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Synthesis and Preliminarily Cytotoxicity to A549, HCT116 and MCF-7 Cell Lines of Thieno[2,3-d]pyrimidine Derivatives Containing Isoxazole Moiety

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Abstract: Under the guidance of our previous achievements, and in order to extend this small molecular library. In current work, other 21 novel structures of thieno[2,3-d]pyrimidines containing isoxazole-moiety (1a-1u) were firstly synthesized and the cytotoxicity to A549, HCT116 and MCF-7 cell lines was evaluated using the MTT method. The results showed that most target compounds exhibited good to excellent cytotoxicity to A549, HCT116 and MCF-7 cell lines, especially 6-Methyl-4-{[3-(4-chlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-d]-pyrimidine (1e) exhibited a broad-spectrum and the most potent cytotoxicity to A549, HCT116 and MCF-7 cell lines (IC50s: 2.79, 6.69 and 4.21×10^{-3} μM, respectively) as compared with the reference drug gefitinib (IC50s: 17.90, 21.55 and 20.68 μM, respectively). 1e can be seen as the best drug candidates for development of anticancer drugs.

Key words: Isoxazole moiety; Thieno[2,3-d]pyrimidine derivatives; Synthesis; Anticancer activity

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1. Introduction

Cancer is the leading cause of death after cardiovascular disease, and the relative mortality rate caused by cancer is still very high even in developed countries, statically evident around 6-7 million new cases per year [1]. Lung cancer is the number one cause of cancer related death globally, with an estimated 13% of total cases and accounting for 18% of total deaths worldwide in 2008[2]. In 2010, there were 222,520 new lung cancer patients diagnosed and 157,300 died [3]. However, the non-small cell lung cancer (NSCLC) is usually diagnosed in advanced stages and accounts for 85% of all lung cancer diagnoses in the United States [4]. In China, it is reported that lung cancer has replaced the liver cancer as the number one cause of death among people with malignant tumors in 2008 [5].

Breast cancer is also the most common cancer among American women. It was reported that one in every eight women in the United States was found to have breast cancer. According to American Cancer Society, nearly 40,000 women had died of breast cancer in 2011, and about 232,340 new cases of invasive breast cancer will be diagnosed each year. Thus, cancer has been a great threat to human survival, which prompts us to search for new drugs for treatment of cancer.

Since 1994, Fry et al. found 4-anilino quinazoline(PD153035) as a specific EGFR-TKI. Quinazoline derivatives have been regarded as one of important drug scaffolds in the field of anticancer discovery. For many years, different class of quinazolines have been reported and some of them have been used in clinical or in clinical trails as epidermal growth factor receptor tyrosine kinase inhibitors

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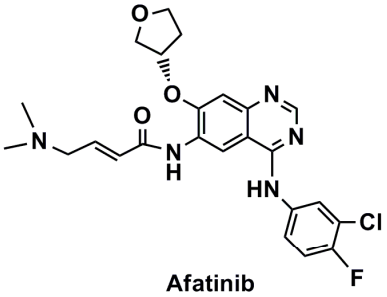
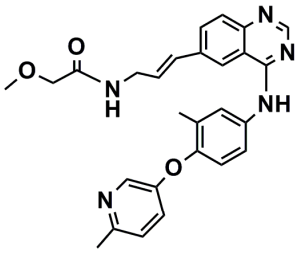
(EGFR-TKIs) (**Fig. 1**).



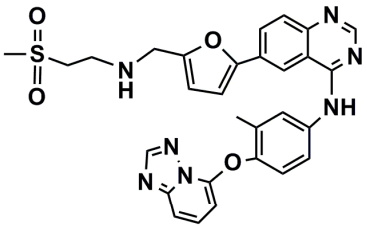
Afatinib

Targets:EGFR,EGFR mutant forms, HER2
IC_{50}s: 0.5nM (EGFR(wt)),0.4nM EGFR(L858R), 10nM EGFR(L858R/T790M) ,14nM(HER2).[6]



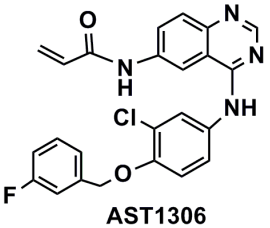
CP-724714

Target: HER2
IC_{50}: 10 nM [7]



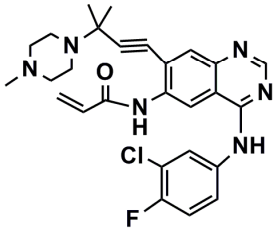
ARRY380

Target:HER2
IC_{50} : 8 nM [8]



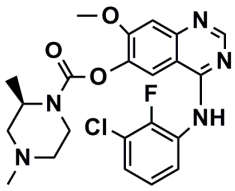
AST1306

Targets: EGFR,Erb2;
and EGFR mutant T790M/L858R
IC_{50}s: 0.5 nM (EGFR) and 3 nM (HER2) [9]



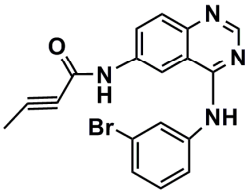
AV-412

Targets: EGFR, ErbB2 and EGFR mutant L858R/T790M
IC_{50}s: 0.51 nM (EGFR L858R); 0.79 nM (EGFRT790M); 19 nM (ErbB2) [10]



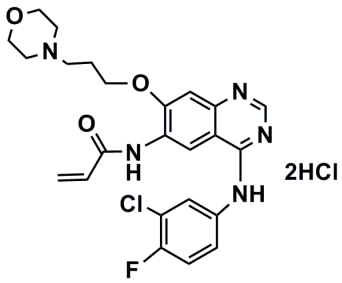
AZD3759

Target: EGFR
IC_{50}s: 0.3 nM (EGFRTK wild type); 0.2 nM (L858R mutant); 0.2 nM (Exon 19Del enzymes) [11]



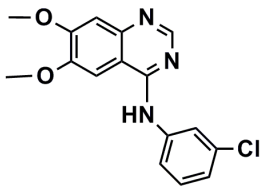
CL-387785

Target: EGFR
IC_{50}: 120~370 pM [12]



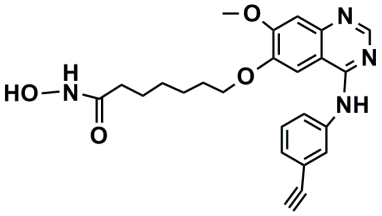
Canertinib dihydrochloride

Targets: EGFR, HER2
IC_{50}s: 1.5nM(EGFR) 0.9nM(HER2) [13]



AG1478

Target:EGFR
IC_{50} : 3 nM [14]



CUDC-101

Targets: EGFR, HER2, HDAC
IC_{50}s: 4.4 nM(HDAC),2.4 nM(EGFR), 15.7 nM(HER2) [15]

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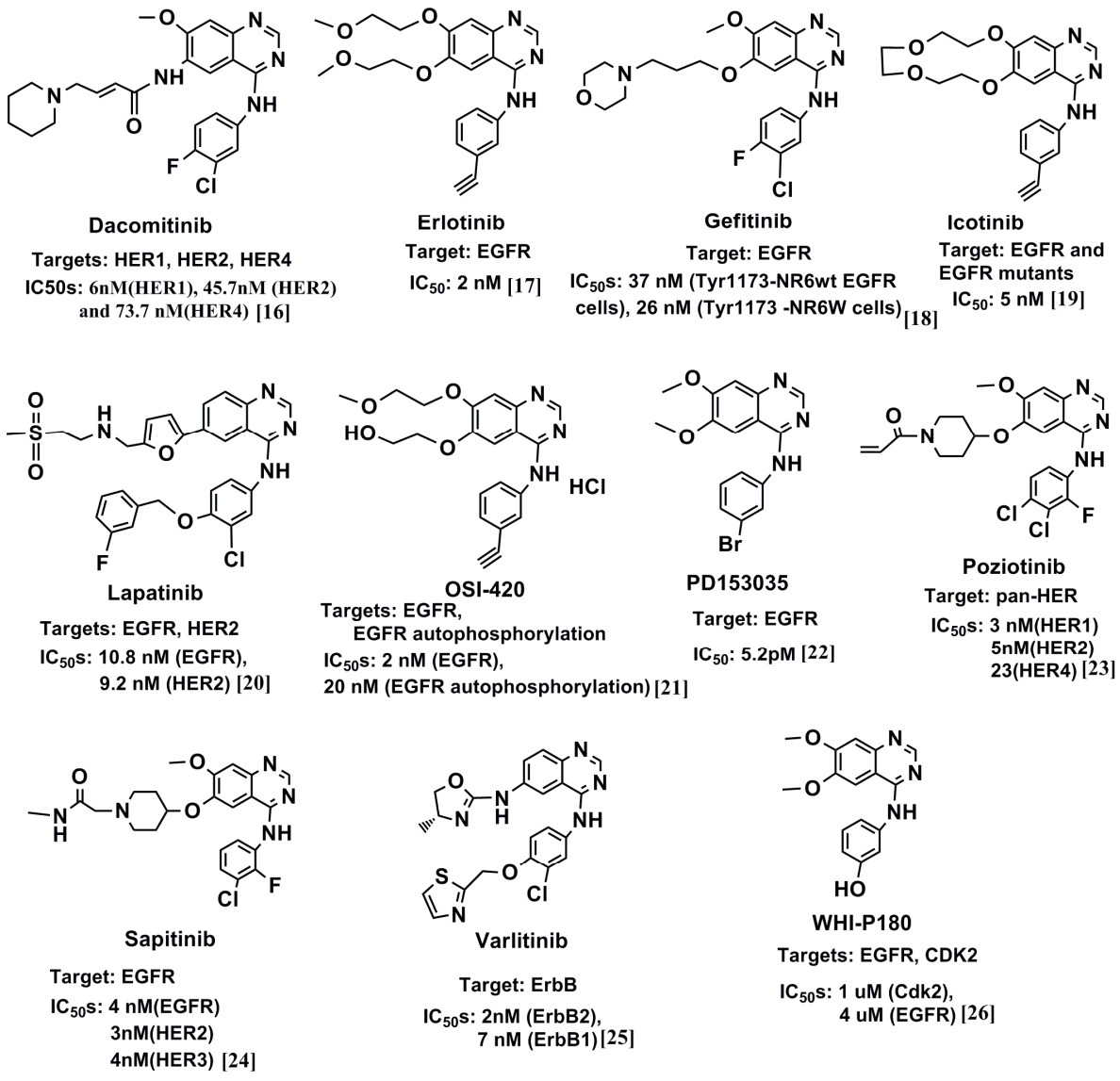

