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Synthesis and Evaluation of Biological Activities of Thieno-[2,3-*d*]-pyrimidine Derivatives

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A series of novel thieno[2,3-*d*]pyrimidine derivatives were designed, synthesized and evaluated for their biological ability. 2-Amino-4,7-dihydro-5*H*-thieno[2,3-*d*]pyrano-3-cyanonitrile was synthesized by Gewald reaction using pyranone as substrate, malonitrile and sulfur powder as raw materials and triethylamine as base at room temperature. Subsequent amination with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) gave *N*'-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-*N*,*N*-dimethylmethanimidamide. In this article, the 23 thieno[2,3-*d*] pyrimidines were obtained by Dimroth rearrangement condensation with different anilines, 16 of them being new compounds. Meanwhile, all compounds were assessed for breast cancer MDA-MB-231 cell line bioactivity with paclitaxel (PTX) as the positive control, and all compounds in the series showed inhibitory effects on breast cancer cell MDA-MB-231. The IC_{50} of compound **I** against MDA-MB-231 is 27.6 μM, which is similar to that of PTX with an IC_{50} of 29.3 μM. This means that compound **I** exhibits comparable inhibitory activity to PTX in MDA-MB-231 cells and may be considered as a potential anti-MDA-MB-231 inhibitor.

Keywords: thieno[2,3-*d*]pyrimidine derivatives, Dimroth rearrangement, Gewald reaction, antitumor, microwave synthesis

Introduction

Cancer is an enemy of human health, and the enormous disease burden caused by cancer is not only among the vital causes of death globally but also a major impediment to increasing human life expectancy.[1] 2.26 million new cases regarding breast cancer were reported worldwide in 2020.[2] There are many ways to classify breast cancer based on immunohistochemistry into three receptors: estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2).[3] Breast cancer is broadly categorized into Luminal A,[4] Luminal B,[5] triple-negative types, as well as HER2 overexpression. The triple-negativity with regards to breast cancer is demonstrated by the fact that all three receptors, ER, PR, and HER2, are negative in cancerous tissues,[6] which is one of the most important diseases in gynecological clinics due to its close association with malignant tumors of the female reproductive tract. Triple-negative breast cancer denotes pathologic subtype of breast cancer, having the highest lethality and recurrence rates of all breast cancer pathologic subtypes. This makes it a very challenging disease.

Thieno[2,3-*d*]pyrimidine allows the design of biotargeted agents that inhibit triple-negative breast cancer cells, including epidermal growth factor receptor suppression (EGFR), tyrosine kinase (TKI), as well as vascular endothelial growth factor suppression (VEGF).[7]

At present, the EGFR tyrosine kinase small molecule inhibitors that have been listed and entered clinical trials are mainly **I** (gefitinib), **II** (erlotinib) and **III** (lapatinib), **IV** (CI1033), **V** (EKB-2569) and **VI** (PKI-66) (Figure 1).[8] They have a pyrimidine nucleus of the quinazoline nucleus and can therefore be considered bioisomers of the quinazoline nucleus.

Thieno[2,3-*d*]pyrimidine derivatives can be regarded as bioelectronic equivalents of quinazoline due to their unique ring structure. Thieno[2,3-*d*]pyrimidine derivatives have pyrimidine rings in their structure, which refers to the building blocks of RNA as well as DNA.[9] These compounds can be served as highly active drug targets with significant pharmacological activity and can be utilized in the

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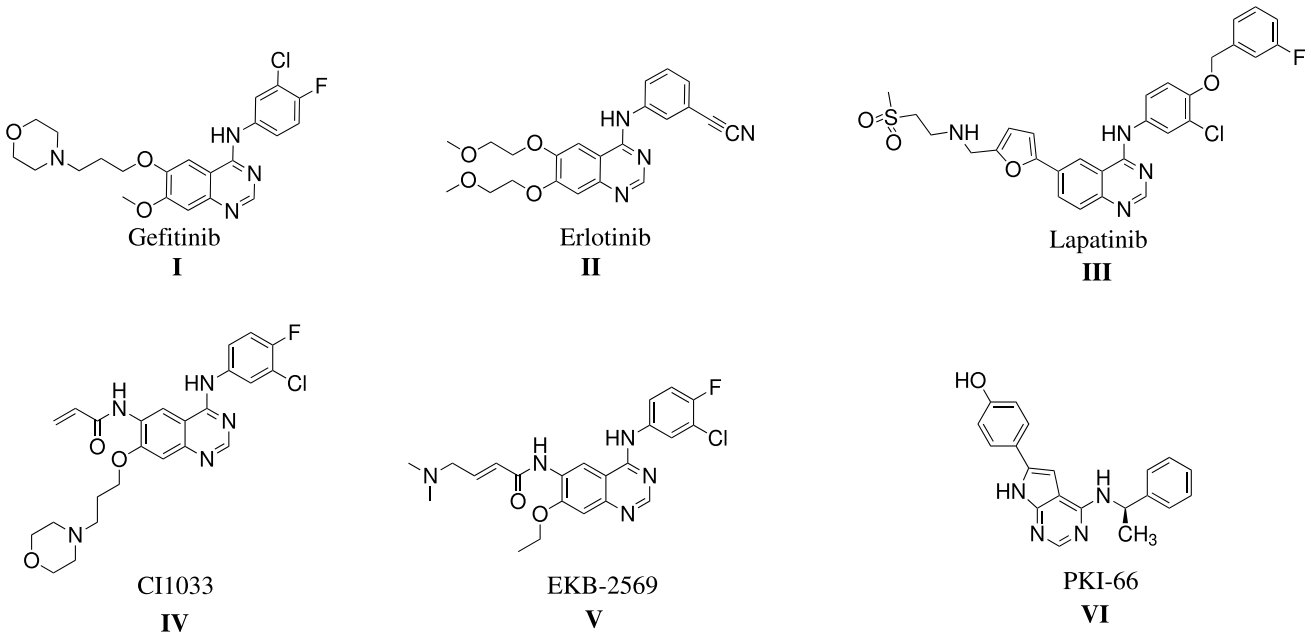


Figure 1. Chemical structure for anticancer biological evaluation drugs with quinazoline.

development of targeted antitumor therapies,[10] antidiabetic,[11] antimicrobial,[12] and anti-inflammatory drugs.[13] In 2017, Saddik *et al.*[14] synthesized a series of 5-amino-2-ethylthio-4-phenyl-6-substituted thieno[2,3-*d*]pyrimidine derivatives, and cell activity assay was carried out, finding that the synthesized compounds **VII** could effectively inhibit the activity of tumor cells. In 2018, Yong *et al.*[15] synthesized a series of thieno[2,3-*d*]pyrimidine derivatives by reacting 5-substituted-4-chlorothienopyrimidine and (3-(substituted-phenyl)-isoxazole-5-yl)-methanol in dried isopropyl alcohol with triethylamine as catalyst. Among them, 6-methyl-4-{[3-(4-chlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (**VIII**) was the most potent inhibitors of tumor cells. In 2022, Elmongy *et al.*[16] synthesized a series of thieno[2,3-*d*]pyrimidine derivatives, and monitored the inhibitory activity of the compound **IX** against breast cancer cells and non-small cell lung cancer, with inhibitory activity ranging from 43 to 87% (Figure 2).

