; (iii) $CH(OEt)_3$, Ac_2O, reflux, 6 h; (iv) RNH_2, 40 °C, 8 h; (v) $NH_2NH_2 \cdot H_2O$, reflux, 3-4 h; (vi) ArCHO, CH_3COOH.

Nuclear magnetic resonance spectral studies

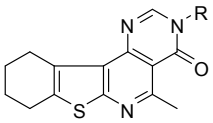
All the structures of the products (**5a-e**, **7a-l**) were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectra. Assignments of the signals were based on the chemical shifts and intensity patterns. The IR spectra of all the target compounds showed typical absorption bands at 1655-1673 (C=O) and 1552-1570 cm^{-1} (C=C). In the ^{1}H NMR spectra, three protons of 5-Me group in pyrimidine appeared at δ 2.94-3.33 ppm as singlet; eight protons of CH_2 group of tetrahydrobenzo[4',5']thieno group appeared at δ 1.82-3.34 ppm as multiplet; a singlet signal seen at δ 8.07-8.85 ppm accounted for CH of pyrimidine group. The ^{1}H NMR spectra of compounds **7a-l** showed a singlet at δ 8.91-9.99 ppm belonged to N=CH group of Schiff base unit.

^{13}C NMR spectra further confirmed structures. All the target compounds showed a signal at 161.6-166.5 ppm due to C=O, and the methyl carbon displayed a signal at 22.9-27.3 ppm. Therefore, on the basis of above information, the compounds were characterized as tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidine derivatives.

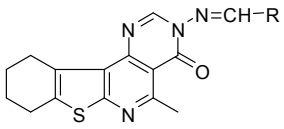
Preliminary *in vitro* antitumor screening

Compounds **5a-e** and **7a-l** were evaluated for their *in vitro* antitumor activities against KB human oral carcinoma cells and CNE2 human nasopharyngeal carcinoma cells by MTT assay, and 5-FU was used as the reference drug. As shown in Table **1**, comparing with 5-FU, some compounds (**7a**, **7c**, **7j**) exhibited more or similar potent cytotoxicity against KB and CNE2 cell lines. Among them compound **7a** (8.18μM vs 13.71μM) showed the best inhibitory activities. Interestingly, different substituents on N of the pyrimidine ring obviously affected the inhibitory activity of compounds. Compared to 5-FU, alkyl-substituted compounds (**5a-e**) diminished potency. However, replacement of R groups by Schiff base (e.g., potency in the order **7a** > **7j** > **7c**) seems to enhance potency. This result suggested that Schiff base might be required for the strong antitumor activities. However, **7b, 7d-i, 7k-l** still displayed very weak antitumor activities. This result suggested that aryl-system of Schiff base also had an effect on the inhibitory activity. An increased activity was observed when phenolic group (**7a**, **7c**, **7j**) was introduced, and *o*-phenolic group (**7a**) showed the best activity, being approximately 6-fold, 4-fold higher than compound **1**. Replacing *o*-phenolic group with other groups such as 4-fluorophenyl, 4-chlorophenyl reduced the antitumor activity.

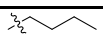
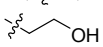
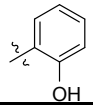
Table 1 *In vitro* antitumor activities for compounds **5a-e** and **7a-l**.

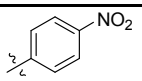
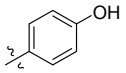
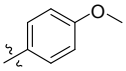
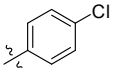
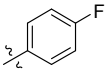
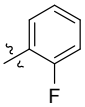
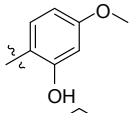
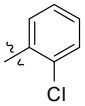
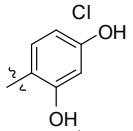
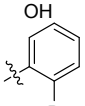
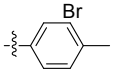


5a-e



7a-l

Compound	R	IC_{50}[a](μM)	
		KB	CNE2
5a		>50	>50
5b		>50	>50
5c		>50	>50
5d		>50	>50
5e		>50	>50
7a		8.18	13.71

7b		>50	>50
7c		28.31	32.80
7d		>50	>50
7e		>50	>50
7f		>50	>50
7g		>50	>50
7h		>50	>50
7i		>50	>50
7j		14.87	18.07
7k		>50	>50
7l		>50	>50
5-FU	---	9.85	17.2

All data are presented as mean values of three independent experiments. Coefficients of variation were < 10%.

[a]IC_{50}: concentration of the test compound that inhibits 50% of cell growth.

