

UDC 577.322

## Identification of 4-methoxythieno[2,3-d]pyrimidines as FGFR1 Inhibitors

I. M. Kotey, M. V. Protopopov, S. A. Starosyla, A. O. Balanda, L. V. Pletnova,  
A. O. Prykhod'ko, V. G. Bdzhola, S. M. Yarmoluk

Institute of Molecular Biology and Genetics, NAS of Ukraine  
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143  
[sergiy@yarmoluk.org.ua](mailto:sergiy@yarmoluk.org.ua)

**Aim.** To identify novel FGFR1 inhibitors using virtual screening approach. **Methods.** We used methods of organic synthesis, molecular docking via the Autodock 4.2.6 program package and *in vitro* biochemical tests with  $\gamma$ -<sup>32</sup>P. **Results.** *In vitro* experiments showed that 9 of 23 tested compounds possess inhibitory activity against FGFR1 with IC<sub>50</sub> values in the range from 0.9 to 5.6  $\mu$ M. **Conclusions.** Nine FGFR1 inhibitors were developed. The mode of compounds binding with the ATP-acceptor site was determined using molecular docking methods and the dependence of the compounds' activity on the substituents R<sup>1</sup>, R<sup>4</sup> and R<sup>5</sup> was evaluated.

**Keywords:** Fibroblast growth factor receptor 1, molecular docking, organic synthesis, *in vitro* testing, 4-methoxythieno[2,3-d]pyrimidin.

### Introduction

Fibroblast growth factor receptor 1 (FGFR1) is a receptor tyrosine kinase. Normally this enzyme is involved in tissue repair, hematopoiesis, angiogenesis and embryonic development. However, due to the FGFR role in regulating cell growth and survival, amplification and/or mutation of this protein kinase drives a number of cancer types, including gastric cancer, multiple myeloma, breast cancer and non-small cell lung cancer (NSCLC). Such inhibitors of FGFR1 as AZD4547, E3810, G141 and E7090 showed anticancer potential and now

are in clinical trials. This means that FGFR1 is an appealing target for the drug development [1-4].

The main aim of this work was the search for new FGFR1 inhibitors among 4-methoxythieno[2,3-d]pyrimidine derivatives. Earlier using molecular docking of the collection of the Department of medicinal chemistry (Institute of molecular biology and genetics, NAS of Ukraine) we identified several classes of FGFR1 inhibitors, such as N-phenyl-naphthostyryl-1-sulfonamides [5], N-phenylthieno[2,3-d]pyrimidin-4-amines [6], quinazolines [7], oxindoles [8], (1H-benzoimidazol-2-yl)-

phenyl-1,2-dihydro-pyrrol-3-ones [9] and flavones [10].

## Materials and methods

### *Molecular docking*

*Preparation of ligands and receptor molecules.* Autodock 4.2.6 programs package was used for the receptor-based flexible docking [11]. Ligands were prepared by Vega ZZ (command line) [12] and MGL Tools 1.5.6 [11]. The incoming formats of receptor and ligands data were converted into PDBQT-format with Vega ZZ using AUTODOCK force field. This format contains the coordinates of the atoms and partial charges. Hydrogen atoms were removed from nonpolar atoms. The receptor was prepared using MGL Tools and AutoGrid [11].

We have used docking parameters reported earlier [13].

*Flexible docking.* The catalytic subunit of protein kinase FGFR1 complex with inhibitor (PDB code 3GQI) was used for molecular docking via Autodock [14]. Water molecules, ions, and ligands were removed from the PDB file.

### *Visual analysis*

A visual analysis of the results of molecular docking (interaction of compounds with the amino acid residues of FGFR1 ATP-binding site) was carried out in the Discovery Studio Visualizer 4.0 (<http://accelrys.com/>).

### *In vitro testing*

The FGFR1 kinase assays with recombinant cytoplasmic domain of the FGFR1 tyrosine kinase (Millipore, Cat. N. 14-582) were performed in a total volume of 30  $\mu$ l containing 10 mM

MOPS (pH 7.2), 0.1 mM sodium orthovanadate, 0.2 mM EDTA, 0.002 % Brij 35, 0.2 mg/ml BSA, 0.02 %  $\beta$ -mercaptoethanol, 250  $\mu$ M of peptide substrate (KKKSPGEYVNIEFG, GenScript), various concentrations of inhibitor dissolved in DMSO (final DMSO concentration in probe less than 1 %) and 10 mU of enzyme. The reaction was initiated by the addition of ATP (50  $\mu$ M ATP, 25 mM MgAc containing 0.1  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] ATP per probe) and samples incubated at 30°C for 25 min. The reaction was terminated by the addition of 5 % phosphoric acid and the precipitation of material onto phosphocellulose filters “Whatman P81”. Filters were washed three times with 0.75 % phosphoric acid and the incorporation of [ $^{32}$ P] into the peptide substrate was determined by counting the radioactivity retained on the filters in a PerkinElmer scintillation counter. As negative control an equal volume of dimethyl sulfoxide (DMSO) was added to the reaction mixture. Inhibition percentage was calculated as a ratio of the substrate-incorporated radioactivity in the presence of inhibitor to the radioactivity incorporated in control reactions, i.e. in the absence of inhibitor. The concentration of compound that inhibited enzymatic activity by 50 % (IC<sub>50</sub>) was determined graphically.