A series of thieno[2,3-*d*]pyrimidine derivatives were synthesized in consideration of the important cytotoxic activity of thieno[2,3-*d*]pyrimidine skeleton compounds.

In this study, we are interested in the cytotoxic activity of the widely reported thieno[2,3-*d*]pyrimidine derivatives via different synthetic methods. There are two main synthesis routes of thieno[2,3-*d*]pyrimidine derivatives reported in the literature: (*i*) first, construct the thiophene ring, and then synthesize starting from the thiophene ring; (*ii*) synthesis from pyrimidine ring.[17-21] There are more research on the former, but relatively few on the latter. The first synthetic route was found: Tolan *et al.*[22] showed that the reaction of substituted 2-aminothiophene-3-carbonitrile (**X**) and substituted acetic acid (RCOOH) in 1,4-dioxane and phosphoryl trichloride afforded the corresponding scaffold **XI** (Figure 3). Isakhanyan *et al.*[23] introduced the synthesis of thieno[2,3-*d*] pyrimidine-4-one derivatives (**XIII**) using a cyclization reaction between 2-aminothiophene-3-carbonitrile (**XII**) and acyl chloride in 1,4-dioxane by refluxing in the presence of concentrated HCl in ethanol (Figure 4). The second synthetic route was found: Brough *et al.*[24] prepared 2-amino-4-chloro-thieno[2,3-*d*]pyrimidine-6-carboxylic acid ethyl ester (**XIV**) with 2-amino-4,6-dichloro-5-pyrimidine carbaldehyde (**XV**) and ethyl-2-mercaptoacetate as raw materials under alkaline conditions (Figure 5). Eissa *et al.*[25] reacted ethyl chloroacetate with 4-mercapto-2-methyl-sulfanyl-6-naphthalen-1-yl-pyrimidine-5-carbonitrile (**XVI**) to obtain an intermediate compound **XVII**, and

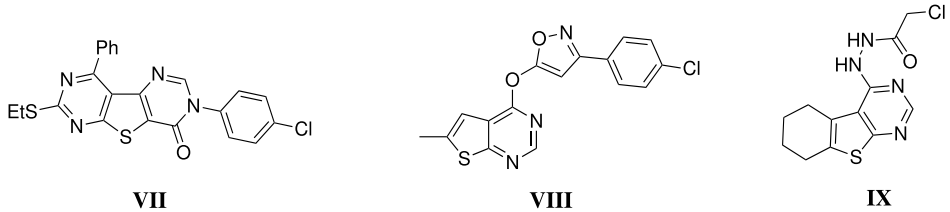


Figure 2. Thieno[2,3-*d*]pyrimidine derivatives in the literature.

5-amino-thieno[2,3-*d*]pyrimidine (**XVIII**) was obtained by intramolecular rearrangement under alkaline conditions (Figure 6).

In recent years, microwave-assisted organic synthesis has received increasing attention as an efficient and portable tool for drug synthesis. Microwave synthesis is suitable for a wide range of reactions, greatly reducing the reaction time, improving the reaction efficiency, and reducing the synthesis of by-products. Microwave irradiation technique can be used for the synthesis of thieno[2,3-*d*]pyrimidine derivatives.

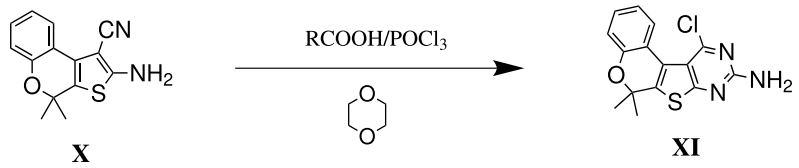
Interestingly, a compound with a similar structure (Figure 7, compound **XIX**) was developed as a novel anticancer drug with VEGFR-2 kinase inhibitory activity,[26] this compound is a pyridino[2,3-*d*]thieno pyrimidine scaffold, which is not extensively reported as anti-triple-negative breast cancer compounds. In this paper, a series of thieno[2,3-*d*]pyrimidine derivatives were designed and synthesized as potential anti-triple-negative breast cancer compounds based on bioisosteric modification strategy with the major aim to improve and enhance the drug activation properties by introducing highly polar groups.

Benzothieno[2,3-*d*]pyrimidine derivative (**XIX**, a novel VEGFR-2 inhibitor) was the lead compound. This series was designed to bear the essential pharmacodynamic fragments for anticancer activity, aiming at maintaining the same binding interaction between the N-1 nitrogen of the fused pyrimidine scaffolds and the hinge region NH. The structure (Figure 7) was modified: the five membered ring was replaced by hexatomic ring, and the nonpolar cyclohexyl moiety was replaced by its oxygenated isostere congener derivative. The substituted aromatic group was replaced by various substituting groups, which were either electron-withdrawing or electron-donating, resulting in compounds **a**-**w** (Scheme 1).

Two problems were solved in this study: (*i*) a series of thieno[2,3-*d*]pyrimidine derivatives **a-w** obtained by microwave irradiation technology (Figure 8); (*ii*) to evaluate the cytotoxic activity of the synthesized compounds against MDA-MB-231 cell.

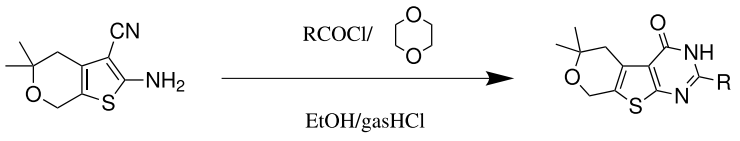
Results and Discussion

Scheme 1 summarizes the synthesis strategy used in this study. 2-Amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carbonitrile was synthesized by a modified Gewald



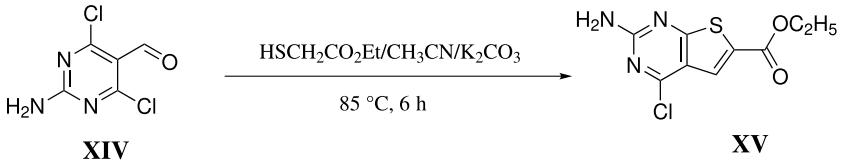
X **XI**

Figure 3. Route for synthesizing compound **XI** containing thieno[2,3-*d*]pyrimidine.



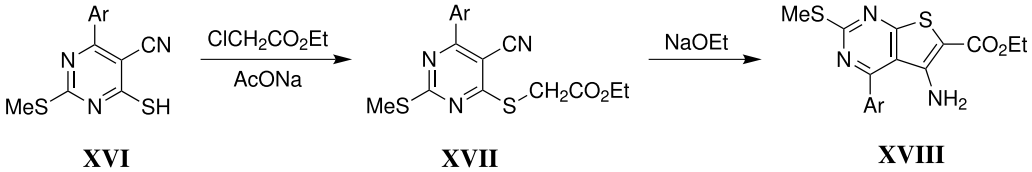
XII **XIII**

Figure 4. Route for synthesizing compound **XIII** containing thieno[2,3-*d*]pyrimidine.



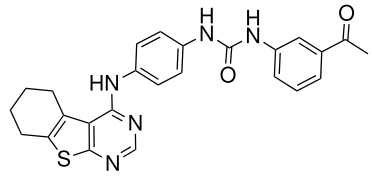
XIV **XV**

Figure 5. Route for synthesizing compound **XV** containing thieno[2,3-*d*]pyrimidine.



