Medicinal Chemistry Research (2019) 28:1000–1009
https://doi.org/10.1007/s00044-019-02354-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Discovery of 7-bromo-1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-c] pyrazole-3-carboxamide derivatives as the potential epidermal growth factor receptors for tyrosine kinase inhibitors

Qian Xie[1] · Jingjing Wang[1] · Miao Liu[1] · Xin Wang[1] · Di Jiao[1] · Qingqing Ma[1] · Zhe Jin[1] · Qingguo Meng[1,2] · Chun Hu[1]

Received: 21 February 2019 / Accepted: 30 April 2019 / Published online: 10 May 2019

Abstract

Small molecule tyrosine kinase inhibitors (TKIs) targeting at epidermal growth factor receptor (EGFR) in recent years have made great progress in the treatment of advanced non-small cell cancer (NSCLC). Although as the first-line treatment for sensitizing EGFR mutation-positive metastatic NSCLC, gefitinib has also behaved quite a lot of side effect and EGFR tolerance. Herein, a novel series of 7-bromo-1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-c]pyrazole-3-carboxamide derivatives were designed and synthesized, and screened for their inhibitory activity on the EGFR high-expressing human lung adenocarcinoma cell line A549 and human large cell lung cancer cell line NCI-H460 by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay. The calculated IC_{50} values were reported. Compound **8h** demonstrated the most potent inhibitory activity ($IC_{50} = 9.57 \pm 2.20\ \mu mol\ L^{-1}$ for A549 and $IC_{50} = 13.04 \pm 1.21\ \mu mol\ L^{-1}$ for NCI-H460), comparable to the positive-control gefitinib ($IC_{50} = 8.58 \pm 1.65\ \mu mol\ L^{-1}$ for A549 and $IC_{50} = 18.66 \pm 5.01\ \mu mol\ L^{-1}$ for NCI-H460). Conclusively, 7-bromo- 1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-c]pyrazole-3-carboxamide derivatives as the EGFR–TKIs were discovered, and could be used as potential leading compounds for further research.

Keywords EGFR Inhibitors · Heterocycle · Synthesis · Anticancer activities

Introduction

Lung cancer is the leading cause of cancer death worldwide (Parkin et al. 2005). Eighty percent of lung cancers belong to non-small cell lung cancer (NSCLC), and 70% of NSCLC is diagnosed at an advanced stage, which is difficult to be cured by surgery or radiotherapy (Schiller et al. 2002). With the deeper study of the molecular mechanism of lung cancer, small molecule tyrosine kinase inhibitors (TKIs) targeting at epidermal growth factor receptor (EGFR) in recent years have made great progress in the treatment of advanced NSCLC (Sharma et al. 2007). Studies have found that most NSCLC cancer tissues express ligands such as EGF and TGFα, and activating EGFR mutations are present in 5–15% of NSCLC in the United States and Europe and in 44–55% of NSCLC in east Asia (Kohno et al. 2015).

Targeting activating EGFR mutations with TKIs showed a promising response and prolonged patients' lives in phase III trials. Gefitinib showed superiority to paclitaxel/carboplatin in the Iressa Pan-Asia Study (IPASS) on advanced EGFR-mutant lung adenocarcinoma (Mok et al. 2009; Fukuoka et al. 2011). The National Comprehensive Cancer Network (NCCN) guideline for NSCLC has suggested using gefitinib for first-line treatment of sensitizing EGFR mutation-positive metastatic NSCLC after 2016 (NCCN, 2016). Although occurring at low frequency, progressive respiratory dysfunction, including acute interstitial pneumonia, is the most-severe adverse effect of gefitinib

Supplementary information The online version of this article (https://doi.org/10.1007/s00044-019-02354-0) contains supplementary material, which is available to authorized users.

✉ Chun Hu
chunhu@syphu.edu.cn

[1] Key Laboratory of Structure-based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China

[2] Department of Pharmacy, Yantai Univeristy, Yantai 264005, China

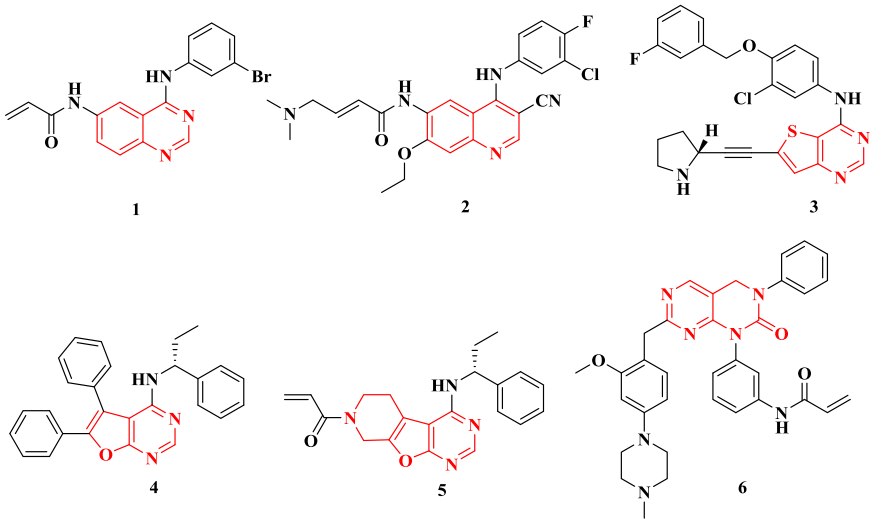


Fig. 1 The structure of some EGFR–TKIs

(Inoue et al. 2003), which has limited the therapeutic benefit of gefitinib. Moreover, ~50% of patients have acquired EGFR-resistant mutations after treatment with gefitinib, which also occurred in the treatment with other EGFR–TKIs (Pao et al. 2005). Consequently, discovering more-effective EGFR–TKIs with lower side effects is still the current hot spot in the research and development of drugs.

It can be found that the structure of most small molecules with EGFR-inhibitory activity reported in the literatures possess heterocyclic scaffold, such as quinazoline, quinoline, thienopyrimidine, furopyrimidine, and tetrahydropyridothiophenepyrimidine, and the side chain with substituted amine group (Fig. 1) (Coumar et al. 2010; Wissner & Mansour, 2008; Wood et al. 2008; Wu et al. 2010; Zhou et al. 2011). Previous studies in our laboratory have shown that 4,5-dihydro-1*H*-thieno[2',3':2,3]thiepino [4,5-c]pyrazole-3-carboxamide derivatives might be potent EGFR inhibitors and showed remarked antitumor activity (Ke et al. 2018). Based on the principle of scaffold hopping (Guo, 2008), hybridization, and bioisosterism, a novel heterocyclic scaffold, thieno[3',2':5,6]thiopyrano[4,3-c] pyrazole, was designed as the target scaffold. The molecular docking has showed that the interaction of the target thieno [3',2':5,6]thiopyrano[4,3-c]pyrazole derivatives with EGFR was similar to the interation of gefitinib with EGFR. Bromination is a frequent modification in medicinal chemistry and can play a significant improvement on the biological activity (Neumann et al. 2008), and introduction of the bromine atom at the parent ring was considered preferentially. As known, the piperazine groups have often appeared in the structures of EGFR inhibitors (Adly et al. 2018; Ke et al. 2018; Sun et al. 2015; Zhang et al. 2016), and the molecular docking results also showed that the target compounds with piperazine side chain have stronger affinity and selectivity for EGFR than that of gefitinib.