### *Chemistry*

The thieno[2,3-d]pyrimidine derivatives were synthesized from corresponding carbonyl compounds according to Scheme 1. Initially substituted 2-amino-3-carbomethoxythiofenones (**2**) were obtained by Gewald reaction (in case of acetophenone [the] synthesis was carried out in two stages: firstly ethyl cyanoacetate was condensed with the corresponding acetophenone and then the product was cyclized with

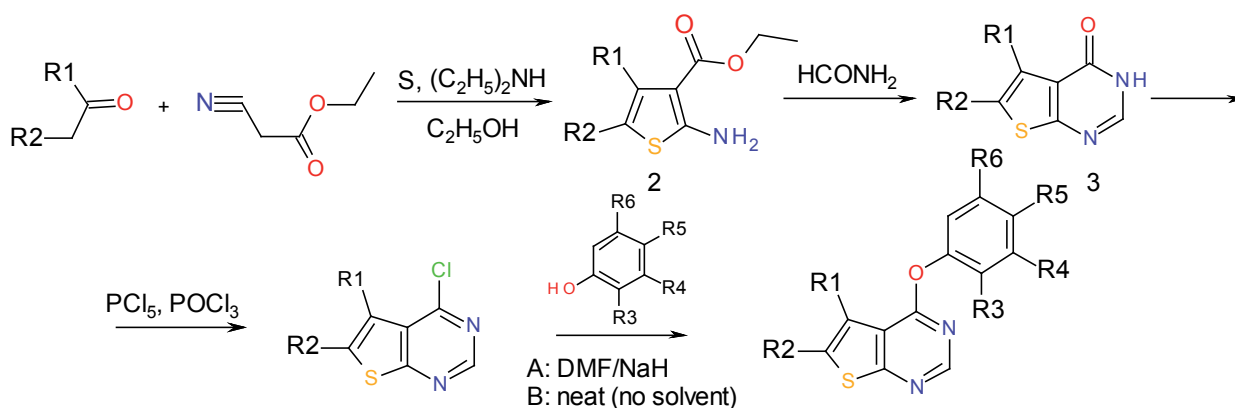
sulfur) (method 1). Then thienopyrimidinones (**3**) were obtained by condensation of 2-amino-3-carbomethoxythiophenes with formamide (method 2). The various 4-chloro-thieno[2,3-d]pyrimidines (**4**) were synthesized after interaction of thienopyrimidinones with  $\text{PCl}_5$  (method 3) and used as a building block for further formation of combinatorial rows. The final compounds were obtained by chlorine substitution in previous molecules with corresponding phenols (method 4a) or protected aminophenols (method 4b).

### Experimental section

Starting materials and solvents were purchased from commercial suppliers and were used without further purification. The progress of the reaction and purity of obtained compounds were controlled by TLC on “Kieselgel 60  $F_{254}$ ” plates (Merck). The structures of synthesized compounds were confirmed by  $^1\text{H}$  NMR spectrometry and liquid chromatography-mass spectra (LC-MS) analysis. Nuclear magnetic resonance spectra were recorded on a Varian Mercury VRX-400 spectrometer using

$\text{DMSO-d}_6$  as solvent and tetramethylsilane as internal standard. Chemical shift values ( $\delta$ ) are quoted in ppm and coupling constants ( $J$ ) in Hz. Liquid chromatography-mass spectra (LC-MS) analyses were performed using the Agilent 1100 LC/MSD SL (Agilent Technologies) separations module and Mass Quad G1956B mass detector with electrospray ionization (Agilent Technologies). HPLC is performed using Zorbax SB-C18 (Agilent Technologies), Rapid Resolution HT Cartridge 4.6x30 mm 1.8- $\mu$  (Agilent Technologies P/N:823975-902) i.d. column, at a temperature of 40 °C with gradient elution of 0–100 %  $\text{CH}_3\text{CN}$  (with 1 mL/L  $\text{HCOOH}$ ):  $\text{H}_2\text{O}$  (with 1 mL/L  $\text{HCOOH}$ ) at a flow rate of 3 mL/min and a run time of 2.8 min. Compounds were detected at 215 nm using a Diode Array G1315B detector. All tested compounds had  $\geq 90$  % purity as determined by this method. All purified synthetic intermediates had  $\geq 95$  % purity as determined by this method.

Method 1. A mixture of 107 mL (1 mol) of ethyl cyanoacetate, 32 g (1 mol) of elemental sulfur and 1 mol of the corresponding ketone



**Scheme 1.** General synthesis of compounds 5-27.

in 200 ml of ethanol was stirred at room temperature. 80 ml of diethylamine were added to this mixture for 12 h. The reaction mixture was left overnight; the precipitate was filtered, washed by aqueous alcohol (1:1) and dried in air [15]. Yield was 50-88 %.

Method 2. 2 mol of formamide were added to 1 mol 2-aminothiophene and the mixture was heated to 160-170°C for 24 h. The hot mixture was diluted with isopropyl alcohol and cooled. The solid product was collected by filtration, washed with isopropyl alcohol and water and dried at 60°C [16]. Yield was 85-90 %.