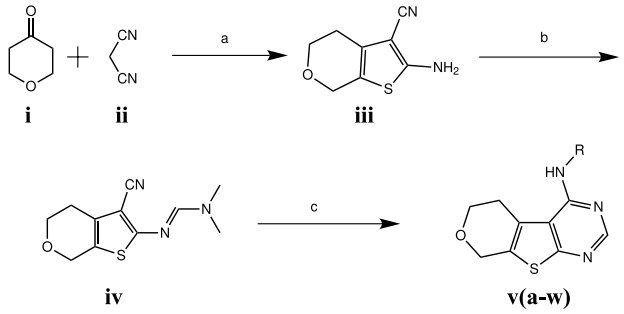
XVI **XVII** **XVIII**

Figure 6. Route for synthesizing compound **XVIII** containing thieno[2,3-*d*]pyrimidine.



XIX

Figure 7. Compound **XIX**.[26]



a: R = Ph
b: R = 2-MeC_6H_4
c: R = 3-MeC_6H_4
d: R = 4-MeC_6H_4
e: R = 2-MeOC_6H_4
f: R = 3-MeOC_6H_4
g: R = 4-MeOC_6H_4
h: R = 2-FC_6H_4
i: R = 3-FC_6H_4
j: R = 4-FC_6H_4
k: R = 2-ClC_6H_4
l: R = 3-ClC_6H_4
m: R = 3-BrC_6H_4
n: R = 4-BrC_6H_4
o: R = 3-NCC_6H_4
p: R = 3-CH≡CC_6H_4
q: R = 3-F_3CC_6H_4
r: R = 3-$(CH_3)_3$CC_6H_4
s: R = 3,4-$Cl_2C_6H_3$
t: R = 3,4-$F_2C_6H_3$
u: R = 3,5-$F_2C_6H_3$
v: R = 3-Cl-4-FC_6H_3
w: R = 2-Me-3-ClC_6H_3

Scheme 1. Synthetic route of target compounds **a-w**. Reagents and conditions: (a) aldehyde (**i**, 10 mmol), nitrile (**ii**, 10 mmol), sulfur (10 mmol), triethylamine (10 mmol), ethyl alcohol 10 mL, room temperature, 5 h (yield 75%); (b) **iii** (10 mmol), DMF-DMA (25 mmol), 70 °C and irradiation at 200 W, 20 min (yield 95%); (c) **iv** (4.25 mmol), amine (5.1 mmol), acetic acid (10 mL), 120 °C and irradiation at 200 W, 1 h.

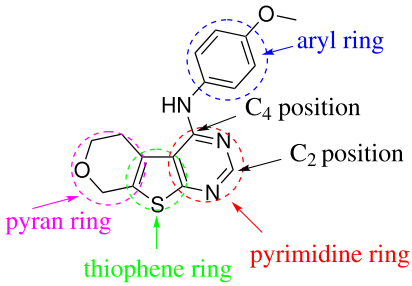


Figure 8. Main structure of synthesized compounds (for example: new compound **g**).

method using the alkali catalyst triethylamine (TEA) as a catalyst and pyranone, malononitrile, and sulfur in ethanol. In order to show the advantages of the current work, we will use triethylamine as base catalyst. Compared to other base catalysts, this method is inexpensive and environmentally friendly, no heating is required, and the reaction can be carried out at room temperature. The product precipitates naturally without the need to pass through the column due to the high polarity of the medium.

Regarding the synthesis of the intermediate compound **iv** (*N*'-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-yl) *N*,*N*-dimethylmethanimidamide), Zhan *et al.*[27] reported that the reaction of 2-amino-5-bromothiophene-3-carbonitrile and *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) were reacted to obtain (*E*)-*N*'-(5-bromo-3-cyanothiophen-2-yl)-*N*,*N*-dimethylformimidamide. In 2004, Yoon *et al.*[28] explored a parallel reaction under microwave conditions. Based on the above information, we can react 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carbonitrile with DMF-DMA under microwave irradiation to obtain compound **iv** in 95% yield. This method has short reaction time, high yield and simple post-treatment.

Compounds **a-w** (Table 1) were synthesized by acid-catalyzed Dimroth rearrangement between compound **iv** and a suitable aromatic amine under microwave radiation. The results showed that *p*-substituted aromatic amines reacted with compound **iv** in high yields. However, under the same reaction condition, *o*-substituted aromatic amines and compound **iv** could only form trace amounts of the desired compounds. The reaction yield of mesoaniline was slightly lower for halogen atoms (e.g., **g**, **h**, **i**). The weaker the electronegativity the easier the reaction is to carry out and the higher the yield (e.g., **g**, **h**, **i**). This new synthetic route involves only three steps, making the process simple and efficient. Therefore, the preparation of thieno[2,3-*d*] pyrimidine-4-amine by microwave reaction is convenient and in high yield. The proposed mechanism for this rearrangement **l** is described in Scheme 2.

The inhibitory effect of thienopyrimidine derivatives on different cancer cells was detected using the MTT method.[29] The MTT method can be referred to as the MTT colorimetric method, and its range of applications can be used to detect cell survival and growth. Once the cellular activity evaluation of 23 synthesized thienopyrimidine analogs was done, antitumor cellular activity tests of new compounds were performed to screen compounds with good targeting drug activity against triple-negative breast cancer cells (Table 2). Correspondingly, it was initially screened at a compound concentration of 50 μmol L^{-1} using paclitaxel (PTX) as a positive control, and compounds with similar or stronger results than PTX were further tested for their half maximal inhibitory concentration (IC_{50}).

By calculating and organizing the obtained data, compounds **a-w** were tested for biological activity against MDA-MB-231 at a concentration of 50 μmol L^{-1}, and it was found that all compounds of the series had different degrees of inhibitory effects on MDA-MB-231. Note that compounds with different substituents attached at different positions on the benzene ring resulted in significantly different rates of inhibition of cancer cells, suggesting

Table 1. Preparation of the compounds **a-w**

Compound	R	mp / °C	Yield[a] / %
a	Ph	157.0-158.0	50
b	2-MeC_6H_4	188.2-189.7	47
c[b]	3-MeC_6H_4	172.0-173.0	79
d	4-MeC_6H_4	174.2-175.5	62
e[b]	2-$MeOC_6H_4$	205.3-206.8	70
f[b]	3-$MeOC_6H_4$	130.0-131.2	83
g[b]	4-$MeOC_6H_4$	170.0-171.3	59
h[b]	2-FC_6H_4	219.8-221.4	73
i	3-FC_6H_4	162.2-163.1	41
j	4-FC_6H_4	190.6-191.9	84
k[b]	2-ClC_6H_4	170.9-171.7	29
l[b]	3-ClC_6H_4	168.5-169.7	59
m[b]	3-BrC_6H_4	186.6-188.1	70
n[b]	4-BrC_6H_4	188.9-190.0	58
o[b]	3-NCC_6H_4	233.3-234.7	23
p[b]	3-CH≡CC_6H_4	212.2-213.9	76
q[b]	3-$F_3CC_6H_4$	185.0-186.0	18
r[b]	3-$(CH_3)_3CC_6H_4$	182.6-184.0	18
s	3,4-$Cl_2C_6H_3$	183.5-184.7	63
t[b]	3,4-$F_2C_6H_3$	186.3-187.8	74
u[b]	3,5-$F_2C_6H_3$	197.6-199.0	34
v	3-Cl-4-FC_6H_3	219.9-221.5	75
w[b]	2-Me-3-ClC_6H_3	205.3-206.8	75