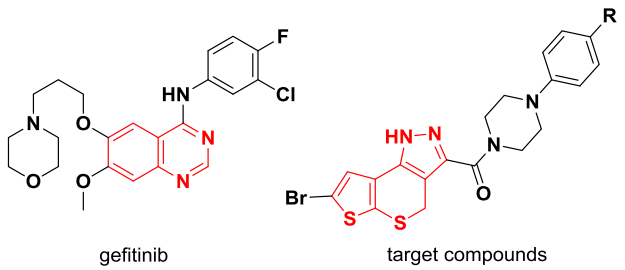


Fig. 2 The structure of gefitinib and designed compounds

Therefore, a novel series of 7-bromo-1,4-dihydrothieno [3',2':5,6]thiopyrano[4,3-c]pyrazole-3-carboxamide derivatives were synthesized in satisfactory yields (Fig. 2). All the target compounds were screened for their antitumor activity on the EGFR high-expressing human lung adenocarcinoma cell line A549 and human large cell lung cancer cell line NCI-H460. The screening results showed that several target compounds exhibited moderate antitumor activity and compound **8h** demonstrated the most potent inhibitory activity.

Results and discussion

Molecular docking studies

A docking study aiming at the characterization of the interaction between the target molecule and EGFR at molecular level was carried out and more-effective EGFR–TKIs were expected to be discovered.

In this study, docking model was selected from Protein Data Bank of Research Collaborator for Structural Bioinformatics (RCSB PDB), and the docking study was prepared by Molegro Virtual Docker (MVD) 2008 (v3.0) (Thomsen & Christensen, 2006). Initially, the docking

studies were carried out on the EGFR–erlotinib complex (PDB code 4HJO) (Park et al. 2012), using the Discovery Studio 2016 client to remove the small-molecule ligand of erlotinib in 4HJO to obtain the three-dimensional crystal structure of EGFR, and then the designed compounds and the positive-control gefitinib were docked to the crystal structure EGFR under MVD software. The detailed docking data will be described below, using compound **8h** with two trifluoromrthyl groups at the 3 and 5 positions of the phenyl ring as an example.

From Fig. 3 and Fig. 4, it can be seen that compound **8h** was inserted inside the protein and the N atom of its pyrazole ring can form a hydrogen bond with the amino acid residue THR830 of EGFR, and similarly the N atom at the N1 position on the quinoline ring of gefitinib also has a hydrogen bond with the amino acid residue of EGFR. At the same time, the amino-acid residue LYS721of EGFR exhibited hydrogen bond interaction with the S atom on the dihydrothiophene ring of **8h** and the N atom at the N1 position on the quinoline ring of gefitinib. The amino-acid residue THR766 of EGFR also has conventional hydrogen bond interaction with the S atom on the dihydrothiophene ring of **8h**. Hence, the importance of dihydrothienopyrazole ring for the binding of small ligand molecules to EGFR is determined. In addition, there is a hydrogen bond interaction with the amino-acid residue SER696 of EGFR on the F atom of the trifluoromethyl group of **8h**. Hence, we can conclude that the type and position of the substituent on the aromatic ring of the aryl piperazine attached to the parent ring will have an effect on the activity of the target compounds. From Fig. 4 and Fig. 5, it can be seen that the amino-acid residues LEU694, LYS721, THR830, ALA719,

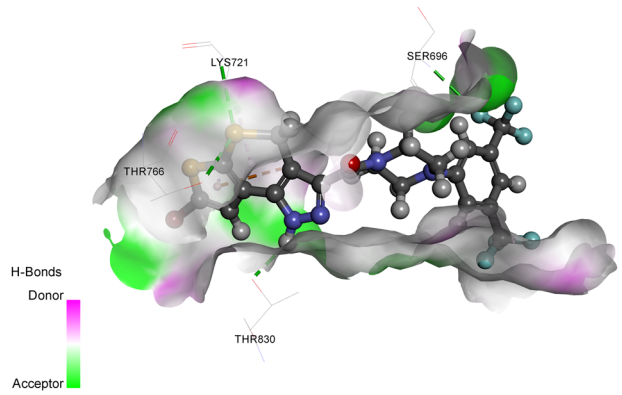


Fig. 3 Interactions of **8h** and the active site of EGFR in H-bonds level

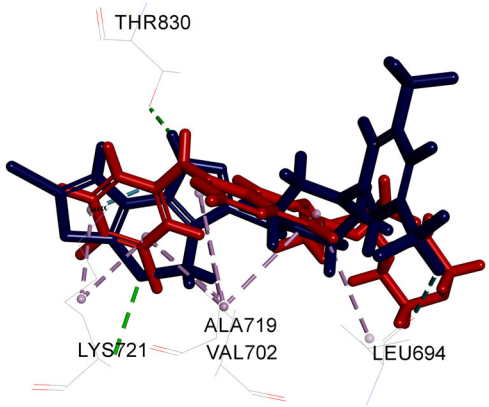


Fig. 5 Interactions of **8h** (blue) and gefinitib (red) with EGFR in a three-dimensional diagram

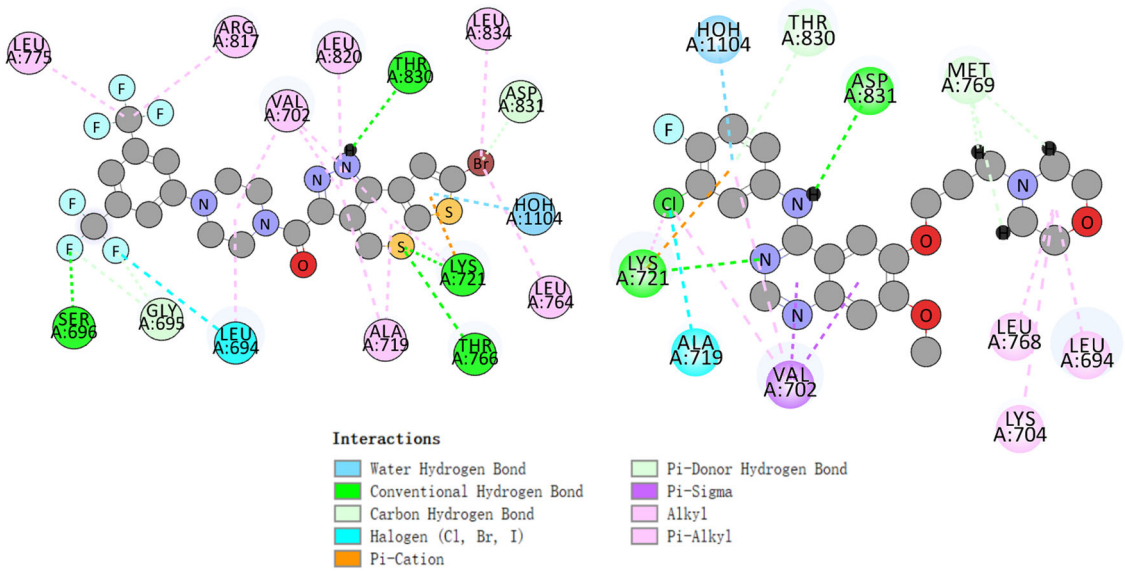
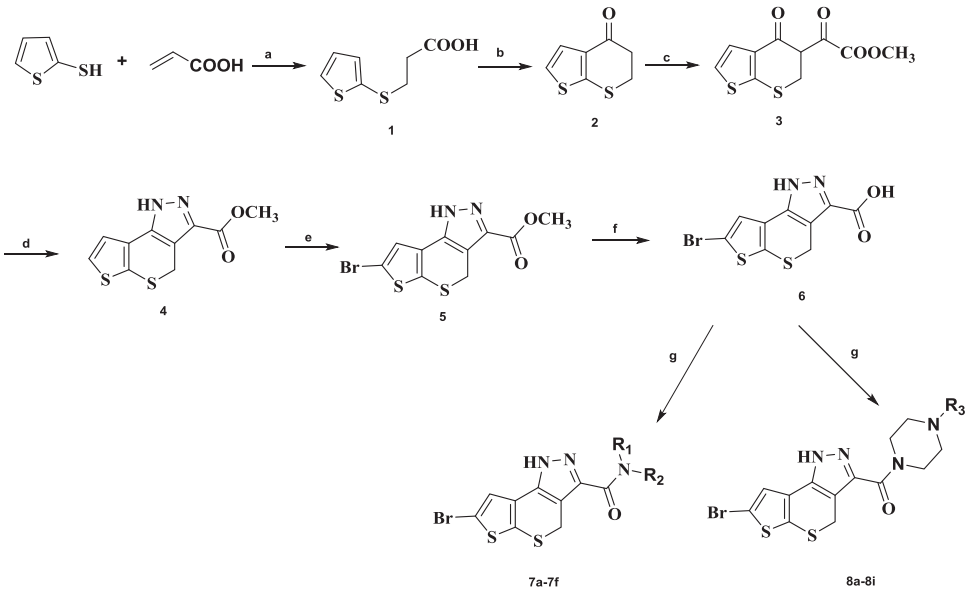