Conclusion

In summary, a novel series of tetrahydrobenzo[4',5']thieno [3',2':5,6]pyrido[4,3-*d*] pyrimidine derivatives were designed, synthesized and studied for their antitumor activities. In the first set (**5a-e**), by replacing the phenyl group with tetrahydrobenzothieno, these five compounds didn't show a tendency to enhance the activity. It was concluded that the presence of the R at the pyrimidine ring with small alkyl groups could not tolerate. Interestingly, in the second set, subsequent modifications of the R led to a new series of Schiff base analogues **7a**, **7c**, **7j**. These compounds displayed modest to potent antitumor activities *in vitro* in comparison with 5-FU. Among them, **7a** was the most active compound with an IC_{50} value of 8.18 μM for KB and 13.71 μM for CNE2. These results also confirmed the hypothesis that the introduction of tetrahydrobenzothieno as well as Schiff base is favorable for antitumor activities. These findings encouraged us to continue developing these novel analogues as potential antitumor agents.

Experimental

General

Melting points were measured on an electrothermal melting point apparatus and were uncorrected. IR spectra were recorded on a NE XUS-470 infrared spectrometer as KBr pellets with absorption in cm^{-1}. ^1H NMR spectra were recorded in $CDCl_3$ or Acetone-d_6 as solvent on a Bruker AVANCE-III 400 spectrometer and resonances were given in ppm relative to TMS. MS spectra were measured with a Finnigan MTA/USA spectrometer (LC-MS). All of the solvents and materials were reagent grade and purified as required. All compounds were routinely checked by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., P. R. China). Column chromatography was performed using silica gel (200-300 mesh) from Qingdao Haiyang Chemical Group Co., China.

General procedure for preparation of (2-4)

A solution of cyclohexanone **1** (4.9 g, 50 mmol), propanedinitrile (5.65 g, 50 mmol), S_8 (1.6 g, 50 mmol), and morpholine (4.25 g, 50 mmol) in ethanol was stirred at 50 °C for 12 h. The solvent was removed under reduced pressure, and the residue was isolated by column chromatography (silica gel, Petroleum ether-Ethyl acetate, 9:1) to yield 2 (6.8g, 76.1%). ^1H NMR (400 MHz, $CDCl_3$): δ 4.54 (2H, br s, NH_2), 2.46 (4H, m, 2×CH_2), 1.74 (4H,

m, 2×CH_2).

The compound **2** (1.78 g, 10 mmol) and $SnCl_4$ (5.2 g, 20 mmol) were added to a stirring solution of ethyl acetoacetate (1.3 g, 10 mmol) in anhydrous toluene (30 mL). The mixture was stirred under nitrogen at room temperature for 0.5 h, and then heated to reflux for 6 h (130 °C). The mixture was added to sat.aq. Na_2CO_3 solution (150 mL, pH 10), and the resulting suspension was extracted with EtOAc (3×50 mL). The combined organic layer were dried with anhydrous Na_2SO_4 and concentrated under reduced pressure, the residue was purified by column chromatography (silica gel, Petroleum ether-Ethyl acetate, 8 : 2) to afford **3** in 64 % yield.

A mixture of **3** (1.45 g, 5 mmol) and triethyl orthoformate (50 mL) was refluxed for 8 h. After cooling, the solvent was concentrated *in vacuo* to afford crude product **4**, which was used directly without any purification in the next step.

General procedure for preparation of (5a-e)

A mixture of the intermediate **4** (0.69 g, 2 mmol) and the corresponding amine (2 mmol) in ethanol (10 mL) was refluxed for 8 h. The reaction solution was cooled, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, Petroleum ether-Ethyl acetate, 9: 1) to give **5a-e**.

3-Butyl-5-methyl-8,9,10,11- tetrahydrobenzo[4',5']thieno [3', 2': 5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one **(5a)**. Yellow powder, yield 64%, m.p. 200.8-203.3 °C; IR (KBr): 1659 (C=O), 1559 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.14 (1H, s, CH, pyrimidinone), 3.98 (2H, t, J = 6.8 Hz, CH_2), 3.20 (2H, m, CH_2), 3.11 (3H, s, CH_3), 2.86 (2H, m, CH_2), 1.87 (4H, m, 2×CH_2), 1.41 (4H, m, 2×CH_2), 0.99 (3H, t, J = 6.8 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.1, 160.9, 157.4, 150.9, 149.1, 136.3, 130.8, 125.7, 112.5, 47.0, 31.3, 27.2, 26.8, 26.0, 22.9, 22.7, 20.0, 13.6; LC-MS: 328.26 $(M+H)^+$.

