Volume 6, Issue 6, 2016, 1641 - 1646 ISSN 2069-5837

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Original Research Article Open Access Journal

Received: 13.10.2016 / Revised: 28.10.2016 / Accepted: 03.11.2016 / Published on-line: 05.11.2016

Design and synthesis of the new scaffolds for anti-tuberculosis compounds using general pharmacophore concept

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ABSTRACT

Using general Ser/Thr pharmacophore approach suggested by us previously we have designed two series of compounds capable to suppress the growth of virulent *Mycobacterium Tuberculosis* strain (H37Rv). We suggest that this inhibition is caused by mycobacterial PknB inhibition.

Keywords: *Ser/Thr kinases, Tuberculosis, Pharmacophore, PknB inhibitors.*

1. INTRODUCTION

Tuberculosis belongs to infectious disease stubbornly resisting treatment. Widespread distribution of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains makes the task of searching for anti-TB drugs with a new mechanism of action extremely important. Among the targets for antibacterial drugs currently under development are enzymes involved in the synthesis of bacteria cell wall, bacterial DNA gyrase, RNA polymerase, and bacterial proteinkinases. Bacterial proteinkinases control key processes in prokaryotic cells, and at the same time significantly differs from the human protein kinases, which makes them attractive targets for selective and safe inhibition. 11 Serine/threonine kinases were identified in *Mycobactrium tuberculosis*, most of them are transmembrane proteins that transmit signals within the cell [1, 2, 3]. PknB and PknA were found to be key kinases for growth of mycobacteria [4]. Despite the ongoing intensive search for new inhibitors of PknB kinase, no inhibitors of this kinase in submicromolar range were found so far [5, 6].

2. EXPERIMENT SECTION

Pyrazin-2-yl-[4-(1-thiazol-4-yl-methyl-piperidin-2-yl)-thiazol-2-yl]-amine (I).

2-(2-Bromo-acetyl)-piperidine-1-carboxylic acid *t*-butyl ester (2a)

To cooled to -78°C solution of N-Boc-2-acetyl-piperidine (**1a**) (20.0 g, 87.8 mmol) in anhydrous THF (150 ml) 1M solution of lithium bis(trimethylsilyl)amide in THF (93 ml, 93.0 mmol) was added dropwise during 20 minutes. Solution was stirred at the same temperature for 1 h, then trimethylchlorosilane (12.1 ml, 95.5 mmol) was added therein and stirring was continued at 0°C for 30 min. Then resulting solution was cooled to -78°C again and bromine (4.50 ml, 87.8 mmol) was added. Reaction mixture was stirred for 30 min, then was allowed to warm to room temperature, poured into 10% solution of $Na_2S_2O_3$ (100 ml) and extracted with ethyl acetate (2x300 ml). Combined organic extracts were washed with saturated solution of NH_4Cl (100 ml), dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography on silica gel (eluent hexane/ethyl acetate, 7:1). Yield (**2a**) 18.6 g (69 %).

2-(2-Aminothiazol-4-yl)-piperidine-1-carboxylic acid *t*-butyl ester (3a).

Suspension of **2a** (8.5 g, 27.8 mmol) and thiourea (2.11 g, 27.8 mmol) in ethanol (60 ml) was refluxed for 1 h. The mixture was concentrated; residue was suspended in 2M NaOH (100 ml) and extracted with methylene chloride (2x200 ml). Combined organic extracts were washed with brine (50 ml), dried over $MgSO_4$ and concentrated in vacuum. Crude product was purified by column chromatography on silica gel (eluent ethyl acetate) to give 4.32 g (55%) compound **3a**. APCI-MS (m/z) 284.2 ($[M+H]^+$, 100%), 228.1 ($[M\text{-}tBu+H]^+$, 60%).

2-[2-(Pyrazin-2-ylamino)-thiazol-4-yl]-piperidine-1-carboxylic acid *t*-butyl ester (4a).