Method 3. A mixture of 0.01 mol of thienopyrimidinone, 11 ml of POCl<sub>3</sub> and 1.5 g PCl<sub>5</sub> was refluxed for 5-6 hours. The solvent was carefully evaporated to dryness. The dry product was dissolved in methylene chloride and poured to a cold 0.5 N NaOH solution. The organic phase was separated and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, then solvent was evaporated in vacuum. The residue was recrystallized from isopropanol [17]. Yield was 70-80 %.

Method 4A. NaH (60 % dispersion in mineral oil) (0.5g, 0.012 mol) was added to solution of 0.011 mol of corresponding phenol in 10 ml of dimethylformamide at 0°C, after 30 minutes at ambient temperature 0.01 mol of substituted 4-chloro-thieno[2,3-d]pyrimidine was added and the mixture was stirred at 80°C for 8 hours. The content of the flask was diluted with water. Precipitate was filtered off and dried at room temperature. The resulting solid was recrystallized from ethanol. Yield was 60-80 %.

Method 4B. A mixture of 0.01 mol N-(4-hydroxy-phenyl)-acetamide (or N-(3-hydroxy-

phenyl)-acetamide) and 0.01 mol of substituted 4-chloro-thieno[2,3-d]pyrimidine was melted at 180°C for 30 min. Saturated sodium carbonate solution was added and the precipitate was filtered, washed with water and dried. The residue was recrystallized from mixture of N,N-dimethylformamide and ethanol (for compounds 6,10,12,23,24). Yield 50-60 %.

**4-(4-methylphenoxy)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (5).** Yellow solid, m.p. 102-104°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.90 (s, 4H, CH<sub>2</sub>), 2.35 (s, 3H, CH<sub>3</sub>), 2.75 (s, 2H, CH<sub>2</sub>), 3.05 (s, 2H, CH<sub>2</sub>), 7.08 (d, J = 8.3 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.22 (d, J = 8.3 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 8.37 (s, 1H, pyrimidine). LC-MS: *m/z* 297.0 [M+H]<sup>+</sup>. Yield 65 %.

**N-{3-[(5-phenylthieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}acetamide (6).** Brown solid, m.p. 190-191°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.03 (s, 3H, CH<sub>3</sub>), 6.82 (s, 1H, pyrimidine), 7.28-7.5 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 7.62 (m, 3H, Ar), 7.8 (s, 1H, Ar), 8.6 (s, 1H, pyrimidine), 9.92 (s, 1H, NH). LC-MS: *m/z* 362.0 [M+H]<sup>+</sup>. Yield 50 %.

**7-[[5-(4-bromophenyl)thieno[2,3-d]pyrimidin-4-yl]oxy]-2H-chromen-2-one (7).** White solid, m.p. 216-217°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 6.43 (m, 1H, CH), 7.18 (m, 1H, CH), 7.36 (s, 1H, pyrimidine), 7.58 (m, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.63 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.75-8.08 (m, 3H), 8.63 (s, 1H, pyrimidine), LC-MS: *m/z* 450.9 [M+H]<sup>+</sup>. Yield 65 %.

**4-(1,3-benzodioxol-5-yloxy)-5-(4-chlorophenyl)thieno[2,3-d]pyrimidine (8).** Yellow solid, m.p. 156-158°C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 6.08 (s, 2H, CH<sub>2</sub>), 6.7 (m, 1H, Ar), 6.92-7.06 (m, 2H, Ar), 7.52 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.72 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.94 (s, 1H, pyrimidine), 8.67 (s, 1H, pyrimidine). LC-MS: *m/z* 383.0 [M+H]<sup>+</sup>. Yield 80 %.

**1-(4-{[5-(4-methylphenyl)thieno[2,3-d]pyrimidin-4-yl]oxy}phenyl)ethanone (9).**

White solid, m.p. 134-135°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.35 (s, 3H, CH<sub>3</sub>), 2.58 (s, 3H, CH<sub>3</sub>), 7.2 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.29 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.53 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.74 (s, 1H, pyrimidine), 8.02 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 8.57 (s, 1H, pyrimidine). LC-MS: *m/z* 361.0 [M+H]<sup>+</sup>. Yield 72 %.

**N-(4-{[5-(4-methylphenyl)thieno[2,3-d]pyrimidin-4-yl]oxy}phenyl)acetamide (10).**

White solid, m.p. 204-205°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.03 (s, 3H, CH<sub>3</sub>), 2.33 (s, 3H, CH<sub>3</sub>), 7.04 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.21 (d, J = 6.3 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.52 (d, J = 6.3 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.58 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.69 (s, 1H, pyrimidine), 8.53 (s, 1H, pyrimidine), 9.91 (s, 1H, NH). LC-MS: *m/z* 376.0 [M+H]<sup>+</sup>. Yield 58 %.

**4-(3-chloro-4-fluorophenoxy)-5-phenylthieno[2,3-d]pyrimidine (11).**

Blue solid, m.p. 143-145°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 7.16-7.2 (m, 1H, Ar), 7.33-7.49 (m, 5H, Ar), 7.65 (d, 2H, Ar), 7.78 (s, 1H, pyrimidine), 8.57 (s, 1H, pyrimidine). LC-MS: *m/z* 357.0 [M+H]<sup>+</sup>. Yield 76 %.