Fig. 1 Chemical structures of quinazolines used in clinical or clinical trial

Isoxazole moiety was usually introduced to the drug molecules to improve their biological activities. Some drug molecules containing isoxazole moiety showed remarkable biological activities [27-33]. In our previous work, we introduced the isxoazole moiety at the 4-position of quinazoline parent ring and synthesized some quinazolines containing isoxazole moiety. The biological evaluation showed that

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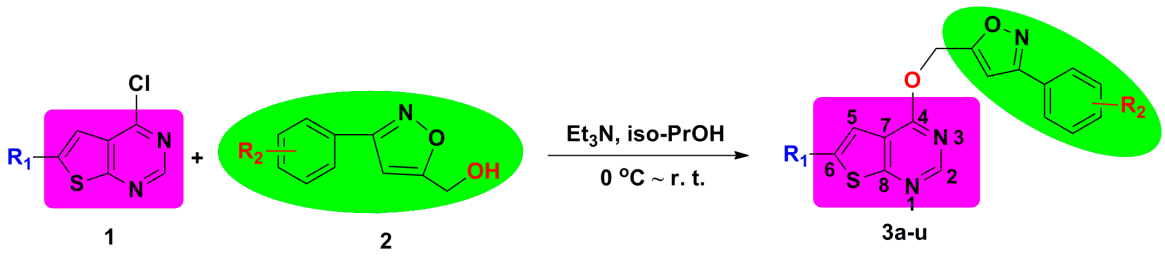
most compounds exhibited significant anticancer activity against MCF-7, A549 and HCT116 cell lines [34, 35].

Thieno[2,3-*d*]pyrimidine derivatives also exhibited a wide range of biological activities: such as antimicrobial [36-39], antiviral [40,41], anti-inflammatory [42,43], antidiabetic [44], antioxidant [45], anxiolytic agents [46] and especially anticancer activities [47-59]. Based on our previous excellent achievements [34, 35], we have replaced the quinazoline parent core with the thieno[2,3-*d*]pyrimidine parent core and synthesized some thieno[2,3-*d*]pyrimidines containing isoxazole moiety. The results showed that most compounds exhibit more active anticancer activity against MCF-7, A549 and HCT116 cell lines [60]. In order to extend this small molecular library and find more active molecules for the development of anticancer agents, in current work, other novel structures of thieno[2,3-*d*]pyrimidines containing isoxazole-moiety (**3a-3u**) (**Scheme 1**) were also synthesized.



1 2 3a-u

$R_1 = CH_3$

Compound No.	R_2
3a	H
3b	4-CH_3
3c	4-OCH_3
3d	2-Cl
3e	4-Cl
3f	2,4-diCl
3g	4-Br

$R_1 = CH_2CH_3$

Compound No.	R_2
3h	H
3i	4-CH_3
3j	4-OCH_3
3k	2-Cl
3l	4-Cl
3m	2,4-diCl
3n	4-Br

$R_1 = C(CH_3)_3$

Compound No.	R_2
3o	H
3p	4-CH_3
3q	4-OCH_3
3r	2-Cl
3s	4-Cl
3t	2,4-diCl
3u	4-Br

Scheme 1. Synthetic route for thieno[2,3-*d*]pyrimidines containing isoxazole-moiety-

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Furthermore, we evaluated their biological activity against lung cancer cell line A549, colorectal cancer cell line HCT116 and breast cancer cell line MCF-7.

2. Results and discussion

2.1 Chemistry

To obtain more potent anticancer agent as well as to study their structure-activity relationship (SAR), different substitution patterns at the 6-position of the thieno[2,3-*d*]pyrimidine core and on the benzene ring of isxoazole moiety were selected based on the substutition's volume and electronic environments, which would affect lipophilicity and steric hindrance, and hence the anticancer activity of the target molecules. Based on these factors, 21 novel structures of thieno[2,3-*d*]pyrimidine derivatives containing isoxazole-moiety were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, IR and mass spectrometry. Their *in vitro* anticancer activity against lung cancer A549, colorectal cancer HCT116 and breast cancer MCF-7 cell lines was preliminarily evaluated.

2.2 Analytical spectral data of compounds 3a-u

The IR spectral data of compounds **3a-u** showed a characteristic absorption band at the 3103-3107 cm^{-1}, which corresponds to the thiophene core, =C-H and Ph-H. The absorption band of the aromatic cycle skeleton, N=C group and the stretching frequency of C=C showed an absorption band at 1548-1567 cm^{-1}. In the ^{1}H NMR spectra, a singlet at 7.26-7.28 ppm in compounds **3a-n** belongs to the proton of the 5-position of the thieno[2,3-*d*]pyrimidine core, while for the compounds **3o-u**

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(6-position of the thieno[2,3-*d*]pyrimidine core was substituted by *tert*-butyl group), the proton signal of the 5-position of the thieno[2,3-*d*]pyrimidine core was showed at 7.44 ppm, which is attributed to the steric effects caused by *tert*-butyl group; a singlet for a proton varying from 8.6-8.8 ppm belonged to the proton of 2-position of the thieno[2,3-*d*]pyrimidine core; a singlet recorded at 7.1-7.3 ppm corresponds to the proton of the isoxazole ring; two protons of CH_2 connected isoxazole ring was recorded as a singlet at 5.8-5.9 ppm. In ^{13}C NMR spectra, the carbon of CH_2 connected isoxazole-ring appeared at 60-61 ppm, the 3-C, 4-C and 5-C of isoxazole ring appeared at 166, 101 and 158 ppm respectively. The 2-C, 4-C, 8-C and 9-C of thieno[2,3-*d*]pyrimidine core appeared at 154, 163, 145 and 127ppm respectively, the 6-C of thieno[2,3-*d*]pyrimidine core of all compounds appeared at 137-138ppm, which indicates that *tert*-butyl group did not affect the chemical shifts of 5-C and 6-C of thieno[2,3-*d*]pyrimidine core. Some carbon atoms in the same chemical environment appeared only one signal. All target compounds were also confirmed by MS according to their molecular formulae, most compounds showed the expected molecular ion signal $[M]^+$, but some showed the quasi-molecular ion signal $[M+H]^+$.

2.3 In vitro anticancer evaluation

We preliminarily evaluated the synthesized compounds **3a-u** for *in vitro* anticancer activity against A549, HCT116 and MCF-7 cell lines. The anticancer efficacy was comparable to the standard drug (gefitinib) (**Table 1)**. It can be seen from the **Table 1**, the half of compounds exhibit good to excellent anticancer activity

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against A549, HCT116 and MCF-7 cell lines. For **3a-g**, it indicates that introducing the methyl and methoxy group to 4-position of the benzene ring of the isoxazole mioety will reduce their anticancer activity (For example, **3a** is the most potent compounds among **3a-c,** and the active sequence is **3a>3b>3c**); while for **3d-g**, it indicates that introducing the halogen atom to 4-position of the benzene ring of the isoxazole moiety will increase their anticancer activity, and 4-Cl substituted derivative is more potent than 4-Br substituted derivative; **3e** is the most potent compound among **3a-g**. In fact, **3e** is 6.4-fold more active than gefitinib against A549, 3.2-fold more active than gefitinib against HCT116 and 4912-fold much more active than gefitinib against MCF-7. For **3h-n** (6-position of thieno[2,3-*d*]pyrimidine core is substituted by ethyl group), the anticancer activity of these compounds exhibited the same trend observed for compounds **3a-g**. However, compounds **3j** and **3k** exhibited much stronger inhibition against A549 cell line and are the most potent ones among **3h-n**. Substance **3j** (IC_{50} =5.50×10^{-4} μM) is 32545-fold more active than gefitinib (IC_{50} = 17.90 μM) against A549 cell line; while **3k** (IC_{50} = 7.14×10^{-3} μM) is 2507-fold more active than gefitinib (IC_{50} = 17.90 μM) against A549 cell line. Furthermore, **3j** and **3k** also exhibited potent anticancer activity against HCT116 cell line. For **3o-u** (6-position of thieno[2,3-*d*]pyrimidine core is substituted by *tert*-butyl group), as evidence from **Table 1**, the anticancer activity against A549 and HCT116 cell lines of these compounds is not remarkable, but the anticancer activity against MCF-7 is: **3o** (IC_{50} = 2.06 μM) is 10-fold more potent than gefitinib (IC_{50} = 20.68 μM) against MCF-7 cell line, **3r** (IC_{50} = 3.90 μM) is 5.3-fold more potent than gefitinib (IC_{50} =

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20.68 μM) against MCF-7 cell line and **3u** (IC_{50} = 5.38 μM) is 3.8-fold more potent than gefitinib (IC_{50} = 20.68 μM) against MCF-7 cell line.

