

A Microwave-Enhanced Synthesis and Biological Evaluation of *N*-Aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines

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A series of *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines were synthesized in moderate to good yield by using a microwave-enhanced conditions. The selected compounds were evaluated for their cytotoxic effects (IC₅₀ values) on human pulmonary carcinoma (A549), murine BALB/c spontaneous colon adenocarcinoma (CT26) and human hepatocellular liver carcinoma (HepG2) cell lines *in vitro*. Amongst these compounds, one compound was found to have the better cytotoxic activity with reference to the standard Erlotinib hydrochloride (Tarceva™) against A549 (IC₅₀ = 16.06 ± 0.09 μM) and HepG2 (IC₅₀ = 15.01 ± 0.31 μM) cell lines. Especially, two compounds showed best cytotoxic effects against CT26 (IC₅₀ = 11.38 ± 0.44 μM) and HepG2 (IC₅₀ = 8.51 ± 0.52 μM) cell lines, respectively. The preliminary structure-activity relationships were disclosed and the thieno[2,3-*d*]pyrimidine skeleton could be exploited to potential antitumor agents in the future.

Keywords: thieno[2,3-*d*]pyrimidine, microwave-enhanced, Gewald reaction, Dimroth rearrangement, biological evaluation

Introduction

In investigation, we found the presence of several marketed anticancer drugs such as gefitinib **I** (Iressa™),¹ vandetanib **II** (Caprelsa™),² lapatinib **III** (Tykerb™),³ erlotinib **IV** (Tarceva™),⁴ tandutinib **V** (MLN518) (phase II clinical trials)⁵ and icotinib **VI** (Conmana™)⁶ (Figure 1), which have a quinazoline nucleus, the thieno[2,3-*d*]pyrimidine core can be considered a bioisostere of this quinazoline core.⁷

In literatures,⁸⁻¹⁰ thieno[2,3-*d*]pyrimidin-4-amine derivatives exhibit a broad range of biological and pharmacological activities associated with their heterocyclic scaffold and have been widely used in the medical field. Thieno[2,3-*d*]pyrimidin-4-amine displays a wide range of biological activity including anti-tumor,¹¹ anti-bacterial,¹²

anti-leukemia,^{13,14} antiviral,¹⁵ anti-inflammatory¹⁶ and anti-diabetic properties.¹⁷ As such, many antitumor agents based on thieno[2,3-*d*]pyrimidine have been reported by researchers. Yang *et al.*¹⁸ synthesized two series of thieno[2,3-*d*]pyrimidine derivatives and evaluated for cytotoxic activity in human pulmonary carcinoma cell line (A549). Of these compounds, one showed the most cytotoxic effect on A549 cells with half maximal inhibitory concentration (IC₅₀) value of 4.87 μM (Figure 2, compound **VII**). Gryshchenko *et al.*¹⁹ reported the synthesis of *N*-phenylthieno[2,3-*d*]pyrimidin-4-amines, and evaluated for their ability to inhibit enzymatic activity of the fibroblast growth factor receptor 1 (FGFR1). In these compounds, compounds **VIII** and **IX** exhibited most effects, with IC₅₀ values of 0.18 and 0.16 μM, respectively. Abbas *et al.*²⁰ synthesized two series of new tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidines (2,3-disubstituted derivatives and 2,4-disubstituted ones) and evaluated for their cytotoxic

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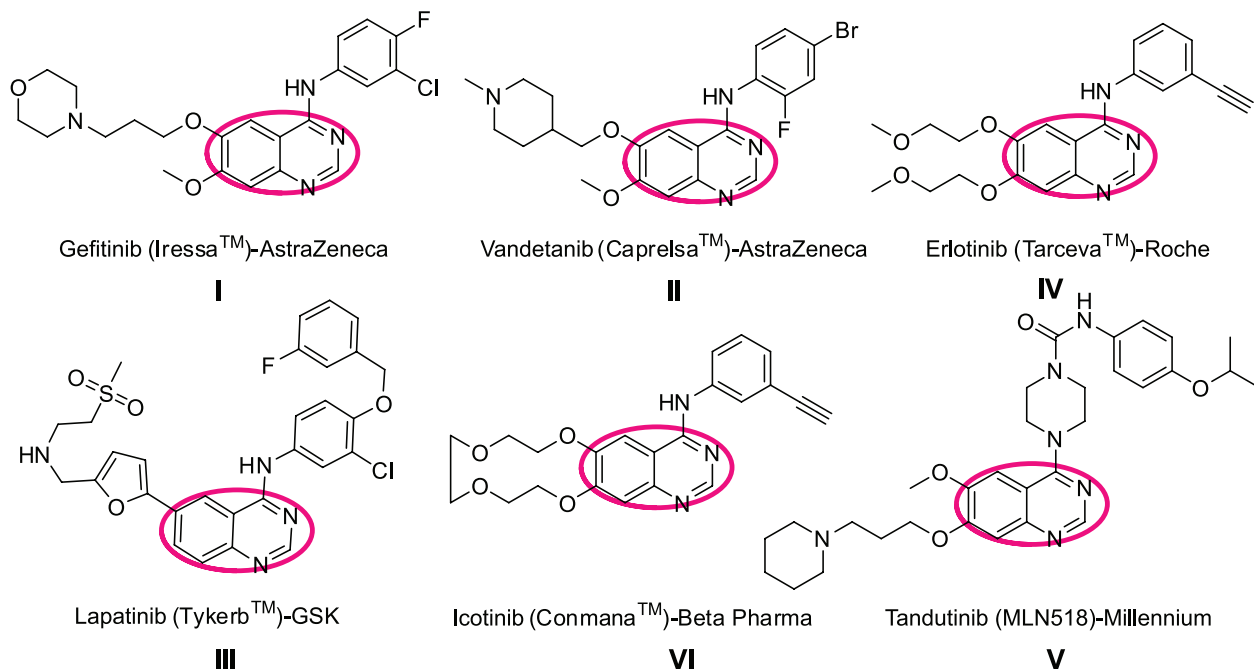


Figure 1. Representative examples of potent anticancer quinazoline drugs.

activity. According to the results, compound **X** was found to have the highest cytotoxic activity against breast MCF-7 with $IC_{50} = 15.01 \mu\text{M}$, and **XI** showed to be the most active one to inhibit human hepatocellular liver carcinoma cell line (HepG2) with $IC_{50} = 1.29 \mu\text{M}$. Besides, compound **XII**²¹ showed the best FLT3 (FMS-like receptor tyrosine kinase 3) cytotoxic activity, with IC_{50} value of $0.065 \mu\text{M}$.

Herein, we take into consideration that the compounds of thieno[2,3-*d*]pyrimidine skeleton express an important biological cytotoxic activity, and intend to synthesize a series of thieno[2,3-*d*]pyrimidine derivatives.

In this study, we are interested in the widely reported cytotoxic activity of thieno[2,3-*d*]pyrimidine derivatives

via different synthetic methods. Four strategies used for the synthesis of various thieno[2,3-*d*]pyrimidine scaffolds have been reported in literatures (Figure 3).^{11,14,22-29} The first method involves the synthesis of thieno[2,3-*d*]pyrimidin-4(3*H*)-one (**XVII**) from compounds **XIV**, **XV** and **XVI**, then, compound **XVII** is converted to 4-chlorothieno[2,3-*d*]pyrimidine (**XIX**), and the target compound **XX** is obtained from **XIX**. The second route is that the compound **XIII** is directly converted to compound **XVII**, and the final compound **XX** can be obtained from **XVII**. The third protocol is the direct synthesis of 4-chlorothieno[2,3-*d*]pyrimidine (**XIX**) from compound **XV**. The fourth method is the synthesis

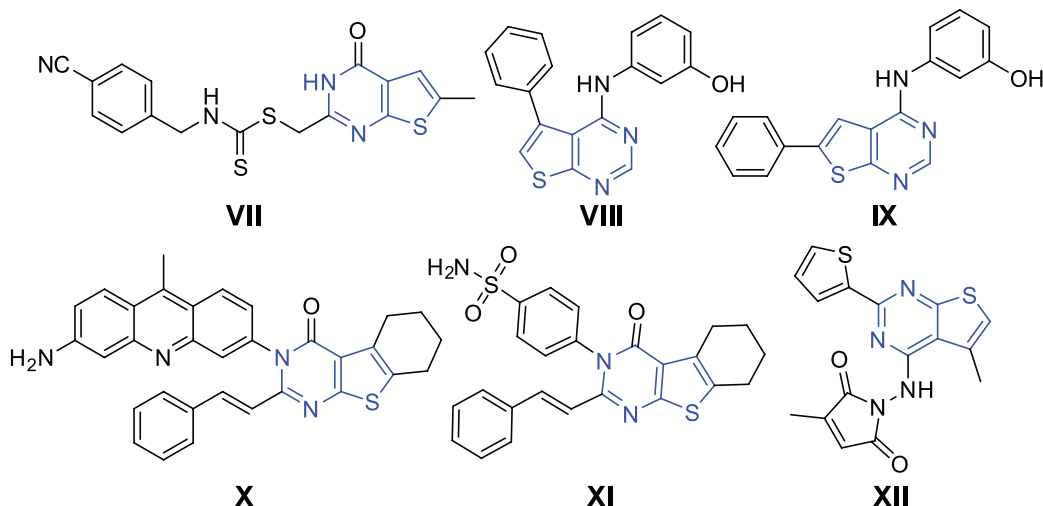


Figure 2. Thieno[2,3-*d*]pyrimidine derivatives in literatures.¹⁸⁻²¹

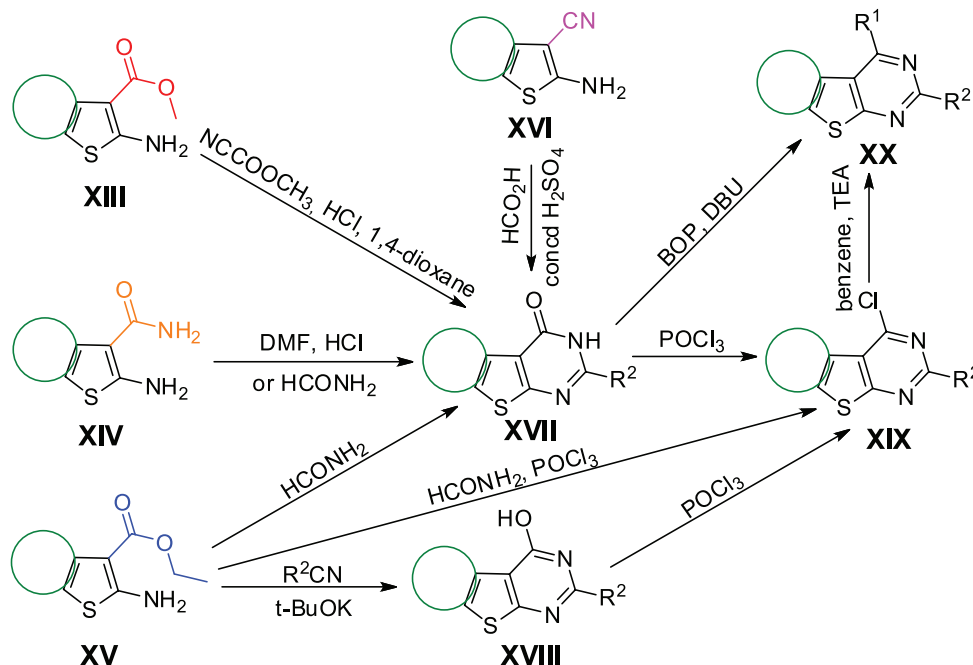


Figure 3. A summary of the synthetic approaches for thieno[2,3-*d*]pyrimidines in literatures.^{11,14,22-29}

of compound **XXVIII** from **XV**, subsequently, compound **XV** is converted to compound **XIX**, therefore, the target compound **XX** can be synthesized by **XV**.