A mixture of compound **3a** (1.7 g, 6.0 mmol), 2-chloropyrazine (0.73 g, 6.4 mmol), 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthen (174 mg, 0.30 mmol, 5% mol), tris(dibenzylidenacetone)dipalladium (0)-chloroform (150 mg, 0.15 mmol, 2.5% mol), sodium *t*-butylate (0.86 g, 9.0 mmol) in toluene (15 ml) was stirred under argon atmosphere in microwave reactor at 130°C for 2 h. Reaction mixture was cooled to room temperature, then water (30 ml) was added and the mixture was extracted with ethyl acetate (2 x 30 ml). Combined organic extracts were over Na_2SO_4 and concentrated in vacuum. Crude product was purified by column chromatography on silica gel (eluent ethyl acetate/hexane 1:1) to give 0.91 g of **4a** (42%). APCI-MS (m/z): 362.3 ($[M+H]^+$, 100%).

(4-Piperidin-2-yl-thiazol-2-yl)-pyrazin-2-yl-amine dihydrochloride (5a).

Compound **4a** (900 mg, 2.49 mmol) was dissolve in ethanol (10 ml), then HCl (3 ml) was added and reaction mixture was stirred at room temperature for 4 h. Solvent was removed in

vacuum, residue was triturated with ethyl acetate to give 750 mg of dihydrochloride **5a** (90%).

APCI-MS (m/z): 262.1 ($[M+H]^+$, 100%). ^{1}H-ЯМР (DMSO-d_6): δ= 1.55-2.15 (m, 6H); 3.00 (br s, 1H); 3.25 (m, 1H); 4.22 (t, 1H); 7.21 (s, 1H); 8.12 (s, 1H); 8.29 (s, 1H); 8.64 (s, 1H); 9.25 (br s, 1H), 9.45 (br s, 1H), 11.00-12.00 (s, 1H).

Pyrazin-2-yl-[4-(1-thiazol-4-yl-methyl-piperidin-2-yl)-thiazol-2-yl]-amine (I).

To suspension of **5a** (94 mg, 0.28 mmol) in methylene chloride (10 ml) triethylamine (116 мкл, 0.83 mmol) and thiazolecarbaldehyde (40 mg, 0.42 mmol) were added. After 15 min sodium triacetoxyborohydride (88 mg, 0.42 mmol) was added therein and stirring was continued at room temperature for 12h. Reaction mixture was poured into saturated solution of potassium carbonate (30 ml) and extracted with methylene chloride (30 ml x 3). Organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography on silica gel (eluent ethyl acetate) to give 68 mg of target product **I** (68%). APCI-MS (m/z): 359.30 ($[M+H]^+$, 100%). ^{1}H NMR δ_H (400 MHz, D_6-DMSO): 1.31 (m, 1H, CH_2), 1.58 (m, 2H, CH_2), 1.75 (m, 2H, CH_2), 2.21 (m, 1H, CH), 3.00 (m, 1H, CH_2), 3.32 (m, 3H, CH_2), 3.82 (m, 1H, CH_2), 7.00 (s, 1H, ArH), 7.45 (s, 1H, ArH), 8.06 (m, 1H, ArH), 8.26 (m, 1H, ArH), 8.42 (s, 1H, ArH), 9.00 (s, 1H, ArH), 11.90 (br s, 1H, ArH).

{4-[1-(6-METHYLPYRIDIN-2-YL-METHYL)-PIPERIDIN-2-YL]-THIAZOL-2-YL}-PYRAZIN-2-YL-AMINE (II)

Compound **II** was obtained in 59% yield as described for compound **I**, using 6-methyl-pyridine-2-carbaldehyde. APCI-MS (m/z): 367.2 ($[M+H]^+$, 100%).

^{1}H NMR δ_H (400 MHz, D_6-DMSO): 1.32 (m, 1H, CH_2), 1.41-1.56 (m, 2H, CH_2), 1.74 (m, 3H, CH_2), 2.02 (t, 1H, CH), 2.46 (s, 3H, CH_3), 2.83 (d, 1H, CH_2), 3.06 (d, 1H, CH_2), 3.33 (m, 1H, CH_2), 3.69 (m, 1H, CH_2), 6.90 (s, 1H, ArH), 6.99 (d, 1H, ArH), 7.22 (d, 1H, ArH), 7.56 (t, 1H, ArH), 8.04 (m, 1H, ArH), 8.24 (m, 1H, ArH), 8.37 (s, 1H, ArH), 11.78 (s, 1H, NH).