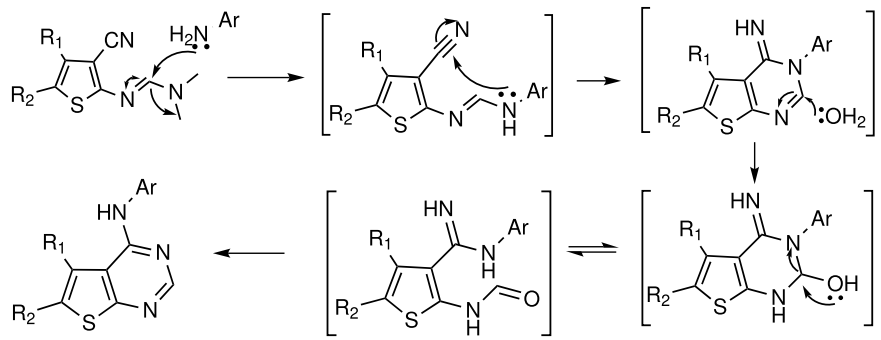
[a]Isolated yield; [b]new compounds. mp: melting point.

Table 2. Results of *in vitro* antitumor activity of compounds **a-w**

Compound	R	Inhibition rate (at 50 μM) / %	
		MDA-MB-231	PTX[a]
a	Ph	23.1	56.4
b	2-MeC_6H_4	57.0	59.0
c	3-MeC_6H_4	38.7	59.0
d	4-MeC_6H_4	24.9	59.0
e	2-$MeOC_6H_4$	33.0	54.3
f	3-$MeOC_6H_4$	37.3	54.3
g	4-$MeOC_6H_4$	33.7	54.3
h	2-FC_6H_4	44.7	54.3
i	3-FC_6H_4	25.1	59.0
j	4-FC_6H_4	41.5	59.0
k	2-ClC_6H_4	41.5	54.3
l	3-ClC_6H_4	58.3	54.3
m	3-BrC_6H_4	32.7	54.3
n	4-BrC_6H_4	34.5	59.0
o	3-NCC_6H_4	43.3	56.6
p	3-CH≡CC_6H_4	43.1	56.6
q	3-$F_3CC_6H_4$	40.4	54.3
r	3-$(CH_3)_3CC_6H_4$	20.8	54.3
s	3,4-$Cl_2C_6H_3$	30.7	54.3
t	3,4-$F_2C_6H_3$	54.0	55.3
u	3,5-$F_2C_6H_3$	11.2	54.3
v	3-Cl-4-FC_6H_3	45.4	54.3
w	2-Me-3-ClC_6H_3	47.3	55.3

[a]Used as a positive control (standard PTX). MDA-MB-231: human breast cancer cells. PTX: paclitaxel.

that the same substituents will have different activities depending on their position on the R benzene ring. Different substituents attached at the identical position pertaining to the R benzene ring exhibited a great difference in the inhibition rate of cancer cells. This indicates that different atoms attached at the same position pertaining to the R benzene ring possessed distinct inhibitory effects on MDA-MB-231. The anticancer activity of compound **l** was slightly higher than that of PTX, which is *meso*-substituted and carries substituents that are more polar halogens. It is hypothesized that its spatial configuration, as well as the substituents it carries, affect the inhibition rate of the compounds against MDA-MB-231 to some extent. Here, the compound **l** was measured for its IC_{50} value and calculated. The IC_{50} of **l** against MDA-MB-231 was obtained as 27.6 μM, while that of PTX against MDA-MB-231 was 29.3 μM, which was observed to be lower than that of PTX for **l** against MDA-MB-231.



Scheme 2. Proposed mechanism for the Dimroth rearrangement.

The cytotoxic activity of MDA-MB-231 was assessed for 23 synthesized compounds, leading to the derivation of the following preliminary structure-activity relationships:

(*i*) Compound **l** had the strongest cytotoxic activity against MDA-MB-231 cell line (IC_{50} = 27.6 μM). The cytotoxic activity of the compounds replaced by electron-withdrawing group (**l**, Ar = *m*-ClPh) is higher than that of the other compounds (IC_{50} = 29.3 μM), which may be due to the presence of p-π conjugation effect (**l**, Ar = *m*-ClPh).

(*ii*) All the synthesized compounds exhibited cytotoxic activity against MDA-MB-231 cell line at the drug concentration of 50 μM. Moreover, all the synthesized compounds showed significant selective cytotoxicity effects on MDA-MB-231.

Conclusions

Gewald, amination, and Dimroth rearrangement reactions were utilized in the synthesis of 23 thieno[2,3-*d*] pyrimidin-4-amines. Moreover, the effect of antitumor activity on breast cancer cell MDA-MB-231 was detected, and it was concluded that all compounds of the series had an inhibitory effect. Compound **l** was screened for stronger inhibition of breast cancer cell MDA-MB-231 than the positive control, and the series of derivatives can be further structurally optimized as well as investigated for mechanism of action to obtain potential anti-breast cancer cellular drugs for clinical studies.

Experimental

Every reagent as well as starting materials, except if noted otherwise, were purchased from MACKLIN unpurified (MACKLIN, Shanghai, China). All reported yields are the maximum observed, they can be up to 8% lower given slight variation in weighing reagents, solvent quality, and manipulation losses. Each melting points were measured using an uncorrected METTLER TOLEDO MP90 (Mettler Toledo, Zurich, Switzerland) melting point apparatus. Using tetramethylsilane (TMS) as the internal standard, ^{1}H nuclear magnetic resonance (NMR) as well as ^{13}C NMR spectra were documented on a Bruker AVANCE II HD 400 MHz (Bruker, Billerica, USA). Mass spectrometry (MS) was performed in an LCMS-2020 with electrospray ionization (ESI, Shimadzu, Kyoto, Japan). Additionally, via KBr pellets, infrared (IR) spectra were captured using a VERTEX 80/Raman II FTIR (Bruker, Karlsruhe, Germany) spectrometer. Meanwhile, a Triple TOF™ 5600+ (AB SCIEX, California, USA) was utilized to record the mass spectra. Next, the Anton Paar Microwave Synthesizer (Anton Paar, Shanghai, China) has been utilized to conduct the microwave-assisted reactions. By employing silica gel GF254 (HAIYANG, Qingdao, China), thin layer chromatography (TLC) was utilized to track the reactions. Further details are available in the Supplementary Information (SI) section.

Experimental data

Synthesis of 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carbonitrile (**iii**)

Pyrantel (1.00 g, 10 mmol), sulfur (0.32 g, 10 mmol), malononitrile (0.66 g, 10 mmol), as well as ethanol (10 mL) were incorporated into a 50 mL round-bottom flask. Triethylamine (1.01 g, 10 mmol) was then slowly introduced. Next, the reaction was mixed for 5 h at room temperature with TLC observation. It was filtered after the reaction. The filter cake was then washed with ethanol to obtain a crude product, which was recrystallized with ethanol to obtain the light pink product **iii**. After drying, it was weighed to be 1.35 g with a yield of 75%.