**N-(3-{[5-(4-methoxyphenyl)thieno[2,3-d]pyrimidin-4-yl]oxy}phenyl)acetamide (12).**

Cream solid, m.p. 160-161°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.03 (s, 3H, CH<sub>3</sub>), 3.77 (s, 3H, CH<sub>3</sub>), 6.96 (d, J = 8.1 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.28-7.37 (m, 3H, Ar), 7.5 (s, 1H, Ar), 7.58 (d, J = 8.1 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.78 (s, 1H, pyrimidine), 8.62 (s, 1H, pyrimidine), 10.06 (s, 1H, NH). LC-MS: *m/z* 392.0 [M+H]<sup>+</sup>. Yield 58 %.

**4-(1,3-benzodioxol-5-yloxy)-5-(4-methylphenyl)thieno[2,3-d]pyrimidine (13).** White solid, m.p. 145-147°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.31 (s, 3H, CH<sub>3</sub>), 2.49 (s, 2H, CH<sub>2</sub>), 6.05

(s, 1H, Ar), 6.89-6.94 (m, 2H, Ar), 7.21 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.56 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.79 (s, 1H, pyrimidine), 8.62 (s, 1H, pyrimidine). LC-MS: *m/z* 363.0 [M+H]<sup>+</sup>. Yield 73 %.

**4-(quinolin-8-yloxy)-5,6,7,8-tetrahydro[1]****benzothieno[2,3-d]pyrimidine (14).**

Yellow solid, m.p. 193-194°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.93 (s, 4H, CH<sub>2</sub>), 2.91 (s, 4H, CH<sub>2</sub>), 7.48-7.72 (m, 3H, Ar), 7.92 (d, J = 8.1 Hz, 1H, Ar, quinoline), 8.18 (s, 1H, Ar, quinoline), 8.39 (d, J = 8.1 Hz, 1H, Ar, quinoline), 8.68 (s, 1H, pyrimidine). LC-MS: *m/z* 334.0 [M+H]<sup>+</sup>. Yield 60 %.

**4-(4-bromophenoxy)-5,6,7,8-tetrahydro[1]****benzothieno[2,3-d]pyrimidine (15).**

Cream solid, m.p. 90-91°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.86 (s, 4H, CH<sub>2</sub>), 2.87 (s, 2H, CH<sub>2</sub>), 3.00 (s, 2H, CH<sub>2</sub>), 7.27 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.65 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 8.47 (s, 1H, pyrimidine). LC-MS: *m/z* 360.9 [M+H]<sup>+</sup>. Yield 72 %.

**4-(4-fluorophenoxy)-5,6,7,8-tetrahydro[1]****benzothieno[2,3-d]pyrimidine (16).**

Cream solid, m.p. 89-91°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.78 (m, 4H, CH<sub>2</sub>), 2.75 (s, 2H, CH<sub>2</sub>), 3.08 (s, 2H, CH<sub>2</sub>), 7.43 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 8.38 (s, 1H, pyrimidine). LC-MS: *m/z* 301.0 [M+H]<sup>+</sup>. Yield 75 %.

**4-(3-methylphenoxy)-5,6,7,8-tetrahydro[1]****benzothieno[2,3-d]pyrimidine (17).**

Brown solid, m.p. 95-96°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.87-1.96 (m, 4H, CH<sub>2</sub>), 2.33 (s, 3H, CH<sub>3</sub>), 2.75 (s, 2H, CH<sub>2</sub>), 3.00 (s, 2H, CH<sub>2</sub>), 7.03-7.11 (m, 3H, Ar), 7.26 (m, 1H, Ar), 8.23 (s, 1H, pyrimidine). LC-MS: *m/z* 297.0 [M+H]<sup>+</sup>. Yield 65 %.

**4-(2,4-dichlorophenoxy)-5,6-****dimethylthieno[2,3-d]pyrimidine (18).**

White solid, m.p. 125°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ:



2.53 (s, 6H, CH<sub>3</sub>), 7.57 (d, 2H, Ar), 7.84 (s, 1H, Ar), 8.50 (s, 1H, pyrimidine). LC-MS: *m/z* 324.9 [M+H]<sup>+</sup>. Yield 62 %.

**4-[3-(trifluoromethyl)phenoxy]-6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-d]pyrimidine (19).** White solid, m.p. 83-84°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.72 (m, 4H, CH<sub>2</sub>), 1.89 (m, 2H, CH<sub>2</sub>), 2.96 (m, 2H, CH<sub>2</sub>), 3.25 (m, 2H, CH<sub>2</sub>), 7.59-7.78 (m, 4H, Ar), 8.48 (s, 1H, pyrimidine). LC-MS: *m/z* 365.0 [M+H]<sup>+</sup>. Yield 71 %.

**dimethyl 5-[(5-(4-methylphenyl)thieno[2,3-d]pyrimidin-4-yl)oxy]isophthalate (20).** Light brown solid, m.p. 103-105°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.33 (s, 3H, CH<sub>3</sub>), 3.12 (s, 3H, CH<sub>3</sub>), 3.89 (s, 3H, CH<sub>3</sub>), 7.32 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.58 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.76 (s, 1H, Ar), 7.98 (s, 2H, Ar), 8.39 (s, 1H, pyrimidine), 8.60 (s, 1H, pyrimidine). LC-MS: *m/z* 435.0 [M+H]<sup>+</sup>. Yield 61 %.