(5-METHYLPYRIDIN-2-YL)-[4-(1-THIAZOL-4-YL-METHYL-PYRROLIDIN-2-YL)-THIAZOL-2-YL]-AMINE (III).

2-(2-Bromoacetyl)-pyrrolidine-1-carboxylic acid *t*-butyl ester (2b)

To solution of N-Boc-2-acetylpyrrolidine (**1b**) (18.7 g, 87.8 mmol) in anhydrous THF (150 ml) cooled to -78°C 1M solution of lithium bis(trimethylsilyl amide) in THF (93 ml, 93.0 mmol) was added dropwise during 20 min. Stirring was continued at the same temperature for 1 h, then trimethylsilyl chloride (12.1 ml, 95.5 mmol) was added and reaction mixture was stirred at 0°C for 30 min. Then reaction mixture was cooled to -78°C again and bromine was added (4.50 ml, 87.8 mmol). Stirring was continued for additional 30 min, then reaction mixture was allowed to warm up to room temperature and poured into 10% solution of $Na_2S_2O_3$ (100 ml). Resulting suspension was extracted with ethyl acetate (2x300 ml). Combined organic layers were washed with saturated NH_4Cl (100 ml), dried over Na_2SO_4 and concentrated. Product was purified by column chromatography on silica gel (eluent hexane/ethyl acetate 7:1) Yield of **2b** 17.2 g (67 %).

2-(2-Aminothiazol-4-yl)-pyrrolidine-1-carboxylic acid *t*-butyl ester (3b).

Suspension of **2b** (8.1 g, 27.8 mmol) and thiourea (2.11 g, 27.8 mmol) in ethanol (60 ml) was refluxed for 1 h. Solvent was removed under reduced pressure, residue was suspended in 2M NaOH (100 ml) and extracted with methylene chloride (2x200 ml). Combined organic layers were washed with saturated NaCl (50 ml), dried over $MgSO_4$ and concentrated. Product was purified by column chromatography on silica gel (eluent ethyl acetate) Yield of **3b** 4.58 g (61 %). APCI-MS (m/z): 270.3 ($[M+H]^+$, 100%), 214.1 ($[M-tBu+H]^+$, 35%).

2-[2-(5-Methylpyridin-2-ylamino)-thiazol-4-yl]-pyrrolidine-1-carboxylic acid *t*-butyl ester (4b).

Suspension of **3b** (1.62 g, 6.0 mmol), 2-bromo-5-methylpyridine (1.1 g, 6.4 mmol), 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthen (174 mg, 0.30 mmol, 5% mol), tris(dibenzylidenacetone)dipalladium (0)-chloroform (150 mg, 0.15 mmol, 2.5% mol), sodium *t*-butylate (0.86 g, 9.0 mmol) in toluene (15 ml) was stirred under argon atmosphere in microwave reactor at 130°C for 2 h. Reaction mixture was cooled to room temperature, then water (30 ml) was added and the mixture was extracted with ethyl acetate (2 x 30 ml). Combined organic extracts were over Na_2SO_4 and concentrated in vacuum. Crude product was purified by column chromatography on silica gel (eluent ethyl acetate/hexane 1:1) to give 1.34 g of **4b** (62%). APCI-MS (m/z): 361.4 ($[M+H]^+$, 100%).

(5-Methylpyridin-2-yl)-(4-pyrrolidin-2-yl-thiazol-2-yl)-amine hydrochloride (5b)

Compound **4b** (1.08 g, 3.0 mmol) was dissolve in ethanol (15 ml), and HCl (3 ml) was added therein. After 4 h at room temperature reaction mixture was concentrated to dryness and residue was triturated with ethyl acetate to give 850 mg **5b** as dihydrochloride (85%).

APCI-MS (m/z): 261.1 ($[M+H]^+$, 100%).

(5-Methylpyridin-2-yl)-[4-(1-thiazol-4-yl-methyl-pyrrolidin-2-yl)-thiazol-2-yl]-amine (III).