Fig. 4 Interactions of **8h** (left) and gefinitib (right) with EGFR in a two-dimensional diagram



Scheme 1 Reagents and conditions: **a** $NaOC_2H_5$/C_2H_5OH, reflux, N_2; then HCl (aq). **b** oxalyl chloride, r.t, 1 h, N_2; then $SnCl_4$, 0 °C, 12 h, N_2. **c** $NaOCH_3$, dimethyl oxalate, r.t, 24 h. **d**) 80% hydrazine hydrate, reflux, 4 h. **e** Br_2, CH_3COOH/H_2O, 0 °C, 24 h. **f** NaOH/H_2O, reflux; 18% HCl(aq). **g** Amines or 1arylpiperazines, EDCI/HOBt, Et_3N, r.t. 24 h

and VAL702 formed some weak interactions between the compound **8h** and gefitinib.

The molecular docking results revealed that the target compound **8h** and its analogs were potential inhibitors of EGFR, and might exhibit similar effect to gefinitib. Therefore, we synthesized a novel series of 7-bromo- 1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxamide derivatives, aiming to assay their inhibitory activity to EGFR.

Synthesis

The synthesis of 7-bromo- 1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-*c*]pyrazole-3-carboxamide derivatives **7a–7f** and **8a–8i** is described in Scheme 1.

4-(Thiophen-2-ylthio)butanoic acid (**1**), obtained by treating 2-mercaptothiophene with γ-butyrolactone under the nitrogen gas, was reacted with oxalyl chloride and tin tetrachloride under nitrogen gas to give **2** (Ponticello et al. 1988) in the yield of 25–47.5%. The reaction of **2** with dimethyl oxalate in dry toluene at room temperature for 24 h in the presence of sodium hydride yielded the compound **3** (Wang et al. 1990) in the field of 60%. A mixture of **3** and hydrazine hydrate in acetic acid was then refluxed for 4 h to yield **4** (Zhao et al. 2002). Then bromine was added to a mixed solution of 95% glacial acetic acid and compound **4** while stirring in an ice bath, and allowed to react at room temperature for 24 h after the completion of the dropwise addition to give **5**. Compound **5** was refluxed in aqueous sodium hydroxide for 2 h, and then 18% hydrochloric acid was used to yield compound **6** in the field of 75–90%. The target compounds **7a–7f** and **8a–8i** were obtained by reacting **6** with amines or piperazine derivatives in the presence of EDCI and HOBt and were purified by column chromatography on silica gel with eluent of ethyl acetate/petroleum ether (1:1).

Bioactivity in cell line

Using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay, and using gefitinib as a positive-control drug, 15 target compounds were evaluated on the EGFR, high-expressing human lung adenocarcinoma cell line A549, and human large cell lung cancer cell line NCI-H460, which were commonly used in EGFR–TKI-related studies for the effect of cell proliferation levels (Mosmann, 1983; Sun et al. 2016; Zhang et al. 2017; Zhou et al. 2016). The antitumor activity of these 15 target compounds was preliminary screened (final concentration of drug is 8 μM). The calculated IC_{50} value, the concentration (mu-gram/mL) of a compound that was able to cause 50% growth inhibition with respect to the control culture, was summarized in Table 1.

In general, the screening results showed that most of the target compounds exhibit remarked antiproliferative activities against A549 cells, and most of the target compounds hardly exhibit any antiproliferative activities against NCI-H460 cells except **7e**, **7f**, **8b**, **8h**, and **8i**.

In A549 cells, the IC_{50} values of compounds **7e**, **7f**, **8c**, **8f**, and **8h** were close to the positive-control drug gefitinib, indicating that these compounds exhibit significant proliferation inhibitions on A549 lung cancer cells; in NCI-H460 lung cancer cells, the IC_{50} values of the compound **8h** were smaller than that of the positive-control drug gefitinib, suggesting that these drugs have a stronger inhibitory effect on proliferation of NCI-H460 lung cancer cells. As shown

Table 1 IC_{50} values of the target compounds **7a–7f** and **8a–8i** against A549 and NCI-H460

No.	Substituents			IC_{50}(μM)	
	R_1	R_2	R_3	A549	NCI-H460
7a	C_2H_5	C_2H_5		25.13±6.91	-67.29±101.53
7b	H			14.31±1.94	-11.14±5.72
7c	H			4.71±28.95	-27.73±15.66
7d	H	C_6H_5		17.66±8.45	-14.65±6.31
7e	H			**11.77±1.10**	28.31±23.13
7f	H			**12.73±4.68**	23.70±15.45
8a				19.12±1.98	-93.76±53.15
8b				3.39±27.14	42.12±34.69
8c				**12.06±2.29**	-43.32±13.66
8d				7.67±38.51	-16.32±9.08
8e				25.43±51.63	-16.12±7.74
8f				**10.57±2.20**	-24.61±17.68
8g				20.30±6.57	-19.69±3.91
8h				**9.57±2.20**	**13.04±1.21**
8i				-72.53±126.56	9.46±72.03
gefitinib				8.58±1.65	18.66±5.01

in Table 1, the compound **8h** displayed the most-potent inhibitory activity ($IC_{50} = 9.57 \pm 2.20\ \mu mol\ L^{-1}$ for A549 and $IC_{50} = 13.04 \pm 1.21\ \mu mol\ L^{-1}$ for NCI-H460), comparable to the positive-control gifitinib ($IC_{50} = 8.58 \pm 1.65\ \mu mol\ L^{-1}$ for A549 and $IC_{50} = 18.66 \pm 5.01\ \mu mol\ L^{-1}$ for NCI-H460).