3-Isobutyl-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3', 2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**5b**). Yellow powder, yield 60%, m.p. 204.6-207.5 °C; IR (KBr): 1659 (C=O), 1559 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (1H, s, CH, pyrimidinone), 3.93 (2H, d, J = 7.2 Hz, CH_2), 3.19 (2H, m, CH_2), 3.08 (3H, s, CH_3), 2.86 (2H, m, CH_2), 2.15 (1H, m, CH), 1.86 (4H, m, 2×CH_2), 0.97 (6H, d, J = 6.4 Hz, 2×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 161.0, 157.3, 150.8, 149.4, 136.2, 130.0, 125.6, 112.4, 54.5, 27.8, 27.1, 26.9, 25.9, 22.9, 22.6, 19.9, 19.9; LC-MS: 328.26 $(M+H)^+$.

5-Methyl-3-propyl-l-8,9,10,11-tetrahydrobenzo[4',5']thieno[3', 2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one(**5c**). Yellow powder, yield 67%, m.p. 195.9-197.8 °C; IR (KBr): 1659 (C=O), 1559 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.12 (1H, s, CH, pyrimidinone), 3.94 (2H, t, J = 7.4 Hz, CH_2), 3.20 (2H, m, CH_2), 3.09 (3H, s, CH_3), 2.88 (2H, m, CH_2), 1.90 (4H, m, 2×CH_2), 1.78 (2H, m, CH_2), 1.0 (3H, t, J = 7.4 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 160.9, 157.3, 150.8, 149.1, 136.2, 130.0, 125.6, 112.5, 48.8, 27.1, 26.9, 25.9, 22.9, 22.6, 22.5, 11.2; LC-MS: 314.21 $(M+H)^+$.

3-Isopropyl-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3', 2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**5d**). Yellow powder, yield 56%, m.p. 200.5-202.4 °C; IR (KBr): 1659 (C=O), 1559 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.26 (1H, s, CH, pyrimidinone), 5.16 (1H, m, CH), 3.22 (2H, m, CH_2), 3.12 (3H, s, CH_3), 2.89 (2H, m, CH_2), 1.90 (4H, m, 2×CH_2), 1.49 (6H, d, J = 6.8 Hz, 2×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.0, 160.6, 157.5, 150.2, 146.3, 136.2, 130.0, 125.5, 112.3, 45.7, 27.2, 27.0, 25.9, 22.9, 22.7, 22.1, 22.1; LC-MS: 314.18 $(M+H)^+$.

3-(2-Hydroxyethyl)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5'] thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**5e**). Yellow powder, yield 46%, m.p. 244.4-247.7 °C; IR (KBr): 1655 (C=O), 1552 (C=C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.43 (1H, s, CH, pyrimidinone), 4.89 (2H, s, OH)4.04 (2H, m, CH_2), 3.69 (2H, m, CH_2), 3.15 (2H, m, CH_2), 2.96 (3H, s, CH_3), 2.83 (2H, m, CH_2), 1.83 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.6, 160.1, 156.3, 151.5, 150.6, 135.2, 129.5, 124.7, 112.1, 58.0, 48.6, 26.7, 26.1, 25.1, 22.2, 21.9; LC-MS: 316.28 $(M+H)^+$.

3-Amino-5-methyl-8,9,10,11- tetrahydrobenzo[4',5']thieno [3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3H)-one (**6**)

A mixture of the intermediate **4** (1.73 g, 5 mmol) and excess of hydrazine hydrate (15 mL) in ethanol (30 mL) was refluxed for 6 h. The solvent was then removed under reduced pressure. The solid product obtained was triturated with water, filtered and recrystallized from ethanol as pale yellow crystals, yield 89%, ^1H NMR (400 MHz, $CDCl_3$): δ 8.37 (1H, s, CH, pyrimidinone), 4.91 (2H, s, NH_2), 3.17 (2H, m, CH_2), 3.08 (3H, s, CH_3), 2.88 (2H, m, CH_2), 1.90 (4H, m, 2×CH_2);^{13}C NMR (125 MHz, $CDCl_3$): δ 163.0, 161.4, 156.9, 150.4, 149.7, 136.5, 130.1, 125.8, 111.8, 27.1, 26.4, 25.9, 22.8, 22.5.