Triethyl amine (0.116 ml, 0.83 mmol) and thiazole-4-carbaldehyde (40 mg, 0.42 mmol) were added to suspension of **5b** (94 mg, 0.28 mmol) in methylene chloride (10 ml), after 15 min triacetoxyborohydride (88 mg, 0.42 mmol) was added therein. Reaction mixture was stirred at room temperature for 12 h, poured into saturated solution of potassium carbonate (30 ml) and extracted with methylene chloride (30 ml). Organic layer was separated, dried over sodium sulfate, concentrated and purified by column chromatography on silica gel (eluent ethyl acetate). Yield of compound **III** - 77 mg (77%). APCI-MS (m/z): 358.2 ($[M+H]^+$, 100%). ^{1}H NMR δ_H (400 MHz, D_6-DMSO): 1.70-1.85 (m, 3H, CH_2), 2.07 (m, 1H, CH_2), 2.17 (s, 3H, CH_3), 2.36 (m, 1H, CH_2), 3.04 (m, 1H, CH), 3.53-3.62 (m, 2H, CH_2), 3.98 (d, 1H, CH_2), 6.74 (s, 1H, ArH), 6.89 (d, 1H, ArH), 7.47 (m, 1H, ArH), 8.06 (s, 1H, ArH), 8.11 (s, 1H, ArH), 8.98 (m, 1H, ArH), 11.16 (br s, 1H, NH).

{6 – METHYL – 2 - [1 - (2H-PYRAZOL – 3 – YLMETHYL) - PIPERIDIN – 2 – YL] – PYRIMIDIN – 4 - YL} - (5-METHYLTHIAZOL – 2 - YL) - AMINE (IV)

2-Carbamoylpiperidine-1-carboxylic acid *t*-butyl ester (8)

To solution of Boc-pipecolin-2-carboxylic acid (**7**) (23.0 g, 100 mmol) in anhydrous acetonitrile (150 ml) TBTU (38.5 g, 120 mmol), triethylamine (21 ml, 150 mmol) and ammonium

carbonate (19.2 g, 200 mmol) were added and reaction mixture was stirred at room temperature for 24 h. When reaction was completed reaction mixture was poured into saturated solution of potassium carbonate (250 ml), extracted with methylene chloride (3 x 250 ml). Combined organic extracts were washed with water (2 x 150 ml), dried over sodium sulfate, concentrated and purified by column chromatography on silica gel (eluent hexane/ethyl acetate) to give 17.6 g (77%) of compound **8**.

2-Carbamimidoylpiperidine-1- carboxylic acid *t*-butyl ester (9)

Triethyloxonium tetrafluoroborate (14.6 g, 77 mmol) was added under stirring to solution of compound **8**) (17.6 g, 77 mmol) in anhydrous methylene chloride (200 ml). Reaction mixture was stirred for 3 h (TLC control), then solvent was removed under reduced pressure at < 40°C and oily residue was treated with 10% ammonia in methanol (150 ml) for 24 h at room temperature. Methanol was removed under reduced pressure at < 40°C, residue was triturated with diethyl ether to give19.2 g (79%) of compound **9**, which was used in the following step without additional purification.

2 - (4 – Hydroxy – 6 – methylpyrimidin – 2 - yl) – piperidine – 1 - carboxylic acid *t*-butyl ester (10)

Sodium *t*-butylate (5.9 g, 61 mmol) in ethanol (150 ml) was added to stirred solution of amidine **9** (19.2 g, 60.8 mmol). After 10 min ethyl acetoacetate (11.7 g, 90 mmol) was added therein. Resulting mixture was refluxed for 10 h, cooled to room temperature, poured into saturated ammonium chloride (300 ml), extracted with methylene chloride (2 x 200 мл). Combined organic extracts were washed with water (2 x 150 ml), dried over sodium sulfate, concentrated and purified by column chromatography on silica gel (eluent ethyl acetate/methylene chloride 1:1) to give **10** (11.4 g, 64%).

2-(4-Chloro-6- methylpyrimidin-2-yl)-piperidine-1- carboxylic acid *t*-butyl ester (11)

To solution of intermediate **10** (10 g, 34.1 mmol) and N,N,-dimethylaniline (37 g, 306 mmol) in anhydrous toluene (200 ml) phosphorus oxychloride (15.6 g, 102 mmol) was added. Reaction mixture was refluxed for 3 h, cooled to room temperature and left overnight. Reaction mixture was poured into cold water (300 ml), organic layer was separated, washed with 1N HCl (2 x 70 ml), and then with water (1 x 70 ml), dried over sodium sulfate, concentrated and purified by column chromatography on silica gel (eluent ethyl acetate/hexane 1:3) to give **11** (5.7 g, 54%).