Microwave irradiation technology in organic synthesis is a powerful tool for drug synthesis as it can speed up the reactions under mild conditions allowing the rapid preparation of compounds.³⁰ As mentioned above, microwave irradiation technology has been successfully applied to the synthesis of *N*-arylthieno[2,3-*d*]pyrimidine derivatives. Our recent endeavors involving green chemical techniques have focused on the synthesis of small organic molecular compounds. Then, in earlier work from our lab, we have reported the synthesis of 6-bromopyrido[2,3-*d*]pyrimidine derivatives (**XXI**),³¹ pyrido[2,3-*d*]pyrimidine derivatives (**XXII**),³² cyclopenta[4,5]thieno[2,3-*d*]pyrimidine derivatives (**XXIII**)³³ by microwave irradiation technology (Figure 4).

Interestingly, a compound of similar structure

(Figure 4, compound **XXIV**)³⁴ was evaluated to inhibit EGFR (epidermal growth factor receptor) kinase activity with the enzyme inhibition $\text{IC}_{50} = 2.6 \mu\text{M}$. Herein, thieno[2,3-*d*]pyrimidine skeleton of compounds **XXIII** and **XXIV** were used as a template, and the structure was modified: (Figure 4a) hexatomic ring was linked to thiophene ring instead of five membered ring (compound **XXIII**); (Figure 4b) substituted aromatic group was linked to secondary amine and $-\text{CH}_2$ group was subtracted (compound **XXIV**). Subsequently, compounds **3a-x** (Scheme 1) were obtained.

In this study, two questions would be solved: (i) a series of *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines (**3a-x**) were obtained with the aid of microwave irradiation technology (Figure 5); (ii) the cytotoxic activity of synthesized compounds were evaluated against A549, murine BALB/c spontaneous colon adenocarcinoma cell line (CT26), and HepG2.

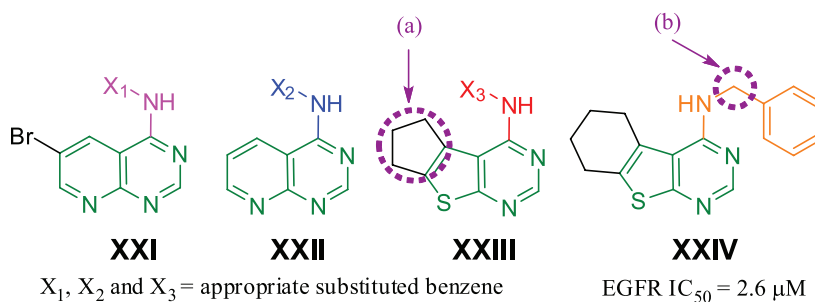
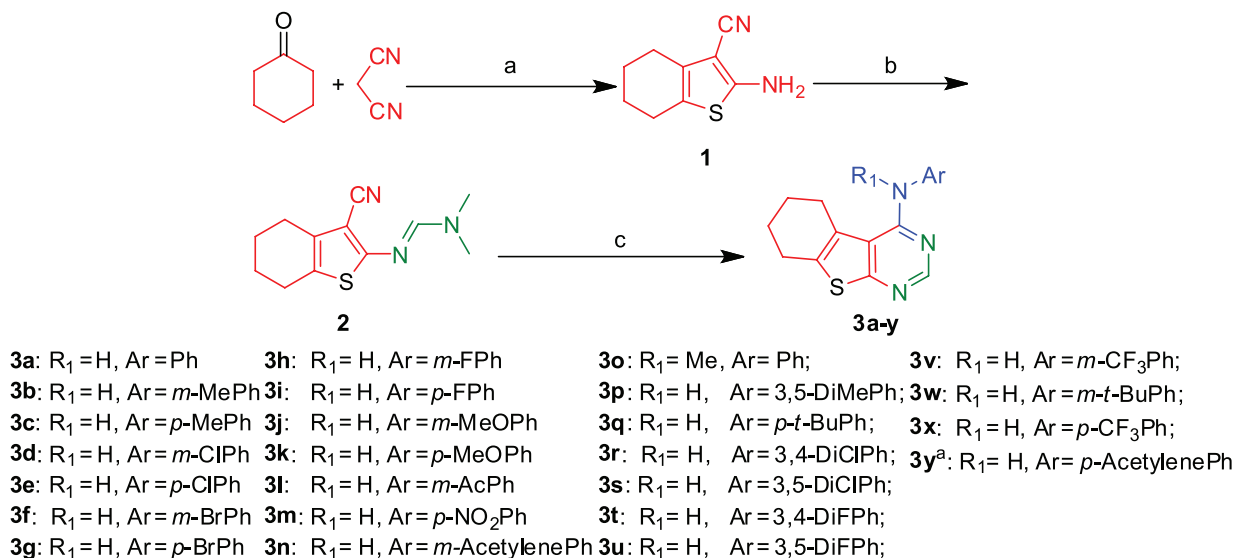


Figure 4. Structures of compounds **XXI**, **XXII**, **XXIII**, **XXIV**. (a) Hexatomic ring was linked to thiophene ring instead of five membered ring (compound **XXIII**); (b) substituted aromatic group was linked to secondary amine and $-\text{CH}_2$ group was subtracted (compound **XXIV**).



Scheme 1. Synthetic route of target compounds **3a-y**. Reagents and conditions: (a) S₈, K₂CO₃, EtOH, microwave irradiation (MWI), 85 °C, 15 min, 95%; (b) DMF-DMA, MWI, 78 °C, 5 min, 98%; (c) ArNH₂, AcOH, MWI, 90 °C, 10 min, 28-83%. ^a**3y**: this compound could not be obtained under the reaction conditions.

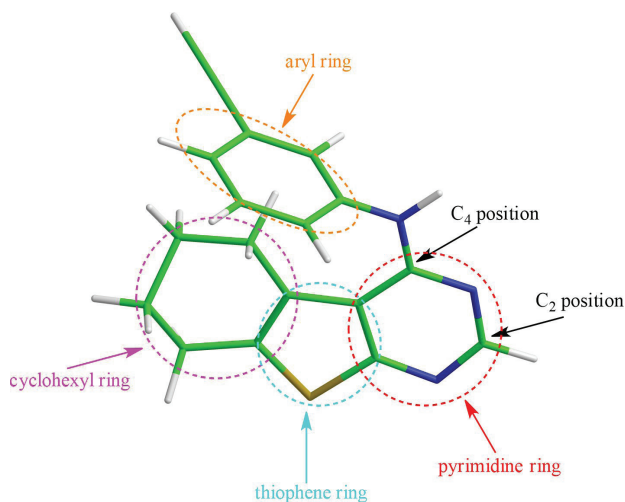


Figure 5. Main structure of synthesized compounds (example: new compound **3n**).

Results and Discussion

Synthesis

The synthetic strategy used in this study is outlined in Scheme 1. 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile was prepared using a modified Gewald procedure via the reaction of cyclohexanone, malononitrile and sulfur in the presence of potassium carbonate (K₂CO₃), which serves as a heterogeneous solid basic catalyst, in ethanol. To show the merits of the present work, we compared the as-obtained results using K₂CO₃ with those previously reported in the literature.³⁵⁻⁴⁰

It was found that our method can achieve the target

product in 95% yield in 15 min of reaction. Compared to the results in the literature,³⁵⁻⁴⁰ our method has a shorter reaction time and high yield. So, under the assistance of the microwave irradiation, K₂CO₃ can act as a cheap, highly efficient, and green catalyst used to prepare compound **1** in high yield over a short reaction time.

As for the synthesis of the intermediate compound **2** (*N'*-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-*N,N*-dimethyl-formimidamide), Loidreau *et al.*⁴¹ have reported that 2-amino-5-bromothiophene-3-carbonitrile reacts with *N,N*-dimethylformamide dimethyl acetal (DMF-DMA) to give *N'*-(3-cyanothiophen-2-yl)-*N,N*-dimethylformimidamide under conventional heating. Meanwhile, Loidreau *et al.*⁴¹ described the synthesis of *N'*-(2-cyano-benzo[*b*]thiophen-3-yl)-*N,N*-dimethylformimidamide in high yield upon the reaction of 3-amino-benzo[*b*]thiophene-2-carbonitrile and DMF-DMA with the aid of microwave irradiation. Inspired by these methods, we attempted to use 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile to react with DMF-DMA under the microwave irradiation and obtained compound **2** in excellent yields (98%). This method not only reduces the reaction time (only 5 min) with good yield, but also has an easy work-up procedure.

The synthesis of compounds **3a-x** (Table 1) involved the acid-catalyzed Dimroth rearrangement between compound **2** and an appropriate aromatic amine under microwave irradiation. It was found that the reaction between *m*, *p*-substituted aromatic amines and compound **2** can easily occur in high yield with a short reaction time. However, the reaction between *o*-substituted aromatic

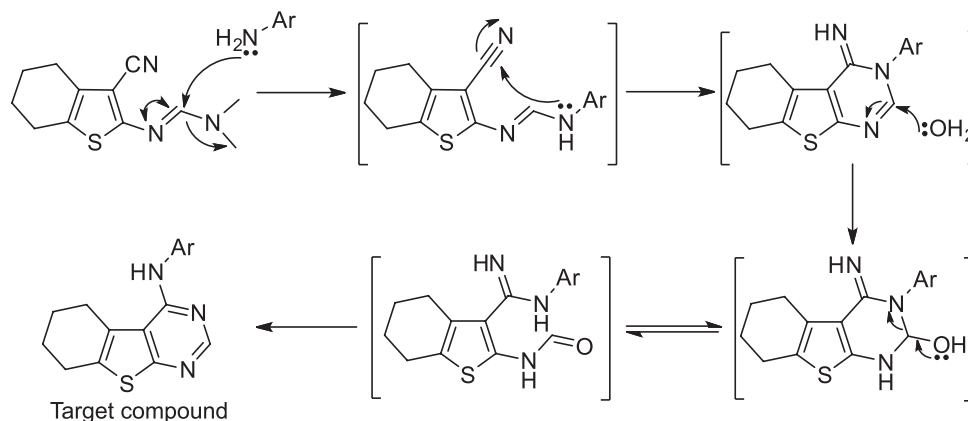
amines and compound **2** only formed trace amounts of the desired compounds under the same reaction conditions. This new synthetic route involves only three steps making this procedure simple and efficient. Hence, this method is an efficient method for the synthesis of *N*-arylthieno-

5,6,7,8-tetrahydro-benzothieno[2,3-*d*]pyrimidin-4-amines. The proposed mechanism for this rearrangement⁴¹ is described in Scheme 2.