General procedure for preparation of (7a-l)

A mixture of compound **6** (0.29 g, 1 mmol), CH_3COOH (1.0 mL) and the appropriate aromatic aldehyde (1 mmol) in ethanol (20 mL) was refluxed for 8 h. After cooling, the solid precipitate was collected and recrystallized from appropriate solvent to give **7a-l**.

3-(2-Hydroxybenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7a**). Yellow powder, yield 57%, m.p. 255.0-255.7 °C; IR(KBr): 1670 (C=O), 1556 (C=C) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6+C_5D_5N): δ 11.06 (1H, s, OH), 9.53 (1H, s, N=CH), 8.59 (1H, s, CH, pyrimidinone), 7.94 (1H, d, J = 8.0 Hz, Ar-H), 7.43 (1H, t, J = 7.8 Hz, Ar-H), 7.11 (1H, d, J = 8.4 Hz, Ar-H), 6.96 (1H, t, J = 7.6 Hz, Ar-H), 3.13 (2H, m, CH_2), 3.04 (3H, s, CH_3), 2.72 (2H, m, CH_2), 1.72 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, DMSO-d_6+C_5D_5N): δ 164.0, 162.2, 158.9, 157.8, 156.9, 149.5, 148.0, 135.7, 134.2, 129.8, 128.7, 125.3, 119.6, 118.4, 116.8, 113.0, 27.0, 26.6, 25.3, 22.4, 22.1; LC-MS: 391.13 $(M+H)^+$.

3-(4-Nitrobenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7b**). Yellow powder, yield 38%, m.p. 238.6-239.4 °C; IR (KBr): 1673 (C=O), 1559 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.61 (1H, s, N=CH), 8.41 (1H, s, CH, pyrimidinone), 7.79 (2H, d, J = 8.4 Hz, Ar-H), 7.45 (2H, d, J = 8.4 Hz, Ar-H), 3.24 (2H, m, CH_2), 3.12 (3H, s, CH_3), 2.87 (2H, m, CH_2), 1.90 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9, 161.3, 159.8, 157.6, 149.0, 148.6, 138.5, 136.9, 131.8, 130.2, 129.8 (2×C), 129.3 (2×C), 126.0, 113.1, 27.2, 26.8, 26.1, 23.0, 22.8; LC-MS: 420.11 $(M+H)^+$.

3-(4-Hydroxybenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7c**). Yellow powder, yield 42%, m.p. 264.8-267.1 °C; IR (KBr): 1670 (C=O), 1561 (C=C) cm^{-1}; ^1H NMR (400 MHz,

DMSO-d_6): δ 10.34 (1H, s, OH), 8.91 (1H, s, N=CH), 8.58 (1H, s, CH, pyrimidinone), 7.86 (2H, d, J = 8.4 Hz, Ar-H), 6.92 (2H, d, J = 8.4 Hz, Ar-H), 3.10 (2H, m, CH_2), 2.94 (3H, s, CH_3), 2.81 (2H, m, CH_2), 1.82 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.5, 161.6, 161.6, 157.4, 156.5, 149.0, 147.8, 135.4, 130.8 (2×C) 129.4, 124.8, 123.1, 115.8 (2×C), 112.6, 26.7, 26.2, 25.1, 22.2, 21.9; LC-MS: 391.09 $(M+H)^+$.

3-(4-Methoxybenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7d**). Yellow powder, yield 53%, m.p. 207.4-208.9 °C; IR (KBr): 1663 (C=O), 1552 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.29 (1H, s, N=CH), 8.34 (1H, s, CH, pyrimidinone), 7.78 (2H, d, J = 8.4 Hz, Ar-H), 6.95 (2H, d, J = 8.8 Hz, Ar-H), 3.85 (3H, s, -OCH_3), 3.20 (2H, m, CH_2), 3.10 (3H, s, CH_3), 2.85 (2H, m, CH_2), 1.89 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.5, 163.4, 163.2, 159.3, 157.5, 149.2, 148.2, 136.5, 130.5 (2×C), 130.2, 125.8, 125.6, 114.5 (2×C), 113.1, 55.4, 27.2, 26.8, 26.0, 22.9, 22.7; LC-MS: 405.13 $(M+H)^+$.