**7-[(5,6-dimethylthieno[2,3-d]pyrimidin-4-yl)oxy]-4-methyl-2H-chromen-2-one (21).** White solid, m.p. 275-276°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.58 (s, 6H, CH<sub>3</sub>), 3.05 (s, 3H, CH<sub>3</sub>), 6.28 (s, 1H, CH), 7.28 (m, 1H, Ar), 7.32 (m, 1H, Ar), 7.80 (m, 1H, Ar), 8.45 (s, 1H, pyrimidine). LC-MS: *m/z* 339.0 [M+H]<sup>+</sup>. Yield 72 %.

**5-phenyl-4-[3-(trifluoromethyl)phenoxy]thieno[2,3-d]pyrimidine (22).** White solid, m.p. 124-126°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 7.34-7.50 (m, 3H, Ar), 7.60 (s, 1H, Ar), 7.63-7.70 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 7.83 (s, 1H, pyrimidine), 8.61 (s, 1H, pyrimidine). LC-MS: *m/z* 373.0 [M+H]<sup>+</sup>. Yield 75 %.

**N-{4-[(5,6-dimethylthieno[2,3-d]pyrimidin-4-yl)oxy]phenyl}acetamide (23).** White solid, m.p. 202-203°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.07 (s, 3H, CH<sub>3</sub>), 2.57 (s, 3H, CH<sub>3</sub>), 3.11 (s, 3H, CH<sub>3</sub>), 7.08 (d, J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.62 (d,

J = 7.6 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 8.37 (s, 1H, pyrimidine), 9.87 (s, 1H, NH). LC-MS: *m/z* 314.0 [M+H]<sup>+</sup>. Yield 56 %.

**N-[3-(6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-yloxy)phenyl]acetamide (24).** Grey solid, m.p. 197-198°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.03 (s, 3H, CH<sub>3</sub>), 2.43-2.58 (m, 2H, CH<sub>2</sub>), 3.03-3.18 (m, 4H, CH<sub>2</sub>), 6.77 (d, 1H, Ar), 7.23 (m, 1H, Ar), 7.38 (d, 1H, Ar), 7.56 (s, 1H, Ar), 8.33 (s, 1H, pyrimidine), 9.78 (s, 1H, NH). LC-MS: *m/z* 326.0 [M+H]<sup>+</sup>. Yield 53 %.

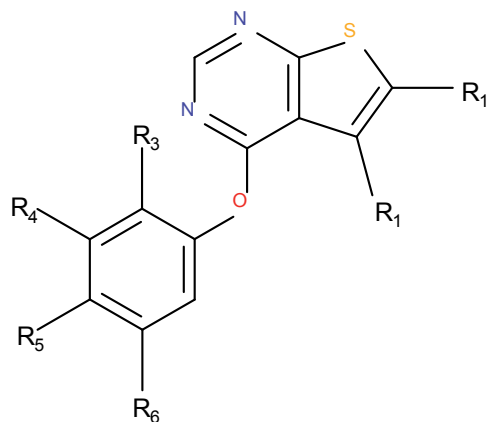
**6-methyl-5-phenyl-4-[3-(trifluoromethyl)phenoxy]thieno[2,3-d]pyrimidine (25).** White solid, m.p. 161-163°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 2.83 (s, 3H, CH<sub>3</sub>), 7.21-7.53 (m, 9H, Ar), 8.43 (s, 1H, pyrimidine). LC-MS: *m/z* 387.0 [M+H]<sup>+</sup>. Yield 78 %.


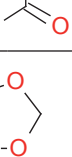


**Methyl 4-[(6-methyl-5-phenylthieno[2,3-d]pyrimidin-4-yl)oxy]benzoate (26).** Grey solid, m.p. 147-148°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 3.20 (s, 3H, CH<sub>3</sub>), 3.87 (s, 3H, CH<sub>3</sub>), 7.17 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 7.33-7.58 (m, 5H, C<sub>6</sub>H<sub>5</sub>), 7.96 (d, J = 7.8 Hz, 2H, C<sub>6</sub>H<sub>4</sub>), 8.53 (s, 1H, pyrimidine). LC-MS: *m/z* 377.0 [M+H]<sup>+</sup>. Yield 69 %.

**7-methyl-4-[3-(trifluoromethyl)phenoxy]-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidine (27).** Yellow solid, m.p. 95-96°C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ: 1.11 (d, 3H, CH<sub>3</sub>), 1.40-1.58 (m, 1H, CH), 1.88-2.04 (m, 2H, CH<sub>2</sub>), 2.88-3.02 (m, 2H, CH<sub>2</sub>), 3.15-3.23 (m, 2H, CH<sub>2</sub>), 7.62-7.78 (m, 4H, Ar), 8.51 (s, 1H, pyrimidine). LC-MS: *m/z* 365.0 [M+H]<sup>+</sup>. Yield 69 %.

