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Research Letter

General Ser/Thr Kinases Pharmacophore Approach for Selective Kinase Inhibitors Search as Exemplified by Design of Potent and Selective Aurora A Inhibitors

Natalya I. Vasilevich*, Elena A. Aksenova, Denis N. Kazyulkin and Ilya I. Afanasyev

Novie Nauchnie Tekhnologii Ltd. (ASINEX Company Group), 20 Geroev Panfilovtsev Str., Moscow 125480, Russia
**Corresponding author: Natalya I. Vasilevich, nvasilevich@asinex.com*

A general pharmachophore model for various types of Ser/Thr kinases was developed. Search for the molecules fitting to this pharmacophore among ASINEX proprietary library revealed a number of compounds, which were tested and appeared to possess some activity against several Ser/Thr kinases such as Aurora A, Aurora B and Haspin. The possibility of performing the fine-tuning of the general Ser/Thr pharmacophore to desired types of kinase to get active and selective inhibitors was exemplified by Aurora A kinase. As a result, several hits in 3–5 nM range of activity against Aurora A kinase with rather good selectivity and ADME properties were obtained.

Key words: kinase, molecular modeling, pharmacophore modeling, phosphatase, structure-based drug design

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Serine–threonine kinases play an important role in signal transduction pathways in both eukaryotic and prokaryotic cells; they act in regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation and embryonic development. Therefore, inhibitors of Ser/Thr kinase activity have a broad range of potential therapeutic uses – from treating cancer to promoting a desired immunosuppressive effect. As these kinases were found in a number of mycobacterial organisms, their inhibitors can be attractive targets to treat bacterial infection (1).

A number of attempts have been done to construct a pharmacophore model for various serine–threonine kinase inhibitors. For example, pharmacophore for STPK inhibitors of tuberculosis (2,3), for mTor kinase inhibitors (4), for Aurora B inhibitors (5), for Aurora A inhibitors (6,7) and for B-Raf inhibitors (8) was developed. All these works were devoted to selected kinase of serine–threonine family, but we surmised that there are a number of common features of all serine–threonine inhibitors so it would be possible to construct a general pharmacophore model which subsequently could be optimized for specific kinds of serine–threonine kinase.

To achieve our goal, we analysed a number of known inhibitors of various serine–threonine kinases including compounds, which have been found both in our laboratory previously and in literature references cited above. For this purpose, we used pharmacophore elucidating functionality of MOE version 2010.10. The results of this work are on Figure 1.

As it is seen from the figure, the general pharmacophore looks like a kind of diamond with two opposite hydrophobic centres, one aromatic centre and a couple of H-bond donor and acceptor projections in one corner. The length of a rhomb side is about 4–5 A.

Application of the general pharmacophore found to ASINEX proprietary library allowed to identify a scaffold including two 5- and 6-member heterocyclic aromatic rings linked through NH group, containing additional pyrrolidine or piperidine group attached to 6-member ring in meta-position to NH group. To confirm their activity against Ser/Thr kinases, a set of compounds belonging to this scaffold was tested against Aurora A, Aurora B and Haspin kinases.

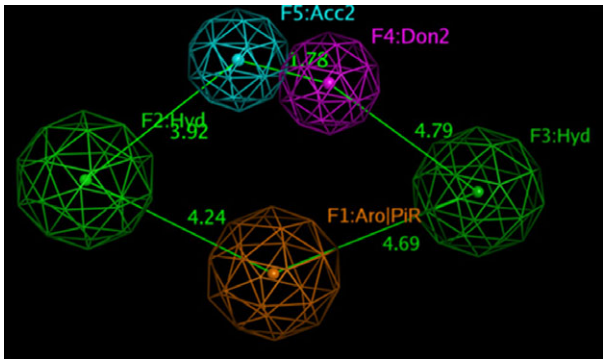

Figure 1: General pharmacophore of serine–threonine kinase inhibitors (MOE 2010.10).

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Table 1: Some results of general Ser/Thr pharmacophore application to ASINEX proprietary library

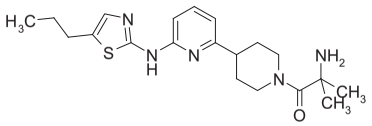
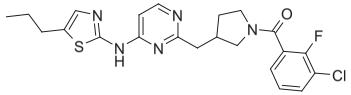
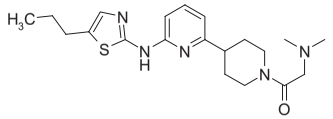
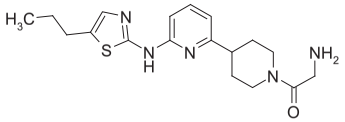
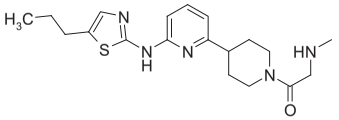
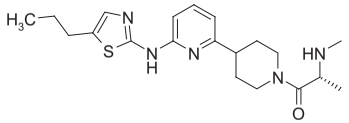
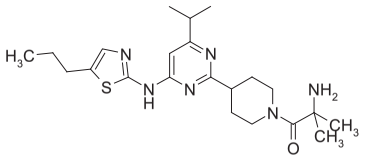
No.	Structure	Aurora A		Aurora B		Haspin	
		I, % (Mean ± SEM, 4 repeats)	IC_{50}, μM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, μM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, μM (Mean ± SEM, 4 repeats)
1		99.0 ± 1.0	2.06 ± 0.15	101.0 ± 0.9	1.45 ± 0.17	96.0 ± 3.2	1.49 ± 0.15
2		98.0 ± 1.1	0.10 ± 0.005	94.0 ± 5.3	3.46 ± 0.36	47.0 ± 4.8	105.2 ± 11.2
3		99.0 ± 2.0	1.95 ± 0.22	89.0 ± 8.1	1.76 ± 0.16	79.0 ± 7.1	7.06 ± 0.68
4		100.0 ± 1.5	2.08 ± 0.25	96.0 ± 4.1	1.72 ± 0.19	104 ± 6.2	0.08 ± 0.008
5		97.0 ± 4.6	1.44 ± 0.13	95.0 ± 6.1	0.91 ± 0.08	100.0 ± 1.5	0.43 ± 0.034
6		99.0 ± 2.0	1.67 ± 0.17	96.0 ± 3.5	1.75 ± 0.18	94.0 ± 6.2	1.12 ± 0.11
7		97.0 ± 3.1	2.79 ± 0.30	94.0 ± 5.3	2.25 ± 0.23	86.0 ± 8.7	3.67 ± 0.37