By comparing with the IC_{50} values of these compounds (**3a-u**) against A549, HCT116 and MCF-7 cell lines, we can also draw some rules: (1) Introducing the isoxazole moiety at the 4-position of thieno[2,3-*d*]pyrimidine core will greatly improve its anticancer activity. (2) Introducing a bulky hydrophobic group at the 6-positions of thieno[2,3-*d*]pyrimidine core will greatly reduce anticancer activity against A549 and HCT116 cell lines. (3) The methyl group is superior than other groups substituted at 6-position of thieno[2,3-*d*]pyrimidine core for anticancer activity.

Overall, **3a**, **3e**, **3g** and **3h** are the most potent compounds among these thieno[2,3-*d*]pyrimidines containing isoxazole-moiety by comparing their IC_{50} values with gefitinib. Consequently, they can be regarded as very promising lead drug candidates for the development of anticancer drugs.

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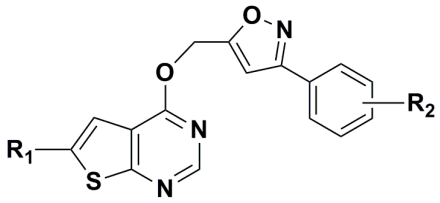
Table 1 *In vitro* anticancer activity of 3a-u



Compd	Substitutents		IC_{50} ±SD (μM)		
	R_1	R_2	A549	HCT116	MCF-7
3a	CH_3	H	118.90±0.0300	22.46±0.0580	6.02±0.0365
3b	CH_3	4-CH_3	144.10±0.0363	53.94±0.0593	13.47±0.0328
3c	CH_3	4-OCH_3	—[a]	3.58×10^{6}±0.0254	1.19×10^{6}±0.0612
3d	CH_3	2-Cl	—[a]	0.35±0.0629	—[a]
3e	CH_3	4-Cl	2.79±0.0138	6.69±0.0486	4.21×10^{-3}±0.0615
3f	CH_3	2,4-diCl	134.90±0.0418	—[a]	50.58±0.0584
3g	CH_3	4-Br	101.30±0.0572	49.13±0.0266	7.77×10^{-4}±0.0345
3h	CH_3CH_2	H	68.58±0.0178	43.22±0.0265	113.8±0.0624
3i	CH_3CH_2	4-CH_3	153.10±0.0840	60.71±0.0291	101.90±0.0535
3j	CH_3CH_2	4-OCH_3	5.50×10^{-4}±0.0997	112.00±0.0654	—[a]
3k	CH_3CH_2	2-Cl	7.14×10^{-3}±0.2631	19.87±0.3442	—[a]
3l	CH_3CH_2	4-Cl	45.48±0.0612	33.26±0.0855	1.08×10^{5}±0.1528
3m	CH_3CH_2	2,4-diCl	64.90±0.0938	21.09±0.0691	250.40±0.00145
3n	CH_3CH_2	4-Br	4.94×10^{2}±0.0221	1.69×10^{6}±0.0563	71.44±0.0408
3o	$(CH_3)_3C$	H	9.53×10^{5}±0.2077	7.45×10^{5}±0.0461	2.06±0.1159
3p	$(CH_3)_3C$	4-CH_3	—[a]	7.50×10^{5}±0.0253	23.50±0.1068

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3q	$(CH_3)_3C$	4-OCH_3	—[a]	$2.19\times10^{6}\pm0.0540$	33.61 ± 0.0051
3r	$(CH_3)_3C$	2-Cl	$7.99\times10^{5}\pm0.2234$	—[a]	3.90 ± 0.0646
3s	$(CH_3)_3C$	4-Cl	$1.59\times10^{2}\pm0.0721$	$3.58\times10^{6}\pm0.0254$	$1.19\times10^{6}\pm0.0612$
3u	$(CH_3)_3C$	4-Br	41.40 ± 0.2725	$1.29\times10^{6}\pm0.0053$	5.38 ± 0.0462
	Gefitinib		17.90 ± 0.0074	21.55 ± 0.0129	20.68 ± 0.0384

[a] The IC_{50} values could not be calculated

3. Conclusion

In this work, we synthesized 21 novel structures of thieno[2,3-*d*]pyrimidines, containing isoxazole moiety and tested them against three tumor cell lines: A549, HCT116 and MCF-7. The results indicate that introducing the isoxazole moiety to the 4-position of thieno[2,3-*d*]pyrimidines will greatly improve anticancer activity. Compounds **3a**, **3e**, **3g** and **3h** exhibited a broad-spectrum and much stronger inhibitory activity against these tumor cell lines, for which they are considered promising drug candidates for development of anticancer drugs.

4. Experimental

4.1 Materials and apparatus

Melting points (°C, uncorrected) were determined on a XT5 micrio melting point apparatus. NMR spectra were recorded on Bruker AVANCE III at 400 MHz for ^{1}H and 100 MHz for ^{13}C, using TMS as internal standard and peak multiplicities was designed as follows: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). The coupling constants, *J*, are reported in Hertz (Hz). The FTIR spectra were recorded on VERTEX70, in KBr discs in the 600-3600 cm^{-1} region. ESI-MS were

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performed on a DECAX-30000 LCQ Deca XP (70 eV). All reactions were monitored by thin layer chromatography (TLC) on silica gel plates at 254 nm under a UV lamp using petrolum/ethyl acetate as eluent. Silica gel (100-200 mesh, Qingdao haiyang chemical Co. Ltd.) was used for column chromatography. MTT{[3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide], Amresco} and dimethyl sulfoxide (DMSO) were purchased from Sigma. Gefitinib was purchased from the Hubei Prosperity Galaxy Chemical Co. Ltd., China. Dulbecco's modified essential medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin were purchased from Invitrogen. The intermediates {3-[(R_2)-substituted-phenyl]-isoxazole-5-yl}-methanol (**2a-g)** were prepared according to our reported procedures [61]. Other chemicals were commercially available, and used without further purification. Isopropyl alcohol (*iso*-PrOH) was dried over anhydrous magnesium sulfate before use.

4.2 General procedure for the preparation of the target compounds 3a-u

General synthesis of **3a**: 6-methyl-4-chloro-thieno[2,3-*d*]pyrimidine (0.184 g, 1 mmol) was added into a 25 mL one-necked round-bottom flask with 5mL of dry *iso*-PrOH, and the mixture was stirred in a cold bath. Then a solution of Et_3N (0.101 g, 1 mmol) and (3-phenyl-isoxazole-5-yl)-methanol (0.175 g, 1 mmol) in 10mL dry *iso*-PrOH was slowly added to the reaction system using a syringe. The mixture was stirred in a cold bath for an additional 30 min, and then temperature was allowed to reach room temperature. After reaction completion (monitored by thin layer chromatography, TLC), the reaction mixture was evaporated under reduced

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pressure, and the residue was purified by column chromatography on silica gel using petroleum ether and ethyl acetate (5:1→2:1) as eluant. Fractions with similar R_f values were combined to obtain the target compound **3a.** Other thieno[2,3-*d*]pyrimidine derivatives containing isoxazole moiety **3b-u** were synthesized using the same process **3a**.

4.2.1 6-Methyl-4-[(3-phenyl-isoxazol-5-yl-)methoxy]-thieno[2,3-*d*]pyrimidine (3a)

White solid; mp. 106-107 °C; yield, 88 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 770; ^{1}H NMR (400 MHz) δ in ppm: 2.61 (s, 3H, CH_3), 5.80 (s, 2H, isoxazole-C$\underline{H}_2$), 7.24 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.51 (d, 2H, J = 2.8 Hz, Ph-H), 7.52-7.53 (m, 1H, Ph-H), 7.88-7.90 (m, 2H, Ph-H), 8.68 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 15.4 (CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.1 (5-C of thieno[2,3-*d*]pyrimidine), 127.0 (9-C of thieno[2,3-*d*]pyrimidine) , 127.5 (two carbon atoms of Ph), 128.3 (Ph), 128.9 (Ph), 129.3 (two carbon atoms of Ph), 138.9 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine) , 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z* , 100 %): 324 ($[M+H]^+$, 20); Anal. calcd. for $C_{17}H_{13}N_3O_2S$: C, 63.16; H, 4.02; N, 13.00. Found: C, 63.20; H, 3.99; N, 12.99 %.