3-(4-Chlorobenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7e**). Yellow powder, yield 56%, m.p. 237.9-238.6 °C; IR (KBr): 1664 (C=O), 1556 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.61 (1H, s, N=CH), 8.41 (1H, s, CH, pyrimidinone), 7.80 (2H, d, J = 8.4 Hz, Ar-H), 7.46 (2H, d, J = 8.8 Hz, Ar-H), 3.25 (2H, m, CH_2), 3.12 (3H, s, CH_3), 2.89 (2H, m, CH_2), 1.90 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9, 161.3, 159.8, 157.6, 149.1, 148.6, 138.5, 136.9, 131.8, 130.2, 129.8 (2×C), 129.3 (2×C), 126.0, 113.1, 27.2, 26.8, 26.1, 23.0, 22.8; LC-MS: 409.13 $(M+H)^+$.

3-(4-Fluorobenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7f**). Yellow powder, yield 36%, m.p. 207.4-209.5 °C; IR (KBr): 1665 (C=O), 1560 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.50 (1H, s, N=CH), 8.39 (1H, s, CH, pyrimidinone), 7.85 (2H, m, Ar-H), 7.15 (2H, m, Ar-H), 3.20 (2H, m, CH_2), 3.10 (3H, s, CH_3), 2.89 (2H, m, CH_2), 1.90 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.2 (d, $^1J_{CF}$ = 252.3 Hz), 163.6, 161.6, 159.5, 157.5, 149.0, 148.4, 136.7, 130.6 (d, $^3J_{CF}$ = 8.9 Hz), 130.6 (d, $^3J_{CF}$ = 8.9 Hz), 130.1, 129.4 (d, $^4J_{CF}$ = 4.0 Hz), 125.8, 116.1 (d, $^2J_{CF}$ = 22.2 Hz), 116.1 (d, $^2J_{CF}$ = 22.2 Hz), 113.0, 27.1, 26.7, 25.9, 22.9, 22.6; LC-MS: 393.17 $(M+H)^+$.

3-(2-Fluorobenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7g**). Yellow powder, yield 38%, m.p. 202.2-205.4 °C; IR (KBr): 1664 (C=O), 1563 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.83 (1H, s, N=CH), 8.41 (1H, s, CH, pyrimidinone), 8.08 (1H, m, Ar-H), 7.48 (1H, m, Ar-H), 7.23 (1H, m, Ar-H), 7.13 (1H, m, Ar-H), 3.23 (2H, m, CH_2), 3.12 (3H, s, CH_3), 2.90 (2H, m, CH_2), 1.93 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.8, 162.5 (d, $^1J_{CF}$ = 242.8 Hz), 159.4, 157.6, 156.6 (d, $^3J_{CF}$ = 5.6 Hz), 148.9, 148.4, 136.7, 133.8 (d, $^3J_{CF}$ = 8.6 Hz), 130.1, 127.2 (d, $^4J_{CF}$ = 2.0 Hz), 125.8, 124.5 (d, $^4J_{CF}$ = 3.5 Hz), 121.1 (d, $^2J_{CF}$ = 9.4 Hz), 116.1 (d, $^2J_{CF}$ = 20.8 Hz), 113.0, 27.1, 26.7, 25.9, 22.9, 22.6; LC-MS: 393.19 $(M+H)^+$.

3-(2-Hydroxyl-4-methoxybenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7h**). Yellow powder, yield 31%, m.p. 243.9-246.9 °C; IR (KBr): 1667 (C=O), 1558 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N): δ 12.21 (1H, s, OH), 9.69 (1H, s, N=CH), 8.85 (1H, s, CH, pyrimidinone), 7.89 (1H, d, J = 8.8 Hz, Ar-H), 6.78 (1H, d, J = 8.0 Hz, Ar-H), 6.67 (1H, dd, J_1 = 2.4 Hz, J_2 = 8.8 Hz, Ar-H), 3.69 (3H, s, -OCH_3), 3.34 (2H, m, CH_2), 3.33 (3H, s, CH_3), 2.75 (2H, m, CH_2), 1.73 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, C_5D_5N): δ 165.8, 165.3, 163.4, 162.3, 158.6, 157.7, 149.6, 148.2, 136.4, 132.2, 130.6, 126.2, 113.8, 112.1, 107.7, 102.0, 55.5, 27.6, 27.3, 26.0, 23.1, 22.8; LC-MS: 421.14 $(M+H)^+$.