Table 1: continued

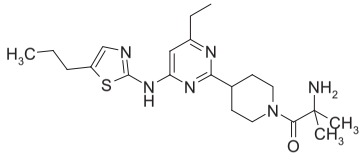
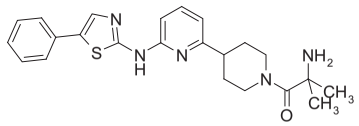
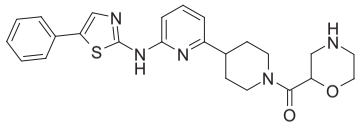
No.	Structure	Aurora A		Aurora B		Haspin	
		I, % (Mean ± SEM, 4 repeats)	IC_{50}, μM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, μM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, μM (Mean ± SEM, 4 repeats)
8		97.0 ± 2.9	3.58 ± 0.43	88.0 ± 7.1	2.05 ± 0.24	77.0 ± 8.5	2.90 ± 0.43
9		84.0 ± 8.0	3.17 ± 0.33	59.0 ± 6.1	8.02 ± 0.80	101.0 ± 5.0	0.27 ± 0.019
10		91.0 ± 9.5	8.38 ± 0.84	93.0 ± 9.5	0.84 ± 0.09	63.0 ± 6.5	7.80 ± 0.81

Table 2: Optimization of compound 2 for Aurora A kinase

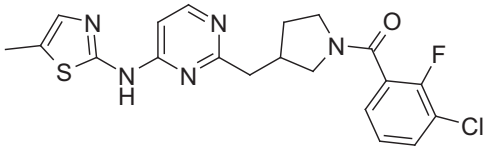
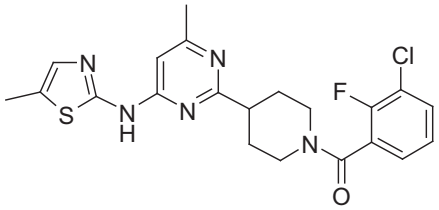
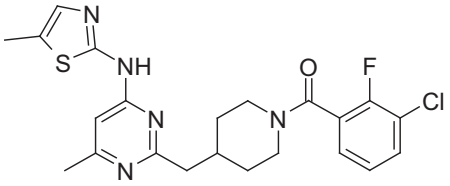
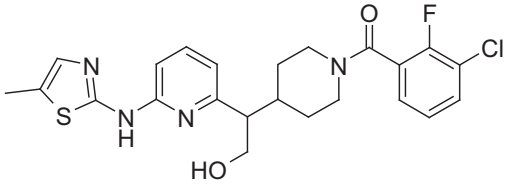
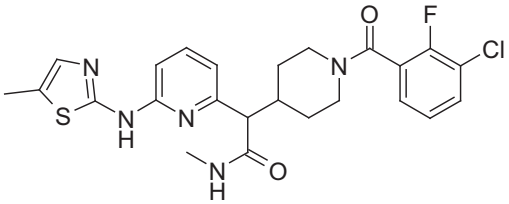
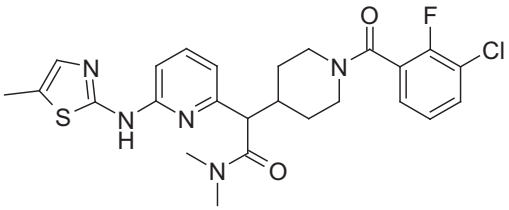
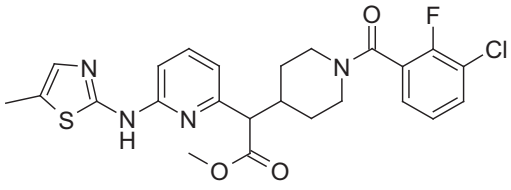
		IC_{50}, nM (Mean ± SEM, 4 repeats)
2		96.0 ± 9.1
11		85.0 ± 9.2
12		12.0 ± 1.1
13		193.6 ± 20.5
14		246.4 ± 21.2
15		418.3 ± 40.5
16		154.1 ± 11.2