By analyzing the effect of side-chain changes on activity, it was found that the target compounds with substituted piperazine side chains (**8a–8i**) are significantly more active than the compounds with other amine side chains (**7a–7f**). The compound which introduced a methyl group or a methoxy group (**8f** and **8g**) at the 4-position of the phenyl ring in the substituted aryl piperazine side chain has high biological activity, but when an electron-withdrawing group (**8e**) at this position was introduced, the biological activity is reduced. It can be seen that the introduction of a smaller electron-donating group at this position is advantageous for enhancing the biological activity. When two trifluoromethyl groups were introduced at the 3 and 5 positions of the phenyl ring, this compound (**8h**) showed strong EGFR-inhibitory activity in human lung adenocarcinoma cell line A549, whereas the EGFR-inhibitory activity of compound **8i** is significantly reduced after removing one trifluoromethyl group. It can be seen that there is a close relationship between the position of the substituent and the biological activity. When one or two electron-withdrawing groups are introduced at different positions of the phenyl ring (such as **8a–8e**), the biological activity of the compound varies greatly. These results suggest that there is an interesting correlation between the position and size of the substituents and the biological activity.

Conclusion

Based on the structure of known EGFR–TKI inhibitors, and the principle of scaffold hopping, hybridization, and bioisosterism, combining structural optimization, a novel series of 7-bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-c] pyrazole-3-carboxamide derivatives, was designed as the target compounds. The binding mode of **8h**, a representative of the target compounds, to EGFR was simulated by Molegro Virtual Docker software. It was found that the N atom of pyrazole ring and the S atom of dihydrothiophene ring can both form a hydrogen bond with the amino-acid residue in the EGFR protein. As the target compounds, these novel structures lay a foundation for the discovery of novel EGFR–TKI inhibitors through synthesis and pharmacological screening studies.

A novel series of 7-bromo-1,4-dihydrothieno[3',2':5,6] thiopyrano[4,3-*c*]pyrazole-3-carboxamide derivatives were successfully synthesized and screened for their inhibitory activity on EGFR high-expressing human lung adenocarcinoma cell line A549 and human large cell lung cancer cell line NCI-H460 using the MTT colorimetric assay. As shown, a structure–activity relationship was clearly discernible, and several of the compounds exhibited moderate antitumor activity. Particularly, compound **8h** demonstrated the most-potent inhibitory activity ($IC_{50} = 9.57 \pm 2.20\ \mu mol\ L^{-1}$ for A549 and $IC_{50} = 13.04 \pm 1.21\ \mu mol\ L^{-1}$ for NCI-H460), comparable to the positive-control gefitinib ($IC_{50} = 8.58 \pm 1.65\ \mu mol\ L^{-1}$ for A549 and $IC_{50} = 18.66 \pm 5.01\ \mu mol\ L^{-1}$ for NCI-H460). It is hoped that these novel compounds, as EGFR–TKIs, could be used as potential leading compounds for further research.

Experimental section

Materials and methods

All the starting materials were commercially available and directly used without further purification. All the reaction progresses were monitored by thin layer chromatography with the silica gel plates (Qingdao Jiyida silica reagent factory, Qingdao, China). The melting points were determined on a melting-point apparatus with microscope (Zhengzhou Mingyi Instrument Equipment Co., Ltd., Zhengzhou, China), and were uncorrected. The infrared (IR) spectra were recorded on a Bruker IFS55 spectrometer (Faellanden, Switzerland) utilizing KBr pellets. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance spectrometer (Karlsruhe, Germany) and with TMS as an internal standard and DMSO-d_6 or $CDCl_3$ as the solvent, chemical shifts (*d* values), and coupling constants (*J* values) were, respectively, given in ppm and Hz. ESI mass spectra were performed on a Waters spectrometer (Massachusetts, USA). High resolution mass spectrometry (HRMS) analyses were performed on an Agilent Technologies 6530 Accurate-Mass Q-TOF Mass Spectrometer (Santa Clara, CA, USA). The purities were determined by high-performance liquid chromatography (HPLC) using an Agilent 1100 series HPLC (Santa Clara, USA).

Synthesis of 4-(thiophen-2-ylthio)butanoic acid (1)

The sodium ethoxide was prepared with sodium (3.34 g, 145 mmol) and ethanol (120 mL). 2-mercaptothiophene (9.80 g, 84 mmol) was added dropwise to this solvent and stirred 30 min at room temperature. γ-Butyrolactone (11.5 mL, 128 mmol) was added and the mixture was refluxed for 19 h under N_2. The mixture was concentrated, and the residue was solved in water. The solution was acidified with 18% hydrochloric acid to pH 1–2, and then extracted three times with EtOAc. The combined organic layer was dried, filtered, and concentrated. The residue was recrystallized from petroleum ether to give **1** (8.08 g, 47.4%) as a white solid. mp. 40–42 °C.

Synthesis of 6,7-dihydrothieno[2,3-b]thiepin-4(5H)-one (2)

To a solution of **1** (4.04 g, 20 mmol) add 2 drops of DMF in CH_2Cl_2 (20 mL), oxalyl chloride (2.81 g, 22 mmol) was added dropwise at room temperature under N_2. After the mixture was stirred for 1 h, it was cooled to 0 °C and a solution of $SnCl_4$ (2.57 g, 10 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The solution was stirred at 0 °C for 12 h, and then H_2O was added. The organic layer was separated and washed with saturated Na_2CO_3 and brine, respectively. After drying and filtration, the resulting organic solution was concentrated to dryness under vacuum. The residue was recrystallized from petroleum ether to yield **2** (0.92 g, 25.0%) as a white solid. mp: 59.0–60.5 °C (lit. 65 °C (Cagniant & Cagniant 1966)). ^{1}H-NMR (600 MHz, $DMSOd_6$): δ 2.77(2H, t, $J = 5.1$ Hz, S-CH_2), 3.47(2H, t, $J = 5.1$ Hz, $CH_2C{=}O$), 7.35(1H, d, $J = 5.4$ Hz, H-7), 7.38 (1H, d, $J = 5.4$ Hz, H-8).

Synthesis of methyl oxo(4-oxo-4,5,6,7-tetrahydrothieno[2,3-b]thiepin-5-yl)acetate (3)

The sodium methoxide was prepared with sodium (0.46 g, 20 mmol) and methanol (20 mL), which were removed by distillation when sodium was dissolved completely. A solution of **2** (1.84 g, 10 mmol) and dimethyl oxalate (2.36 g, 20 mmol) in dry toluene (20 mL) was added to the sodium methoxide. The mixture was stirred for 24 h at room temperature. Then the mixture was poured into 100 mL

water. The aqueous layer was separated, and the organic layer was extracted three times with 10% NaOH (each 15 mL). The combined aqueous layer was acidified with 18% hydrochloric acid to yield **3** (1.62 g, 60.0%) as a red oil.

Synthesis of methyl 4,5-dihydro-1H-thieno[2′,3′:2,3]thiepino[4,5-c]pyrazole-3-carboxylate (4)

A solution of **3** (0.54 g, 2 mmol) and 80% hydrazine hydrate (2 mL, 32 mmol) in acetic acid (10 mL) was refluxed for 4 h. The reaction mixture was poured into ice–water mixture (50 g) and allowed to stand overnight. A precipitate was collected to yield **4** (0.40 g, 75.0%) as a white solid. mp: 94.0–95.7 °C (lit. 94–96 °C (Sun et al. 2011)). ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.97(3H, s, O-CH_3), 4.33(2H, s, S-CH_2), 7.18(1H, d, $J = 5.3$ Hz, H-7), 7.40 (1H, d, $J = 5.3$ Hz, H-8).