Table 1. Preparation of the compounds **3a-x**

Compound	R ¹	Ar	mp/mp literature / °C	Yield ^a / %
3a	H	Ph	177.7-178.7/176-178 ²²	83
3b	H	<i>m</i> -MePh	138.1-139.5/-	69
3c	H	<i>p</i> -MePh	151.4-153.1/141-143 ²³	82
3d	H	<i>m</i> -ClPh	153.4-154.8/168 ⁴²	71
3e	H	<i>p</i> -ClPh	142.2-144.1/136-138 ²²	67
3f	H	<i>m</i> -BrPh	168.6-170.3/169 ⁴²	66
3g	H	<i>p</i> -BrPh	156.1-157.2/154-156 ²²	75
3h	H	<i>m</i> -FPh	123.3-125.0/210.6 ⁴²	69
3i	H	<i>p</i> -FPh	169.4-170.6/167-169 ²²	79
3j	H	<i>m</i> -MeOPh	105.7-106.6/193 ⁴²	66
3k	H	<i>p</i> -MeOPh	122.9-124.3/145-147 ²³	51
3l	H	<i>m</i> -AcPh	142.7-144.1/-	48
3m	H	<i>p</i> -NO ₂ Ph	203.1-204.6/-	56
3n^b	H	<i>m</i> -acetylenePh	183.5-185.4/-	65
3o	Me	Ph	120.6-121.8/-	72
3p	H	3,5-DiMePh	196.5-197.9/-	65
3q	H	<i>p-t</i> -BuPh	144.7-145.9/-	80
3r	H	3,4-DiClPh	170.6-171.8/-	75
3s	H	3,5-DiClPh	216.4-217.8/-	62
3t^b	H	3,4-DiFPh	199.6-200.7/-	73
3u^b	H	3,5-DiFPh	195.2-196.3/-	61
3v	H	<i>m</i> -CF ₃ Ph	121.9-123.0/224-225 ⁴³	53
3w^b	H	<i>m-t</i> -BuPh	108.9-110.1/-	79
3x^b	H	<i>p</i> -CF ₃ Ph	181.9-182.5/-	28

^aIsolated yield; ^bnew compounds.



Scheme 2. Proposed mechanism for the Dimroth rearrangement.

The structures of all compounds **1**, **2** and **3a-x** were confirmed by infrared (IR), ¹H nuclear magnetic resonance (NMR), ¹³C NMR spectroscopy, and high-resolution mass spectrometry (HRMS). The IR spectrum of compound **1** shows bands at 3444 and 2193 cm⁻¹ corresponding to the -NH and -C≡N groups, respectively. For compound **2**, the IR spectrum shows bands at 2199, 1627 and 1106 cm⁻¹ corresponding to the -C≡N, -C=N and -C-N groups, respectively. For the sake of the assignment of ¹H NMR spectra, we chose compound **3h** as the representative example. The ¹H NMR spectrum of the compound **3h** recorded in DMSO-*d*₆ shows the multiplet peaks at δ 7.74-7.60, 7.54-7.43, 7.42-7.30, and 6.96-6.80 assignable to the aryl protons (-CH) and the other multiplets at δ 3.24-3.04, 2.96-2.74, and 1.96-1.75 correspond to alicyclic portions protons (-CH₂). However, there are two peaks at δ 8.38 and 8.13, probably assigned to 2-position hydrogen on the pyrimidine ring (-CH) and the 4-position at pyrimidine ring (-NH), respectively. In order to solve this assignment problem, D-H exchange technology was applied. One drop of D₂O was added into the NMR sample of the compound **3h** and it was found that the peak at δ 8.13 disappeared. Thus, the peak at δ 8.13 was assigned to the hydrogen of -NH and the other peak at δ 8.38 assigned to the -CH signal.

To further confirm the structures of these compounds and provide a basis for studies on the structure activity relationships of these compounds, the crystal structures of compounds **3a** and **3c** were determined using single crystal X-ray diffraction. The molecular structures of the two compounds are illustrated in Figure 6. Colorless single crystals of the compound **3a** (0.35 × 0.20 × 0.10 mm) and **3c** (0.36 × 0.30 × 0.17 mm) were selected for X-ray diffraction analysis. The analysis reveals that the

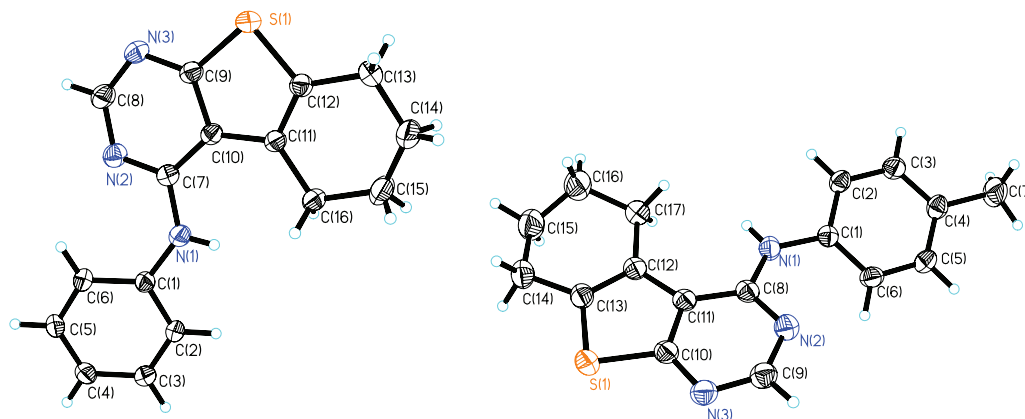


Figure 6. X-ray structures of compounds **3a** (left) and **3c** (right).

thieno[2,3-*d*]pyrimidine ring, which exhibits good coplanar nature, forms a dihedral angle with the benzene ring. The crystal data for **3a**: monoclinic, $a = 10.7726(5) \text{ \AA}$, $b = 8.6739(4) \text{ \AA}$, $c = 15.3075(7) \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 106.897(2)^\circ$, $\gamma = 90^\circ$, $V = 1368.59(11) \text{ \AA}^3$, wavelength = 0.71073 \AA , space group $P2(1)/c$, $Z = 4$, absorption coefficient = 0.229 mm^{-1} , calculated density = 1.366 mg cm^{-3} , θ range for data collection (2.73 to 27.68°), $F(000) = 592$, 3072 unique ($R_{\text{int}} = 0.0190$) were used in all calculations. The final R_1 was 0.0810 ($I > 2\sigma(I)$) and wR_2 was 0.2202 (all data). The crystal data for **3c**: monoclinic, $a = 11.8384(3) \text{ \AA}$, $b = 8.6429(2) \text{ \AA}$, $c = 14.8165(4) \text{ \AA}$, $\alpha = 90^\circ$, $\beta = 98.8890(10)^\circ$, $\gamma = 90^\circ$, $V = 1497.79(7) \text{ \AA}^3$, wavelength = 0.71073 \AA , space group $P2(1)/c$, $Z = 4$, absorption coefficient = 0.213 mm^{-1} , calculated density = 1.310 mg cm^{-3} , θ range for data collection (1.74 to 27.41°), $F(000) = 624$, 3381 unique ($R_{\text{int}} = 0.0201$) were used in all calculations. The final R_1 was 0.0707 ($I > 2\sigma(I)$) and wR_2 was 0.1982 (all data). Crystallographic data were deposited with the Cambridge Crystallographic Data Centre (see Supplementary Information section below).

Biological activity

Taking Erlotinib as reference compound, the synthesized compounds **3a-x** were evaluated for cytotoxic activities of A549, CT26 and HepG2 cell lines using 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. The inhibition (at $25 \mu\text{M}$) of all target compounds and cytotoxic activities (IC_{50} , in μM) for selected compounds were summarized in Table 2.

As showed in Table 2, compounds **3b**, **3d**, **3k**, **3l**, **3q**, **3w** and **3x** displayed cytotoxic activities against A549 with drug concentration of $25 \mu\text{M}$. Among the seven compounds, compounds **3b**, **3d**, **3k** and **3w** showed lower cytotoxicity (at $25 \mu\text{M}$) than Erlotinib ($25.18 \pm 0.28\%$), with inhibition of 9.21 ± 0.12 , 15.88 ± 0.45 , 1.87 ± 0.21

and $17.84 \pm 0.74\%$, respectively. Compounds **3l** and **3x** displayed better cytotoxic activity (37.18 ± 0.26 and $55.84 \pm 0.05\%$) compared with the positive drug Erlotinib. Surprisingly, the most outstanding compound is **3q**, which displayed best cytotoxic activity against A549 with the inhibition of $85.83 \pm 0.31\%$.

Besides, most of target compounds (**3b**, **3d**, **3e**, **3f**, **3g**, **3h**, **3j**, **3m**, **3n**, **3o**, **3p**, **3q**, **3r**, **3s**, **3t**, **3u**, **3v**, **3w** and **3x**) were shown to have cytotoxic activities (at $25 \mu\text{M}$) against CT26. In these compounds, the selected six compounds (**3e**, **3g**, **3o**, **3p**, **3q** and **3u**) demonstrated inapparent cytotoxic activities compared with the positive control Erlotinib ($24.52 \pm 0.13\%$), with inhibition of 2.25 ± 0.19 , 1.65 ± 0.04 , 2.45 ± 0.39 , 1.90 ± 0.20 , 5.96 ± 0.51 and $3.04 \pm 0.59\%$, respectively. In addition, compounds **3b**, **3f**, **3h**, **3m**, **3n** and **3s** inhibited 14.21 ± 0.44 , 13.57 ± 0.55 , 14.79 ± 0.63 , 19.52 ± 0.18 , 10.65 ± 0.23 , $12.95 \pm 0.08\%$ of the cytotoxic activities of tested CT26 cell line at compound concentration of $25 \mu\text{M}$, respectively. Besides, compounds **3d**, **3j**, **3r**, **3t**, **3v**, **3w** and **3x** outperformed the positive control Erlotinib against CT26 cell line, with inhibition of 40.81 ± 0.05 , 49.48 ± 0.06 , 55.51 ± 0.62 , 64.72 ± 0.37 , 55.40 ± 0.17 , 62.16 ± 0.54 and $34.08 \pm 0.01\%$, respectively.