2-[4-Methyl-6-(5-methylthiazol-2-ylamino)pyrimidin-2-yl]-piperidine-1- carboxylic acid *t*-butyl ester (12)

Suspension of chloride **11** (2 g, 6.4 mmol), 5-methyl-thiazol-2-ylamine (0.68 g, 6 mmol), 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthen (174 mg, 0.30 mmol, 5% mol), tris(dibenzylidenacetone)dipalladium (0)-chloroform (150 mg, 0.15 mmol, 2.5% mol), potassium carbonate (0.95 g, 9.0 mmol) in toluene-water mixture (15 ml, 10:1) was stirred under argon atmosphere in microwave reactor at 140°C for 2 h. Reaction mixture was cooled to room temperature, then water (30 ml) was added and the mixture was extracted with ethyl acetate (2 x 30 ml). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuum. Crude product was purified by column chromatography on silica gel (eluent ethyl acetate/hexane 1:2) to give 1.87 g of **12** (80%).

(6-Methyl-2-piperidin-2-ylpyrimidin-4-yl)-(5-methylthiazol-2-yl)-amine (13)

To solution of Boc-derivative **12** (1.87 g, 4.8 mmol) in ethanol (15 ml) 16% HCl in dioxane (6 ml) was added, reaction mixture was stirred for 4 h at room temperature. Solvent was removed under vacuum, residue was adjusted to pH9 with 10% sodium hydroxide solution, extracted with methylene chloride (3 x 100 ml), dried over Na_2SO_4 and concentrated to afford **13**.

{6 – Methyl – 2 - [1 - (2H-pyrazol-3-ylmethyl) – piperidin -2 - yl] – pyrimidin – 4 - yl}-(5-methylthiazol-2-yl)-amine (IV)

To solution of amine **13** (145 g, 0.5 mmol) in anhydrous methylene chloride (10 ml) 2H-pyrazole carbaldehyde (64 mg, 0.6 mmol) and sodium triacetoxyborohydride (127 mg, 0.6 mmol) were added. Reaction mixture was stitted at room temperature for 16 h, then poured into saturated potassium carbonate (20 ml), extracted with methylene chloride (3 x 25 ml), dried over sodium sulfate and concentrated. Product was purified by column chromatography on silica gel (eluent ethyl acetate) to give compound **IV** (96 mg, 52%).

APCI-MS (m/z): 370.10 ($[M+H]^+$, 100%). 1H NMR δH (400 MHz, D_6-DMSO): 1.29 (m, 1H, CH_2), 1.56 (m, 2H, CH_2), 1.71 (m, 2H, CH_2), 1.89 (m, 1H, CH_2), 2.04 (m, 1H, CH_2), 2.34 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.93 (m, 1H, CH_2), 3.29 (d, 1H, CH_2), 3.39 (dd, 1H, CH), 3.50 (d, 1H, CH2), 6.07 (d, 1H, ArH), 6.73 (s, 1H, ArH), 7.06 (q, 1H, ArH), 7.41 (br s, 1H ArH), 11.2 (br s, 1H, NH), 12.3 (br s, 1H, NH).

3. RESULTS SECTION

Among the literature devoted to construction of the pharmacophore model for bacterial PknB kinases a work of Seal *et al*. [7] can be distinguished, in which a new pharmacophore was designed using a combination of docking to known crystallographic models and analysis of known ligands.

As a result of overlay of the resulting PknB pharmacophore on the database of ASINEX compounds consisting of 393000 molecules 45 potentially active structures were selected by authors of this work [7]. Analysis of these potentially active compounds enables us to identify two main clusters: a cluster **A** comprising 19 compounds and consisting of central 2-pyridyl or 2-pyrimidyl-aminothiazole fragment linked to two aromatic rings and a cluster **B** comprising 15 compounds and consisting of two aromatic (possibly bicyclic) systems linked through NH-group. The remaining 11 compounds belong to different chemical classes.

Earlier general pharmacophore model for Ser/Thr kinases was constructed in our laboratory [8]. Our approach was based on consumption that all Ser/Thr kinases share common structural features in their active site that can be identified as general pharmacophore shown in Figure 2a. The general pharmacophore consists of three hydrophobic areas, one of which (brown) should be aromatic, and projections of donor (pink) and acceptor (blue) hydrogen bonds.