Synthesis of 7-bromo-1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-c]pyrazole-3-carboxylic acid methyl ester (5)

A mixture of **4** (2.52 g, 0.01 mol), 50 mL glacial acetic acid, and 5 mL water was placed in a round-bottom flask, and bromine (2.08 g, 0.013 mol) was added dropwise with stirring in an ice bath. Then the mixture was reacted at room temperature for 24 h. The reaction liquid was poured into 200 mL ice–water, and a white solid was precipitated, and filtered, to give a white solid (2.52 g). ^{1}H-NMR (400 MHz, $DMSOd_6$) δ 3.86 (s, 3H, -O-CH_3), 4.38 (s, 2H, S-CH_2),7.47 (s, 1H, Ar-H). ESI-MS: m/z (pos) 330.9 $C_{10}H_7BrN_2O_2S_2$ $[M+H]^+$ (calcd 329.9).

Synthesis of 7-bromo-1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-c]pyrazole-3-carboxylic acid (6)

A 100 mL round bottom flask was charged with **5** (0.62 g, 2 mmol), 20 mL water, and 0.4 g sodium hydroxide, and heated to reflux for 2 h. The reaction mixture was adjusted to pH 2 with 18% hydrochloric acid, and a white solid (0.57 g) was precipitated in the field of 90.0%. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 4.37 (s, 2H, S-CH_2), 7.39 (s, 1H, Ar-H), 12.92 (s, 1H, -OH). ESI-MS: m/z (pos) 316.9 $C_9H_5BrN_2O_2S_2$ $[M+H]^+$ (calcd 315.9).

General procedure for the synthesis of 7-bromo- 1,4-dihydrothieno[3′,2′:5,6]thiopyrano[4,3-c]pyrazole-3-carboxamids (7a–7f and 8a–8i)

A mixture of **6** (1.01 g, 4 mmol), amines or piperazine derivative (4 mmol), 1ethyl-3-(3dimethylaminopropyl)carbodiimide hydrochloride (1.00 g, 5 mmol), 1hydroxybenzotriazole (0.10 g, 0.7 mmol), 1 mL Et_3N and absolutely dry CH_2Cl_2 (20 mL) was stirred for 24 h at room temperature. The mixture was filtered and the filtrate was washed with 20 mL of 1N HCl, saturated Na_2CO_3 and brine, respectively. Then the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography on silica with EtOAc-petroleum (3:1, v/v) as the eluent to yield product as a white solid.

***N*,*N*-Diethyl-7-bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-c]pyrazole-3-carboxamide (7a)**: white solid, yield 50%. mp. 147–148 °C; IR (KBr, cm^{-1}): 3212, 2958, 2917, 2849, 1735, 1583, 1535, 1508, 1485, 1461, 1375, 1215, 1159, 967, 849; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.25(m, 6H, 2CH_3), 3.54(m, 4H, 2N-CH_2), 4.12(s, 2H, S-CH_2), 7.72(s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.83 (C=O), 143.03 (C-1), 135.57 (C-2), 131.65 (C-6), 127.55, 126.07, 110.25, 108.89 (Ar-C), 43.30, 43.10 (H_2C-N-CH_2), 26.19 (C-3), 15.17, 13.30(2CH_3); HRMS (ESI) m/z (neg): 369.969569, $C_{13}H_{13}BrN_3OS_2$ $[M-H]^-$(calcd. 369.968890); purity (HPLC): 98.53%.

***N*-tert-Butyl-7-bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-c]pyrazole-3-amide (7b)**: white solid, yield 75%. mp. 205–207 °C; IR (KBr, cm^{-1}): 3364, 3201, 2919, 1638, 1553, 1526, 1492, 1450, 1385, 1296, 1220, 1167, 969, 863; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.49(s, 9H, $C(CH_3)_3$), 4.49(s, 2H, S-CH2), 7,21(s, 1H, H-10), 7.40(s, 1H, NHC=O); HRMS (ESI) m/z (neg): 369.969569, $C_{13}H_{13}BrN_3OS_2$ $[M-H]^-$(calcd. 369.968890); purity (HPLC): 98.02%.

***N*-Isopropyl-7-bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-c]pyrazole-3-amide (7c)**: white solid, yield 69%. mp. 189–190 °C; IR (KBr, cm^{-1}): 3400.5, 3207.5, 2974.5, 2921.3, 1637.4, 1556.3, 1528.3, 1452.1, 1384.6, 1175.4, 967.5, 836.5; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 1.14(d, 6H, $J = 6.6$ Hz, 2CH_3), 4.48–4.52(m, 1H, N-CH), 4.39(s, 2H, S-CH_2), 7.50(s, 1H, H-10), 8.08(s, 1H, NHC=O); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 161.38 (C=O), 141.96 (C-1), 136.54 (C-2), 131.99 (C-6), 129.10, 126.34, 109.28, 108.80 (Ar-H), 67.84 (N-CH), 30.28 (C-3), 14.35, 11.26 (2CH_3); HRMS (ESI) m/z (neg): 355.953471, $C_{12}H_{11}BrN_3OS_2$ $[M-H]^-$(calcd. 355.953240); purity (HPLC): 97.80%.

***N*-Phenyl-7-bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-c]pyrazole-3-amide (7d)**: white solid, yield 30%. mp. 190–191 °C; IR (KBr, cm^{-1}): 3443.8, 2919.8, 2850.5, 1730.5, 1612.4, 1555.1, 1441.0, 1384.2, 1296.2, 1114.0, 1038.4, 778.5; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 4.42 (s, 2H, S-CH_2), 7.36–7.43 (m, 5H, Ar-H), 7.42 (s, 1H, H-10), 8.82 (s, 1H, NHC=O), 13.64 (s, 1H, NH); MS (ESI) m/z (neg): 390.1, $C_{15}H_8BrN_3OS_2$ $[M-H]^-$(calcd. 389.9).

***N*-Benzyl-7-bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano[4,3-c]pyrazole-3-amide (7e)**: white solid, yield 56%. mp 98–100 °C; IR (KBr, cm^{-1}): 3421.9, 2920.9,