Interestingly, all of synthesized compounds were shown to have cytotoxic activities (at $25 \mu\text{M}$) against HepG2. In these compounds, **3m** ($8.45 \pm 0.59\%$) and **3o** ($2.72 \pm 0.35\%$) demonstrated the lowest cytotoxic activities. Whereas, ten compounds (**3a**, **3c**, **3f**, **3g**, **3h**, **3k**, **3l**, **3p**, **3s** and **3u**) depicted moderate cytotoxic effects, with the inhibition of 18.24 ± 0.38 , 18.72 ± 0.08 , 23.38 ± 0.44 , 20.64 ± 0.15 , 16.28 ± 0.04 , 19.17 ± 0.63 , 17.48 ± 0.32 , 21.29 ± 0.07 , 14.58 ± 0.39 and $19.88 \pm 0.69\%$, respectively. There are four compounds (**3e**, **3i**, **3n** and **3t**) which exhibited almost similar cytotoxic activities (27.37 ± 0.55 , 28.69 ± 0.29 , 27.49 ± 0.11 and $26.81 \pm 0.09\%$) compared with the positive drug

Table 2. Cytotoxic activities of the compounds **3a-x**

Compound	R ¹	Ar	Inhibition (at 25 μ M) / %			IC ₅₀ / μ M			
			A549	CT26	HepG2	A549	CT26	HepG2	AML-12 ^a
3a	H	Ph	NA ^b	NA	18.24 \pm 0.38	NT ^c	NT	NT	NT
3b	H	<i>m</i> -MePh	9.21 \pm 0.12	14.21 \pm 0.44	33.57 \pm 0.23	NT	NT	NT	NT
3c	H	<i>p</i> -MePh	NA	NA	18.72 \pm 0.08	NT	NT	NT	NT
3d	H	<i>m</i> -ClPh	15.88 \pm 0.45	40.81 \pm 0.05	33.94 \pm 0.11	NT	45.71 \pm 0.23	29.81 \pm 0.11	NT
3e	H	<i>p</i> -ClPh	NA	2.25 \pm 0.19	27.37 \pm 0.55	NT	NT	NT	NT
3f	H	<i>m</i> -BrPh	NA	13.57 \pm 0.55	23.38 \pm 0.44	NT	NT	NT	NT
3g	H	<i>p</i> -BrPh	NA	1.65 \pm 0.04	20.64 \pm 0.15	NT	NT	NT	NT
3h	H	<i>m</i> -FPh	NA	14.79 \pm 0.63	16.28 \pm 0.04	NT	NT	NT	NT
3i	H	<i>p</i> -FPh	NA	NA	28.69 \pm 0.29	NT	NT	NT	NT
3j	H	<i>m</i> -MeOPh	NA	49.48 \pm 0.06	47.98 \pm 0.06	NT	26.92 \pm 0.18	22.35 \pm 0.36	NT
3k	H	<i>p</i> -MeOPh	1.87 \pm 0.21	NA	19.17 \pm 0.63	NT	NT	NT	NT
3l	H	<i>m</i> -AcPh	37.18 \pm 0.26	NA	17.48 \pm 0.32	70.90 \pm 0.22	NT	NT	NT
3m	H	<i>p</i> -NO ₂ Ph	NA	19.52 \pm 0.18	8.45 \pm 0.59	NT	NT	NT	NT
3n	H	<i>m</i> -acetylenePh	NA	10.65 \pm 0.23	27.49 \pm 0.11	NT	NT	NT	NT
3o	Me	Ph	NA	2.45 \pm 0.39	2.72 \pm 0.35	NT	NT	NT	NT
3p	H	3,5-DiMePh	NA	1.90 \pm 0.20	21.29 \pm 0.07	NT	NT	NT	NT
3q	H	<i>p-t</i> -BuPh	85.83 \pm 0.31	5.96 \pm 0.51	61.53 \pm 0.26	16.06 \pm 0.09	NT	15.01 \pm 0.31	> 100
3r	H	3,4-DiClPh	NA	55.51 \pm 0.62	35.76 \pm 0.43	NT	22.91 \pm 0.16	36.21 \pm 0.22	NT
3s	H	3,5-DiClPh	NA	12.95 \pm 0.08	14.58 \pm 0.39	NT	NT	NT	NT
3t	H	3,4-DiFPh	NA	64.72 \pm 0.37	26.81 \pm 0.09	NT	19.05 \pm 0.05	NT	95.50 \pm 0.83
3u	H	3,5-DiFPh	NA	3.04 \pm 0.59	19.88 \pm 0.69	NT	NT	NT	NT
3v	H	<i>m</i> -CF ₃ Ph	NA	55.40 \pm 0.17	63.64 \pm 0.17	NT	22.39 \pm 0.32	14.02 \pm 0.15	61.66 \pm 0.59
3w	H	<i>m-t</i> -BuPh	17.84 \pm 0.74	62.16 \pm 0.54	32.94 \pm 0.54	> 100	11.38 \pm 0.44	> 100	56.23 \pm 0.71
3x	H	<i>p</i> -CF ₃ Ph	55.84 \pm 0.05	34.08 \pm 0.01	66.66 \pm 0.03	21.38 \pm 0.69	> 100	8.51 \pm 0.52	> 100
Erlotinib ^d	–	–	25.18 \pm 0.28	24.52 \pm 0.13	29.83 \pm 0.21	> 100	51.99 \pm 0.19	43.05 \pm 0.09	> 100

^aAML-12: murine non-transformed hepatocyte cell line; ^bNA: no cytotoxic activity (at 25 μ M); ^cNT: IC₅₀ value was not measured; ^dused as a positive control (standard Erlotinib hydrochloride). A549: human pulmonary carcinoma cell line; CT26: murine BALB/c spontaneous colon adenocarcinoma cell line; HepG2: human hepatocellular liver carcinoma cell line.

Erlotinib (29.83 \pm 0.21%). Besides, compounds **3b**, **3d**, **3j** and **3w** (33.57 \pm 0.23, 33.94 \pm 0.11, 47.98 \pm 0.06 and 32.94 \pm 0.54%) showed better cytotoxic activities than the positive drug. The most promising compounds **3q**, **3v** and **3x** exhibited the best cytotoxic effects against HepG2 cell line with inhibition of 61.53 \pm 0.26, 63.64 \pm 0.17 and 66.66 \pm 0.03%, respectively.

According to the analysis of above activity data, five compounds (**3b**, **3d**, **3q**, **3w** and **3x**) exhibited cytotoxic activities against all selected tumor cell lines (A549, CT26 and HepG2). Especially, compound **3q** depicted better cytotoxic effects against A549 and HepG2 cell lines. In addition, **3t** and **3x** displayed the best cytotoxic effects against CT26 and HepG2 cell lines, respectively.

Three (**3l**, **3q** and **3x**), seven (**3d**, **3j**, **3r**, **3t**, **3v**, **3w** and **3x**) and seven compounds (**3d**, **3j**, **3q**, **3r**, **3v**, **3w** and

3x), which have better cytotoxic activities compared to Erlotinib (at 25 μ M), were subsequently severally selected to investigate the IC₅₀ values (Table 2) of cytotoxic activity against A549, CT26 and HepG2 cell lines, respectively.

The present study investigated the effect of several different structures and from the results of cytotoxic activities of the compounds, the following preliminary structure-activity relationships can be derived:

(i) The compound **3q** showed the most outstanding cytotoxic activity (IC₅₀ = 16.06 \pm 0.09 μ M) and the next most promising compound was **3x**, which displayed good cytotoxic activity (IC₅₀ = 21.38 \pm 0.69 μ M) against A549 cell line. Compound **3l** showed moderate cytotoxic effects (IC₅₀ = 70.90 \pm 0.22 μ M). Based on this (Figure 7), it is possible to surmise: when introducing a group with strong electron-donating (**3q**, Ar = *p-t*-BuPh) or strong electron-

withdrawing (**3x**, Ar = *p*-CF₃Ph) into the 4-position, the cytotoxic activity (A549) of compounds could be boosted. It was also observed that the presence of π - π conjugate effect (**3l**, Ar = *m*-AcPh) played an important role in the cytotoxic effect.

(ii) Subsequently, the introduction of strong electron-withdrawing group (**3r**, **3t** and **3v**, Ar = 3,4-DiClPh, 3,4-DiFPh and *m*-CF₃Ph, respectively) led to a better cytotoxic activity (IC₅₀ = 22.91 \pm 0.16, 19.05 \pm 0.05, 22.39 \pm 0.32 μ M, respectively) against CT26 cell line (Figure 8). Furthermore, the introduction of strong electron-donating (**3w**, Ar = *m*-*t*-BuPh) also improves the cytotoxic effect (IC₅₀ = 11.38 \pm 0.44 μ M) of the compound. Another interesting phenomenon was found that when introducing a double chlorine or a double fluorine (**3r**, Ar = 3,4-DiClPh; **3t**, Ar = 3,4-DiFPh) into the 3,4-position, the resulting activity was higher than that of compounds **3s** and **3u** (Ar = 3,5-DiClPh; Ar = 3,5-DiFPh) with drug concentration of 25 μ M. Besides, compounds **3d** and **3j** also displayed good cytotoxic activity (IC₅₀ = 45.71 \pm 0.23 and 26.92 \pm 0.18 μ M, respectively) against CT26 cell line, which might be due to the presence of *p- π conjugate effect (**3j**, Ar = *m*-MeOPh).*

(iii) Meanwhile, compounds substituted with bulky electron-withdrawing (**3x**, Ar = *p*-CF₃Ph; **3v**, Ar = *m*-CF₃Ph)

or strong electron-donating groups (**3q**, Ar = *p*-*t*-BuPh) exhibited far superior cytotoxic activity (IC₅₀ = 8.51 \pm 0.52, 14.02 \pm 0.15 and 15.01 \pm 0.31 μ M, respectively) than other compounds (Figure 9). Moreover, the cytotoxic activity against HepG2 cell line was enhanced because of *p*- π conjugate effect (**3d**, Ar = *m*-ClPh; **3j**, Ar = *m*-MeOPh), with IC₅₀ values of 29.81 \pm 0.11 and 22.35 \pm 0.36 μ M, respectively.

(iv) To sum up, all of the synthesized compounds exhibited cytotoxic activities against HepG2 cell line with drug concentration of 25 μ M, most of target compounds were shown to have cytotoxic activities against CT26 cell line, while only seven compounds demonstrated cytotoxic activities against A549 cell line. In general, it was found that all synthesized compounds showed significant selective cytotoxic effect against the HepG2.

The effects of selected compounds (IC₅₀ values of selected compounds > IC₅₀ values of Erlotinib against A549, CT26 and HepG2) on cell viability were assessed. The detailed results are provided in Figures 10, 11 and 12, respectively.

In relation to selectivity index (SI), Erlotinib showed SI > 1.92 and 2.32 for CT26 and HepG2 cells (Table 3, entry 1), respectively. Compound **3v** showed moderate SI = 2.75 for CT26 cell line (Table 3, entry 4) and

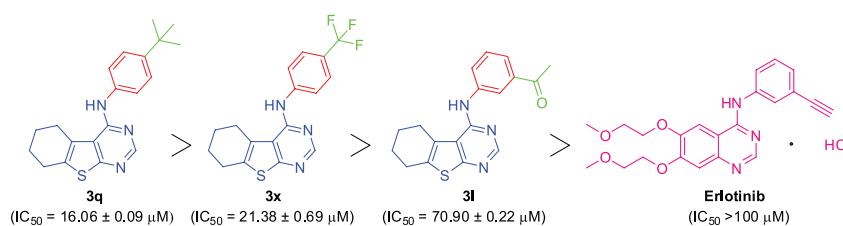


Figure 7. Comparison of cytotoxic activities (IC₅₀ values) against A549 cell line.