Along with the structural features pointed out in general pharmacophore and common to all Ser/Thr kinases every particular kinase possesses very individual structural features providing the selectivity of its ligands. We have previously

demonstrated the possibility to adapt the general pharmacophore to individual kinases by the example of the kinase Aurora A [8, 9]. Interestingly that Aurora A inhibitors (Figure 1, **C**) found by means of general pharmacophore concept included aryl-amino-thiazole fragment (red oval), which present in compounds of cluster **A** selected by Seal *et al*. [7] However, according to our model the third aromatic ring linked to thiazole should be placed in a position different to those predicted by Seal *et al*. and introduced through more flexible system of bonds.

In frame of our anti-tuberculosis program an attempt to adjust the general pharmacophore to PknB kinase was performed. Then synthetic schemes to achieve the constructed compounds were developed and all synthesized compounds were tested against virulent strain H37Rv.

Optimization of the general pharmacophore for bacterial PknB kinase led to chemical scaffolds **D** and **E** with the third aromatic cycle connected to aryl-amino-thiazole fragment through flexible 2-piperidinyl- or 2-pyrrolidinyl- linker (Figure 1). It should be noted that the linker and the third aromatic ring can be connected to either side of pharmacophore aryl-amino-thiazole moiety affording compounds with thiazole ring in the middle (**D**) or in the side (**E**) position. Both of these scaffolds can be overlay onto general pharmacophore model (Figure 2).

General synthetic scheme for scaffold **D** compounds is shown on Scheme 1 and exemplified by synthesis of compounds **I-III**.

N-Boc-2-Acetylpiperidine or N-Boc-2-acetylpyrrolidine was converted to corresponding bromoacetyl derivative **2** by consequent treatment with lithium bis(trimethylsilyl)amide, Me_3SiCl, and then with bromine. Compound **2** was coupled with thiourea to give aminothiazoles **3**, which entered into Buchwald-Hartwig reaction with chloropyridine or chloropyrimidine resulting compounds **4**. After Boc-deprotection imine **5** was reductively alkylated by corresponding aldehyde to afford desired compounds with general formula **6**, including **I-III.**

General synthetic scheme for scaffold **E** compounds is depicted on Scheme 2 and exemplified by synthesis of compound **IV**.

Cyclic amino acids such as Boc-proline, Boc-pipecoline-2-carboxylic acid or morpholino-3-carcoxylic acid were converted into corresponding amide **8** with ammonium carbonate in acetonitrile in presence of carbonyldiimidazole and triethylamine. Amide **8** was consequently treated with triethyloxonium tetrafluoroborate in dichloromethane and then after solvent removing with 10% ammonia in methanol to give amidine **9**. Crude amidine **9** was put into cyclization reaction with ethylacetoacetate resulting in bicyclic 4-hydroxy-6-methylpyrimidine derivative **10**. Then hydroxyl-group was replaced for clorine by phosphorus oxychloride treatment, and resulting compound **11** was coupled with heteroaromatic amines under Buchwald-Hartwig conditions. After Boc-deprotection imine **13** was reductively alkylated with various aromatic aldehydes to afford final compounds **14,** including compound **IV.**
A series of compounds of both scaffolds were tested for their ability to suppress the growth of a virulent strain *Mycobacterium Tuberculosis* (H37Rv) in liquid medium as it is described in the literature [10].

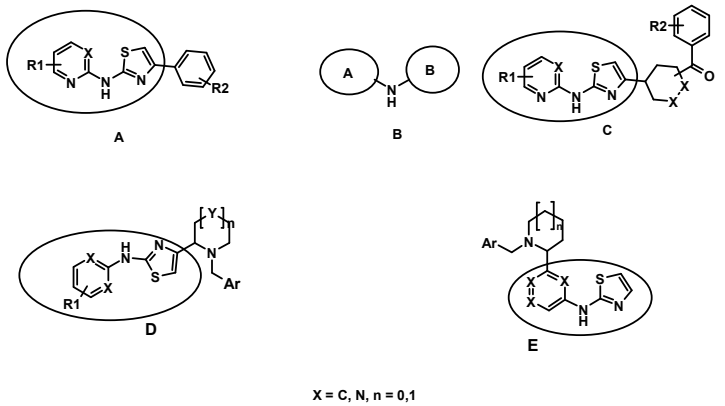


Figure 1. Chemical scaffolds for Ser/Thr kinase inhibitors: A and B – scaffolds for potential PknB inhibitors identified by Seal *et al*. [7], C-scaffold for AuroraA inhibitors designed by means of general pharmacophore model, D and E – scaffolds for potential PknB inhibitors designed by means of general pharmacophore model.