2851.7, 1629.7, 1446.5, 1384.2, 1274.3, 1114.1, 850.9; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 4.40 (d, 2H, Ar-CH), 4.42 (s, 2H, S-CH_2), 7. 36–7.43(m, 5H, Ar-H), 7.42 (s, 1H, H-10), 8.82(s, 1H, NHC=O), 13.64(s, 1H, NH); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.34 (C=O), 140.19 (C-1), 136.50 (C-11), 133.96 (C-2), 131.72 (C-6), 129.12, 128.69, 127.81, 127.45, 127.17, 127.01, 126.27, 109.45, 108.88 (Ar-H), 42.34 (NH-CH_2), 25.85 (C-3); HRMS (ESI) m/z (neg): 403.953743, $C_{16}H_{11}BrN_3OS_2$ [M-H]$^-$(calcd. 403.953240); purity (HPLC): 98.68%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(2-methylphenyl)piperazine (7f): white solid, yield 48%. mp.167–170 °C; IR (KBr, cm^{-1}): 3442.6, 2920.4, 2851.6, 1621.6, 1491.9, 1443.3, 1384.0, 1327.3, 1274.9, 1224.2, 1126.5, 1021.8, 1000.2, 762.3; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 4.22(s, 2H, S-CH_2), 4.60(d, 2H, N-CH), 6.29(d, 1H, $J = 6$ Hz, H-19), 6.33(d, 1H, $J = 6$ Hz, H-21), 7.20(dd, 1H, $J = 6$ Hz, 6 Hz, H-20), 7.25(s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.24 (C=O), 153.01 (C-1), 142.30 (C-11), 141.61 (C-14), 136.54 (C-2), 131.76 (C-6), 127.47, 126.19, 110.91, 109.42, 108.89, 107.19 (Ar-H), 35.65 (NH-CH_2), 25.88 (C-3); HRMS (ESI) m/z (neg): 393.932823, $C_{14}H_9BrN_3O_2S_2$ [M-H]$^-$(calcd. 393.932504); purity (HPLC): 99.41%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(3-chloro-4-fluorophenyl) piperazine (8a): white solid, yield 36%. mp. 120 °C; IR (KBr, cm^{-1}): 3233, 2924, 2853, 1745, 1609, 1586, 1502, 1464, 1382, 1223, 1154, 998; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.16(t, 4H, $J = 6.0$ Hz, Ar-N(CH_2)$_2$), 3.95(t, 4H, $J =$ 6.0 Hz, CON(CH_2)$_2$), 4.26(s, 2H, S-CH_2), 6.76–6.80(m, 1H, H-23), 6.95(dd, 1H, $J = 6.3$ Hz, $J = 2.4$ Hz, H-27), 7.05 (dd, 1H, $J = 8.7$ Hz, $J = 9$ Hz, H-26), 7.25 (s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.00 (C=O), 152.65 (C-17), 150.28 (C-1), 148.72 (C-14), 131.84 (C-2), 130.09 (C-6), 126.26, 120.14, 119.96, 117.62, 117.41, 117.20, 116.50, 108.96 (Ar-H), 49.55, 48.97 (C-11, C-12), 46.52, 41.97 (C-10, C-13), 29.49 (C-3); HRMS (ESI) m/z (neg): 510.948158, $C_{19}H_{14}BrClFN_4OS_2$ [M-H]$^-$(calcd. 510.947045); purity (HPLC): 99.74%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(2-fluorophenyl)piperazine (8b): white solid, yield 39%. mp. 78–80 °C; IR (KBr, cm^{-1}): 3221, 2921, 2851, 1720, 1641, 1500, 1446, 1394, 1385, 1287, 1240, 1151, 999; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.11 (t, 4H, $J = 10.2$ Hz, Ar-N(CH_2)$_2$), 4.02(t, 4H, $J = 10.2$ Hz, CON(CH_2)$_2$), 4.24(s, 2H, S-CH_2), 7.01–7.04(m, 3H, Ar-H), 7.23(s, 1H, H-10), 7.38(d, 1H, $J = 7.8$ Hz, Ar-H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 161.62 (C=O), 156.68 (C-15), 140.01 (C-1), 139.92 (C-2), 126.27, 125.34, 125.31, 123.34, 123.26, 120.12, 120.09, 116.56, 116.36, 108.97 (Ar-H), 51.18, 50.73 (C-11, C-12), 46.99, 42.32 (C-10, C-13), 25.90 (C-3); HRMS (ESI) m/z (neg): 476.986542, $C_{19}H_{15}BrFN_4OS_2$ [M-H]$^-$(calcd. 476.986017); purity (HPLC): 99.12%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(3-bromophenyl)piperazine (8c): white solid, 0.82 g, yield 38%. mp. 150–151 °C; IR (KBr, cm^{-1}): 3418.7, 3217.5, 2920.0, 2850.7, 1590.4, 1480.6, 1442.9, 1384.6, 1224.6, 1157.9, 999.9, 938.2, 764.3; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.20–3.27(m, 4H, Ar-N(CH_2)$_2$), 3.98–4.04(m, 4H, CON(CH_2)$_2$), 4.26(s, 2H, S-CH_2), 6.85(m, 1H, Ar-H), 7.00–7.03(m, 1H, Ar-H), 7.05–7.09(m, 1H, Ar-H), 7.11–7.14(m, 1H, Ar-H), 7.23 (s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 161.68 (C=O), 152.58 (C-14), 132.17 (C-1), 131.97 (C-2), 131.61 (C-18), 131.23 (C-6), 129.12, 126.28, 123.00, 121.87, 118.26, 114.87, 110.41, 108.96 (Ar-H), 48.74, 48.37 (C-11, C-12), 46.52, 42.02 (C-10, C-13), 25.92 (C-3); HRMS (ESI) m/z (neg): 536.906767, $C_{19}H_{15}Br_2N_4OS_2$ [M-H]$^-$ (calcd. 536.905951); purity (HPLC): 99.62%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(3,4-difluorophenyl)piperazine (8d): white solid, yield 44%. mp. 190–192 °C; IR (KBr, cm^{-1}): 3444.2, 2919.8, 1656.7, 1560.4, 1417.7, 1384.3, 1276.6, 1116.2; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 3.12–3.16 (m, 4H, Ar-N(CH_2)$_2$), 3.70–3.74(t, 4H, CON (CH_2)$_2$), 4.27(s, 2H, S-CH_2), 6.72–6.77(m, 1H, Ar-H), 7.00–7.04 (m, 1H, Ar-H), 7.25 (d, 1H, $J = 9.6$ Hz, Ar-H), 7.45(s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 160.06 (C=O), 151.31 (C-16), 149.02 (C-1), 148.71 (C-14), 148.63 (C-17), 144.71 (C-2), 142.22 (C-6), 126.26, 117.84, 117.67, 112.02, 108.94, 105.38, 105.17 (Ar-H), 49.46, 48.86 (C-11, C-12), 46.47, 41.97 (C-10, C-13), 26.01 (C-3); HRMS (ESI) m/z (neg): 494.976868, $C_{19}H_{14}BrF_2N_4OS_2$ [M-H]$^-$(calcd. 494.976595); purity (HPLC): 99.44%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(4-fluorophenyl) piperazine (8e): white solid. mp. 185–186 °C; IR (KBr, cm^{-1}): 3443, 2922, 2852, 1605, 1507, 1443, 1384, 1225, 1116; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.17–3.23 (m, 4H, Ar-N(CH_2)$_2$), 4.02–4.05 (m, 4H, CON(CH_2)$_2$), 4.24 (s, 2H, S-CH_2), 7.0–7.04 (m, 4H, Ar-H), 7.24 (s, 1H, H-10); ^{13}C-MR (101 MHz, DMSO-d_6) δ 162.01 (C=O), 157.93 (C-17), 148.18 (C-1), 142.34 (C-14), 135.82 (C-2), 131.87 (C-6), 127.47, 126.15, 118.22, 118.14, 115.91, 115.70, 110.66, 108.96 (Ar-H), 50.31, 49.71 (C-11, C-12), 46.72, 42.14 (C-10, C-13), 26.09 (C-3); HRMS (ESI) m/z (neg): 476.986845, $C_{19}H_{15}BrFN_4OS_2$ [M-H]$^-$(calcd. 476.986017); purity (HPLC): 98.58%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(4-trimethoxyphenyl) piperazine (8f): white solid, yield 44%. mp. 90 °C; IR (KBr, cm^{-1}): 2922, 1730, 1618, 1511, 1446, 1379, 1255, 1224, 1186, 1137, 1096, 1034, 992, 816; ^{1}H-NMR (600 MHz, $CDCl_3$)): δ3.12–3.15(m, 4H, Ar-N(CH_2)$_2$), 3.96–4.03(m, 7H, CON