Synthesis of *N*'-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-*N*,*N*-dimethylmethanimidamide (**iv**)

Compound 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*] pyran-3-carbonitrile **iii** (1.80 g, 10 mmol), *N*,*N*-dimethyl-formamide dimethyl acetal DMF-DMA (4.17 mL, 25 mmol), and magnets were incorporated to a 30 mL microwave tube. Afterward, the reaction temperature was set to 70 °C. Note that the reaction time was set to 10 min as well as the microwave power was modified to 200 W. Once the reaction was concluded, the temperature was brought down to room level. Subsequently, it was streamed into 20 mL of ice water and filtered. Then, ice water was utilized to wash the filter cake to give the yellow solid **iv**. After drying, it was weighed to give 2.35 g in 95% yield.

Synthesis of thieno[2,3-*d*]pyrimidin-4-amine derivatives (**a-w**)

The yellow solid compound *N*'-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-*N*,*N*-dimethylmethanimidamide **iv** (1.00 g, 4.25 mmol), aniline with different substituent groups (5.1 mmol) and 10 mL of acetic acid as well as magnets were added in a 30 mL microwave tube. Next, they were all mixed well. The tube was put into a microwave synthesizer and set aside to react for 1 h. TLC was used to track the completion of the reaction. The majority of the acetic acid evaporated under lower pressure at the final stage of the reaction. They were then left to stand

overnight in a refrigerator. The target compounds **a-w** were filtered and washed with ether.

Structural characterization of compounds **a-w**

N-Phenyl-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**a**)

White solid, 0.60 g, 50%, mp 157.0-158.0 °C; IR (KBr) ν / cm^{-1} 3434, 3059, 2982, 2838, 1598, 1563, 1497, 1445, 1355, 977, 781, 756, 692; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (s, 1H), 8.20 (s, 1H), 7.66 (d, *J* 7.5 Hz, 2H), 7.36 (t, *J* 8.0 Hz, 2H), 7.10 (t, *J* 7.3 Hz, 1H), 4.83 (s, 2H), 3.97 (t, *J* 5.48 Hz, 2H), 3.23 (t, *J* 5.63 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 155.0, 152.4, 139.1, 130.6, 128.4, 124.6, 123.4, 122.2, 116.2, 64.7, 63.9, 25.9.

N-(*o*-Tolyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**b**)

White solid, 0.59 g, 48%, mp 188.2-189.7 °C; IR (KBr) ν / cm^{-1} 3403, 2987, 2826,1557, 1511, 1470, 1419, 1303, 977, 874, 754; ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.48 (s, 1H), 7.89 (d, *J* 8.0 Hz, 1H), 7.29 (t, *J* 7.9 Hz, 2H), 7.16 (t, *J* 7.3 Hz, 2H), 6.82 (s, 1H), 4.90 (t, *J* 2.0 Hz, 2H), 4.13 (t, *J* 5.5 Hz, 2H), 3.13-3.17 (m, 2H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 154.6, 152.2, 131.1, 124.4, 118.6, 118.5, 118.4, 117.0, 116.8, 116.2, 111.4, 111.1, 64.7, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{16}H_{16}N_3OS$ $[M + H]^+$: 298, found: 298.

N-(*m*-Tolyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**c**)

White solid, 1.00 g, 79%, mp 172.0-173.0 °C; IR (KBr) ν / cm^{-1} 3457, 2978, 2846, 2822, 1617, 1573, 1514, 1485, 1375, 973, 886, 781, 690; ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.52 (s, 1H), 7.46 (d, *J* 7.4 Hz, 1H), 7.42 (s, 1H), 7.28 (t, *J* 7.7 Hz, 1H), 6.97 (t, *J* 7.6 Hz, 2H), 4.89 (t, *J* 2.0 Hz, 2H), 4.12 (t, *J* 5.5 Hz, 2H), 3.16-3.24 (m, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 155.0, 152.4, 139.0, 137.7, 130.6, 128.3, 124.6, 124.2, 122.6, 119.2, 116.2, 64.7, 63.9, 25.9, 21.2; MS (ESI) *m/z*, calcd. for $C_{16}H_{16}N_3OS$ $[M + H]^+$: 298, found: 298.

N-(*p*-Tolyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**d**)

White solid, 0.78 g, 62%, mp 174.2-175.5 °C; IR (KBr) ν / cm^{-1} 3445, 2974, 2861, 2844, 1608, 1565, 1507, 1439, 973, 872, 812, 783, 690; ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.48 (s, 1H), 7.48 (d, *J* 8.3 Hz, 2H), 7.19 (d, *J* 8.7 Hz, 2H), 6.90 (s, 1H), 4.84 (t, *J* 1.8 Hz, 1H), 4.10 (t, *J* 5.5 Hz, 2H), 3.13 (t, *J* 5.3 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 155.2, 152.4, 136.4, 132.6, 130.4, 128.8, 124.6, 122.4, 116.0, 64.7, 63.9, 26.0, 20.5; MS (ESI) *m/z*, calcd. for $C_{16}H_{16}N_3OS$ $[M + H]^+$: 298, found: 298.

N-(2-Methoxyphenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**e**)

White solid, 1.10 g, 83%, mp 205.3-206.8 °C; IR (KBr) ν / cm^{-1} 3445, 2974, 2861, 2844, 1608, 1565, 1507, 1439, 973, 872, 812, 783, 690; ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 154.4, 152.6, 149.0, 131.1, 128.1, 123.8, 123.4, 120.8, 120.5, 116.0, 110.9, 64.6, 63.8, 56.2, 25.8; MS (ESI) *m/z*, calcd. for $C_{16}H_{16}N_3O_2S$ $[M + H]^+$: 314, found: 314.

N-(3-Methoxyphenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**f**)

Yellow solid, 0.78 g, 59%, mp 130.0-131.2 °C; IR (KBr) ν / cm^{-1} 3447, 2995, 2978, 2870, 2832, 1602, 1563, 1516, 1454, 1280, 998, 876, 748; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.16 (s, 1H), 7.32-7.35 (m, 1H), 7.26 (d, *J* 5.5 Hz, 2H), 6.64-6.71 (m, 1H), 4.84 (s, 2H), 3.98 (t, *J* 5.6 Hz, 2H), 3.76 (s, 3H), 3.20-3.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 159.9, 155.4, 152.8, 140.7, 131.2, 129.6, 125.0, 116.8, 114.6, 109.1, 108.2, 65.2, 64.3, 55.5, 26.3; MS (ESI) *m/z*, calcd. for $C_{16}H_{16}N_3O_2S$ $[M + H]^+$: 314, found: 314.

N-(4-Methoxyphenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**g**)

White solid, 0.94 g, 71%, mp 170.0-171.3 °C; IR (KBr) ν / cm^{-1} 3459, 2984, 2921, 2828, 1598, 1561, 1501, 1443, 1256, 977, 874, 824, 775, 701; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.07 (s, 1H), 7.50 (d, *J* 9.0 Hz, 2H), 6.94 (d, *J* 9.2 Hz, 2H), 4.83 (s, 2H), 3.97 (t, *J* 4.0 Hz, 2H), 3.76 (s, 3H), 3.21 (t, *J* 5.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 156.4, 155.8, 153.0, 132.2, 130.6, 125.2, 125.0, 116.1, 114.0, 65.2, 64.3, 55.6, 26.4; MS (ESI) *m/z*, calcd. for $C_{16}H_{16}N_3O_2S$ $[M + H]^+$: 314, found: 314.