4.2.2 6-Methyl-4-[(3-*p*-tolyl-isoxazol-5-yl-)methoxy]-thieno[2,3-*d*]pyrimidine(3b)

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White solid; mp. 111-113 °C; yield, 90 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 812; ^{1}H NMR (400 MHz) δ in ppm: 2.36 (s, 3H, Ph-CH_3), 2.61 (s, 3H, CH_3), 5.79 (s, 2H, isoxazole-CH_2), 7.20 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.33 (d, 2H, J = 8.0 Hz, Ph-H), 7.78 (d, 2H, J = 8.0 Hz, Ph-H), 8.67 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm:14.9 (CH_3), 21.3 (Ph-CH_3), 65.7 (isoxazole-CH_2), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 125.7 (two carbons of Ph), 125.2 (Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 129.5 (two carbons of Ph), 131.7 (Ph), 138.9 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0(3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 338 ($[M+H]^+$, 100); Anal. calcd. for $C_{18}H_{15}N_3O_2S$: C, 64.09; H, 4.45; N, 12.46. Found: C, 64.11; H, 4.47; N, 12.38 %.

4.2.3 6-Methyl-4-{[3-(4-methoxyphenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3c)

White solid; mp. 109-112 °C; yield, 91 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 835; ^{1}H NMR (400 MHz) δ in ppm: 2.61 (s, 3H, CH_3), 3.80 (s, 3H, Ph-OCH_3), 5.78 (s, 2H, isoxazole-CH_2), 7.06 (d, 2H, J = 6.8 Hz, Ph-H), 7.17 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.84 (d, 2H, J = 8.8 Hz, Ph-H), 8.67 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm:14.9 (CH_3), 55.8 (Ph-OCH_3), 65.7 (isoxazole-CH_2), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 114.8 (two carbons of Ph),

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120.2 (Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.5 (two carbons of Ph), 138.9(6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 160.6 (Ph), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z* , 100 %): 352 ([M]$^+$, 100); Anal. calcd. for $C_{18}H_{15}N_3O_3S$: C, 61.36; H, 4.26; N, 11.93. Found: C, 61.22; H, 4.29; N, 11.91 %.

4.2.4 6-Methyl-4-{[3-(2-chlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3d)

White solid; mp. 98-101 ^oC; yield, 78 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 867; ^1H NMR (400 MHz) δ in ppm: 2.61 (s, 3H, CH_3), 5.83 (s, 2H, isoxazole-C$\underline{H}_2$), 7.13 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.47-7.51 (m, 1H, Ph-H), 7.54-7.58 (m, 1H, Ph-H), 7.64-7.67 (m, 1H, Ph-H), 7.70-7.72 (m, 1H, Ph-H), 8.68 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 14.9 (CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.9 (Ph), 129.9 (Ph), 130.1 (Ph), 130.6 (Ph), 132.2 (Ph), 132.5 (Ph), 138.9 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 357 ([M]$^+$, 50); Anal. calcd. for $C_{17}H_{12}ClN_3O_2S$: C, 57.14; H, 3.36; N, 11.76. Found: C, 57.11; H, 3.38; N, 11.75 %.

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4.2.5 6-Methyl-4-{[3-(4-chlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3e)

White solid; mp. 105-106 ºC; yield, 82 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 832; ^{1}H NMR (400 MHz) δ in ppm: 2.61 (s, 3H, CH_3), 5.81 (s, 2H, isoxazole-C$\underline{H}_2$), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.27 (s, 1H, H of isoxazole-ring), 7.59 (d, 2H, J = 8.4 Hz, Ph-H), 7.92 (d, 2H, J = 8.4 Hz, Ph-H), 8.67 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 14.9 (CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.3 (Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.9 (two carbons of Ph), 129.5 (two carbons of Ph), 134.3 (Ph), 138.9 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 160.6 (Ph), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 379 ($[M+23]^+$,100); Anal. calcd. for $C_{17}H_{12}ClN_3O_2S$: C, 57.14; H, 3.36; N, 11.76. Found: C, 57.11; H, 3.38; N, 11.75 %.

4.2.6 6-Methyl-4-{[3-(2,4-dichlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3f)

White solid; mp. 99-100 ºC; yield, 72 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 856; ^{1}H NMR (400 MHz) δ in ppm: 2.61 (s, 3H, CH_3), 5.83 (s, 2H, isoxazole-C$\underline{H}_2$), 7.15 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.58-7.61 (m, 1H, Ph-H), 7.75 (d, 1H, J = 8.4 Hz, Ph-H), 7.85 (d, 1H, J = 2.0 Hz, Ph-H), 8.68 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C

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NMR (100 MHz) δ in ppm: 14.9 (CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 127.4 (Ph), 128.0 (Ph), 130.3 (Ph), 130.9 (Ph), 133.6 (Ph), 135.7 (Ph), 138.9 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100%): 393 ($[M+H]^+$,70); Anal. calcd. for $C_{17}H_{11}Cl_2N_3O_2S$: C, 52.04; H, 2.80; N, 10.71. Found: C, 52.07; H, 3.00; N, 10.50 %.

4.2.7 6-Methyl-4-{[3-(4-bromophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3g)

White solid; mp. 129-131 ^oC; yield, 72 %; IR (KBr, cm^{-1}) υ: 3103, 1567, 1546, 1442, 1309, 1026, 834; ^1H NMR (400 MHz) δ in ppm: 2.61 (s, 3H, CH_3), 5.81 (s, 2H, isoxazole-C$\underline{H}_2$), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.27 (s, 1H, H of isoxazole-ring), 7.74 (d, 2H, J = 8.8 Hz, Ph-H), 7.86 (d, 2H, J = 6.4 Hz, Ph-H), 8.67 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 14.9 (CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 123.3 (Ph), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 127.3 (Ph), 128.3 (two carbons of Ph), 132.2 (two carbons of Ph), 138.9 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 160.6 (Ph), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS(*m/z*, 100 %): 441 ($[M+39]^+$, 30); Anal. calcd. for $C_{17}H_{12}BrN_3O_2S$: C, 50.74; H,2.99; N, 10.45. Found: C,

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50.76; H, 3.00; N, 10.48 %.

4.2.8 6-Ethyl-4-[(3-phenyl-isoxazol-5-yl-)-methoxy-]-thieno[2,3-*d*]pyrimidine (3h)

White solid, mp. 115-116 °C; yield, 81 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 775; ^{1}H NMR (400 MHz) *δ* in ppm: 1.34 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.2 Hz), 2.92 (q, 2H, $C\underline{H}_2CH_3$, *J* = 0.8 Hz), 5.81 (s, 2H, isoxazole-$C\underline{H}_2$), 7.25 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.51-7.53 (m, 3H, Ph-H), 7.88-7.91 (m, 2H, Ph-H), 8.68 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) *δ* in ppm: 16.4 (CH_2-$\underline{C}H_3$), 23.5 ($\underline{C}H_2$-CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.1 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine) , 127.5 (two carbon atoms of Ph), 128.2 (Ph), 128.7 (Ph), 129.3(two carbon atoms of Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine) , 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 337 ($[M]^+$, 10); Anal. calcd. for $C_{18}H_{15}N_3O_2S$: C, 64.09; H,4.45; N, 12.46. Found: C, 64.07; H, 4.50; N, 12.41 %.

4.2.9 6-Ethyl-4-[(3-*p*-tolyl-isoxazol-5-yl-)methoxy]-thieno[2,3-*d*]pyrimidine (3i)

White solid; mp. 112-113 °C; yield, 88 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 845; ^{1}H NMR (400 MHz) *δ* in ppm: 1.34 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.6 Hz), 2.36 (s, 3H, Ph-$C\underline{H}_3$), 2.97 (q, 2H, $C\underline{H}_2CH_3$, *J* = 1.2 Hz), 5.79 (s, 2H, isoxazole-$C\underline{H}_2$), 7.21 (s, 1H, H of isoxazole-ring), 7.27 (s, 1H, 5-H of

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thieno[2,3-*d*]pyrimidine), 7.31 (d, 2H, J = 7.6 Hz, Ph-H), 7.79 (d, 2H, J = 8.4 Hz, Ph-H), 8.68 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 16.1 (CH_2-$\underline{C}H_3$), 21.5 (Ph-$\underline{C}H_3$), 23.1 ($\underline{C}H_2$-CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.1 (5-C of thieno[2,3-*d*]pyrimidine), 125.3 (Ph), 125.7 (two carbons of Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 129.5 (two carbons of Ph), 131.7 (Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine) , 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 352 ($[M+H]^+$, 10); Anal. calcd. for $C_{19}H_{17}N_3O_2S$: C, 64.96; H,4.45; N, 12.46. Found: C, 64.07; H, 4.50; N, 12.41 %.

4.2.10 6-Ethyl-4-{[3-(4-methoxyphenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3j)

White solid; mp. 114-116 ^{o}C; yield, 86 %; IR (KBr, cm^{-1}) υ: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 845; ^{1}H NMR (400 MHz) δ in ppm: 1.32 (t, 3H, $CH_2C\underline{H}_3$, J = 7.6 Hz), 2.97 (q, 2H, $C\underline{H}_2CH_3$, J = 1.2 Hz), 3.93 (s, 3H, Ph-O$C\underline{H}_3$), 5.78 (s, 2H, isoxazole-$C\underline{H}_2$), 7.08 (d, 2H, J = 8.8 Hz, Ph-H), 7.18 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.83 (d, 2H, J = 8.8 Hz, Ph-H), 8.67 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 16.1 (CH_2-$\underline{C}H_3$), 23.1 ($\underline{C}H_2$-CH_3), 55.8 (OCH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 114.8 (two carbons of Ph), 120.5 (Ph), 125.1 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.5 (two carbons of Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of

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thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 160.6 (Ph), 163.3 (4-C of thieno[2,3-*d*]pyrimidine) , 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 367 ($[M]^+$, 5), 368 ($[M+H]^+$, 10); Anal. calcd. for $C_{19}H_{17}N_3O_3S$: C, 62.12; H, 4.63; N, 11.44. Found: C, 62.10; H, 4.68; N, 11.39 %.