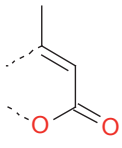
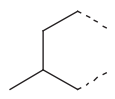
## Results and Discussion

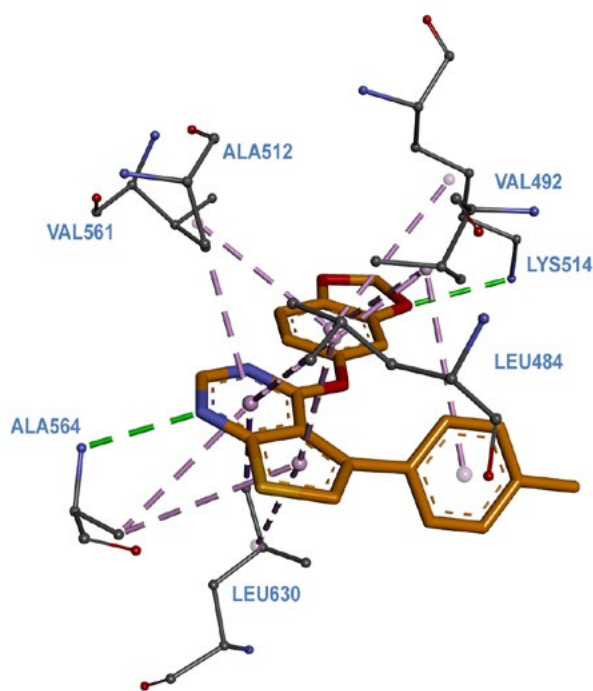
Previously N-phenylthieno[2,3-d]pyrimidin-4-amines were identified as FGFR1 inhibitors [6]. In this study 4-methoxythieno[2,3-d]py-

Table 1. Structure, *in vitro* inhibitory activity toward FGFR1 and IC<sub>50</sub> of studied compounds.

Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	Residual activity of FGFR1 at, %		IC <sub>50</sub> , μM
							33 μM	10 μM	
5	-(CH <sub>2</sub> ) <sub>4</sub> -		H	H	Me	H	33.8	41.7	4.47
6	phenyl	H	H	H	NAc	H	24.5	N/A	2.3
7	4-bromophenyl	H	H			H	14.7	40.3	2.45
8	4-chlorophenyl	H	H			H	29.6	30.1	2.14
9	4-methylphenyl	H	H	H	Ac	H	26.9	N/A	5
10	4-methylphenyl	H	H	H	NAc	H	27.7	N/A	2.5
11	phenyl	H	H	Cl	F	H	21.8	33,1	3.55
12	4-methoxyphenyl	H	H	H	NAc	H	19.3	N/A	5.6
13	4-methylphenyl	H	H			H	15.1	17.4	0.9
14	-(CH <sub>2</sub> ) <sub>4</sub> -				H	H	N/A	86,5	N/A
15	-(CH <sub>2</sub> ) <sub>4</sub> -		H	H	Br	H	52	N/A	N/A
16	-(CH <sub>2</sub> ) <sub>4</sub> -		H	H	F	H	50.4	N/A	N/A
17	-(CH <sub>2</sub> ) <sub>4</sub> -		H	Me	H	H	65.3	N/A	N/A
18	Me	Me	Cl	H	Cl	H	88.2	N/A	N/A
19	-(CH <sub>2</sub> ) <sub>5</sub> -		H	CF <sub>3</sub>	H	H	64.3	N/A	N/A
20	4-methylphenyl	H	H	COOMe	H	COOMe	53.2	83.7	N/A

Ended Table 1

Compound	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	R <sup>6</sup>	Residual activity of FGFR1 at, %		IC <sub>50</sub> , μM
							33 μM	10 μM	
21	Me	Me	H			H	N/A	N/A	N/A
22	phenyl	H	H	CF <sub>3</sub>	H	H	70.4	N/A	N/A
23	Me	Me	H	H	NAc	H	65.8	N/A	N/A
24	-(CH <sub>2</sub> ) <sub>3</sub> -		H	NAc	H	H	N/A	N/A	N/A
25	phenyl	Me	H	CF <sub>3</sub>	H	H	53.5	90.5	N/A
26	phenyl	Me	H	H	COOMe	H	42.7	50	N/A
27			H	CF <sub>3</sub>	H	H	80	N/A	N/A



**Fig. 1.** The complex of compound **13** with ATP-acceptor site of FGFR1 obtained with Autodock 4.2.6. H-bonds are shown by green dashed lines, hydrophobic interactions – purple dashed lines.

rimidines have been synthesized and tested toward FGFR1 kinases.

Twenty three 4-methoxythieno[2,3-d]pyrimidines were evaluated for ability to inhibit the FGFR1 activity using <sup>32</sup>P-ATP radiolabeled assay. This method provides direct measurement of the enzyme activity. The results are summarized in Table 1. Nine compounds showed inhibitory activity with IC<sub>50</sub> value from 0.9 to 5.6 μM.

To understand the activity of the compounds and make some conclusions about SAR we have performed molecular docking and analyzed the complexes of studied compounds with FGFR1 ATP-binding pocket to propose their binding mode. The complex of the most active found compound with the ATP-acceptor site of FGFR1 is shown in Fig. 1.

