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Rational design and synthesis of novel Syk-kinase inhibitors

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Molecular modeling and subsequent synthesis of novel Syk-kinase inhibitors, 7*H*-pyrrolo[2,3-*d*]pyrimidine and 1,3,5-triazine derivatives, have been carried out. The best of the obtained compounds demonstrated to inhibit Syk-kinase activity at IC_{50} = 230±10 nM.

Syk-kinase represents one of the key mediators of immune system activation in response to exposure to an antigen.[1] A number of recent publications[2,3] demonstrate that Syk-kinase inhibitors are effective in treating various autoimmune diseases such as rheumatoid arthritis. Furthermore, as the activity of Syk-kinase plays critical role in B-lymphocite maturation,[4] the use of its inhibitors in treating hematological disorders related to uncontrolled growth of B-cells. Clinical trials of the Syk-kinase inhibitor, [6-({5-fluoro-2-[(3,4,5-trimetoxyphenyl)amino]pyrimidin-4-yl}-amino)-2,2-dimethyl-3-oxo-1,3-dihydro-4*H*-pyrid[3,2-*b*][1,4]-oxazin-4-yl]methylphosphoric acid (fostamatinib), have proven its efficacy in treating rheumatoid arthritis. Interestingly, fostamatinib is a prodrug which was elaborated in order to overcome unsatisfactory pharmacokinetic properties of tamatinib (R-406).[2] Thus, the development of novel Syk-kinase inhibitors with improved pharmacokinetic parameters is of great importance.

In the present work, a rational search for the compounds capable of blocking the active site of Syk-kinase was performed using molecular docking method.† The latter method uses full atom spatial models of biological macromolecules in order to calculate the optimum geometry of the complex and to estimate ligands binding energy based on molecular-mechanical potentials.[5,6] The majority of the known Syk-kinase inhibitors were developed using the fragment-based approach,[7] *i.e.* their structure consists of a number of linked fragments (Figure 1). This approach allows one to synthesize the required number of building blocks and then to link them combinatorially yielding a large and diverse library of presumably active compounds.‡

Structural filtration methodology[8] was employed in order to increase the accuracy of the docking-based rational search for inhibitors. The analysis of the X-ray structural data for the known Syk-kinase–inhibitor complexes has shown that active ligands form a pair of correlated hydrogen bonds with carbonyl and amide groups of Ala451 residue in all cases (Figure 2). Presumably, these hydrogen bonds are characteristic of the binding mode of active ligands. Therefore, according to the idea of the structural filtration, only the ligands forming the above-mentioned hydrogen bonds were selected from the docking results, while other ligands were considered inactive.

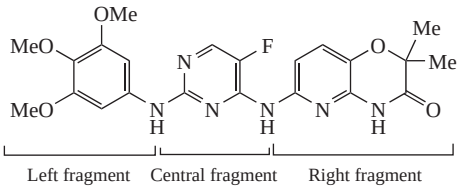


Figure 1 Syk-kinase inhibitor molecule decomposed into fragments. Compound tamatinib is given as an example.

As a result of the molecular docking study and the subsequent structural filtration, a set consisting of 18 presumable Syk-kinase inhibitors was formed. Currently, two compounds from this set, 2,2-dimethyl-6-({4-[(3,4,5-trimetoxyphenyl)amino]pyrimidin-4-yl}amino)-2*H*-pyrid[3,2-*b*][1,4]oxazin-3(4*H*)-one **1** and methyl 2-methyl-2-[(2-nitropyridin-3-yl)oxy]propanoate **2**, were synthesized (Schemes 1 and 2).§ Dose-dependent Syk-kinase inhibi-

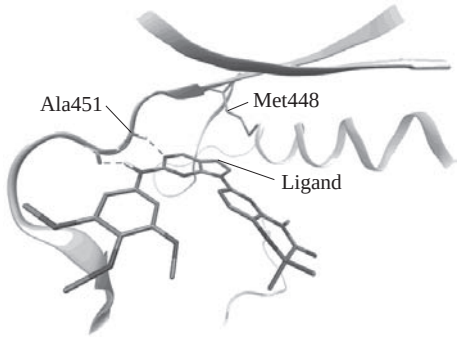
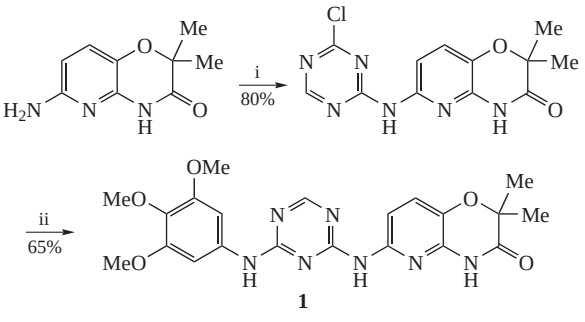


Figure 2 Binding of compound **2** to active site of Syk-kinase predicted with molecular modeling.