4.2.11 6-Ethyl-4-{[3-(2-chlorophenyl)-isoxazol-5-yl-]-methoxy}-thieno[2,3-*d*]pyrimidine (3k)

White solid; mp. 100-101 ^oC; yield, 76 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 845; ^1H NMR (400 MHz) *δ* in ppm: 1.33 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.6 Hz), 2.97 (q, 2H, $C\underline{H}_2CH_3$, *J* = 1.2 Hz), 5.83 (s, 2H, isoxazole-$C\underline{H}_2$), 7.13 (s, 1H, H of isoxazole-ring), 7.27 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.47-7.58 (m, 2H, Ph-H), 7.64-7.66 (m, 1H, Ph-H), 7.67-7.72 (m, 1H, Ph-H), 8.69 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) *δ* in ppm: 16.1 (CH_2-$\underline{C}H_3$), 23.1 ($\underline{C}H_2$-CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.9 (Ph), 129.9 (Ph), 130.1 (Ph), 130.6 (Ph), 132.2 (Ph), 132.5 (Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-d]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 372 ($[M+H]^+$, 15); Anal. calcd. for $C_{18}H_{14}ClN_3O_2S$: C, 58.22; H,3.77; N, 11.32. Found: C, 58.17; H, 3.80; N, 11.31 %.

4.2.12 6-Ethyl-4-{[3-(4-chlorophenyl)-isoxazol-5-yl-]-methoxy}-

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thieno[2,3-*d*]pyrimidine (3l)

White solid; mp. 144-145 ºC; yield, 78 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 845; ^{1}H NMR (400 MHz) *δ* in ppm: 1.32 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.6 Hz), 2.96 (q, 2H, $C\underline{H}_2CH_3$, *J* = 1.2 Hz), 5.81 (s, 2H, isoxazole-$C\underline{H}_2$), 7.26 (s, 1H, H of isoxazole-ring), 7.28 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.60 (d, 2H, *J* = 8.8 Hz, Ph-H), 7.93 (d, 2H, *J* = 8.8 Hz, Ph-H), 8.68 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) *δ* in ppm: 16.1 (CH_2-$\underline{C}H_3$), 23.1 ($\underline{C}H_2$-CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.3 (Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.9 (two carbons of Ph), 129.5 (two carbons of Ph), 134.3 (Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 389 ($[M+18]^+$, 5); Anal. calcd. for $C_{18}H_{14}ClN_3O_2S$: C, 58.22; H,3.77; N, 11.32. Found: C, 58.17; H, 3.80; N, 11.31%.

4.2.13 6-Ethyl-4-{[3-(2,4-dichlorophenyl)-isoxazol-5-yl-]-methoxy}-thieno[2,3-*d*]pyrimidine (3m)

White solid; mp. 94-96 ºC; yield, 70 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 845; ^{1}H NMR (400 MHz) *δ* in ppm: 1.33 (t, 3H, $CH_2C\underline{H}_3$, *J* = 7.6 Hz), 2.95 (q, 2H, $C\underline{H}_2CH_3$, *J* = 1.2 Hz), 5.83 (s, 2H, isoxazole-$C\underline{H}_2$), 7.16 (s, 1H, H of isoxazole-ring), 7.26 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.58-7.60 (m, 1H, Ph-H), 7.75 (d, 1H, *J* = 8.4 Hz, Ph-H), 7.85 (s, 1H, Ph-H), 8.69 (s,

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1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 16.1 (CH_2-$\underline{C}H_3$), 23.1 ($\underline{C}H_2$-CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 127.4 (Ph), 128.0 (Ph), 130.3 (Ph), 130.9 (Ph), 133.6 (Ph), 135.7 (Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 406 ($[M]^+$,15); Anal. calcd. for $C_{18}H_{13}Cl_2N_3O_2S$: C, 53.20; H,3.20; N, 10.34. Found: C, 53.21; H, 3.23; N, 10.31 %.

4.2.14 6-Ethly-4-{[3-(4-bromophenyl)-isoxazol-5-yl-]-methoxy}- thieno[2,3-*d*]pyrimidine (3n)

White solid; mp. 148-149 ^{o}C; yield, 75 %; IR (KBr, cm^{-1}) υ: 3111, 1571, 1548, 1512, 1461, 1429, 1372, 1322, 1036, 846; ^{1}H NMR (400 MHz) δ in ppm: 1.32 (t, 3H, $CH_2C\underline{H}_3$, J = 7.6 Hz), 2.96 (q, 2H, $C\underline{H}_2CH_3$, J = 1.2 Hz), 5.81 (s, 2H, isoxazole-$C\underline{H}_2$), 7.27 (s, 1H, H of isoxazole-ring), 7.28 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.74 (d, 1H, J = 8.4 Hz, Ph-H), 7.86 (d, 1H, J = 8.4 Hz, Ph-H), 8.66 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 16.1 (CH_2-$\underline{C}H_3$), 23.1 ($\underline{C}H_2$-CH_3), 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 123.3 (Ph), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 127.3 (Ph), 128.3 (two carbons of Ph), 132.2 (two carbons of Ph), 137.3 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of

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thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 416 ($[M]^+$, 5); Anal. calcd. for $C_{18}H_{14}BrN_3O_2S$: C, 51.92; H,3.37; N, 10.10. Found: C, 51.93; H, 3.39; N, 10.09 %.

2.4.15 6-*Tert*-butyl-4-[(3-phenyl-isoxazol-5-yl-)-methoxy-]-thieno[2,3-*d*]pyrimidine (3o)

White solid; mp. 140-141 °C; yield, 75 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 775; ^1H NMR (400 MHz) *δ* in ppm: 1.45 [s, 9H, $(CH_3)_3C$], 5.82 (s, 2H, isoxazole-$C\underline{H}_2$), 7.18 (s, 1H, H of isoxazole-ring), 7.44 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.65-7.68 (m, 3H, Ph-H), 7.78-7.80 (m, 2H, Ph-H), 8.78 (s, 1H, 2-H of thieno[2,3-d]pyrimidine); ^{13}C NMR (100 MHz) *δ* in ppm: 32.1 ($3CH_3$), 40.4 [$(CH_3)_3\underline{C}$], 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 127.5 (two carbons of Ph), 128.2 (Ph), 128.7 (Ph), 129.2 (two carbons of Ph), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 365 ($[M]^+$, 25); Anal. calcd. for $C_{20}H_{19}N_3O_2S$: C, 65.75; H,5.21; N, 11.51. Found: C, 65.70; H, 5.24; N, 11.49 %.

2.4.16 6-*Tert*-butyl-4-[(3-*p*-tolyl-isoxazol-5-yl-)methoxy]-thieno[2,3-*d*]pyrimidine (3p)

White solid; mp. 148-151 °C; yield, 75 %; IR (KBr, cm^{-1}) *υ*: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 810; ^1H NMR (400 MHz) *δ* in ppm: 1.44 [s, 9H,

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$(CH_3)_3C$], 2.36 (s, 3H, Ph-CH_3), 5.82 (s, 2H, isoxazole-CH_2), 7.19 (s, 1H, H of isoxazole-ring), 7.33 (d, 2H, J = 8.0 Hz, Ph-H), 7.43 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.78 (d, 2H, J = 8.0 Hz, Ph-H), 8.78 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 21.5 (Ph-CH_3), 32.1 ($3CH_3$), 40.4 [$(CH_3)_3$C], 65.7 (isoxazole-CH_2), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 125.2 (Ph), 125.7 (two carbons of Ph), 129.5 (two carbons of Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 131.7 (Ph), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 379 ($[M]^+$, 50); Anal. calcd. for $C_{21}H_{21}N_3O_2S$: C, 66.49; H,5.54; N, 11.08. Found: C, 66.47; H, 5.56; N, 11.05 %.

2.4.17 6-*Tert*-butyl-4-{[3-(4-methoxyphenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3q)

White solid; mp. 121-123 ^{o}C; yield, 75 %; IR (KBr, cm^{-1}) υ: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 823; ^{1}H NMR (400 MHz) δ in ppm: 1.45 [s, 9H, $(CH_3)_3$C], 3.93 (s, 3H, Ph-OCH_3), 5.82 (s, 2H, isoxazole-CH_2), 7.08 (d, 2H, J = 8.8 Hz, Ph-H), 7.17 (s, 1H, H of isoxazole-ring), 7.44 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.84 (d, 2H, J = 8.8 Hz, Ph-H), 8.78 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 32.1 ($3CH_3$), 40.4 [$(CH_3)_3$C], 55.8 (Ph-OCH_3), 65.7 (isoxazole-CH_2), 101.5 (4-C of isoxazole-ring),114.9 (two carbons of Ph), 120.5 (Ph), 125.0 (5-C of

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thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.5 (two carbons of Ph), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 160.6 (Ph), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.0 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 395 ([M]$^+$, 40); Anal. calcd. for $C_{21}H_{21}N_3O_3S$: C, 63.80; H,5.32; N, 10.63. Found: C, 63.79; H, 5.35; N, 10.61 %.