N-(2-Fluorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**h**)

Yellow solid, 0.53 g, 41%, mp 219.8-221.4 °C; IR (KBr) ν / cm^{-1} 3436, 2980, 2910, 2824, 1596, 1559, 1501, 1435, 1346, 1235, 975, 827, 746, 697; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (s, 1H), 8.21 (s, 1H), 7.80-7.88 (m, 1H), 7.31-7.39 (m, 3H), 4.84 (s, 2H), 4.00 (t, *J* 5.5 Hz, 2H), 3.19 (t, *J* 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 155.2, 152.5, 130.8, 127.0, 126.6, 126.2, 124.4, 124.3, 116.0, 115.6, 115.4, 64.6, 63.8, 26.0; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}FN_3OS$ $[M + H]^+$: 302, found: 302.

N-(3-Fluorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**i**)

Yellow solid, 1.08 g, 84%, mp 162.2-163.1 °C; IR (KBr) ν / cm^{-1} 3445, 2972, 2879, 2844, 1606, 1582, 1505, 1448, 1280, 979, 864, 680; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.34 (s, 1H), 7.67 (dt, *J* 11.9, 2.4 Hz, 1H), 7.46 (d, *J* 9.2 Hz, 1H), 7.38-7.46 (m, 1H), 6.90 (td, *J* 8.5, 2.7 Hz, 1H), 4.84 (s, 2H), 4.04 (q, *J* 8.2 Hz, 2H), 3.23 (t, *J* 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 154.6, 152.2, 141.1, 141.0, 131.2, 130.0, 124.6, 117.4, 116.6, 109.6, 108.2, 64.8, 63.9, 25.8; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}FN_3OS$ $[M + H]^+$: 302, found: 302.

N-(4-Fluorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**j**)

White solid, 0.39 g, 31%, mp 190.6-191.9 °C; IR (KBr) ν / cm^{-1} 3409, 3067, 2925, 2840, 1611, 1561, 1503, 1441, 1282, 977, 876, 833, 777, 692; ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.49 (s, 1H), 7.52-7.56 (m, 2H), 7.09-7.17 (m, 2H), 6.92 (s, 1H), 4.88 (t, *J* 2.0 Hz, 2H), 4.11 (t, *J* 5.5 Hz, 2H), 3.14-3.22 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 159.7, 157.3, 155.0, 152.4, 135.2, 130.6, 124.6, 116.0, 115.1, 64.7, 63.8, 26.0; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}FN_3OS$ $[M + H]^+$: 302, found: 302.

N-(2-Chlorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**k**)

White solid, 0.80 g, 59%, mp 170.9-171.7 °C; IR (KBr) ν / cm^{-1} 3428, 3048, 3007, 2844, 1594, 1565, 1499, 1445, 1297, 1086, 990, 789, 738, 684; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.20 (s, 1H), 8.07 (dd, *J* 8.1, 1.6 Hz, 1H), 7.55 (dd, *J* 8.0, 1.5 Hz, 1H), 7.40 (td, *J* 7.7, 1.5 Hz, 1H), 7.23 (td, *J* 7.7, 1.6 Hz, 1H), 4.84 (t, *J* 1.9 Hz, 2H), 4.01 (t, *J* 5.5 Hz, 2H), 3.22-3.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 155.0, 152.4, 135.8, 131.0, 129.4, 127.6, 127.5, 126.2, 126.0, 124.2, 116.0, 64.6, 63.8, 26.2; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}ClN_3OS$ $[M + H]^+$: 318, found: 318.

N-(3-Chlorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**l**)

Yellow solid, 1.08 g, 80%, mp 168.5-169.7 °C; IR (KBr) ν / cm^{-1} 3416, 2987, 2933, 2838, 1600, 1557, 1503, 1478, 1095, 979, 876, 864, 682; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.33 (s, 1H), 7.84 (t, *J* 2.1 Hz, 1H), 7.64 (d, *J* 8.1 Hz, 1H), 7.37 (t, *J* 8.1 Hz, 1H), 7.13 (d, *J* 8.5 Hz, 1H), 4.84 (t, *J* 2.1 Hz, 2H), 3.98 (t, *J* 5.5 Hz, 2H), 3.23-3.31 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 154.6, 152.2, 140.7, 132.7, 131.2, 130.0, 124.6, 122.8, 121.1, 120.2, 116.5, 64.7, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}ClN_3OS$ $[M + H]^+$: 318, found: 318.

N-(3-Bromophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**m**)

Yellow solid, 0.90 g, 58%, mp 186.6-188.1 °C; IR (KBr) ν / cm^{-1} 3418, 2991, 2927, 2832, 1600, 1555, 1505, 1445, 1268, 1091, 992, 874, 857, 682; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.31 (s, 1H), 7.96 (t, *J* 1.9 Hz, 1H), 7.69 (d, *J* 8.1 Hz, 1H), 7.31 (t, *J* 8.0 Hz, 1H), 7.26 (d, *J* 8.0 Hz, 1H), 4.84 (s, 2H), 3.98 (t, *J* 5.5 Hz, 2H), 3.24 (d, *J* 5.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 154.5, 152.2, 140.8, 131.2, 130.3, 125.7, 124.5, 124.0, 121.2, 120.6, 116.5, 64.7, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}BrN_3OS$ $[M + H]^+$: 362; 364, found: 362; 364.

N-(4-Bromophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**n**)

Yellow solid, 0.27 g, 18%, mp 188.9-190.0 °C; IR (KBr) ν / cm^{-1} 3459, 2976, 2877, 2838, 1604, 1557, 1503, 1437, 1070, 971, 880, 822, 777, 690; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H), 8.29 (s, 1H), 7.65 (d, *J* 9.0 Hz, 2H), 7.53 (d, *J* 8.7 Hz, 2H), 4.84 (s, 2H), 3.97 (t, *J* 5.6 Hz, 2H), 3.19-3.25 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 154.6, 152.2, 138.6, 131.2, 131.0, 124.6, 123.8, 116.4, 115.0, 64.7, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{15}H_{13}BrN_3OS$ $[M + H]^+$: 362; 364, found: 362; 364.

3-((5,8-Dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-yl)amino)-benzonitrile (**o**)

Yellow solid, 1.00 g, 76%, mp 233.3-234.7 °C; IR (KBr) ν / cm^{-1} 3436, 2976, 2939, 2836, 2225, 1613, 1559, 1511, 1485, 1303, 983, 862, 672; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.44 (s, 1H), 8.16 (s, 1H), 7.98 (d, *J* 7.7 Hz, 1H), 7.47-7.60 (m, 2H), 4.84 (s, 2H), 3.98 (t, *J* 5.5 Hz, 2H), 3.23 (t, *J* 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 154.4, 152.2, 140.2, 131.4, 129.8, 126.6, 126.4, 124.6, 124.5, 118.8, 116.6, 111.2, 64.7, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{16}H_{12}N_4OS$ $[M + H]^+$: 309.05, found: 309.08.