Table 2: continued

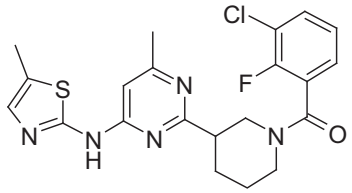
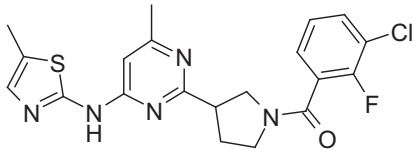
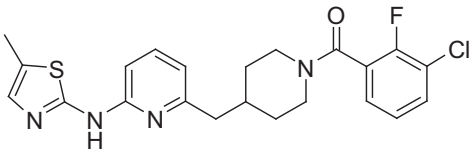
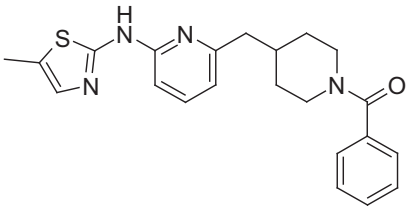
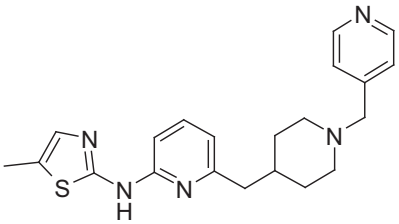
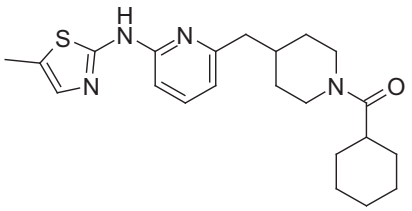
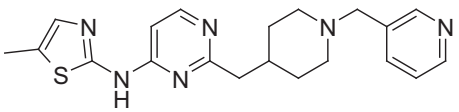
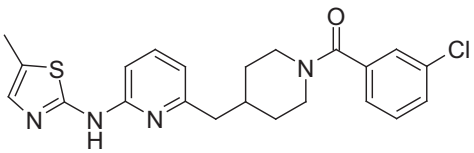
		IC_{50}, nM (Mean ± SEM, 4 repeats)
17		70.4 ± 6.5
18		20.5 ± 1.9
19		6.0 ± 0.3
20		265.0 ± 22.4
21		292.2 ± 24.3
22		166.8 ± 15.6
23		60.0 ± 5.4
24		22.3 ± 0.15