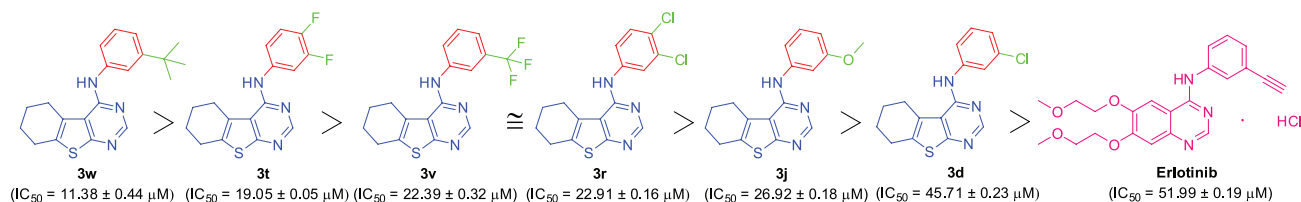


Figure 8. Comparison of cytotoxic activities (IC₅₀ values) against CT26 cell line.

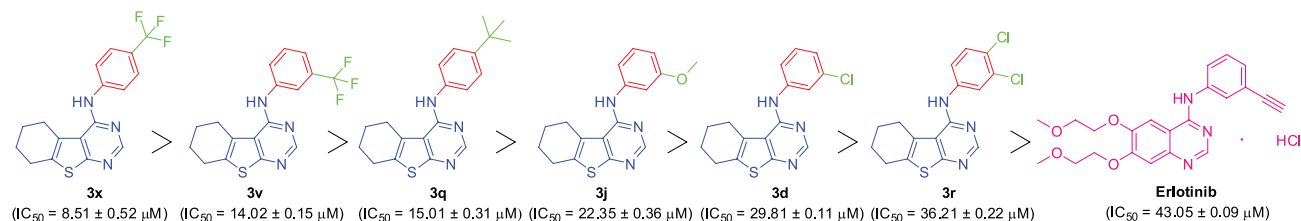


Figure 9. Comparison of cytotoxic activities (IC₅₀ values) against HepG2 cell line.

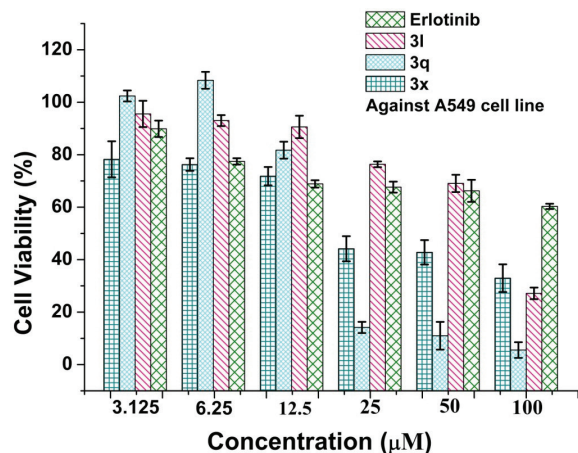


Figure 10. Cell viability of selected compounds toward A549 cell line.

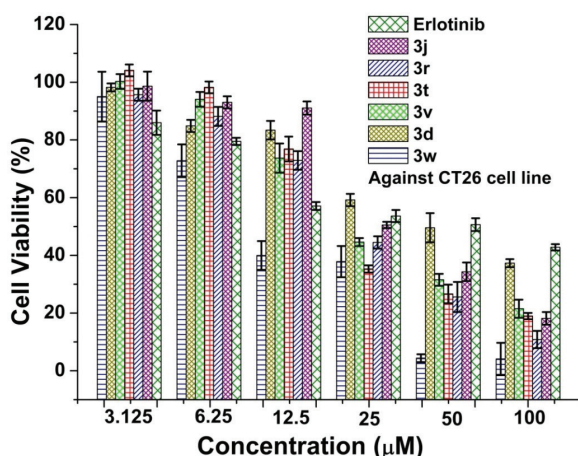


Figure 11. Cell viability of selected compounds toward CT26 cell line.

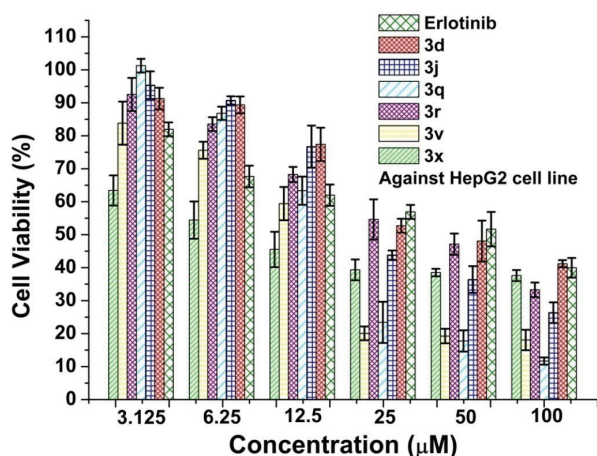


Figure 12. Cell viability of selected compounds toward HepG2 cell line.

compounds **3t**, **3w** and **3v** showed good selectivity index for CT26 cell line (Table 3, entry 3, 5; SI = 5.01, 4.94) and HepG2 cell line (Table 3, entry 4; SI = 4.40). Besides, compound **3x** also displayed good SI > 4.68 for A549 cell line and lower cytotoxicity (Table 3, entry 6), with SI > 11.75 for HepG2 cell line.

Table 3. Selectivity index (SI)^a for selected compounds

entry	Compound	Selectivity index		
		AML-12 vs. A549	AML-12 vs. CT26	AML-12 vs. HepG2
1	Erlotinib ^b	NC ^c	> 1.92	> 2.32
2	3q	> 6.23	NC	> 6.66
3	3t	NC	5.01	NC
4	3v	NC	2.75	4.40
5	3w	NC	4.94	NC
6	3x	> 4.68	NC	> 11.75

^aSI, selectivity index: IC₅₀ on normal cells / IC₅₀ on cancer cells;

^bpositive control: standard Erlotinib hydrochloride of AML-12 cell;

^cNC: not calculated. AML-12: murine non-transformed hepatocyte cell line; A549: human pulmonary carcinoma cell line; CT26: murine BALB/c spontaneous colon adenocarcinoma cell line; HepG2: human hepatocellular liver carcinoma cell line.

Conclusions

In conclusion, we have synthesized and evaluated a library of thieno[2,3-*d*]pyrimidine derivatives. Amongst these compounds (**3a-x**), **3q** was found to have better cytotoxic activity with reference to the standard Erlotinib hydrochloride (TarcevaTM) against A549 (IC₅₀ = 16.06 ± 0.09 µM) and HepG2 (IC₅₀ = 15.01 ± 0.31 µM) cell lines. Especially, **3w** and **3x** showed best cytotoxic effects against CT26 (IC₅₀ = 11.38 ± 0.44 µM) and HepG2 (IC₅₀ = 8.51 ± 0.52 µM) cell lines, respectively. Compound **3x** also showed good selectivity index (SI > 11.75). Furthermore, additional preliminary structure-activity relationships could be responsible for the found potent and selective activity of new thieno[2,3-*d*]pyrimidine compounds. Besides, the synthesized compounds (**3a-x**) would be evaluated for their enzyme activities and protein activities in following work and the more complete structure-activity relationships would be disclosed.

Experimental

General remarks

All melting points were measured on an MP90 apparatus and are uncorrected. IR spectra were recorded in a VERTEX 80/Raman II (Bruker, Switzerland, KBr pellets). The ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded in an Avance III HD (Bruker, Switzerland) spectrometer with tetramethylsilane (TMS) as internal reference in CDCl₃ or dimethyl sulfoxide (DMSO-*d*₆) as the solvent. Only discrete or characteristic signals for the ¹H NMR are reported.

The MS spectra were obtained on a Triple-TOF™ 5600+ spectrometer (AB SCIEX, USA). An X-ray crystallographic analysis was performed with a Bruker SMART APEX II (Bruker, Switzerland). The structure of the single crystals was refined by a Shelx-97.⁴⁴ All reactions were monitored by thin layer chromatography (TLC) with ethyl acetate:hexane (1:4, v/v) as the eluent. All reagents and solvents had been purchased from commercial sources and commonly purified before their usage.

General procedure for preparation of 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**1**)

Cyclohexanone (0.98 g, 10 mmol), malononitrile (0.66 g, 10 mmol), sulfur powder (0.35 g, 11.0 mmol), K₂CO₃ (0.28 g, 2.0 mmol) and 20 mL of dry ethanol were mixed and placed in a 50 mL pressure tube. The mixture was subjected to microwave irradiation (CEM Explorer Hybrid, USA, 100 W, 200 psi, 85 °C) for 15 min. After the reaction, the insoluble material was filtered out, and the solvent was removed through evaporation under reduced pressure. The crude product was washed with water and recrystallized from ethanol to give yellowish crystals of 1.69 g (9.50 mmol), in 95% yield; mp 146.8-148.0 °C; ¹H NMR (400 MHz, CDCl₃) δ 4.61 (s, 2H), 2.59-2.39 (m, 4H), 1.90-1.69 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 159.87, 132.37, 120.67, 115.43, 88.82, 24.52, 24.13, 23.37, 22.12; IR (KBr) ν / cm⁻¹ 3444, 3327, 2907, 2193, 1618, 1520; HRMS (ESI) *m/z*, calcd. for C₉H₁₀N₂S [M + H]⁺: 179.0637; found: 179.0638.

Preparation of *N'*-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-*N,N*-dimethylformimidamide (**2**)

Compound **1** (1.78 g, 10 mmol) and DMF-DMA (3.57 g, 30 mmol) were added into a 1-necked 20 mL pressure tube, and then the mixture was subjected to microwave irradiation (CEM Explorer Hybrid, USA, 100 W, 200 psi, 78 °C) for 5 min. After the reaction, the DMF-DMA was evaporated in vacuum and the crude compound was re-crystallized from ethyl acetate to give the target compound of 2.29 g (9.80 mmol), in 98% yield; mp 125.8-126.9 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.67 (s, 1H), 3.08 (s, 6H), 2.61-2.52 (m, 4H), 1.87-1.74 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 164.07, 153.85, 133.25, 124.39, 116.30, 97.11, 40.59, 34.95, 24.75, 24.44, 23.39, 22.27; IR (KBr) ν / cm⁻¹ 2930, 2199, 1627, 1508, 1106; HRMS (ESI) *m/z*, calcd. for C₁₂H₁₅N₃S [M + H]⁺: 234.1059; found: 234.1099.

Preparation of *N*-aryl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amines (**3a-x**)

Compound **2** (2.33 g, 10 mmol), appropriate aniline (11 mmol) and acetic acid (8 mL) as the solvent as well as catalyst were added into a 1-necked 20 mL pressure tube at room temperature. The mixture was subjected to microwave irradiation (CEM Explorer Hybrid, USA, 100 W, 200 psi, 90 °C) for 10 min. After reaction, the acetic acid was evaporated in a vacuum and the crude product was recrystallized from ethyl acetate to give target compounds (**3a-x**).