$(CH_2)_2$, O-CH_3), 4.28(s, 2H, S-CH_2), 6.85(dd, 2H, $J = 7.2$ Hz, 2.4 Hz, Ar-H), 6.91(dd, 2H, $J = 7.2$ Hz, 2.4 Hz, Ar-H), 7.24 (s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.00 (C=O), 153.78 (C-17), 145.61 (C-1), 142.39 (C-14), 135.80 (C-2), 131.86 (C-6), 129.11, 127.48, 126.15, 118.49, 114.75, 110.61, 108.96, 99.98 (Ar-H), 55.65 (O-CH_3), 51.01, 50.43 (C-11, C-12), 46.87, 42.25 (C-10, C-13), 26.08 (C-3); HRMS (ESI) m/z (neg): 489.006763, $C_{20}H_{18}BrN_4O_2S_2$ $[M-H]^-$(calcd. 489.006004); purity (HPLC): 98.96%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(4-methylphenyl)piperazine (8g): white solid, yield 30%. mp. 185–186 °C; IR (KBr, cm^{-1}): 3441, 2920, 2852, 1610, 1513, 1445, 1384, 1156, 1023, 813, 619; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 3.11–3.16(m, 4H, Ar-N $(CH_2)_2$), 3.95–4.03(m, 7H, CON$(CH_2)_2$, Ar-CH_3), 4.28(s, 2H, S-CH_2), 6.85(dd, 2H, $J = 7.2$ Hz, 2.4 Hz, H-10), 6.91(dd, 2H, $J = 7.2$ Hz, 2.4 Hz, H-10), 7.24 (s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.01 (C=O), 149.21 (C-1), 142.37 (C-14), 135.81 (C-2), 131.86 (C-6), 129.88, 128.68, 127.48, 126.67, 126.15, 116.77, 116.64, 110.63, 108.94 (Ar-H), 50.06, 49.44 (C-11, C-12), 46.75, 42.16 (C-10, C-13), 26.10 (C-3), 20.54 (CH_3); HRMS (ESI) m/z (neg): 473.011806, $C_{20}H_{18}BrN_4OS_2$ $[M-H]^-$(calcd. 473.011089); purity (HPLC): 97.21%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(3,5-trifluoromethylphenyl) piperazine (8h): white solid, yield 45%. mp. 220–221 °C; IR (KBr, cm^{-1}): 3421.2, 2918.2, 1610.2, 1581.2, 1485.5, 1400.4, 1275.0, 1175., 1131.4, 995.3, 963.6, 862.4; ^{1}H-NMR (600 MHz, DMSO-d_6): δ 3.40–3.48(m, 4H, Ar-N $(CH_2)_2$), 3.75–3.78(m, 2H, $CONCH_2$), 4.11–4.14(m, 2H, $CONCH_2$), 4.31(s, 2H, S-CH_2), 7.33(s, 1H, Ar-H), 7.45(s, 1H, H-10), 7.50(s, 2H, Ar-H), 13.64(s, 1H, NH); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 162.12 (C=O), 151.85 (C-14), 142.27 (C-1), 135.85 (C-2), 131.89, 131.74 (C-16, C-18), 131.42 (C-6), 131.10, 130.01 ($2CF_3$), 127.45, 126.16, 125.40, 122.69, 114.88, 110.77, 108.95 (Ar-H), 48.03, 47.35 (C-11, C-12), 46.24, 41.87 (C-10, C-13), 26.10 (C-3); HRMS (ESI) m/z (neg): 594.970282, $C_{21}H_{14}BrF_6N_4OS_2$ $[M-H]^-$(calcd. 594.970208); purity (HPLC): 98.63%.

1-(7-Bromo-1,4-dihydrothieno[3',2':5,6]thiopyrano [4,3-c]pyrazole-3-formyl)-4-(3-trifluoromethylphenyl) piperazine (8i): white solid, yield 45%. mp. 185–186 °C; IR (KBr, cm^{-1}): 3444.9, 3219.1, 2918.2, 2849.8, 1591.1, 1444.4, 1384.4, 1349.9, 1319.6, 1269.0, 1222.1, 1163.5, 1112.2, 1075.2, 999.6, 946.4, 864.1, ^{1}H-NMR (600 MHz, DMSO-d_6): δ 3.26–3.34(m, 8H, N$(CH_2CH_2)_2$N), 4.27(s, 2H, S-CH_2), 7.09 (d, 1H, $J = 7.7$ Hz, Ar-H), 7.24 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.43 (t, 1H, $J = 8.0$ Hz, Ar-H), 7.50 (s, 1H, H-10); ^{13}C-NMR (101 MHz, DMSO-d_6) δ162.08 (C=O), 151.47 (C-14), 142.32 (C-1), 135.83 (C-2), 131.89 (C-6), 130.54 (C-16), 130.48 (CF_3), 127.46, 126.15, 123.52, 119.47, 115.42, 111.77, 110.71, 108.96 (Ar-H), 48.74, 48.09 (C-11, C-12), 46.47, 42.00 (C-10, C-13), 26.09 (C-3); HRMS (ESI) m/z (neg): 526.983766, $C_{20}H_{15}BrF_3N_4OS_2$ $[M-H]^-$(calcd. 526.982823); purity (HPLC): 99.72%.

Molecular docking

The complexes for the enzyme were those deposited in the RCSB Protein Data Bank for the EGFR (PDB code: 4HJO) after eliminating the inhibitor (erlotinib) and water molecules. The missing residues were built and the polar hydrogen atoms acid residues were added. Molecular docking was carried out using MVD. The docking was set at 1500 maximum iterations, with a simplex evolution population size of 50 and a minimum of 10 runs. The schematic diagrams of interactions between 4HJO and the small molecules were analyzed by PyMOL 1.7.

Bioactivity in cell line

In order to determine the anticancer activity of our target compounds in vitro, the MTT assay was used. A549 cells, Hela cells, SW480 cells, HepG2 cells, and HL7702 cells were harvested in the logarithmic growth phase and seeded in 96-well plates at a density of 8000 cells per well, and cultured at 37 °C in humidified atmosphere containing 5% CO_2 in Dulbecco's modified Eagle's medium (or RPMI-1640) with 10% fetal bovine serum for 24 h before any treatments. Tested compounds were dissolved in DMSO and diluted in the culture fluid to get various concentrations. The cells were treated with target compounds subsequently and incubated overnight. Then 20 μL of MTT (5 mg/mL) was added in each well and after 4 h incubation, the medium was removed immediately and MTT formazan was solubilized in 150 μL DMSO. The optical densities were measured with a microplate reader (Bio-Tek instruments, INC. USA) at 490 nm. Inhibitory effects were determined as IC_{50} value.