Table 2: continued

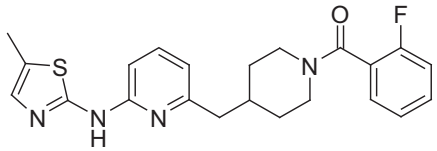
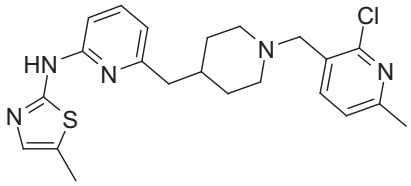
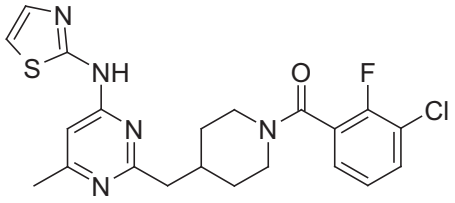
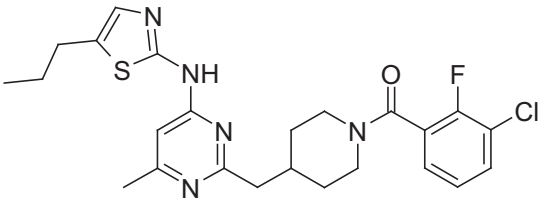
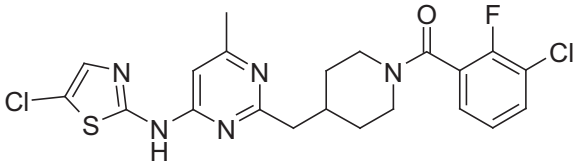
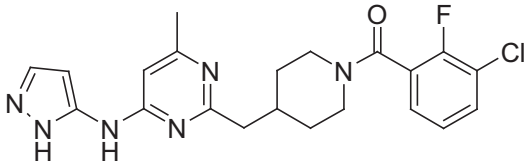
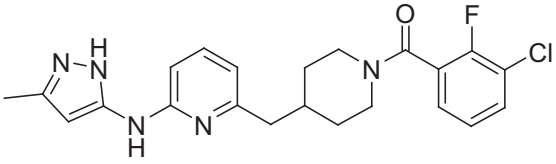
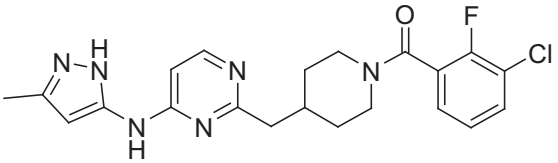
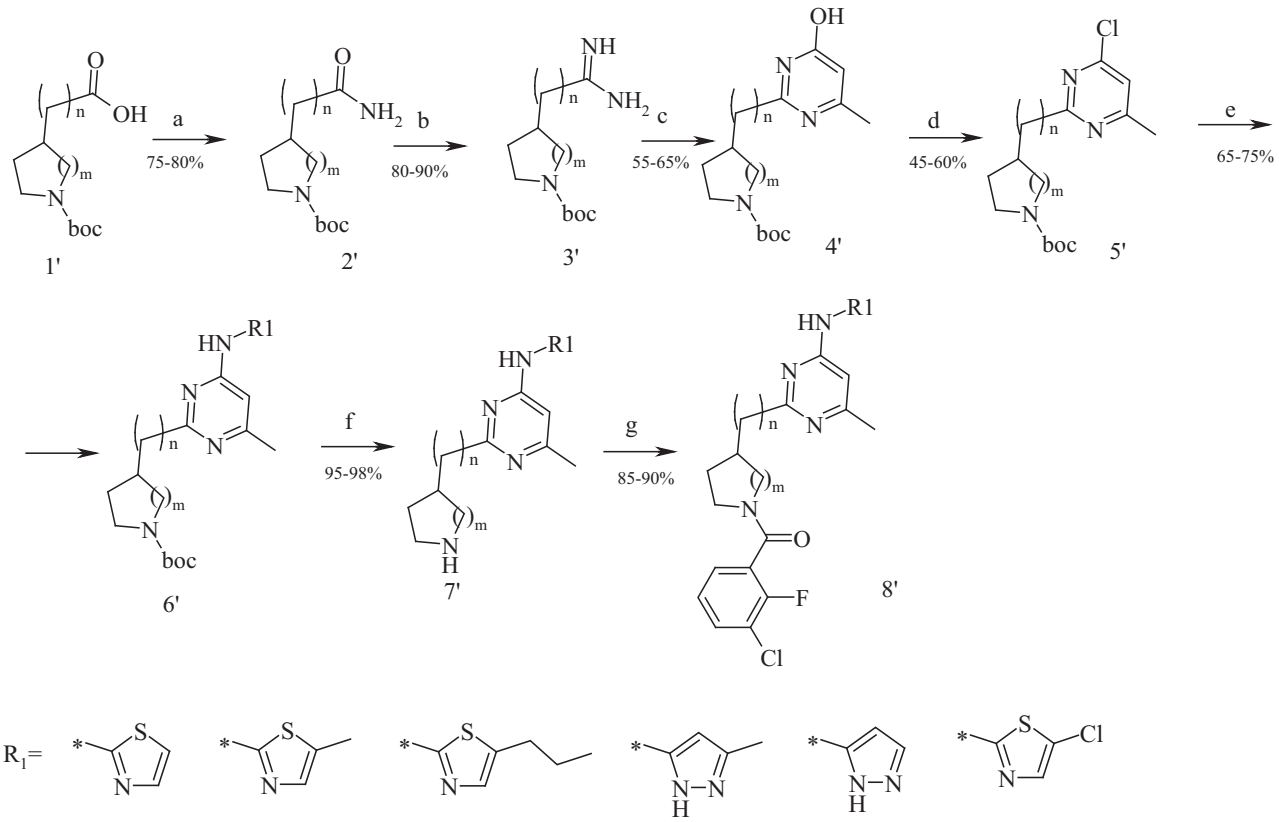
		IC_{50}, nM (Mean ± SEM, 4 repeats)
25		95.2 ± 9.1
26		96.1 ± 8.9
27		35.1 ± 0.36
28		103.2 ± 10.1
29		4.1 ± 0.35
30		5.3 ± 0.46
31		17.5 ± 2.1

Table 2: continued

		IC_{50}, nM (Mean ± SEM, 4 repeats)
32		3.5 ± 0.29



n=0, 1; m=1, 2

Scheme 1: Synthesis of compounds **11**, **12**, **17**, **18**, **27**, **28**, **29**, **30**.