N-Phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3a**)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.38 (s, 1H), 8.13 (s, 1H), 7.67-7.65 (m, 2H), 7.39-7.32 (m, 2H), 7.13-7.07 (m, 1H), 3.14 (m, 2H), 2.83 (m, 2H), 1.86 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.24, 155.35, 152.56, 139.67, 133.41, 128.90, 127.09, 123.80, 122.58, 117.25, 25.83, 25.55, 22.63, 22.48; IR (KBr) ν / cm⁻¹ 3426, 3044, 2921, 1602, 1563, 1500, 1440, 1213, 972, 748, 697, 498; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₅N₃S [M + H]⁺: 282.1059; found: 282.1063.

N-(*m*-Tolyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3b**)

¹H NMR (400 MHz, CDCl₃) δ 8.49 (s, 1H), 7.50-7.40 (m, 2H), 7.30-7.22 (m, 1H), 7.11 (s, 1H), 6.96 (d, *J* 7.5 Hz, 1H), 3.12-3.02 (m, 2H), 2.90-2.81 (m, 2H), 2.39 (s, 3H), 2.06-1.88 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 166.23, 155.10, 152.65, 139.00, 138.35, 134.59, 128.93, 124.94, 124.80, 121.93, 118.51, 116.65, 26.56, 25.56, 22.60, 22.44, 21.59; IR (KBr) ν / cm⁻¹ 3450, 2944, 1610, 1565, 1482, 1372, 1165, 777, 517; HRMS (ESI) *m/z*, calcd. for C₁₇H₁₇N₃S [M + H]⁺: 296.1216; found: 296.1219.

N-(*p*-Tolyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3c**)

¹H NMR (400 MHz, CDCl₃) δ 8.46 (s, 1H), 7.50 (d, *J* 5.5 Hz, 2H), 7.19 (d, *J* 8.2 Hz, 2H), 7.07 (s, 1H), 3.16-2.98 (m, 2H), 2.94-2.74 (m, 2H), 2.35 (s, 3H), 2.11-1.80 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 166.15, 155.31, 152.71, 135.70, 134.42, 133.97, 129.62, 124.87, 121.86, 116.53, 26.56, 25.55, 22.60, 22.45, 20.94; IR (KBr) ν / cm⁻¹ 3421, 2918, 1599, 1560, 1505, 1222, 977, 815, 496; HRMS (ESI) *m/z*, calcd. for C₁₇H₁₇N₃S [M + H]⁺: 296.1216; found: 296.1212.

N-(3-Chlorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3d**)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.45 (s, 1H, -CH),

8.24 (s, 1H), 7.86 (t, *J* 2.0 Hz, 1H), 7.73-7.53 (m, 1H), 7.36 (t, *J* 8.1 Hz, 1H), 7.26-6.98 (m, 1H), 3.25-3.03 (m, 2H), 2.98-2.69 (m, 2H), 1.93-1.68 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.54, 154.82, 152.37, 141.30, 133.90, 133.21, 130.41, 126.96, 123.11, 121.49, 120.55, 117.53, 25.74, 25.56, 22.61, 22.42; IR (KBr) *v* / cm⁻¹ 3444, 2944, 1599, 1558, 1502, 1437, 1219, 1129, 984, 875, 762, 679, 515; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₄ClN₃S [M + H]⁺: 316.0670; found: 316.0668.

N-(4-Chlorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3e**)

¹H NMR (400 MHz, CDCl₃) δ 8.48 (s, 1H), 7.73-7.50 (m, 2H), 7.44-7.29 (m, 2H), 7.11 (s, 1H), 3.17-2.95 (m, 2H), 2.96-2.74 (m, 2H), 2.07-1.86 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 166.46, 154.76, 152.43, 137.09, 135.09, 129.06, 128.94, 124.59, 122.48, 116.70, 26.58, 25.57, 22.57, 22.41; IR (KBr) *v* / cm⁻¹ 3451, 2933, 1606, 1560, 1495, 1434, 1092, 979, 832, 497; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₄ClN₃S [M + H]⁺: 316.0670; found: 316.0673.

N-(3-Bromophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3f**)

¹H NMR (400 MHz, CDCl₃) δ 8.44 (s, 1H), 7.87 (d, *J* 1.8 Hz, 1H), 7.53-7.46 (m, 1H), 7.18-7.10 (m, 2H), 7.07 (s, 1H), 3.03-2.94 (m, 2H), 2.83-2.73 (m, 2H), 1.99-1.81 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 166.15, 154.55, 152.17, 139.85, 135.39, 130.27, 126.71, 124.55, 123.70, 122.65, 119.39, 116.79, 26.53, 25.57, 22.53, 22.38; IR (KBr) *v* / cm⁻¹ 3446, 2945, 1597, 1500, 1469, 1439, 1378, 1131, 992, 763, 668, 520; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₄BrN₃S [M - H]⁻: 358.0019; found: 358.0020.

N-(4-Bromophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3g**)

¹H NMR (400 MHz, CDCl₃) δ 8.41 (s, 1H), 7.59-7.44 (m, 2H), 7.44-7.35 (m, 2H), 7.06 (s, 1H), 3.04-2.95 (m, 2H), 2.84-2.72 (m, 2H), 2.00-1.79 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 166.04, 154.69, 152.17, 137.53, 135.26, 131.99, 124.61, 122.77, 116.76, 116.54, 26.54, 25.57, 22.54, 22.39; IR (KBr) *v* / cm⁻¹ 3451, 2932, 1605, 1558, 1496, 1432, 1222, 1125, 829, 803; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₄BrN₃S [M - H]⁻: 358.0019; found: 358.0017.

N-(3-Fluorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3h**)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.46 (s, 1H), 8.28 (s, 1H), 7.74-7.60 (m, 1H), 7.54-7.43 (m, 1H), 7.42-7.30 (m, 1H), 6.96-6.80 (m, 1H), 3.24-3.04 (m, 2H), 2.96-2.74 (m, 2H), 1.96-1.75 (m, 4H); ¹H NMR (400 MHz, DMSO-*d*₆-D₂O) δ 8.44 (s, 1H), 7.72-7.63 (m, 1H), 7.50-7.43 (m, 1H),

7.41-7.32 (m, 1H), 6.94-6.84 (m, 1H), 3.20-3.04 (m, 2H), 2.90-2.76 (m, 2H), 1.94-1.77 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.47, 163.73, 161.34, 154.78, 152.38, 141.44, 134.06, 130.42, 126.98, 117.72 (d, *J* 2.6 Hz), 117.56, 109.91, 108.63, 25.68, 25.55, 22.58, 22.41; IR (KBr) *v* / cm⁻¹ 3452, 2936, 1611, 1577, 1506, 1447, 1377, 1139, 990, 763; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₄FN₃S [M - H]⁻: 298.0820; found: 298.0825.

N-(4-Fluorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3i**)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.35 (s, 1H), 8.13 (s, 1H), 7.70-7.59 (m, 2H), 7.24-7.14 (m, 2H), 3.16-3.07 (m, 2H), 2.87-2.76 (m, 2H), 1.91-1.80 (m, 4H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 166.21, 160.11, 157.73, 155.46, 152.54, 135.90 (d, *J* 2.6 Hz), 133.32, 127.07, 125.02, 117.03, 115.55, 115.33, 25.85, 25.53, 22.63, 22.46; IR (KBr) *v* / cm⁻¹ 3428, 2921, 1614, 1569, 1504, 1438, 1205, 1128, 955; HRMS (ESI) *m/z*, calcd. for C₁₆H₁₄FN₃S [M - H]⁻: 298.0820; found: 298.0823.

N-(3-Methoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3j**)

¹H NMR (400 MHz, CDCl₃) δ 8.50 (s, 1H), 7.47-7.42 (m, 1H), 7.32-7.24 (m, 1H), 7.15 (s, 1H), 7.14-7.10 (m, 1H), 6.72-6.65 (m, 1H), 3.85 (s, 3H), 3.13-3.02 (m, 2H), 2.91-2.79 (m, 2H), 2.07-1.87 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 166.29, 160.25, 154.97, 152.55, 139.71, 134.79, 129.74, 124.73, 116.73, 113.38, 109.27, 107.34, 55.39, 26.55, 25.57, 22.59, 22.42; IR (KBr) *v* / cm⁻¹ 3443, 2925, 1608, 1565, 1486, 1156, 1043, 779, 679, 521; HRMS (ESI) *m/z*, calcd. for C₁₇H₁₇N₃OS [M + H]⁺: 312.1165; found: 312.1161.

N-(4-Methoxyphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3k**)

¹H NMR (400 MHz, CDCl₃) δ 8.44 (s, 1H), 7.56-7.41 (m, 2H), 7.02 (s, 1H), 6.97-6.87 (m, 2H), 3.83 (s, 3H), 3.14-2.97 (m, 2H), 2.94-2.77 (m, 2H), 2.06-1.83 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 176.04, 165.75, 156.79, 155.66, 152.67, 134.42, 131.03, 124.93, 124.21, 116.42, 114.38, 55.57, 26.57, 25.53, 22.59, 22.45, 20.74; IR (KBr) *v* / cm⁻¹ 3418, 2935, 1604, 1564, 1503, 1221, 1026, 829, 552; HRMS (ESI) *m/z*, calcd. for C₁₇H₁₇N₃OS [M + H]⁺: 312.1165; found: 312.1159.

1-(3-((5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)amino)phenyl)ethan-1-one (**3l**)

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.40 (s, 1H), 8.32 (s, 1H), 8.22-8.18 (m, 1H), 8.00-7.95 (m, 1H), 7.71-7.66 (m, 1H), 7.53-7.46 (m, 1H), 3.18-3.12 (m, 2H), 2.86-2.79 (m,

2H), 2.60 (s, 3H), 1.90-1.83 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 198.18, 166.48, 155.17, 152.44, 140.21, 137.67, 133.65, 129.15, 127.21, 127.10, 123.69, 121.76, 117.45, 27.28, 25.81, 25.56, 22.64, 22.45; IR (KBr) ν / cm^{-1} 3443, 2939, 1566, 1508, 1442, 1360, 1262, 964, 775, 680, 534; HRMS (ESI) m/z , calcd. for $\text{C}_{18}\text{H}_{17}\text{N}_3\text{OS}$ [$\text{M} + \text{H}$] $^+$: 324.1165; found: 324.1162.

N-(4-Nitrophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3m**)

^1H NMR (400 MHz, CDCl_3) δ 8.60 (s, 1H), 8.36-8.18 (m, 2H), 8.03-7.84 (m, 2H), 7.60 (s, 1H), 3.18-3.07 (m, 2H), 2.96-2.82 (m, 2H), 2.09-1.89 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 167.17, 153.73, 152.02, 144.82, 142.66, 136.49, 125.23, 124.16, 119.33, 117.35, 26.59, 25.64, 22.52, 22.35; IR (KBr) ν / cm^{-1} 3439, 2932, 2846, 1585, 1557, 1502, 1435, 1326, 1237, 1181, 1116, 851, 778, 534, 490; HRMS (ESI) m/z , calcd. for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_2\text{S}$ [$\text{M} + \text{H}$] $^+$: 327.0910; found: 327.0915.