† *Molecular docking* was performed using Lead Finder 1.1.15 (available at *www.moltech.ru*)[11] with default parameters. Full-atom spatial model of Syk-kinase was prepared from PDB ID 3FQS[12] using Model Builder program[13] from Lead Finder package. Ligand binding energies were estimated with dG-scoring function of Lead Finder.[14]

‡ *Ligand library preparation*. In the preparation of fragment library 32 novel central scaffolds, 4 right and 8 left fragments were used. The example of decomposing a known Syk-kinase inhibitor into fragments is shown in Figure 1. Thus, a virtual combinatorial library containing 1024 structures was generated. Spatial structure optimization was carried out with Corina 3.4. For each molecule the pK_a values of all ionizable groups were calculated, and the ionization states were then set to those corresponding to physiological pH 7.0.

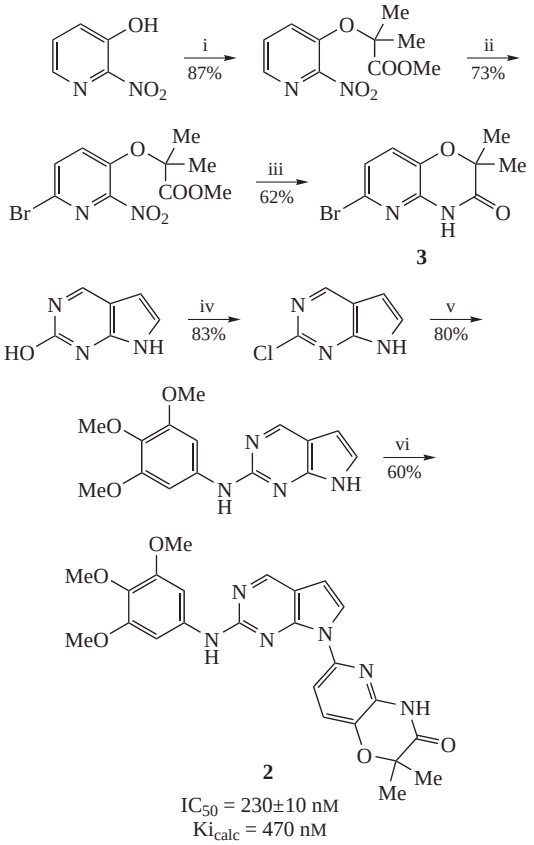
§ For synthetic procedures and characteristics of compounds synthesised, see Online Supplementary Materials.



1

IC_{50} = 1000±30 nM
Ki_{calc} = 750 nM

Scheme 1 *Reagents and conditions*: i, 2,4-dichloro-1,3,5-triazine, DMF, 60 °C, 8 h; ii, 3,4,5-$(MeO)_3C_6H_2NH_2$, DMF, 110 °C, 6 h.



3

2

IC_{50} = 230±10 nM
Ki_{calc} = 470 nM

Scheme 2 *Reagents and conditions*: i, NaH, DMF, then $Me_2C(Br)CO_2Me$; ii, Br_2, AcOH; iii, $SnCl_2$, HCl, H_2O/MeOH, then $NaHCO_3$; iv, $P(O)Cl_3$, $PhNMe_2$, 60 °C; v, 3,4,5-$(MeO)_3C_6H_2NH_2$, DMF, 110 °C, 6 h; vi, **3**, DMF, 60 °C, 8 h.

tion curves and the corresponding IC_{50} values were obtained for both compounds.¶ Synthesis and IC_{50} determination for the rest 16 presumably active compounds are planned to be conducted shortly.

Seemingly, the comparison of the inhibition efficacy for known inhibitors tamatinib (IC_{50}, 41 nM)[9] and 5-fluoro-2,4-bis(3-hydroxyphenylamino)pyridine (IC_{50}, 226 nM)[10] with those obtained in the present work shows that compound **2** has remarkable potency. The latter compound was selected for the future Syk-kinase binding optimization.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2012.03.006.

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¶ *Syk-kinase inhibition assay.* Peptide substrate was dissolved in reaction buffer to the target concentration of 0.2 mg ml^{-1}. Recombinant Syk-kinase solution was added to the target concentration of 2 nM, and also the tested compound to the given concentration (1 nM–10 μM). ^{33}P-ATP solution was then added (10 μM, final specific activity 0.01 μCi). The reaction mixture was incubated for 120 min, then poured on an ion-exchange membrane which was then washed with excessive amount of phosphoric acid. The conversion was then determined based on the radioactivity of reaction products.

IC_{50} values were recalculated into inhibition constants Ki based on the concurrent inhibition equation: $Ki = IC_{50}/(1 + [ATP]/Km)$, where [ATP] is ATP concentration used in the experiment, and Km is a Michaelis constant for enzymatic reaction (according to the published data,[15] Km = 75 μM).