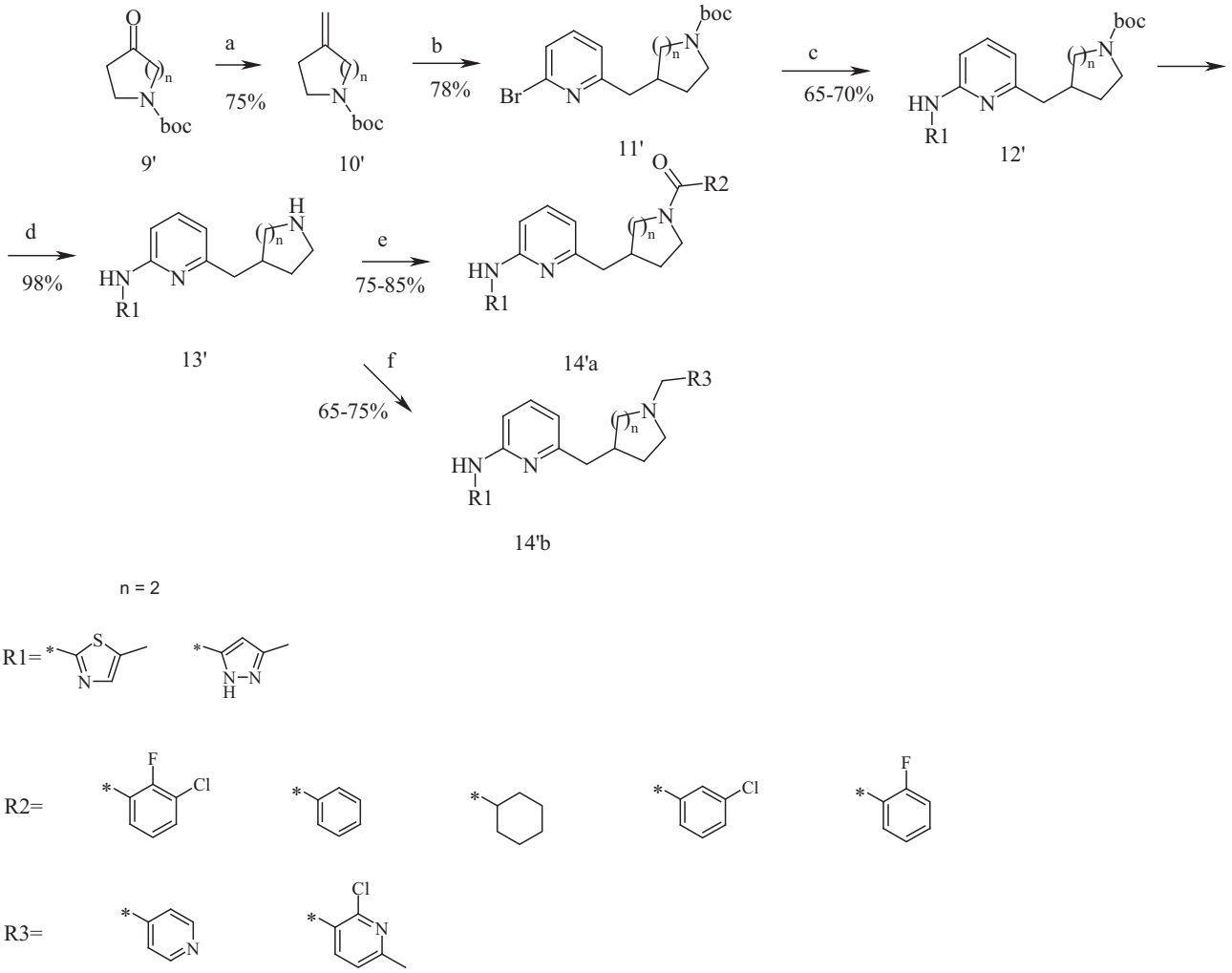
Conditions: (a) $(NH_4)_2CO_3$, TBTU, Et_3N, CH_3CN, RT, 8 h; (b) triethyloxonium tetrafluoroborate, CH_2Cl_2, RT, 2 h; NH_3/MeOH, RT, 16 h; (c) CH_3COCH_2-COOEt, NaO^tBu, EtOH, RF, 10 h; (d) $POCl_3$, N,N-dimethylaniline, toluene, RF, 4 h; (e) R_1NH_2, Na_2CO_3, Pd_2dba_3, Xantphos, toluene/H_2O, MW, 140 °C, 2 h; (f) HCl(aq)/MeOH, RT, 4 h; and (g) 3-chloro-2-fluoro-benzoic acid, TBTU, Et_3N, CH_3CN, RT, 8 h.

Noteworthy, none of these compounds were identified previously in scientific literature as Ser/Thr kinases inhibitors.

Indeed, some of these compounds revealed activity against all three Ser/Thr kinases, though not very high (Table 1).

To confirm the possibility of fine-tuning of the general pharmacophore for selected type of kinases, we chose Aurora A kinase and tried to optimize the scaffold that we identified in the screen to be more selective for this target.

Among the primary set of ten compounds depicted in Table 1, we found compound **2**, which possessed the best inhibitory activity towards Aurora A kinase – about 95 nM and attempted to optimize this compound for Aurora A kinase.



Scheme 2: Synthesis of compounds **19**–**22**, **24**–**26**, **31**.