Acknowledgements This work was supported by the National Science Foundation of China (NSFC) for the grant No. 21342006 and the Program for Innovative Research Team of the Ministry of Education of China for the grant No. IRT_14R36. We thank Molegro ApS for kindly providing a free evaluation copy of their software package and Schrödinger, LLC for allowing the use of PyMOL Molecular Graphics System.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals, performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

References

Adly ME, Gedawy EM, El-Malah AA, El-Telbany FA (2018) Synthesis of novel thieno[2,3-d]pyrimidine derivatives and rvaluation of Theircytotoxicity and EGFR inhibitory activity. Anticancer Agents Med Chem 18(5):747–756

Cagniant P, Cagniant D (1966) Contibution àl'étude des hétérocycles soufrés condensés.Dérivés du dihydro-5,6 4H thiéno-[2,3-b]-thiopyranne, du dihydro-5,6 7H thiéno [3,2-b]-thiopyranne et de la tétrahydro-5,6,7,8 thiéno[3,2-b]-thiépinne. Bull Soc Chim Fr 7:2172–2179

Coumar MS, Chu CY, Lin CW, Shiao HY, Ho YL, Reddy R, Lin WH, Chen CH, Peng YH, Leou JS, Lien TW, Huang CT, Fang MY, Wu SH, Wu JS, Chittimalla SK, Song JS, Hsu JT, Wu SY, Liao CC, Chao YS, Hsieh HP (2010) Fast-forwarding hit to lead: aurora and epidermal growth factor receptor kinase inhibitor lead identification. J Med Chem 53(13):4980–4988

Fukuoka M, Wu YL, Thongprasert S, Sunpaweravong P, Leong SS, Sriuranpong V, Chao TY, Nakagawa K, Chu DT, Saijo N, Duffield EL, Rukazenkov Y, Speake G, Jiang H, Armour AA, To KF, Yang JC, Mok TS (2011) Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 29:2866–2874

Guo ZR (2008) Strategy of molecular drug design: pharmacophore and scaffold hopping. Chin J Med Chem 18:147–157

Inoue A, Saijo Y, Maemondo M, Gomi K, Tokue Y, Kimura Y, Ebina M, Kikuchi T, Moriya T, Nukiwa T (2003) Severe acute interstitial pneumonia and gefitinib. Lancet 361:137–139

Ke J, Lu Q, Wang X, Sun R, Jin Z, Zhan X, Hu J, Wan DCC, Hu C (2018) Discovery of 4,5-Dihydro-1H-thieno[2′,3′:2,3]thiepino [4,5-c]pyrazole-3-carboxamide derivatives as the potential epidermal growth factor receptors for tyrosine kinase inhibitors. Molecules 23:1980

Kohno T, Nakaoku T, Tsuta K, Tsuchihara K, Matsumoto S, Yoh K, Goto K (2015) Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. Transl Lung Cancer Res 4:156–164

Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. New Engl J Med 361:947–957

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immun Methods 65:55–63

National Comprehensive Cancer Network. Non-small Cell Lung Cancer (Version 2, 2016). Available at: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf. Accessed 2015-12-01

Neumann CS, Fujimori DG, Walsh CT (2008) Halogenation strategies in natural product biosynthesis. Chem Biol 15:99–109

Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2(3):e73

Park JH, Liu Y, Lemmon MA, Radhakrishnan R (2012) Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain. Biochem J 448:417–423

Parkin DM, Bray FB, Pisani P (2005) Global cancer statistics. CA Cancer J Clin 55(2):74–108

Ponticello GS, Freedman MB, Habecker CN, Habecker CN, Holloway MK, Amato JS, Conn RS, Baldwin JJ (1988) Utilization of α, β-unsaturated acids as Michael acceptors for the synthesis of thieno[2,3-b]thiopyrans. J Org Chem 1988 53:9–13

Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH (2002) Comparison of four chemotherapy regimens for advanced nonsmall cell lung cancer. New Engl J Med 346:92–98

Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 7(3):169–181

Sun B, Zhang J, Yin XE, Xu Y, Zhang FR, Huang YS, Wang JH, Wang GQ, Hu C (2016) Synthesis, characterization and biological activity of thieno[2,3-d]pyrimidine derivatives as the epidermal growth factor receptor inhibitors. Lat Am J Pharm 35:570–577

Sun J, Wang XY, Lv PC, Zhu HL (2015) Discovery of a series of novel phenylpiperazine derivatives as egfr tk inhibitors. Sci Rep 5:13934

Sun R, Song J, Zhao H, Yan CL, Zhang AJ, Koirala D, Li DW, Hu C (2011) Synthesis and anti-tumor activity of 1,4-dihydrothieno [3',2':5,6]thiopyrano[4,3-c]pyrazole-3-carboxamide derivatives. J Chin Pharm Sci 20:26–34

Thomsen R, Christensen MH (2006) MolDock: a new technique for high-accuracy molecular docking. J Med Chem 49(11):3315–3321

Wang JJ, Yin BZ, Jiang GJ, Imafuku K (1990) Synthesis of isochroman-fused pyrazolone and pyrimidine derivatives. J Heterocycl Chem 27:1181–1184

Wissner A, Mansour TS (2008) The development of HKI-272 and related compounds for the treatment of cancer. Arch Pharm 2008 341(8):465–477

Wood ER, Shewchuk LM, Ellis B, Brignola P, Brashear RL, Caferro TR, Dickerson SH, Dickson HD, Donaldson KH, Gaul M, Griffin RJ, Hassell AM, Keith B, Mullin R, Petrov KG, Reno MJ, Rusnak DW, Tadepalli SM, Ulrich JC, Wagner CD, Vanderwall DE, Waterson AG, Williams JD, White WL, Uehling DE (2008) 6-Ethynylthieno[3,2-d]- and 6-ethynylthieno[2,3-d]pyrimidin-4-anilines as tunable covalent modifiers of ErbB kinases. Proc Natl Acad Sci USA 105(8):2773–2778

Wu CH, Coumar MS, Chu CY, Lin WH, Chen YR, Chen CT, Shiao HY, Rafi S, Wang SY, Hsu H, Chen CH, Chang CY, Chang TY, Lien TW, Fang MY, Yeh KC, Chen CP, Yeh TK, Hsieh SH, Hsu JT, Liao CC, Chao YS, Hsieh HP (2010) Design and synthesis of tetrahydropyridothieno[2,3-d]pyrimidine scaffold based epidermal growth factor receptor (EGFR) kinase inhibitors: the role of side chain chirality and Michael acceptor group for maximal potency. J Med Chem 53(20):7316–7326

Zhang HQ, Gong FH, Li CG, Zhang C, Wang YJ, Xu YG, Sun LP (2016) Design and discovery of 4-anilinoquinazoline-acylamino derivatives as EGFR and VEGFR-2 dual TK inhibitors. Eur J Med Chem 109:371–379

Zhang L, Deng XS, Zhang C, Meng GP, Wu JF, Li XS, Zhao QC, Hu C (2017) Design, synthesis and cytotoxic evaluation of a novel series of benzo[d]thiazole-2-carboxamide derivatives as potential EGFR inhibitors. Med Chem Res 26(9):2180–2189

Zhao Y, Wu XR, Yin JG, Wang JJ (2002) Synthesis of isochromano [4,3-C]pyrazole substituted with biheterocycle at 3-position. J Yantai Univ Nat Sci Eng Ed 15:113–117

Zhou W, Ercan D, Jänne PA, Gray NS (2011) Discovery of selective irreversible inhibitors for EGFR-T790M. Bioorg Med Chem Lett 21(2):638–643

Zhou XF, Guo JL, Ji YM, Pan GF, Liu T, Zhu H, Zhao JP (2016) Reciprocal negative regulation between EGFR and DEPTOR plays an important role in the progression of lung adenocarcinoma. Mol Cancer Res 14:448–457