Proposed binding mode of the studied compounds belongs to the type I protein kinases inhibitors. The thieno[2,3-d]pyrimidine core is located in adenine binding region and forms



hydrogen bond with the amino acid residue Ala564 of the hinge region. Most R<sup>3</sup>-R<sup>6</sup> substituents are extended towards hydrophobic region I and form hydrophobic interactions with Ala512 and Val561 and hydrogen bonds with Lys514 or Asp641. R<sup>1</sup>-R<sup>2</sup> substituents are oriented to hydrophobic region II and form hydrophobic interactions with Val492 and/or Leu630 and/or Leu484. As exceptions were the compounds **14**, **15**, **16**, **19** and **24** with 5- and 6-membered cycles at R<sup>1</sup>-R<sup>2</sup>. R<sup>1</sup>-R<sup>2</sup> substituents of these compounds are oriented to the hydrophobic region I and R<sup>3</sup>-R<sup>6</sup> substituents are oriented to the outer of binding site.

The Structure Activity Relationship (SAR) study showed that R<sup>1</sup> of the most active compounds is phenyl. Mostly it is a hydrophobic 4-substituted phenyl. The compound **10** with 4-methylphenyl (more hydrophobic) at R<sup>1</sup> has two-fold higher activity than compound **12** with 4-methoxyphenyl at R<sup>1</sup>. IC<sub>50</sub> values were 2.5 and 5.6 μM respectively. But the compound **8** with 4-chlorophenyl (more hydrophobic) at R<sup>1</sup> has lower activity than compound **13** with 4-methylphenyl. IC<sub>50</sub> values were 2.14 and 0.9 μM, respectively. Therefore, the order of potency for the substituents at R<sup>1</sup> could be proposed as following: 4-methylphenyl >4-chlorophenyl >4-methoxyphenyl.

SAR study of substituents at R<sup>3</sup>-R<sup>6</sup> showed that the most active compounds have hydrogen bond acceptor at positions R<sup>4</sup> and/or R<sup>5</sup>. Compounds **8** and **13** contain dioxymethan and have IC<sub>50</sub> values of 2.14 and 0.9 μM, respectively.

## Conclusions

Nine new inhibitors of FGFR1 were discovered among 23 4-methoxythieno[2,3-d]py-

rimidine derivatives. SAR study showed that the most profitable substituent at R<sup>1</sup> is 4-methylphenyl and hydrogen bond acceptors at R<sup>4</sup> and/or R<sup>5</sup> are important for revealing the inhibitory activity toward FGFR1. The most potent compound in this series showed the IC<sub>50</sub> value of 0.9 μM. The studied compounds are good candidates for the further development of effective FGFR1 inhibitors and potential anti-cancer drugs.

## Funding

This work was supported by a grant from the Project of the National Academy of Sciences (NAS) of Ukraine № 0117U000204 “New approaches for target-oriented search of potential antitumor and anti-infectious agents to overcome the problem of drug resistance”.

## REFERENCES

1. Yu T, Yang Y, Liu Y, Zhang Y, Xu H, Li M, Ponnusamy M, Wang K, Wang JX, Li PF. A FGFR1 inhibitor patent review: progress since 2010. *Expert Opin Ther Pat.* 2017;**27**(4):439–454.
2. Chioni AM, Grose R. FGFR1 cleavage and nuclear translocation regulates breast cancer cell behavior. *J Cell Biol.* 2012;**197**(6):801–17.
3. Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, Ullrich RT, Menon R, Maier S, Soltermann A, Moch H, Wagener P, Fischer F, Heynck S, Koker M, Schöttle J, Leenders F, Gabler F, Dabow I, Querings S, Heukamp LC, Balke-Want H, Ansén S, Rauh D, Baessmann I, Altmüller J, Wainer Z, Conron M, Wright G, Russell P, Solomon B, Brambilla E, Brambilla C, Lorimier P, Solberg S, Brustugun OT, Engel-Riedel W, Ludwig C, Petersen I, Sängler J, Clement J, Groen H, Timens W, Sietsma H, Thunnissen E, Smit E, Heideman D, Cappuzzo F, Ligorio C, Damiani S, Hallek M, Beroukhim R, Pao W, Klebl B, Baumann M, Buettner R, Ernestus K, Stoelben E, Wolf J, Nürnberg P, Per-