3-(2-Cholobenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7i**). Yellow powder, yield 46%, m.p. 211.2-212.3 °C; IR(KBr): 1663 (C=O), 1571 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.99 (1H, s, N=CH), 8.43 (1H, s, CH, pyrimidinone), 8.16 (1H, d, J = 8.4 Hz, Ar-H), 7.44 (2H, m, 2×Ar-H), 7.34 (1H, m, Ar-H), 3.22 (2H, m, CH_2), 3.12 (3H, s, CH_3), 2.88 (2H, m, CH_2), 1.91 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.5, 159.7, 159.4, 157.6, 148.9, 148.5, 136.7, 136.3, 133.0, 130.7, 130.1, 130.1, 127.7, 127.2, 125.8, 113.0, 27.1, 27.0, 26.0, 22.8, 22.6; LC-MS: 409.12 $(M+H)^+$.

3-(2,4-Dihydroxylbenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7j**). Yellow powder, yield 29%, m.p. 290.8-293.5 °C; IR (KBr): 1663 (C=O), 1570 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67 (1H, s, OH), 10.40 (1H, s, OH), 9.03 (1H, s, N=CH), 8.62 (1H, s, CH, pyrimidinone), 7.63 (H, d, J = 8.4 Hz, Ar-H), 6.42 (1H, dd, J_1 = 2.0 Hz, J_2 = 10.4 Hz, Ar-H), 6.40 (1H, d, J = 2.0 Hz, Ar-H), 3.04 (2H, m, CH_2), 2.90 (3H, s, CH_3), 2.80 (2H, m, CH_2), 1.79 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ 165.1, 163.3, 161.6, 160.8, 157.4, 156.7, 149.2, 147.8, 135.5, 131.0, 129.6, 124.9, 112.7, 109.8, 108.6, 102.5, 26.9, 26.6, 25.3, 22.3, 22.0; LC-MS: 407.14 $(M+H)^+$.

3-(2-Bromobenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7k**). Yellow powder, yield 43%, m.p. 243.6-245.8 °C; IR (KBr): 1668 (C=O), 1560 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.88 (1H, s, N=CH), 8.38 (1H, s, CH, pyrimidinone), 8.09 (1H, dd, J_1 = 1.8 Hz, J_2 = 7.8 Hz, Ar-H), 7.59 (1H, dd, J_1 = 1.4 Hz, J_2 = 7.8 Hz, Ar-H), 7.30 (2H, m, Ar-H), 3.22 (2H, m, CH_2), 3.07 (3H, s, CH_3), 2.83 (2H, m, CH_2), 1.86 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 162.1, 159.3, 157.6, 148.9, 148.5, 136.8, 133.3 (2×C), 132.0, 130.1, 128.1, 127.7, 126.1, 125.9, 113.0, 27.1, 26.7, 25.9, 22.7, 22.5; LC-MS: 453.05 $(M+H)^+$.

3-(4-Methbenzylideneamino)-5-methyl-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**7l**). Yellow powder, yield 58%, m.p. 203.5-206.8 °C; IR (KBr): 1660 (C=O), 1550 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (1H, s, N=CH), 8.36 (1H, s, CH, pyrimidinone), 7.71 (2H, d, J = 8.0 Hz, Ar-H), 7.23 (2H, d, J = 8.0 Hz, Ar-H), 3.16 (2H, m, CH_2), 3.07 (3H, s, CH_3), 2.39 (3H, s, CH_3), 2.83 (2H, m, CH_2), 1.88 (4H, m, 2×CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.1, 163.0, 159.2, 157.4, 149.1, 148.3, 143.2, 136.6, 130.1, 129.9, 129.6 (2×C), 128.7 (2×C), 125.9, 113.0, 27.1, 26.8, 25.9, 22.8, 22.5, 21.7; LC-MS: 389.19 $(M+H)^+$.

Antitumor activity [20-21]

The MTT assay was used to evaluate the in vitro cytotoxicity of these synthesized compounds against different cancer cell lines. This method is based on the reduction of the soluble 3-(4,5-

dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide(MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells.

Cells were plated into 96-well plate with initial cell number of 2×10^4 per well. After 24 h incubation at 37 ^oC in atmosphere of 5% CO_2, cells were treated with test samples in 10 μL serum free medium and cultured at 37 ^oC for 72 hours. Control cells were treated with serum free medium. Then 20 μL MTT-solution (5 mg/mL) was added to each well and incubated for 4 hours. The supernatant was carefully removed from each well, and 100 μL DMSO was added to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After cells were centrifuged for 5 min (2000 rpm), the absorbance of each well was detected in a microplate reader at 570 nm. The IC_{50} values were calculated according to the dose-dependent curves (Bliss's software).

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