N-(3-Ethynylphenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**p**)

Yellow solid, 0.25 g, 19%, mp 212.2-213.9 °C; IR (KBr) ν / cm^{-1} 3453, 3277, 3053, 2978, 2840, 1604, 1553, 1499, 1470, 1448, 977, 876, 789, 680; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 8.26 (s, 1H), 7.83 (s, 1H), 7.73 (d, *J* 8.2 Hz, 1H), 7.36 (t, *J* 7.9 Hz, 1H), 7.19 (d, *J* 7.7 Hz, 1H), 4.83 (s, 2H), 4.20 (s, 1H), 3.97 (t, *J* 5.6 Hz, 2H), 3.3 (t, *J* 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 155.8, 152.7, 137.4, 133.7, 130.3, 130.0, 126.4, 126.1, 125.6, 124.6, 115.6, 64.7, 64.0, 63.8, 26.2, 18.0; MS (ESI) *m/z*, calcd. for $C_{17}H_{14}N_3OS$ $[M + H]^+$: 308.05, found: 308.09.

N-(3-(Trifluoromethyl)phenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**q**)

Yellow solid, 0.54 g, 36%, mp 185-186 °C; IR (KBr) ν / cm^{-1} 3430, 3077, 2984, 2869, 1598, 1563, 1518, 1450, 1328, 1120, 965, 800, 662; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 2H), 8.07 (s, 1H), 8.00 (d, *J* 8.1 Hz, 1H), 7.58 (t, *J* 7.9 Hz, 1H), 7.42 (d, *J* 7.8 Hz, 1H), 4.84 (s, 2H), 3.98 (t, *J* 5.6 Hz, 2H), 3.25 (t, *J* 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 154.6, 152.2, 140.0, 131.2, 129.5, 129.1, 125.6, 124.6, 122.8, 119.4, 117.9, 116.6, 64.8, 63.9, 25.8; MS (ESI) *m/z*, calcd. for $C_{16}H_{13}F_3N_3OS$ $[M + H]^+$: 352.00, found: 352.07.

N-(3-(*tert*-Butyl)phenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**r**)

White solid, 0.94 g, 65%, mp 182.6-184 °C; IR (KBr) ν / cm^{-1} 3457, 3024, 2980, 2958, 2852, 1611, 1559, 1489, 1439, 1301, 948, 855, 796, 690; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39 (s, 1H), 8.17 (s, 1H), 7.59 (t, *J* 2.0 Hz, 1H), 7.54 (d, *J* 7.9 Hz, 1H), 7.28 (t, *J* 7.9 Hz, 1H), 7.13 (d, *J* 7.8 Hz, 1H), 4.83 (s, 2H), 3.98 (t, *J* 5.5 Hz, 2H), 3.24 (t, *J* 5.6 Hz, 2H), 1.30 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 155.2, 152.4, 151.1, 138.7, 130.5, 128.0, 124.6, 120.5, 119.8, 119.2, 116.2, 64.7, 63.9, 34.4, 31.2, 25.9; MS (ESI) *m/z*, calcd. for $C_{19}H_{22}N_3OS$ $[M + H]^+$: 340.10, found: 340.15.

N-(3,4-Dichlorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**s**)

Yellow solid, 1.01 g, 67%, mp 183.5-184.7 °C; IR (KBr) ν / cm^{-1} 3457, 3070, 2972, 2850, 1594, 1555, 1495, 1445, 979, 1093, 808, 785, 738, 697; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (s, 1H), 8.38 (s, 1H), 8.02 (d, *J* 2.6 Hz, 1H), 7.69 (dd, *J* 8.8, 2.6 Hz, 1H), 7.58 (d, *J* 8.8 Hz, 1H), 4.84 (s, 2H), 3.97 (t, *J* 5.6 Hz, 2H), 3.25 (t, *J* 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 154.2, 152.2, 139.4, 131.4, 130.6, 130.2, 124.5, 124.4, 122.8, 121.8, 116.6, 64.7, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{15}H_{12}Cl_2N_3OS$ $[M + H]^+$: 351.95, found: 352.00.

N-(3,4-Difluorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**t**)

Yellow solid, 0.45 g, 33%, mp 186.3-187.8 °C; IR (KBr) ν / cm^{-1} 3440, 3063, 2970, 2828, 1637,1580, 1514, 1443, 1278, 1157, 981, 864, 802, 775, 692; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 1H), 8.04 (s, 1H), 7.54 (d, *J* 6.4 Hz, 1H), 7.28 (d, *J* 7.3 Hz, 1H), 7.24 (t, *J* 7.4 Hz, 1H), 7.16 (t, *J* 7.3 Hz, 1H), 4.83 (s, 2H), 3.99 (t, *J* 5.5 Hz, 1H), 3.20 (t, *J* 3.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 155.8, 152.7, 137.4, 133.7, 130.3, 130.0, 126.4, 125.6, 124.6, 115.6, 64.7, 64.0, 26.2, 18.0; MS (ESI) *m/z*, calcd. for $C_{15}H_{12}F_2N_3OS$ $[M + H]^+$: 320.05, found: 320.07.

N-(3,5-Difluorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**u**)

White solid, 1.07 g, 79%, mp 197.6-199 °C; IR (KBr) ν / cm^{-1} 3453, 3119, 2974, 2894, 1606, 1577, 1565, 1474, 1318, 1157, 990, 822, 659; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.46 (s, 1H), 7.50 (dd, *J* 9.9, 2.3 Hz, 2H), 6.89 (t, *J* 9.3 Hz, 1H), 4.84 (s, 2H), 3.97 (t, *J* 5.6 Hz, 2H), 3.22 (t, *J* 5.5 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 163.6, 161.2, 154.1, 152.1, 142.0, 131.7, 124.4, 116.8, 104.0, 103.8, 97.8, 64.8, 63.8, 25.8; MS (ESI) *m/z*, calcd. for $C_{15}H_{12}F_2N_3OS$ $[M + H]^+$: 320.05, found: 320.07.

N-(3-Chloro-4-fluorophenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**v**)

Yellow solid, 0.77 g, 54%, mp 219.9-221.5 °C; IR (KBr) ν / cm^{-1} 3385, 3055, 2974, 2834, 1598, 1577, 1495, 1441, 1266, 1202, 1091, 994, 876, 843, 781, 701, 686; ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.53 (s, 1H), 7.84 (dd, *J* 6.4, 2.7 Hz, 1H), 7.45 (ddd, *J* 8.9, 4.1, 2.7 Hz, 1H), 7.15 (t, *J* 8.7 Hz, 1H), 6.89 (s, 1H), 4.89 (t, *J* 2.0 Hz, 2H), 4.13 (t, *J* 5.5 Hz, 2H), 3.14-3.22 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 154.6, 152.2, 136.3, 131.0, 124.5, 123.8, 122.8, 118.8, 118.7, 116.6, 116.2, 64.7, 63.8, 25.9; MS (ESI) *m/z*, calcd. for $C_{15}H_{12}ClFN_3OS$ $[M + H]^+$: 336, found: 336.