2.4.18 6-*Tert*-butyl-4-{[3-(2-chlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3r)

White solid; mp. 121-122 ^oC; yield, 75 %; IR (KBr, cm^{-1}) υ: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 860; ^{1}H NMR (400 MHz) δ in ppm: 1.49 [s, 9H, $(CH_3)_3C$], 5.87 (s, 2H, isoxazole-C$\underline{H}_2$), 7.12 (s, 1H, H of isoxazole-ring), 7.44 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.48-7.58 (m, 2H, Ph-H), 7.67 (d, 1H, J = 8.0 Hz, Ph-H), 7.73 (d, 1H, J = 7.2 Hz, Ph-H), 8.79 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 32.1 ($3CH_3$), 40.4 [$(CH_3)_3\underline{C}$], 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 128.9 (Ph), 129.9 (Ph), 130.1 (Ph), 130.8 (Ph), 132.2 (Ph), 132.5 (Ph), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.5 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 399 ([M]$^+$, 15); Anal. calcd. for $C_{20}H_{18}ClN_3O_2S$: C, 60.15; H,4.51; N, 10.53. Found: C, 60.10; H, 4.56; N, 10.55 %.

2.4.19 6-*Tert*-butyl-4-{[3-(4-chlorophenyl)-isoxazol-5-yl-]-methoxy-}-

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thieno[2,3-*d*]pyrimidine (3s)

White solid; mp. 141-142 °C; yield, 75 %; IR (KBr, cm^{-1}) υ: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 840; ^{1}H NMR (400 MHz) δ in ppm: 1.45 [s, 9H, $(CH_3)_3C$], 5.85 (s, 2H, isoxazole-$C\underline{H}_2$), 7.26 (s, 1H, H of isoxazole-ring), 7.44 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.60 (d, 2H, J = 8.4 Hz, Ph-H), 7.94 (d, 2H, J = 8.4 Hz, Ph-H), 8.78 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 32.1 ($3CH_3$), 40.4 [$(CH_3)_3\underline{C}$], 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.3 (Ph), 128.9 (two carbons of Ph), 129.3 (two carbons of Ph), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 134.3 (Ph), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.5 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 399 ($[M]^+$, 70); Anal. calcd. for $C_{20}H_{18}ClN_3O_2S$: C, 60.15; H,4.51; N, 10.53. Found: C, 60.10; H, 4.56; N, 10.55 %.

2.4.20 6-*Tert*-butyl-4-{[3-(2,4-dichlorophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3t)

White solid; mp. 135-136 °C; yield, 75 %; IR (KBr, cm^{-1}) υ: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 851; ^{1}H NMR (400 MHz) δ in ppm: 1.49 [s, 9H, $(CH_3)_3C$], 5.87 (s, 2H, isoxazole-$C\underline{H}_2$), 7.12 (s, 1H, H of isoxazole-ring), 7.44 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.75 (d, 1H, J = 8.4 Hz, Ph-H), 7.85 (d, 1H, J = 2.0 Hz, Ph-H), 8.79 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 32.1 ($3CH_3$), 40.4 [$(CH_3)_3\underline{C}$], 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of

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isoxazole-ring), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 127.4 (Ph), 128.0 (Ph), 130.3 (Ph), 130.9 (Ph), 133.6 (Ph), 135.8 (Ph), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 162.5 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 434 ([M]$^+$, 60); Anal. calcd. for $C_{20}H_{17}Cl_2N_3O_2S$: C, 55.30; H,3.92; N, 9.68. Found: C, 55.31; H, 3.97; N, 9.71 %.

2.4.21 6-*Tert*-butyl-4-{[3-(4-bromophenyl)-isoxazol-5-yl-]-methoxy-}-thieno[2,3-*d*]pyrimidine (3u)

White solid; mp. 146-148 ^oC; yield, 75 %; IR (KBr, cm^{-1}) υ: 3109, 1571, 1549, 1510, 1461, 1431, 1363, 1322, 1036, 875; ^{1}H NMR (400 MHz) δ in ppm: 1.45 [s, 9H, $(CH_3)_3C$], 5.85 (s, 2H, isoxazole-C$\underline{H}_2$), 7.27 (s, 1H, H of isoxazole-ring), 7.44 (s, 1H, 5-H of thieno[2,3-*d*]pyrimidine), 7.74 (d, 2H, J = 8.8 Hz, Ph-H), 7.87 (d, 2H, J = 8.8 Hz, Ph-H), 8.78 (s, 1H, 2-H of thieno[2,3-*d*]pyrimidine); ^{13}C NMR (100 MHz) δ in ppm: 32.1 ($3CH_3$), 40.4 [$(CH_3)_3\underline{C}$], 65.7 (isoxazole-$\underline{C}H_2$), 101.5 (4-C of isoxazole-ring), 123.1 (Ph), 127.2 (Ph), 128.3 (two carbons of Ph), 132.1 (two carbons of Ph), 125.0 (5-C of thieno[2,3-*d*]pyrimidine), 126.5 (9-C of thieno[2,3-*d*]pyrimidine), 138.1 (6-C of thieno[2,3-*d*]pyrimidine), 145.8 (8-C of thieno[2,3-*d*]pyrimidine), 154.9 (2-C of thieno[2,3-*d*]pyrimidine), 158.9 (5-C of isoxazole-ring), 163.3 (4-C of thieno[2,3-*d*]pyrimidine), 166.3 (3-C of isoxazole-ring); ESI-MS (*m/z*, 100 %): 444 ([M]$^+$, 68); Anal. calcd. for $C_{20}H_{18}BrN_3O_2S$: C, 54.05; H,4.05; N, 9.46. Found: C, 54.08; H, 4.10; N, 9.51 %.

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4.3 Anticancer evaluation

The anticancer activity of **3a-u** was evaluated against MCF-7, HCT116 and A549 cell lines using the MTT method. The cancer cell lines were cultured in Dulbecco's modified Essential Medium (DMEM) supplemented with 10% fetal bovine serum and 1.5 % antibiotic/antimycotic (Life Technologies, Invitrogen, USA) and maintained in CO_2 incubator at 37 ^oC, at 5 % CO_2 and 95 % atmospheric humidity. The mixture of DMSO, PBS and DMEM was used as a negative control and Gefitinib was used as the reference drug. The detailed process was reported earlier [34,35,60] .

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of intrest.

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DISCLOSURE

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REFERENCES

[1] Cozzi, P.; Mongelli, N.; Suarato, A. Recent anticancer cytotoxic agents. *Curr. Med. Chem. Anticancer Agents*. 2004, 4, 93.

[2] Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D. Global cancer

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

statistics. *CA Cancer J. Clin.*, 2011, 61, 69.

[3] Jemal. A.; Siegel, R.; Xu, J.; Ward, E. Cancer statistics, 2010. *CA Cancer J. Clin.*, 2010, 60, 277.

[4] Soria, J. C.; Mok, T. S.; Cappuzzo, F.; Jänne, P. A. EGFR-mutated oncogene-addicted non-small cell lung cancer: current trends and future prospects. *Cancer Treat. Rev.*, 2012, 38, 416.

[5] She, J.; Yang, P.; Hong, Q. Y.; Bai, C.X. Lung cancer in China: Challenges and interventions. *Chest*, 2013, 143, 1117.

[6] Li, D.; Ambroglo, L.; Shimamura, T.; Kubo, S.; Takahashi, M.; Chlrleac, L. R.; Padera, R. F.; Shapiro, G. I.; Baum, A.; Himmeisbach, F.; Rettlg, W. J.; Meyerson, M.; Solca, F.; Greullch, H.; Wong, K. K. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer model. *Oncogene*, 2008, 27, 4702.

[7] Jani, J. P.; Finn, R. S.; Campbell, M.; Coleman, K. G.; Connell, R. D.; Currier, N.; Emerson , E. O.; Floyd, E.; Harriman, S.; Kath, J. C.; Morris, J.; Moyer, J. D.; Pustilnik, L. R.; Rafidi, K.; Ralston, S.; Rossi, A. M.; Steyn, S. J.; Wagner, L.; Winter, S. M.; Bhattacharya, S. K. Discovery and pharmacologic characterization of CP-724714, a selective ErbB2 tyrosine kinase inhibitor. *Cancer Res.*, 2007, 67, 9887.

[8]http://wenku.baidu.com/link?url=QTT84lEXwJYAWeYRReQl__HSMNj6z4CqGk1uVby_yjpeySde5H8VF18Xd_SYeL_LVigR0Ka8iZCGgJA1bpUh7wYJq87U4Doi5GCijtuKR1_

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

[9] Xie, H.; Lin, L.; Tong, L. AST1306, a novel irreversible inhibitor of the epidermal growth factor receptor 1 and 2, exhibits antitumor activity both in vitro and in vivo. *PLoS One.*, 2011, 6, e21487.

[10] Suzuki, T.; Fujii, A.; Ohya, J.; Amano, Y.; Kitano, Y.; Abe, D.; Nakamura, H. Pharmacological characterization of MP-412 (AV-412), a dual epidermal growth factor receptor and ErbB2 tyrosine kinase inhibitor. *Cancer Sci.*, 2007, 98, 1977.