- ner S, Thomas RK. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med*. 2010;**2**(62):62ra93.
4. Sahores A, May M, Sequeira GR, Fuentes C, Jacobsen B, Lanari C, Lamb CA. Targeting FGFR with BGJ398 in Breast Cancer: Effect on Tumor Growth and Metastasis. *Curr Cancer Drug Targets*. 2018;**18**(10):979–987.
  5. Gryshchenko AA, Levchenko KV, Bdzholo VG, Ruban TP, Lukash LL, Yarmoluk SM. Design, synthesis and biological evaluation of naphthostyryl derivatives as novel protein kinase FGFR1 inhibitors. *J Enzyme Inhib Med Chem*. 2015;**30**(1):126–32.
  6. Gryshchenko AA, Bdzholo VG, Balanda AO, Briukhovetska NV, Kotey IM, Golub AG, Ruban TP, Lukash LL, Yarmoluk SM. Design, synthesis and biological evaluation of N-phenylthieno[2,3-d]pyrimidin-4-amines as inhibitors of FGFR1. *Bioorg Med Chem*. 2015;**23**(9):2287–93.
  7. GGryshchenko AA, Bdzholo VG, Pletnyova LV, Chepurna RV, Zhitnetsky IV, Yarmoluk SM. Quinazolone inhibitors of protein kinase FGFR1. *Ukr Bioorg Acta* 2010; **8**(2):63–8.
  8. Gryshchenko AA, Bdzholo VG, Borovikov OV, Kukhareno OP, Pletnyova LV, Yarmoluk SM. Search for FGFR1 inhibitors among oxindole derivatives. *Ukr Bioorg Acta*; **2009**; **7**(2):64–8
  9. Gryshchenko AA, Tarnavskiy SS, Levchenko KV, Bdzholo VG, Volynets GP, Golub AG, Ruban TP, Vygranenko KV, Lukash LL, Yarmoluk SM. Design, synthesis and biological evaluation of 5-amino-4-(1H-benzimidazol-2-yl)-phenyl-1,2-dihydropyrrrol-3-ones as inhibitors of protein kinase FGFR1. *Bioorg Med Chem*. 2016;**24**(9):2053–9.
  10. Grischenko AA, Bdzholo VG, Kukhareno OP, Yarmoluk SM. Search for FGFR1 inhibitors among chromone derivatives. *Ukr Bioorg Acta*; **2009**; **7**(1):50–6.
  11. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem*. 2009;**30**(16):2785–91.
  12. Pedretti A, Villa L, Vistoli G. VEGA--an open platform to develop chemo-bio-informatics applications, using plug-in architecture and script programming. *J Comput Aided Mol Des*. 2004;**18**(3):167–73.
  13. Syniugin AR, Ostrynska OV, Chekanov MO, Volynets GP, Starosyla SA, Bdzholo VG, Yarmoluk SM. Design, synthesis and evaluation of 3-quinoline carboxylic acids as new inhibitors of protein kinase CK2. *J Enzyme Inhib Med Chem*. 2016;**31**(sup4):160–9.
  14. Bae JH, Lew ED, Yuzawa S, Tomé F, Lax I, Schlessinger J. The selectivity of receptor tyrosine kinase signaling is controlled by a secondary SH2 domain binding site. *Cell*. 2009;**138**(3):514–24.
  15. Sabnis RW, Rangnekar DW, Sonawane ND. 2-aminothiophenes by the gewald reaction. *J Heterocycl Chem*. 1999;**36**(2):333–45.
  16. Golub AG, Bdzholo VG, Briukhovetska NV, Balanda AO, Kukhareno OP, Kotey IM, Ostrynska OV, Yarmoluk SM. 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**(3):870–6.
  17. Ostrynska OV, Balanda AO, Bdzholo VG, Golub AG, Kotey IM, Kukhareno OP, Gryshchenko AA, Briukhovetska NV, Yarmoluk SM. Design and synthesis of novel protein kinase CK2 inhibitors on the base of 4-aminothieno[2,3-d]pyrimidines. *Eur J Med Chem*. 2016;**115**:148–60.

#### Ідентифікація інгібіторів FGFR1 серед 4-метокситієно[2,3-d]піримідинів.

І. М. Котей, М. В. Протопопов, С. А. Старосила, А. О. Баланда, Л. В. Плетньова, А. О. Приходько, В. Г. Бджола та С. М. Ярмолук

**Мета.** Пошук нових інгібіторів протеїнкінази FGFR1. **Методи.** В роботі використовувались методи органічного синтезу, молекулярний докінг програмним пакетом Autodock 4.2.6 та біохімічне тестування *in vitro* із використанням  $\gamma$ -<sup>32</sup>P АТФ. **Результати.** Експерименти *in vitro* показали, що 9 з 23 сполук проявляють інгібувальну активність щодо FGFR1 зі значенням IC<sub>50</sub> в межах від 0.9 до 5.6  $\mu$ М. **Висновки.** Було розроблено дев'ять інгібіторів FGFR1. За допомогою методів молекулярного моделювання було визначено спосіб зв'язування

із АТФ-акцепторним сайтом та вивчено залежність активності сполук від замісників R<sup>1</sup>, R<sup>4</sup> та R<sup>5</sup>.

Ключові слова. Протеїнкіназа FGFR1, молекулярний докінг, органічний синтез, *in vitro* тестування.

#### **Идентификация ингибиторов FGFR1 среди 4-метокситиено[2,3-d]пиримидинов**

И. М. Котей, Н. В. Протопопов, С. А. Старосила, Л. В. Плетнёва, А. А. Приходько, А. А. Баланда, В. Г. Бджола и С. М. Ярмолюк

**Цель.** Поиск новых химических соединений со способностью ингибировать протеинкиназу FGFR1.

**Методы.** В работе использовались методы органического синтеза, молекулярного докинга программным

пакетом Autodock 4.2.6 и биохимическое тестирование *in vitro*, используя  $\gamma$ -<sup>32</sup>P АТФ. **Результаты.** Биохимическое тестирование показало, что 9 из 23 исследуемых соединений ингибируют протеинкиназу FGFR1 в диапазоне значений IC<sub>50</sub> от 0.9 до 5.6  $\mu$ M. **Выводы.** Было разработано девять ингибиторов FGFR1. С помощью молекулярного докинга было определено положение лигандов в АТФ-акцепторном сайте и изучено зависимость активности от структуры заместителей R<sup>1</sup>, R<sup>4</sup> та R<sup>5</sup>.

**Ключевые слова:** Протеинкиназа FGFR1, молекулярный докинг, органический синтез, *in vitro* тестирование, 4-метокситиено[2,3-d]пиримидин.

Received 10.10.2018