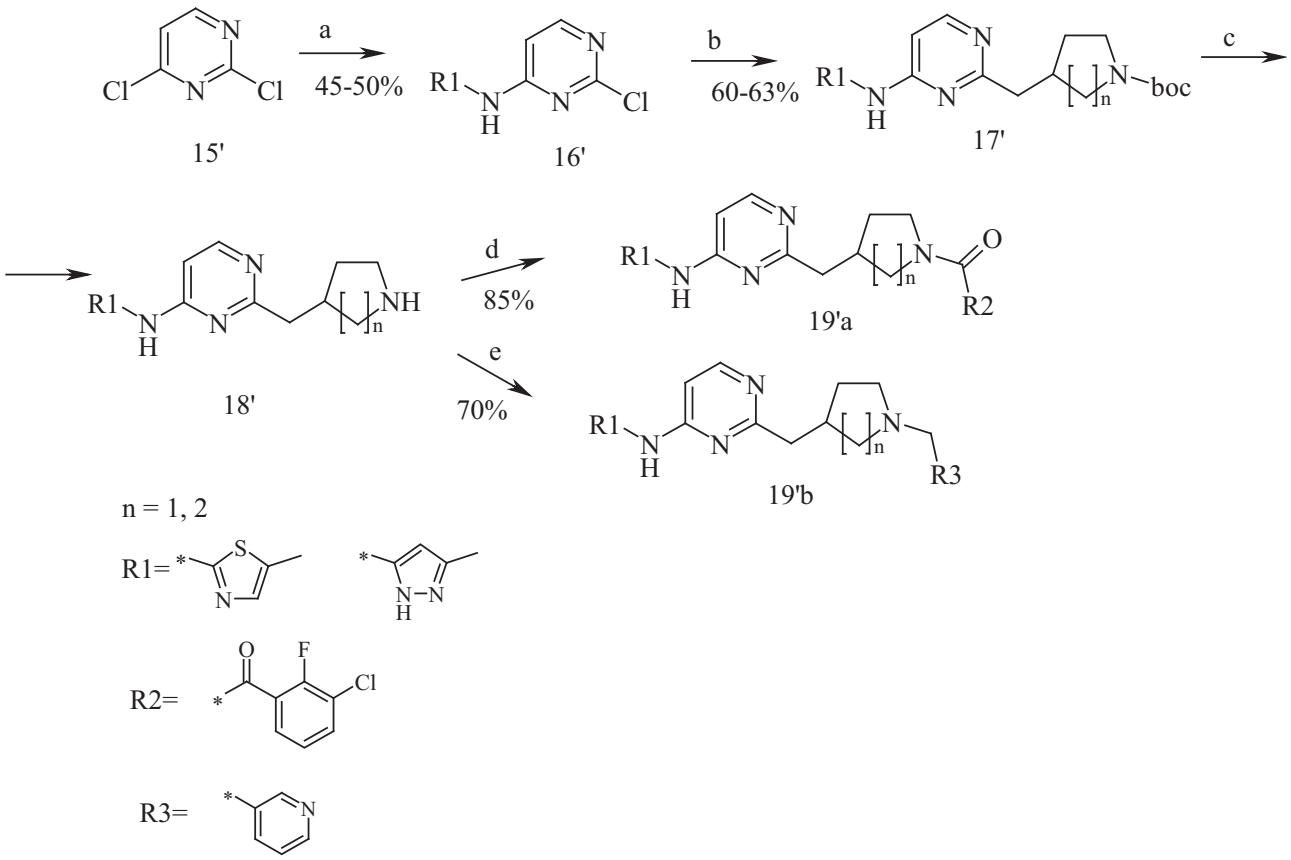
Conditions: (a) $MePh_3P^+I^-$, NaH, THF; (b) 2,6-dibromo-pyridine, 9-BBN, $Pd(Ph_3P)_4$, K_2CO_3, THF, RF; (c) R_1NH_2, Na_2CO_3, Pd_2dba_3, Xantphos, toluene/H_2O, MW, 140 °C, 2 h; (d) HCl(aq)/MeOH, RT, 4 h; (e) R_2COOH, TBTU, Et_3N, CH_3CN, RT, 8 h; and (f) R_3CHO, $NaBH(OAc)_3$, CH_3CN, RT, 8 h.

With this goal, a library of analogues of compound **2** was prepared (Table 2).

Compounds **11**, **12**, **17**, **18**, **27**–**30** were synthesized in accordance with Scheme 1. Amides **2′** were prepared from corresponding Boc-protected amino acids **1′** were converted into amidine salts **3′** by reaction with triethyloxoniun tetrafluoroborate followed by treatment with solution of ammonia in methanol. Cyclization of the amidine obtained with acetoacetic ester in ethanol under reflux gave 6-methyl-pyrimidones **4′** in 55–60% yield. These pyrimidones **4′** were treated with three equivalents of phosphorus oxychloride and nine equivalents of dimethylaniline in toluene to give chlorides **5′** in 45–50% yield. Buchwald reaction with aromatic amines (2-aminothiazoles or 3-aminopyrazoles) led to new derivatives **6′**; this reaction was performed in toluene–water mixture with 2.5% mol Pd_2dba_3 и 5% mol xantphos and potassium carbonate using microwave reactor (yield 65–75%) .

For compound **29** with 5-chloro-2-thiazole moiety intermediate **6′** (where R_1 – unsubstituted 2-aminothiazole) was treated with N-chlorosuccinimide in dichloroethane at room temperature (yield 70%).

To synthesize compounds **30** and **32**, containing 3-aminopyrazole moiety, corresponding 3-amino-1H-pyrazoles (or 3-amino-5-methyl-1H-pyrazoles) were protected by tosyl group via reaction with tosyl chloride and sodium hydrocarbonate in acetonitrile. This protective group was easily removed from intermediates **6′** by sodium hydroxide in methanol treatment.



Scheme 3: Synthesis of compounds **2**, **23**, **32**.

Conditions: (a) R_1NH_2, Na_2CO_3, Pd_2dba_3, Xantphos, toluene/H_2O, MW, 140 °C, 2 h; (b) alkene (10′), 9-BBN, $Pd(Ph_3P)_4$, K_2CO_3, THF, RT; (c) HCl (aq)/MeOH, RT, 4 h; (d) R_2COOH, TBTU, Et_3N, CH_3CN, RT, 8 h; (e) R_3CHO, $NaBH(OAc)_3$, CH_3CN, RT, 8 h.