[11] Zeng, Q. B.; Wang, J. B.; Cheng, Z. Q.; Chen, K.; Johnstrom, P.; Varnas, K.; Li, Y. Z.; Yang, Z. F.; Zhang, X. L. Discovery and Evaluation of Clinical Candidate AZD3759, a Potent, Oral Active, Central Nervous System-Penetrant, Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor. *J. Med. Chem.*, 2015,58, 8200.

[12] Discafani, C.M.; Carroll, M. L.; Floyd, M. B.; Hollander, I. J.; Husain, Z.; Johnson, B. D.; Kitchen, D.; May, M. K.; Malo, M .S.; Minnick, A. A.; Nilakantan, R.; Shen, R.; Wang, Y. F.; Wissner, A.; Greenberger, L. M. Irreversible inhibition of epidermal growth factor receptor tyrosine kinase with in vivo activity by N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide (CL-387,785). *Biochem Pharmacol.*, 1999, 57, 917.

[13] Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Hollis, H. D.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-d]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.*, 2000, 43, 1380.

[14] Levitzki, A.; Gazit, A. Tyrosine kinase inhibition: an approach to drug development. *Science*, 1995, 267,1782.

[15] Cai, X.; Zhai, H. X.;Wang, J.; Forrester, J.; Qu, H.; Yin, L.; Lai, C. J.; Bao, R. D.; Qian, C. G. Discovery of 7-(4-(3-Ethynylphenylamino)-7-methoxyquinazolin-6-yloxy)-N-hydroxyhepta namide (CUDC-101) as a Potent Multi-Acting HDAC, EGFR, and HER2 Inhibitor for the Treatment of Cancer. *J. Med. Chem.*, 2010, 53 ,2000.

[16] Jänne, P. A.; Boss, D. S.; Camidge, D. R.; Britten, C. D.; Engelman, J. A.; Garon, E. B.; Guo, F.; Wong, S.; Liang, J.; Letrent, S.; Millham, R.; Taylor, I.; Eckhardt, S. G.; Schellens, J. H. Phase I dose-escalation study of the pan-HER inhibitor, PF299804, in patients with advanced malignant solid tumors. *Clin Cancer Res.*, 2011,17, 1131.

[17] Higgins, B.; Kolinsky, K.; Smith, M.; Beck, G.; Rashed, M.; Adames, V.; Linn, M.; Wheeldon, E.; Gand, L.; Birnboeck, H.; Hoffmann, G. Antitumor activity of erlotinib (OSI-774, Tarceva) alone or in combination in human non-small cell lung cancer tumor xenograft models. *Anticancer Drugs*, 2004, 5,503.

[18] Pedersen, M. W.; Pedersen, N.; Ottesen, L. H.; Poulsen, H. S. Differential response to gefitinib of cells expressing normal EGFR and the mutant EGFRvIII. *Br. J. Cancer*, 2005, 93, 915.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

[19] Tan, F. L.; Shen, X. Y.; Wang, D. C.; Xie, G. J.; Zhang, X. D.; Ding, L. M.; Hu, Y. Y.; He, W.; Wang, Y. P.; Wang, Y. X. Icotinib (BPI-2009H), a novel EGFR tyrosine kinase inhibitor, displays potent efficacy in preclinical studies. *Lung Cancer*, 2012, 76,177.

[20] Karajannis, M. A.; Legault, G.; Hagiwara, M.; Ballas, M. S.; Brown, K.; Nusbaum, A. O.; Hochman, T.; Goldberg, J. D.; Koch, K. M.; Golfinos, J. G.; Roland, J. T.; Allen, J. C. Phase II trial of lapatinib in adult and pediatric patients with neurofibromatosis type 2 and progressive vestibular schwannomas. *Neuro Oncol.*, 2012,14, 1163.

[21] Meany, H. J.; Fox, E.; Mccully, C.; Tucker, C.; Balis, F. M. The plasma and cerebrospinal fluid pharmacokinetics of erlotinib and its active metabolite (OSI-420) after intravenous administration of erlotinib in non-human primates. *Cancer Chemother. Pharmacol.*, 2008, 62, 387.

[22] Bos, M.; Mendelsohn, J.; Kim, Y. M.; Albanell, J.; Fry, D. W.; Baselga, J. PD153035, a tyrosine kinase inhibitor, prevents epidermal growth factor receptor activation and inhibits growth of cancer cells in a receptor number-dependent manner. *Clin Cancer Res.*, 1997, 3, 2099.

[23] Nam, H. J.; Kim, H. P.; Yoon, Y. K.; Hur, H. S.; Song, S. H.; Kim, M. S.; Lee, G. S.; Han, S. W.; Im, S. A.; Kim, T. Y.; Oh, D. Y.; Bang, Y. J. Antitumor activity of HM781-36B, an irreversible Pan-HER inhibitor, alone or in combination with cytotoxic chemotherapeutic agents in gastric cancer. *Cancer Lett.*, 2011,302, 155.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

[24] Mu, Z. M.; Klinowska, T.; Dong, X. S.; Foster, E.; Wmack, C.; Fernandezand, S. V.; Cristofanilli, M. AZD8931, an equipotent, reversible inhibitor of signaling by epidermal growth factor receptor (EGFR), HER2, and HER3: preclinical activity in HER2 non-amplified inflammatory breast cancer models. *J. Exp. Clin. Cancer Res.*, 2014, 33, 47.

[25]http://checkorphan.org/news/aslan-pharmaceuticals-receives-orphan-drug-designation-from-the-fda-for-aslan001-varlitinib-in-cholangiocarcinoma

[26]An, R.; Hagiya, Y.; Tamura, A.; Li, S.; Saito, H.; Tokushima, D.; Ishikawa, T. Cellular phototoxicity evoked through the inhibition of human ABC transporter ABCG2 by cyclin-dependent kinase inhibitors in vitro. *Pharm Res.*, 2009, 26, 449.

[27] Hamama, W. S.; Ibrahim, M. E.; Zoorob, H. H. Advances in the chemistry of aminoisoxazole. *Synthetic. Commun.*, 2013, 43, 2393.

[28] Kumbhare, R. M.; Kosurkar, U. B.; Ramaiah, M. J.; Dadmal,T. L.; Pushapavalli, S. N. C. V. L.; Pal-Bhadra, M. Syntheisis and biological evaluation of novel triazoles and isoxazoles linked 2-phenyl benzothiazole as potential anticancer agents. *Bioorg. Med. Chem. Lett.*, 2012, 22, 5424.

[29] Gao, Z. L.; Hurst, W. J.; Czechtizky,W.; Hall, D.; Moindrot, N.; Nagorny, R.; Pichat, P.; Stefany, D.; Hendrix, J. A.; George, P. G.; Selenium nanoparticles induced membrane bio-mechanical property changes in MCF-7 cells by disturbing membrane molecules and F-actin. *Bioorg. Med. Chem. Lett.*, 2013, 23, 6296.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

[30] Ostacolo, G.; Ambrosino, P.; Russo, R.; Monte, M. L.; Soldovieri, M. V.; Laneri, S.; Sacchi, A.; Visoli, G.; Taglialatela, M.; Calignano, A. Isoxazole derivatives as potent transient receptor potential melastatin type 8 (TRPM8) agonists. *Eur. J. Med. Chem.*, 2013, 69, 659.

[31] North, E. J.; Scherman, M. S.; Bruhn, D. F.; Scarborough, J. S.; Maddox, M. M.; Jones,V.; Grzegorzewicz, A.; Yang, L.; Hess, T.; Morisseau, C.; Jackson, M.; McNeil, M. R.; Lee, R. E. Design, synthesis and anti-tuberculosis activity of 1-adamantyl-3-heteroaryl ureas with improved in vitro pharmacokinetic properties. *Bioorg. Med. Chem.*, 2013, 21, 2587.

[32] Kankala, S.; Kankala, R. K.; Gundepaka, P.; Thota, N.; Nerella, S.; Gangula, M. R.; Guguloth, H.; Kagga, M.; Vadde, R.; Vasam, C. S.; Regioselective synthesis of isoxazole–mercaptobenzimidazole hybrids and their in vivo analgesic and anti-inflammatory activity studies. *Bioorg. Med. Chem. Lett.*, 2013, 23, 1306.

[33] Bargiotti, A.; Musso, L.; Dallavalle, S.; Merlini, L.; Gallo, G.; Ciacci, A.; Giannini, G.; Cabri,W.; Penco, S.; Vesci, L.; Castorina, M.; Milazzo, F. M.; Cervoni, M. L.; Barbarino, M.; Pisano, C.; Giommarelli, C.; Zuco, V.; Cesare, M. D.; Zunino, F. Isoxazolo(aza)naphthoquinones: A new class of cytotoxic Hsp90 inhibitors. *Eur. J. Med. Chem.*, 2012, 53, 64.

[34] Yong, J. P.; Lu, C. Z.; Wu, X. Y. Potential anticancer agents. I. Synthesis of isoxazole moiety containing quinazoline derivatives and preliminarily in vitro anticancer activity. *Anti-cancer Agents Med. Chem.*, 2015, 15, 131.

[35] Yong, J. P.; Lu, C. Z.; Wu, X. Y. Synthesis and biological evaluation of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

quinazoline derivatives as potential anticancer agents(II). *Anti-cancer Agents Med. Chem.*, 2015, 15, 1326.

[36] Rashad, A. E.; Shamroukh, A. H.; Abdel-Megeid, R. E.; El-Sayed, W. A. Synthesis, Reactions, and Antimicrobial Evaluation of Some Polycondensed Thienopyrimidine Derivatives. *Synthetic. Commun.*, 2010, 40, 1149.