N-(3-Ethynylphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3n**)

^1H NMR (400 MHz, CDCl_3) δ 8.50 (s, 1H), 7.80-7.76 (m, 1H), 7.76-7.68 (m, 1H), 7.37-7.29 (m, 1H), 7.26-7.22 (m, 1H), 7.13 (s, 1H), 3.10 (s, 1H), 3.09-3.02 (m, 2H), 2.91-2.81 (m, 2H), 2.05-1.87 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.39, 154.66, 152.38, 138.63, 135.04, 129.04, 127.46, 124.62, 124.20, 122.81, 121.50, 116.71, 83.37, 26.52, 25.56, 22.54, 22.38; IR (KBr) ν / cm^{-1} 3443, 3240, 2939, 1559, 1502, 1438, 1133, 991, 889, 774, 650, 519; HRMS (ESI) m/z , calcd. for $\text{C}_{18}\text{H}_{15}\text{N}_3\text{S}$ [$\text{M} + \text{H}$] $^+$: 306.1059; found: 306.1055.

N-Methyl-*N*-phenyl-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3o**)

^1H NMR (400 MHz, DMSO- d_6) δ 8.29 (s, 1H), 7.49-7.37 (m, 4H), 7.29-7.21 (m, 1H), 3.48 (s, 3H), 2.62-2.54 (m, 2H), 2.50-2.47 (m, 2H), 1.84-1.71 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 163.24, 153.16, 143.97, 133.15, 130.00, 126.11, 125.79, 121.11, 116.01, 98.08, 34.80, 24.65, 24.40, 23.27, 22.21; IR (KBr) ν / cm^{-1} 3448, 3074, 2942, 2835, 2208, 1616, 1494, 1352, 1224, 1134, 977, 756, 688; HRMS (ESI) m/z , calcd. for $\text{C}_{17}\text{H}_{17}\text{N}_3\text{S}$ [$\text{M} + \text{H}$] $^+$: 296.1216; found: 296.1222.

N-(3,5-Dimethylphenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3p**)

^1H NMR (400 MHz, DMSO- d_6) δ 8.39 (s, 1H), 7.97 (s, 1H), 7.31 (s, 2H), 6.74 (s, 1H), 3.16-3.10 (m, 2H), 2.87-2.77 (m, 2H), 2.28 (s, 6H), 1.90-1.80 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.10, 155.38, 152.65, 139.50,

137.92, 133.30, 127.07, 125.37, 120.10, 117.18, 25.84, 25.54, 22.62, 22.48, 21.55; IR (KBr) ν / cm^{-1} 3451, 2942, 2837, 1614, 1560, 1505, 1450, 1164, 1127, 1042, 820, 519; HRMS (ESI) m/z , calcd. for $\text{C}_{18}\text{H}_{19}\text{N}_3\text{S}$ [$\text{M} + \text{H}$] $^+$: 310.1372; found: 310.1377.

N-(4-(*tert*-Butyl)phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3q**)

^1H NMR (400 MHz, CDCl_3) δ 8.40 (s, 1H), 7.50-7.43 (m, 2H), 7.37-7.31 (m, 2H), 7.06 (s, 1H), 3.03-2.95 (m, 2H), 2.83-2.74 (m, 2H), 2.00-1.81 (m, 4H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ 155.25, 152.38, 147.38, 135.49, 134.74, 126.01, 124.87, 121.47, 116.61, 34.45, 31.40, 26.53, 25.55, 22.59, 22.42; IR (KBr) ν / cm^{-1} 3417, 2954, 1598, 1560, 1501, 1435, 1316, 1237, 982, 822; HRMS (ESI) m/z , calcd. for $\text{C}_{20}\text{H}_{23}\text{N}_3\text{S}$ [$\text{M} - \text{H}$] $^-$: 336.1540; found: 336.1543.

N-(3,4-Dichlorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3r**)

^1H NMR (400 MHz, DMSO- d_6) δ 8.39 (s, 1H), 8.25 (s, 1H), 7.97 (d, J 2.5 Hz, 1H), 7.63 (dd, J 8.8, 2.5 Hz, 1H), 7.52 (d, J 8.8 Hz, 1H), 3.12-2.98 (m, 2H), 2.83-2.69 (m, 2H), 1.85-1.71 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.69, 154.64, 152.34, 140.03, 134.16, 131.05, 130.62, 126.99, 124.84, 123.29, 122.23, 117.67, 25.72, 25.58, 22.62, 22.40; IR (KBr) ν / cm^{-1} 3447, 2936, 1596, 1554, 1493, 1434, 1374, 1266, 1128, 990, 805; HRMS (ESI) m/z , calcd. for $\text{C}_{16}\text{H}_{13}\text{Cl}_2\text{N}_3\text{S}$ [$\text{M} - \text{H}$] $^-$: 348.0134; found: 348.0130.

N-(3,5-Dichlorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3s**)

^1H NMR (400 MHz, DMSO- d_6) δ 8.50 (s, 1H), 8.38 (s, 1H), 7.84 (d, J 1.9 Hz, 2H), 7.25 (t, J 1.9 Hz, 1H), 3.17-3.07 (m, 2H), 2.91-2.79 (m, 2H), 1.92-1.79 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.83, 154.45, 152.30, 142.46, 134.39, 134.11, 127.00, 122.27, 119.89, 117.90, 25.69, 25.59, 22.62, 22.39; IR (KBr) ν / cm^{-1} 3444, 3103, 2943, 2845, 1598, 1502, 1446, 1226, 1121, 1000, 837, 811; HRMS (ESI) m/z , calcd. for $\text{C}_{16}\text{H}_{13}\text{Cl}_2\text{N}_3\text{S}$ [$\text{M} - \text{H}$] $^-$: 348.0134; found: 348.0138.

N-(3,4-Difluorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno [2,3-*d*]pyrimidin-4-amine (**3t**)

^1H NMR (400 MHz, DMSO- d_6) δ 8.52 (s, 1H), 8.42 (s, 1H), 7.56-7.47 (m, 1H), 6.94-6.84 (m, 1H), 3.17-3.08 (m, 2H), 2.88-2.81 (m, 2H), 1.92-1.83 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 166.86, 164.03, 163.88, 161.62, 161.47, 154.47, 152.29, 142.57, 134.49, 126.93, 117.94, 104.51, 104.22, 98.18, 25.63 (d, J 6.4 Hz), 22.62, 22.39;

IR (KBr) ν / cm^{-1} 3449, 3097, 2936, 2853, 1615, 1561, 1509, 1476, 1441, 1148, 1109, 1030, 989, 827; HRMS (ESI) m/z , calcd. for $\text{C}_{16}\text{H}_{13}\text{F}_2\text{N}_3\text{S}$ $[\text{M} - \text{H}]^-$: 316.0725; found: 316.0720.

N-(3,5-Difluorophenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3u**)

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.50 (s, 1H), 8.38 (s, 1H), 7.56-7.46 (m, 2H), 6.87 (tt, J 9.3, 2.3 Hz, 1H), 3.12 (m, 2H), 2.84 (m, 2H), 1.85 (m, 4H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 166.83, 154.45, 152.30, 142.46, 134.39, 134.11, 127.00, 122.27, 119.89, 117.90, 25.64 (d, J 10.1 Hz), 22.62, 22.39; IR (KBr) ν / cm^{-1} 3449, 3097, 2937, 2853, 1615, 1561, 1509, 1476, 1441, 1148, 1108, 988, 826; HRMS (ESI) m/z , calcd. for $\text{C}_{16}\text{H}_{13}\text{F}_2\text{N}_3\text{S}$ $[\text{M} - \text{H}]^-$: 316.0725; found: 316.0728.

N-(3-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3v**)

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.37 (s, 1H), 8.33 (s, 1H), 8.01 (s, 1H), 7.96-7.88 (m, 1H), 7.56-7.46 (m, 1H), 7.38-7.30 (m, 1H), 3.13-3.03 (m, 2H), 2.81-2.72 (m, 2H), 1.83-1.75 (m, 4H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 166.66, 154.90, 152.38, 140.64, 134.01, 129.93, 129.55, 127.06, 126.03, 123.35, 119.79 (d, J 3.6 Hz), 118.36 (d, J 4.0 Hz), 117.61, 25.75, 25.58, 22.63, 22.42; IR (KBr) ν / cm^{-1} 3445, 3095, 2947, 2870, 1567, 1510, 1442, 1327, 1164, 1110, 988, 781; HRMS (ESI) m/z , calcd. for $\text{C}_{17}\text{H}_{14}\text{F}_3\text{N}_3\text{S}$ $[\text{M} - \text{H}]^-$: 348.0788; found: 348.0782.

N-(3-(*tert*-Butyl)phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3w**)

^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.35 (s, 1H), 8.08 (s, 1H), 7.63-7.57 (m, 1H), 7.57-7.49 (m, 1H), 7.33-7.22 (m, 1H), 7.16-7.05 (m, 1H), 3.19-3.09 (m, 2H), 2.88-2.72 (m, 2H), 1.90-1.80 (m, 4H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 166.19, 155.49, 152.59, 151.55, 139.29, 133.22, 128.40, 127.12, 120.83, 120.25, 119.69, 117.17, 34.92, 31.63, 25.84, 25.55, 22.65, 22.49; IR (KBr) ν / cm^{-1} 3453, 3355, 2937, 1603, 1559, 1501, 1438, 1374, 1198, 1131, 992, 784; HRMS (ESI) m/z , calcd. for $\text{C}_{20}\text{H}_{23}\text{N}_3\text{S}$ $[\text{M} + \text{H}]^+$: 338.1685; found: 338.1696.

N-(4-(Trifluoromethyl)phenyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**3x**)

^1H NMR (400 MHz, CDCl_3) δ 8.47 (s, 1H), 7.75 (d, J 8.5 Hz, 2H), 7.55 (d, J 8.6 Hz, 2H), 7.22 (s, 1H), 3.02 (t, J 6.0 Hz, 2H), 2.80 (dd, J 8.1, 3.9 Hz, 2H), 2.00-1.76 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.74, 154.37, 152.25, 141.76, 135.64, 126.31 (d, J 3.8 Hz), 124.4, 120.21, 116.99, 26.58, 25.60, 22.55, 22.38; IR (KBr)

ν / cm^{-1} 3448, 2933, 2846, 1610, 1563, 1501, 1438, 1324, 1241, 1162, 1107, 1066, 983, 838; HRMS (ESI) m/z , calcd. for $\text{C}_{17}\text{H}_{14}\text{F}_3\text{N}_3\text{S}$ $[\text{M} + \text{H}]^+$: 350.0933; found: 350.0912.

Biological evaluation

Cytotoxicity assay *in vitro*

At first, the cytotoxic activities of target compounds **3a-x** were determined against A549, CT26 and HepG2 cell lines *in vitro* according to MTT assay with Erlotinib as the positive control at 25 μM . Then, the selected compounds, which have better cytotoxic effects compared to Erlotinib (at 25 μM), were subsequently severally selected to investigate the IC_{50} values of cytotoxic activity against three tumor cell lines tested at six concentrations (3.125, 6.25, 12.5, 25, 50 and 100 μM). MTT was dissolved at 4 mg mL^{-1} in phosphate-buffered saline (PBS).