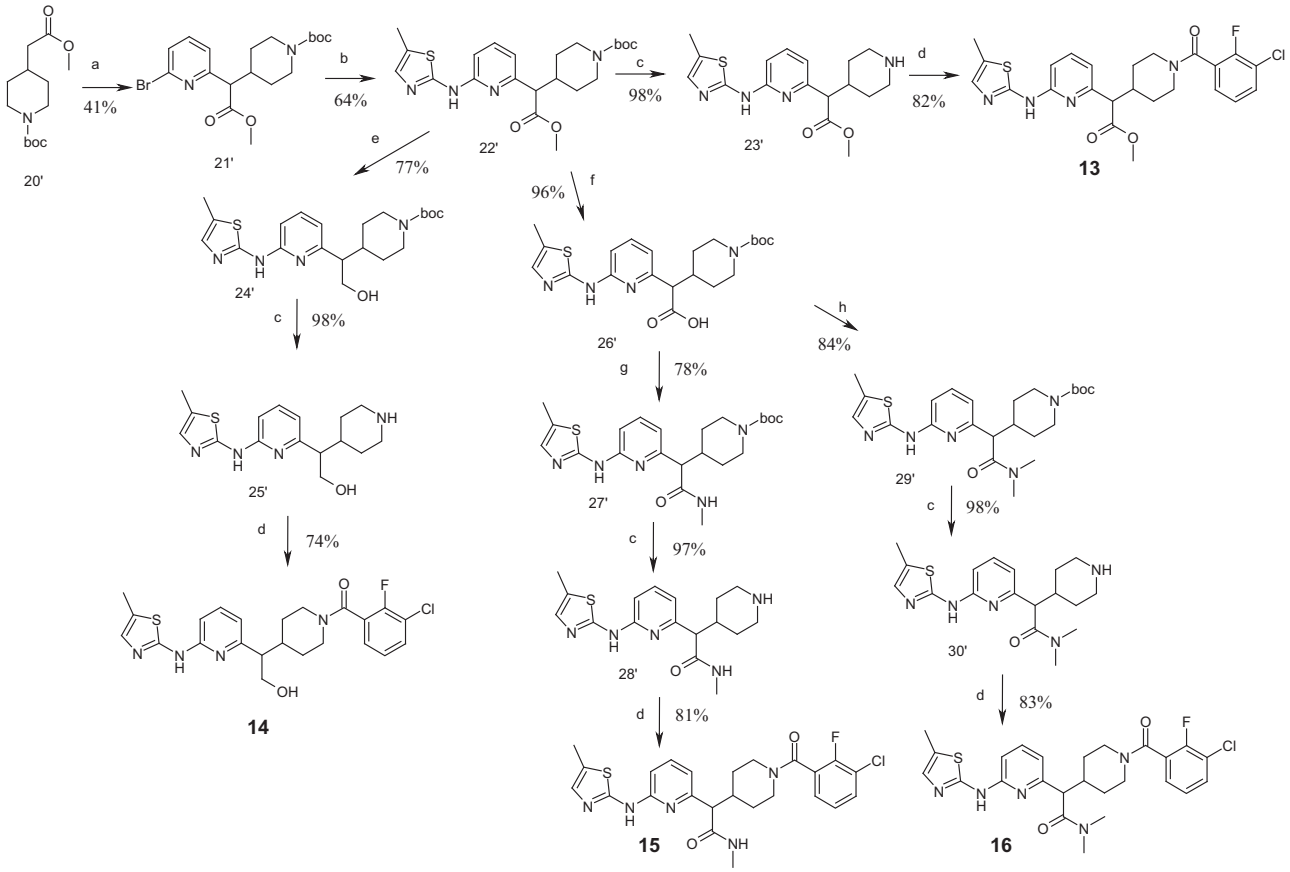
After Boc-deprotection amines **7′** were acylated by 3-chloro-2-fluorobenzoic acid using TBTU as a coupling reagent (yields 85% and more).

Synthesis of compounds **19**–**22**, **24**–**26**, **31** is shown in Scheme 2. Alkene **10′** was obtained from ketone **9′** via reaction with methyltriphenylphosphonium iodide and sodium hydride in 75% yield. Subsequent treatment with 9-BBN in THF, and 2,6-dibromopyridine in Suzuki reaction conditions gave bromide **11′** in 78% yield. Buchwald reaction led to intermediates **12′** (65–75% yields). Compounds **19**, **20**, **22**, **24**, **23** and **31** were prepared by acylation reactions using TBTU as a coupling reagent. Compounds **21** and **26** were obtained by reductive amination reaction in 65–75% yield.

Compounds **2**, **23**, **32** were synthesized as shown in Scheme 3. Buchwald reaction with 2,4-dichloropyrimidines **15′** in conditions described above gave a mixture of regioisomers that were separated by column chromatography in 45–50% yield of target compound **16′**. This compound was involved into Suzuki reaction with alkenes **10′** and 9-BBN followed by Boc-deprotection led to compound **18′** which was further acylated or alkylated to give the target compounds **2**, **23**, **32**.

Compounds **13**–**16** were prepared in accordance with Scheme 4. Intermediate **21′** was obtained by reaction between compound **20′** and 2,6-dibromopyridine in presence of NaHMDS in THF at 0 °C in 41% yield. Buchwald reaction with 2-amino-5-methylthiazole gave compound **22′** (yield 64%), which was easily converted into alcohol **24′** (77%) or amides **27′** and **29′** (78–84%). Following Boc-deprotection and acylation led to compounds **13**–**16** in 74–83% yields.

On the first step, we kept methyl substituent on thiazole ring and 2-fluoro-3-chloro-benzoyl substituent on aliphatic nitrogen (compounds **11**–**19**) varying both 6-member aromatic ring and an aliphatic N-containing cycle tethered to the aromatic one either directly or through methylene linker. We found that the best compound in this series was compound **19**.



Scheme 4: Synthesis of compounds **13–16**.