[37] Dewal, M. B.; Wani, A. S.; Vidaillac, C.; Oupicky, D.; Rybak, M. J.; Firestine, S. M. Thieno[2,3-*d*]pyrimidinedione derivatives as antibacterial agents. *Eur. J. Med. Chem.*, 2012, 51, 145.

[38] Aly, H. M.; Saleh, N. M.; Elhady, H. A. Design and synthesis of some new thiophene, thienopyrimidine and thienothiadiazine derivatives of antipyrine as potential antimicrobial agents. *Eur. J. Med. Chem.*, 2011, 46, 4566.

[39] Salahuddin, M.; Kakad, S.; Shantakumar, S. M. Synthesis of some novel thieno[2,3-d]pyrimidines and their antibacterial activity. *E-J.Chem.*, 2009, 6, 801.

[40] Rashad, A. E.; Ali, M. A. Synthesis and Antiviral Screening of Some Thieno[2,3-*d*]Pyrimidine Nucleosides. *Nucleos. Nucleot. Nucl.*, 2006, 25, 17.

[41] Rashad, A. E.; Shamroukh, A. H.; Abdel-Megeid, R. E.; Mostafa, A.; El-Shesheny, R.; Kandeil, A.; Ali, M. A.; Banert, K. Synthesis and screening of some novel fused thiophene and thienopyrimidine derivatives for anti-avian influenza virus (H5N1) activity. *Eur. J. Med. Chem.*, 2010, 45, 5251.

[42] Alagarsamy, V.; Meena, S.; Ramseshu, K.V.; Solomon,V. R.; Thirumurugan, K.; Dhanabal, K.; Murugan, M. Synthesis, analgesic, anti-inflammatory, ulcerogenic index and antibacterial activities of novel

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

2-methylthio-3-substituted-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-4(3*H*)-ones. *Eur. J. Med. Chem.*, 2006, 41, 1293.

[43] Ashour, H. M.; Shaaban, O. G.; Rizk, O. H.; El-Ashmawy, I. M. Synthesis and biological evaluation of thieno [2′,3′:4,5]pyrimido[1,2-b][1,2,4]triazines and thieno[2,3-d][1,2,4]triazolo[1,5-a]pyrimidines as anti-inflammatory and analgesic agents. *Eur. J. Med. Chem.*, 2013, 62, 341.

[44] Deng, J. F.; 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. The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes. *Eur. J. Med. Chem.*, 2011, 46, 70.

[45] Kotaiah, Y.; Harikrishna, N.; Nagaraju, K.; Rao, C. V. Synthesis and antioxidant activity of 1,3,4-oxadiazole tagged thieno[2,3-*d*]pyrimidine derivatives. *Eur. J. Med. Chem.*, 2012, 58, 340.

[46] Amr, A. E. E.; Sherif, M. H.; Assy, M. G.; Al-Omar, M. A.; Ragab, I. Antiarrhythmic, serotonin antagonist and antianxiety activities of novel substituted thiophene derivatives synthesized from 2-amino-4,5,6,7-tetrahydro-N-phenylbenzo[b]thiophene-3-carboxamide. *Eur. J. Med. Chem.*, 2010, 45, 5935.

[47] Dai,Y.; Guo, Y.; Frey, R. R.; Ji, Z.; Curtin, M. L.; Ahmed, A. A.; Albert, D. H.; Arnold, L.; Arries, S. S.; Barlozzari, T.; Bauch, J. L.; Bouska, J. J.; Bousquet, P. F.; Cunha, G. A.; Glaser, K. B.; Guo, J.; Li, J.; Marcotte, P. A.; Marsh, K. C.;

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

Moskey, M. D.; Pease, L. J.; Stewart, K. D.; Stoll, V. S.; Tapang, P.; Wishart, N.; Davidsen, S. K.; Michaelides, M. R. Thienopyrimidine ureas as novel and potent multitargeted receptor tyrosine kinase inhibitors. *J. Med. Chem.*, 2005, 48, 6066.

[48] Horiuchi, T.; Chiba, J.; Uoto, K.; Soga, T. Discovery of novel thieno[2,3-*d*]pyrimidin-4-yl hydrazone-based inhibitors of Cyclin D1-CDK4: Synthesis, biological evaluation, and structure–activity relationships. *Bioorg. Med. Chem. Lett.*, 2009, 19, 305.

[49] Rheault,T. R.; Caferro, T. R.; Dickerson, S. H.; Donaldson, K. H.; Gaul, M. D.; Goetz, A. S.; Mullin, R. J.; McDonald, O. B.; Petrov, K. G.; Rusnak, D. W.; Shewchak, L. M.; Spehar, G. M.; Truesdale, A. T.; Vanderwall, D. E.; Wood, E. R.; Uehling, D. E. Thienopyrimidine-based dual EGFR/ErbB-2 inhibitors. *Bioorg. Med. Chem. Lett.*, 2009, 19, 817.

[50] Pédeviscqm, S.; Gravier, D.; Casadebaig, F.; Hou, G.; Gissot, A.; De Giorgi, F.; Ichas, F.; Cambar, J.; Pometan, J. P. Synthesis and study of antiproliferative activity of novel thienopyrimidines on glioblastoma cells. *Eur. J. Med. Chem.*, 2010, 45, 2473.

[51] Golub, A. G.; Bdzohla, V. G.; Briukhovetska, N. V.; Balanda, A. O.; Kukharenko, O. P.; Kotey, I. M.; Ostrynska, O. V.; Yarmoluk, S. M. Synthesis and biological evaluation of substituted (thieno[2,3-d]pyrimidin-4-ylthio)carboxylic acids as inhibitors of human protein kinase CK2. *Eur. J. Med. Chem.*, 2011, 46, 870.

[52] Lou, J.; Liu, Z.; Li,Y.; Zhou, M.; Zhang, Z.; Zheng, S.; Wang, R.; Li, J.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

Synthesis and anti-tumor activities of *N*′-benzylidene-2-(4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)acetohydrazone derivatives. *Bioorg. Med. Chem. Lett.*, 2011, 21, 6662.

[53] Zhao, A.; Gao, X.; Wang, Y.; Ai, J.; Wang, Y.; Chen, Y.; Geng, M.; Zhang, A.; Discovery of novel c-Met kinase inhibitors bearing a thieno[2,3-*d*]pyrimidine or furo[2,3-*d*]pyrimidine scaffold. *Bioorg. Med. Chem.*, 2011, 19, 3906.

[54] Becker, T.; Sellmer, A.; Eichhorn, E.; Pongratz, H.; Schächtele, C.; Totzke, F.; Kelter, G.; Krumbach, R.; Fiebig, H.; Böhmer, F.; Mhboobi, S. Novel inhibitors of epidermal growth factor receptor: (4-(Arylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(1*H*-indol-2-yl)methanones and (1*H*-indol-2-yl)(4-(phenylamino)thieno[2,3-*d*]pyrimidin-6-yl)methanones. *Bioorg. Med. Chem.*, 2012, 20, 125.

[55] Safinaz, A. E.; Nagwa, A. M.; Riham, G. F.; Yahya, A. A. Synthesis, antitumor and antibacterial activities of some novel tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine derivatives. *Eur. J. Med. Chem.*, 2013, 65, 195.

[56] Liu, Z. J.; Wu, S. S.; Wang, Y.; Li, R. J.; Wang, J.; Wang, L. H.; Zhao, Y. F.; Gong, P. Design, synthesis and biological evaluation of novel thieno[3,2-d]pyrimidine derivatives possessing diaryl semicarbazone scaffolds as potent antitumor agents. *Eur. J. Med. Chem.*, 2014, 87, 782.

[57] Liu, Z. J.; Wang, Y.; Lin, H. F.; Zuo, D. Z.; Wang, L. H.; Zhao, Y. F.; Gong, P. Design, synthesis and biological evaluation of novel thieno[3,2-d]pyrimidine

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 24 March 2017 doi:10.20944/preprints201703.0190.v1

derivatives possessing diaryl urea moiety as potent antitumor agents. *Eur. J. Med. Chem.*, 2014, 85, 215.

[58] Steffen, B.; Jacob, K. S.; Synne, L.; Unni, N.; Geir, B.; Eirik, S.; Helge, H. B. Structure-activity study leading to identification of a highly active thienopyrimidine based EGFR inhibitor. *Eur. J. Med. Chem.*, 2014, 75, 354.

[59] Manal, K.; Abdelhameid, M. K.; Eman, K.; Labid, M. B. Synthesis of some novel thieno[2,3-d]pyrimidines as potential cytotoxic small molecules against breast cancer. *Chem. Pharm. Bull.*, 2013, 61, 637.

[60] Yong, J. P.; Lu, C. Z.; Wu, X. Y. Synthesis of Isoxazole Moiety Containing Thieno[2,3-d]pyrimidine Derivatives and Preliminarily in vitro Anticancer Activity (Part II). *Anti-cancer Agents Med. Chem.*, 2015, 15, 1148.

[61] Liu, L. J.; Yong, J. P.; Dai, X. J.; Jia, J.; Wang, X. Z.; Wang, J. W. Synthesis of novel isoxazole contained glycyrrhetinic acid derivatives. *Chem. J. Chin. Univ.*, 2006, 27, 1669.

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