Firstly all substances were tested in a single concentration 20 μM and then minimum inhibitory concentration (MIC) required for complete suppression of proliferation of *Mycobacterium Tuberculosis* in liquid medium was determined for compounds showing more than 60% inhibition.

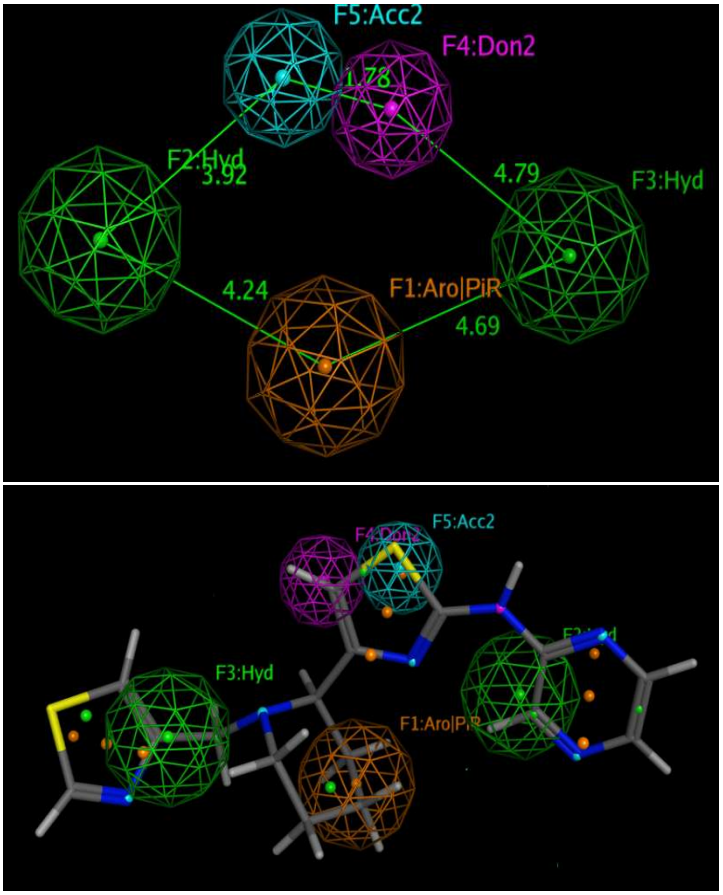
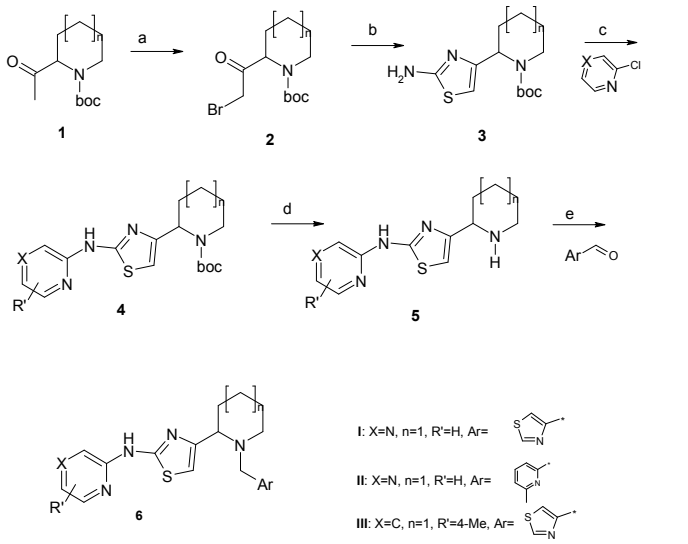
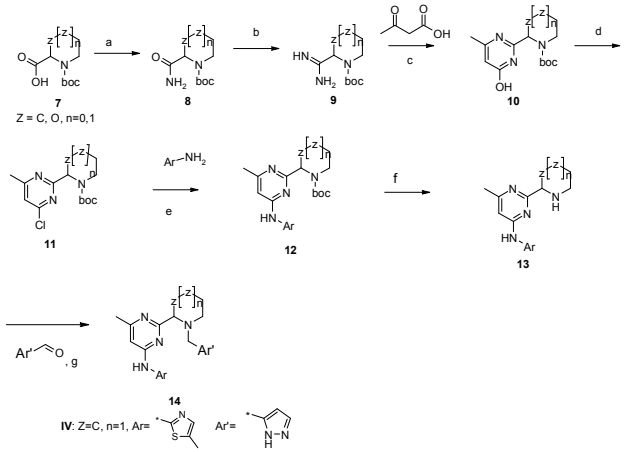