N-(3-Chloro-2-methylphenyl)-5,8-dihydro-6*H*-pyrano[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-amine (**w**)

Yellow solid, 0.93 g, 66%, mp 205.3-206.8 °C; IR (KBr) ν / cm^{-1} 3461, 3170, 3081, 2966, 2850, 1639, 1580, 1501, 1443, 1285, 1016, 971, 822, 746, 707; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28 (s, 2H), 7.39 (dd, *J* 17.0, 7.9 Hz, 2H), 7.26 (t, *J* 7.9 Hz, 1H), 4.83 (s, 2H), 3.99 (t, *J* 5.6 Hz, 2H), 3.20 (t, *J* 5.6 Hz, 2H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 159.4, 156.0, 152.8, 139.4, 133.9, 133.0, 130.5, 127.2, 126.3, 124.9, 115.8, 64.9, 64.1, 26.4, 15.6; MS (ESI) *m/z*, calcd. for $C_{16}H_{15}ClN_3OS$ $[M + H]^+$: 332.00, found: 332.06.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Yue Guo was responsible for conceptualization, methodology, formal analysis, investigation, writing-original draft, data curation, conceptualization; Chenglu Qian for conceptualization, resources, writing-review and editing, conceptualization, validation; Rongrong Yang and Zhongfei Han for experiment and analysis (data curation, investigation); Guoxiang Sun for conceptualization, supervision, writing-review and editing, resources, software, validation, visualization; Yaquan Sun for conceptualization, supervision, writing-review and editing, resources, project administration, formal analysis funding acquisition.

References

1. Cox, T. R.; *Nat. Rev. Cancer*. **2021**, *21*, 217. [Crossref]
2. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Parkin, D. M.; Pineros, M.; Znaor, A.; Bray, F.; *Int. J. Cancer*. **2021**, *149*, 778. [Crossref]
3. Mohanty, S. S.; Sahoo, C. R.; Padhy, R. N.; *Genes Dis*. **2022**, *9*, 648. [Crossref]
4. Gao, J. J.; Swain, S. M.; *Oncologist* **2018**, *23*, 556. [Crossref]
5. Tran, B.; Bedard, P. L.; *Breast Cancer Res*. **2011**, *13*, 221. [Crossref]
6. Goldner, M.; Pandolfi, N.; Maciel, D.; Lima, J.; Sanches, S.; Ponde, N.; *Expert Rev. Anticancer Ther.* **2021**, *21*, 1237. [Crossref]
7. Yang, R. N.; Li, Y. Y.; Wang, H.; Qin, T. L.; Yin, X. M.; Ma, X. L.; *Mol. Biomed.* **2022**, *3*, 8. [Crossref]
8. Ren, X. L.; Wang, X.; Li, Z. Y.; *Zhongguo Xinyao Zazhi.* **2005**, *14*, 821. [Crossref]
9. Othman, I. M.; Gad-Elkareem, M. A.; Snoussi, M.; Aouadi, K.; Kadri, A.; *J. Mol. Struct.* **2020**, *1219*, 128651. [Crossref]
10. Wang, R. F.; Yu, S. J.; Zhao, X. X.; Chen, Y. X.; Yang, B. W.; Wu, T. X.; Hao, C. Z.; Zhao, D. M.; Cheng, M. S.; *Eur. J. Med. Chem.* **2020**, *188*, 112024. [Crossref]
11. Nagaraju, B.; Rajeswari, M.; Vedasree, N.; Rao, C. A.; Srinivasa, V. R.; Prabha, T.; Rao, C. V.; Maddila, S.; *ChemistrySelect* **2023**, *8*, e202303786. [Crossref]
12. Tolba, M. S.; Sayed, A. M.; Sayed, M.; Ahmed, M.; *J. Mol. Struct*. **2021**, *1246*, 131179. [Crossref]
13. Chen, L. Z.; Shu, H. Y.; Wu, J.; Yu, Y. L.; Ma, D.; Huang, X.; Liu, M. M.; Liu, X. H.; Shi, J. B.; *Eur J. Med. Chem*. **2021**, *213*, 113174. [Crossref]
14. Saddik, A. A.; Kamal El-Dean, A. M.; El-Sokary, G. H.; Hassan, K. M.; Abbady, M. S.; Ismail, I. A.; Saber, S. H.; *J. Chin. Chem. Soc.* **2017**, *64*, 87. [Crossref]
15. Yong, J. P.; Lu, C. Z.; Wu, X. Y.; *Lett. Drug Des. Discovery* **2018**, *15*, 463. [Crossref]
16. Elmongy, E. I.; Attallah, N. G.; Altwaijry, N.; AlKahtani, M. M.; Henidi, H. A.; *Molecules* **2021**, *27*, 123. [Crossref]
17. Yu, M.; Zeng, M. H.; Pan, Z. P.; Wu, F. B.; Guo, L.; He, G.; *Eur. J. Med. Chem.* **2020**, *189*, 112076. [Crossref]
18. Häcker, H.-G.; de la Haye, A.; Sterz, K.; Schnakenburg, G.; Wiese, M.; Gütschow, M.; *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6102. [Crossref]
19. Catherine, T.; Karanewsky, D. S.; Tang, X. Q.; Li, X. D.; Zhang, F.; Guy, S.; Chen, Q.; Vincent, D.; Richard, F.; Joseph, F.; *US pat.* 20110224155A1, **2011**.
20. Saleeb, M.; Sundin, C.; Aglar, Ö.; Pinto, A. F.; Ebrahimi, M.; Forsberg, Å.; Schüler, H.; Elofsson, M.; *Eur. J. Med. Chem.* **2018**, *143*, 568. [Crossref]
21. Abaee, M. S.; Hadizadeh, A.; Mojtahedi, M. M.; Halvagar, M. R.; *Tetrahedron Lett*. **2017**, *58*, 1408. [Crossref]
22. Tolan, H. E. M.; Shamroukh, A. H.; Rashad, A. E.; Hegab, M. I.; *Pharma Chem*. **2017**, 9. [Link] accessed in October 2024
23. Isakhanyan, A.; Hakobyan, N.; Panosyan, H.; Harutyunyan, A.; *Russ. J. Org. Chem.* **2023**, *59*, 1489. [Crossref]
24. Brough, P. A.; Barril, X.; Borgognoni, J.; Chene, P.; Davies, N. G.; Davis, B.; Drysdale, M. J.; Dymock, B.; Eccles, S. A.; Garcia-Echeverria, C.; Fromont, C.; Hayes, A.; Hubbard, R. E.; Jordan, A. M.; Jensen, M. R.; Massey, A.; Merrett, A.; Padfield, A.; Parsons, R.; Radimerski, T.; Raynaud, F. I.; Robertson, A.; Roughley, S. D.; Schoepfer, J.; Simmonite, H.; Sharp, S. Y.; Surgenor, A.; Valenti, M.; Walls, S.; Webb, P.; Wood, M.; Workman, P.; Wright, L.; *J. Med. Chem.* **2009**, *52*, 4794. [Crossref]
25. Eissa, A. E.; *Chin. J. Chem.* **2008**, *26*, 1509. [Crossref]
26. Aziz, M. A.; Serya, R. A.; Lasheen, D. S.; Abdel-Aziz, A. K.; Esmat, A.; Mansour, A. M.; Singab, A. N. B.; Abouzid, K. A. M.; *Sci. Rep.* **2016**, *6*, 24460. [Crossref]
27. Zhan, D. M.; Li, S. Y.; Zhao, H. L.; Lan, M. B.; *Chin. J. Org. Chem.* **2011**, *31*, 207. [Crossref]
28. Yoon, D. S.; Han, Y.; Stark, T. M.; Haber, J. C.; Gregg, B. T.; Stankovich, S. B.; *Org. Lett.* **2004**, *6*, 4775. [Crossref]
29. Grela, E.; Kozłowska, J.; Grabowiecka, A.; *Acta Histochem.* **2018**, *120*, 303. [Crossref]

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