The three cancer cell lines were cultured in a Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 g mL^{-1} penicillin and 100 g mL^{-1} streptomycin. Cancer cell lines in logarithmic phase were plated at a density of 3×10^3 cells well^{-1} into each well of 96-well microtiter plates with 100 μL of DMEM and incubated at 37 $^\circ\text{C}$ for 24 h in humidified atmosphere containing 5% CO_2 . Then, compounds **3a-x** solution (100 μL) was added into the culture medium and the cancer cells were incubated at same condition (37 $^\circ\text{C}$, 5% CO_2) for 72 h. The MTT solution (20 μL of 4 mg mL^{-1} MTT in PBS) was added into each well and the cells containing target compounds were further incubated at 37 $^\circ\text{C}$ for 4 h in 5% CO_2 . After removing the culture medium, DMSO (200 μL) was then added into each well to dissolve the crystals, shaken mechanically for 10 min, and then optical densities (OD) values at a wavelength of 540 nm were measured on a microplate reader (SpectraMax 190, Molecular Devices, USA). Survival ratios are expressed in percentages with respect to untreated cells.

Supplementary Information

Crystallographic data (excluding structure factors) for the structures in this work were deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 1542488 (**3a**) and CCDC 1542147 (**3c**). Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/rtrieving.html or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

Supplementary data associated with this paper can be found, in the online version, at <http://jbcs.sbgq.org.br> as PDF file.

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References

- Chen, W.-L.; Carlo, C. D.; Devery, D.; McGrath, D. J.; McHugh, P. E.; Kleinsteinberg, K.; Jockenhoevel, S.; Hennink, W. E.; Kok, R. J.; *Int. J. Pharm.* **2018**, *548*, 803.
- Brocklesby, K. L.; Waby, J. S.; Cawthorne, C.; Smith, G.; *Tetrahedron Lett.* **2017**, *58*, 1467.
- Athar, M.; Ranjan, P.; Jha, P. C.; *J. Mol. Graphics Modell.* **2018**, *84*, 160.
- Yamamoto, Y.; Saita, T.; Yamamoto, Y.; Shin, M.; *J. Pharm. Anal.* **2018**, *8*, 119.
- Batchelor, T. T.; Gerstner, E. R.; Ye, X.-B.; Desideri, S.; Duda, D. G.; Peereboom, D.; Lesser, G. J.; Chowdhary, S.; Wen, P. Y.; Grossman, S.; Supko, J. G.; *Neuro-Oncology* **2016**, *19*, 567.
- Cheng, X.-W.; Lv, X.; Qu, H.-Y.; Li, D.-D.; Hu, M.-M.; Guo, W.-Z.; Ge, G.-B.; Dong, R.-H.; *Acta Pharm. Sin. B* **2017**, *7*, 657.
- Habib, N. S.; Soliman, R.; El-Tombary, A. A.; El-Hawash, S. A.; Shaaban, O. G.; *Med. Chem. Res.* **2013**, *22*, 3289.
- Zaher, N. H.; Salem, A. A. M.; Ismail, A. F. M.; *J. Photochem Photobiol., B* **2016**, *165*, 328.
- Wang, T.; Zheng, C.-H.; Liu, S.; Chen, H.-Z.; *Phosphorus, Sulfur Silicon Relat. Elem.* **2010**, *185*, 1543.
- Song, X.-J.; Yang, P.; Gao, H.; Wang, Y.; Dong, X.-G.; Tan, X.-H.; *Chin. Chem. Lett.* **2014**, *25*, 1006.
- Jeon, M. K.; Kim, J. G.; Lee, D. H.; *Bull. Korean Chem. Soc.* **2016**, *37*, 1406.
- El-Sayed, N. N. E.; Abdelaziz, M. A.; Wardakhan, W. W.; Mohareb, R. M.; *Steroids* **2016**, *107*, 98.
- Yang, J. S.; Park, C. H.; Lee, C.; Kim, H.; Oh, C.; Choi, Y.; Kang, J. S.; Yun, J.; Jeong, J. H.; Kim, M. H.; Han, G.; *Eur. J. Med. Chem.* **2014**, *85*, 399.
- Kandeel, M. M.; Refaat, H. M.; Kassab, A. E.; Shahin, I. G.; Abdelghany, T. M.; *Eur. J. Med. Chem.* **2015**, *90*, 620.
- Rashad, A. E.; Ali, M. A.; *Nucleosides, Nucleotides Nucleic Acids* **2006**, *25*, 17.
- Baran, A. U.; Tel, B. C.; Sarıgöl, D.; Öztürk, E. I.; Kazkayasi, I.; Okay, G.; Ertan, M.; Tozkoparan, B.; *Eur. J. Med. Chem.* **2012**, *57*, 398.
- Deng, J.; Peng, L.; Zhang, G.-C.; Lan, X.-B.; Li, C.-F.; Chen, F.-X.; Zhou, Y. Y.; Lin, Z. X.; Chen, L.; Dai, R.-K.; Xu, H. J.; Yang, L.; Zhang, X.-Q.; Hu, W.-H.; *Eur. J. Med. Chem.* **2011**, *46*, 71.
- Yang, C.-R.; Peng, B.; Cao, S.-L.; Ren, T.-T.; Wei, J.; Wang, F.-C.; Li, Y.-S.; Wang, G.; Zheng, L.; Xu, S.-B.; Liao, J.; Wang, H.-L.; Li, J.; Xu, X.-Z.; *Eur. J. Med. Chem.* **2018**, *154*, 324.
- Gryshchenko, A. A.; Bdzholia, V. G.; Balanda, A. O.; Briukhovetska, N. V.; Kotey, I. M.; Golub, A. G.; Ruban, T. P.; Lukash, L. L.; Yarmoluk, S. M.; *Bioorg. Med. Chem.* **2015**, *23*, 2287.
- Abbas, S. E.; Abdel Gawad, N. M.; George, R. F.; Akar, Y. A.; *Eur. J. Med. Chem.* **2013**, *65*, 195.
- Park, C. H.; Lee, C.; Yang, J. S.; Joe, B. Y.; Chun, K.; Kim, H.; Kim, H. Y.; Kang, J. S.; Lee, J. I.; Kim, M. H.; Han, G.; *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2655.
- Bowers, S.; Truong, A. P.; Neitz, R. J.; Neitz, R. J.; Hom, R. K.; Sealy, J. M.; Probst, G. D.; Quincy, D.; Peterson, B.; Chan, W.; Galembo Jr., R. A.; Konradi, A. W.; Sham, H. L.; Tóth, G.; Pan, H.; Lin, M.; Yao, N.; Artis, D. R.; Zhang, H.; Chen, L.; Dryer, M.; Samant, B.; Zmolek, W.; Wong, K.; Lorentzen, C.; Goldbach, E.; Tonn, G.; Quinn, K. P.; Sauer, J. M.; Wright, S.; Powell, K.; Ruslim, L.; Ren, Z.; Bard, F.; Yednock, T. A.; Griswold-Prenner, I.; *Med. Chem. Lett.* **2011**, *21*, 5521.
- Phoujdar, M. S.; Kathiravan, M. K.; Bariwal, J. B.; Shah, A. K.; Jain, K. S.; *Tetrahedron Lett.* **2008**, *49*, 1269.
- Prasad, M. R.; Kishore, D. P.; *Chem. Pharm. Bull.* **2007**, *55*, 776.
- Kassab, A. E.; Gedawy, E. M.; *Eur. J. Med. Chem.* **2013**, *63*, 224.
- Bassetto, M.; Leyssen, P.; Neyts, J.; Yerukhimovich, M. M.; Frick, D. N.; Brancale, A.; *Eur. J. Med. Chem.* **2016**, *123*, 31.
- Pédeboscq, S.; Gravier, D.; Casadebaig, F.; Hou, G.; Gissot, A.; Giorgi, F. D.; Ichas, F.; Cambar, J.; Pometan, J. P.; *Eur. J. Med. Chem.* **2010**, *45*, 2473.
- Tan, Q.; Zhang, Z.-T.; Hui, J.; Zhao, Y.; Zhu, L.; *Bioorg. Med. Chem.* **2014**, *22*, 358.
- Jain, K. S.; Kathiravan, M. K.; Bariwal, J. B.; Chaskar, P. K.; Tompe, S. S.; Arya, N.; *Synth. Commun.* **2013**, *43*, 719.
- Cheng, X.-M.; Liu, X.-W.; *J. Comb. Chem.* **2007**, *9*, 906.
- Sun, Y.-Q.; Zong, C.-Y.; Ji, J.-Y.; Han, Q.; *Chem. Pap.* **2018**, *72*, 2965.
- Zong, C.-Y.; Gu, H.-W.; Zhang, L.-J.; Jin, Y.-D.; Sun, Y.-Q.; *Chin. J. Org. Chem.* **2018**, *38*, 1165.
- Zong, C.-Y.; Zhang, L.-J.; Gu, M.-T.; Sun, Y.-Q.; *Chin. J. Org. Chem.* **2018**, *38*, 1422.
- Wu, C.-H.; Coumar, M. S.; Chu, C.-Y.; Lin, W.-H.; Chen, Y.-R.; Chen, C.-T.; Shiao, H.-Y.; Rafi, S.; Wang, S.-Y.; Hsu, H.; Chen, C.-H.; Chang, C.-Y.; Chang, T.-Y.; Lien, T.-W.; Fang, M.-Y.; Yeh, K.-C.; Chen, C.-P.; Yeh, T.-K.; Hsieh, S.-H.; Hsu, J. T.-A.; Liao, C.-C.; Chao, Y.-S.; Hsieh, H.-P.; *J. Med. Chem.* **2010**, *53*, 7316.

35. Wang, F.-S.; Gao, H.; Yang, P.; Wang, Y.; Song, X.-J.; *Lett. Org. Chem.* **2014**, *11*, 700.
36. Sridhar, M.; Rao, R. M.; Baba, N. H. K.; Kumbhare, R. M.; *Tetrahedron Lett.* **2007**, *48*, 3171.
37. Tayebee, R.; Javadi, F.; Argi, G.; *J. Mol. Catal. A: Chem.* **2013**, *368*, 16.
38. Wang, D.-F.; Gao, H.; Tan, X.-H.; Li, W.-M.; Wang, J.; Wu, Y.-J.; Song, X.-J.; *Adv. Mater. Res.* **2014**, 887, 661.
39. Zhao, D.-D.; Li, L.; Xu, F.; Wu, Q.; Lin, X.-F.; *J. Mol. Catal. B: Enzym.* **2013**, *95*, 29.
40. Abaee, M. S.; Cheraghi, S.; *J. Sulfur Chem.* **2014**, *35*, 261.
41. Loidreau, Y.; Dubouilh-Benard, C.; Marchand, P.; Nourrisson, M. R.; Duflos, M.; Buquet, C.; Corbière, C.; Besson, T.; *J. Heterocycl. Chem.* **2013**, *50*, 1187.
42. Peter, B.; Gyorgy, K.; Laszlo, O.; Zsolt, S.; Frigyes, W.; *WO pat. 2009104026 A1* **2009**.
43. Issa, S. A. A.; *Orient. J. Chem.* **2014**, *30*, 469.
44. Sheldrick, G. M.; *Acta. Cryst.* **2008**, *A64*, 112.

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