Figure 2. a. Pharmacophore model for Ser/Thr kinases. Hydrophobic areas are shown in green, aromatic cycle – in brown, projection of hydrogen bonds acceptor – in blue, and projection of hydrogen bonds donor – in pink; b. Overlay of compound I (scaffold D) onto general pharmacophore model.

For generation of curve for bacterial cells growth the Levenberg–Marquardt algorithm was applied. Simultaneously all active compounds were tested against possible inhibition of proliferation of human HeLa cells CellTiter-Glo® Luminescent Cell Viability Assay.

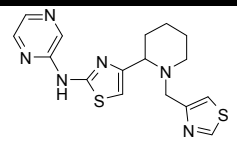
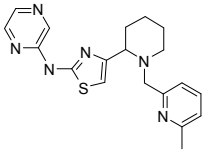
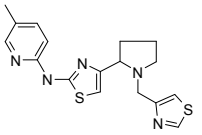
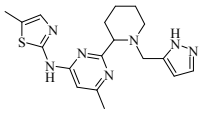


Scheme 1. General synthetic scheme for scaffold D compounds. a) lithium bis(trimethylsilyl)amide, Me_3SiCl, then Br_2; b) thiourea; c) 9,9 dimethyl-4,5-bis(diphenylphosphino)xanthene, tris (dibenzylidenacetone) dipalladium (0)-chloroform, tBuONa, microwave; d) HCl, ethanol; e) $NaHB(OAc)_3$, CH_2Cl_2.



Scheme 2. General synthetic scheme for scaffold E compounds. a) CDI, 60^0C, $(NH_4)_2CO_3$, Et_3N in CH_3CN, then K_2CO_3; b) $Et_3O^+BF_4^-$, CH_2Cl_2, then 10% NH_3 in MeOH; c) t-BuONa, EtOH; d) $POCl_3$, dimathylaniline; e) 9,9 dimethyl - 4,5 – bis (diphenylphosphino) xanthene, tris (dibenzylidenacetone) dipalladium (0) - chloroform, Na_2CO_3, toluene/water, microwave; f) 16% HCl /dioxane, 5 h, rt.; g) $NaHB(OAc)_3$, CH_2Cl_2.

Table 1. Inhibition of H37Rv and HeLa proliferation by compounds from new classes.

	Compound	H37Rv % inhibition at 20 μM	MIC[1]	HeLa % inhibition at 20 μM
I		98%	2 μM	11.5%; 27.7%
II		99%	3 μM	64,3%; 56,4%
III		93%	8 μM	13,7%; 17,8%
IV		90%	1 μM	80%

[1]MIC - minimum inhibitory concentration

The results for some active compounds are shown in Table 1. One can see that compounds of both scaffold **D** and scaffold **E** reveal activity against tuberculosis H37Rv cells with mild inhibition of HeLa cells proliferation.

4. CONCLUSIONS

Using general Ser/Thr pharmacophore approach suggested by us previously we have designed two series of compounds capable to suppress the growth of virulent *Mycobacterium Tuberculosis* strain (H37Rv). We suggest that this inhibition is caused by mycobacterial PknB inhibition and meantime we are studying the detailed mechanism of action of these classes of compounds.

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6. ACKNOWLEDGEMENTS

The authors gratefully acknowledge support from the Ministry of Education and Science of the Russian Federation for funding (agreement 14.576.21.0019 dated July, 27, 2014).