Conditions: (a) 2,6-dibromo-pyridine, NaHMDS, THF, 0 °C; (b) 2-amino-5-methylthiazole, Na_2CO_3, Pd_2dba_3, Xantphos, toluene/H_2O, MW, 140 °C, 2 h; (c) HCl(aq)/MeOH, RT, 4 h; (d) 3-chloro-2-fluoro-benzoic acid, TBTU, Et_3N, CH_3CN, RT, 8 h; (e) $LiAlH_4$, THF, RT, 3 h; (f) NaOH, MeOH/H_2O, RT, 4 h; (g) methyl amine hydrochloride, TBTU, Et_3N, CH_3CN, RT, 8 h; and (h) dimethyl amine hydrochloride, TBTU, Et_3N, CH_3CN, RT, 8 h.

Then, a series of compounds with methylene-4-piperidine linker and various N-acyl and N-alkyl substitution were made (compounds **20–26**). None of these compounds was superior to compound **19**, so the initial 2-fluoro-3-chloro-benzoyl group appeared to be a substituent of choice.

On the last step, we varied thiazole fragment and discovered pyrazole-containing derivatives to be equally or even more active than 4-methyl-thiazole analogues.

Therefore, as a result of our attempts to optimize the general serine–threonine pharmacophore model for particular Aurora A kinase, a number of compounds with excellent potency were obtained. The best three compounds were tested for selectivity against serine/threonine kinases available in house and some of ADME properties. For comparison, one non-receptor tyrosine kinase, RON, was also included. The results are shown in Table 3.

One can see that compound **29**, containing thiazole group, possesses better activity against Aurora A kinase than parent compound **2** (4.1 nM vs. 100 nM); however, its selectivity in comparison with other kinases was approximately equal. Replacement of thiazole group for pyrazole moiety gave compounds **30** and **32**, which appeared to be equally active but more selective that compound **29** (EPHA2: inhibition 70%, 50% and 29%, LYN: 84%, 40% and 10%, RON: 98%, 10% and 37% for compounds **29**, **30** and **32**, correspondingly). Moreover, such a replacement improved a solubility of compounds **30** and **32** to >100 μg/mL.

In conclusion, a general pharmacophore model applicable to various serine–threonine kinases was designed. A possibility of fine-tuning of this pharmacophore to particular types of serine–threonine kinases such as Aurora A kinase was confirmed. As a result, compounds with good potency, selectivity and some ADME parameters were obtained.

Table 3: Selectivity and ADME properties of best compounds

	2		**29**		**30**		**32**	
	I, % (Mean ± SEM, 4 repeats)	IC_{50}, nM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, nM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, nM (Mean ± SEM, 4 repeats)	*I*, % (Mean ± SEM, 4 repeats)	IC_{50}, nM (Mean ± SEM, 4 repeats)
Aurora A	98.0 ± 1.01	100 ± 5.0	93.0 ± 6.0	4.1 ± 0.35	97.0 ± 2.0	5.3 ± 0.46	99.0 ± 0.9	3.5 ± 0.29
Aurora B	94.0 ± 5.3	3480 ± 360.3	97.0 ± 8.1	232.2 ± 24.1	88.0 ± 7.5	18300.0 ± 185.0	99.0 ± 1.0	4182.5 ± 40.1
c-Kit	50.0 ± 3.9		66.0 ± 3.4	17000.00 ± 165	12000.0 ± 115.0	21000 ± 250		
EGFR	24.0 ± 2.4		40.0 ± 5.0				10.0 ± 2.0	
CSK			70.0 ± 8.0	9500.0 ± 101.0	7.0 ± 4.0		15.0 ± 3.0	
EPHA2	90.0 ± 9.4	3000.0 ± 295.0	70.0 ± 9.0	9000.0 ± 985.0	50.0 ± 6.0		29.0 ± 3.0	
GSK-3					98.0 ± 2.0	109.0 ± 12.1	94.0 ± 3.0	292.8 ± 31.2
Haspin	47.0 ± 4.8	105.2 ± 11.2	37.0 ± 4.0		12.0 ± 2.0		25.0 ± 3.0	
JNK3	90.0 ± 9.1	193.0 ± 15.0	99.0 ± 0.5	85.18 ± 9.1	84.0 ± 9.0	1634.0 ± 167	97.0 ± 9.0	572.5 ± 56.1
LYN	85.0 ± 8.0	2530.0 ± 154.0	84.0 ± 8.0	3270.0 ± 324.0	40.0 ± 3.0		10.0 ± 0.8	
PIM1	19.0 ± 1.0		21.0 ± 1.5		3.0 ± 1.0		15.0 ± 1.3	
PLK1	50.0 ± 4.0				28.0 ± 1.2		47.0 ± 4.0	
RON	45.0 ± 4.1		98.0 ± 1.0	970.0 ± 101.0	10.0 ± 1.0		37.0 ± 2.0	
CYP1A2	59.0 ± 5.0		62.0 ± 5.0		11.0 ± 1.0		21.0 ± 1.7	
CYP2C19	91.0 ± 5.7		94.0 ± 4.0		77.0 ± 6.0	9597.0 ± 960.0	95.0 ± 3.0	965.7 ± 94.1
CYP 2C9	100.0 ± 1.0		100.0 ± 2.0		81.0 ± 9.0	3900.0 ± 401	76.0 ± 7.0	326.6 ± 33.0
CYP3A4	100.0 ± 2.0		100.0 ± 1.0		44.0 ± 5.0	15070.0 ± 151	57.0 ± 5.8	596.2 ± 60.1
HERG					5		17	
Solubility (nephelometry)	<10 μg/mL		<10 μg/mL		>100 μg/mL		>100 μg/mL	

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