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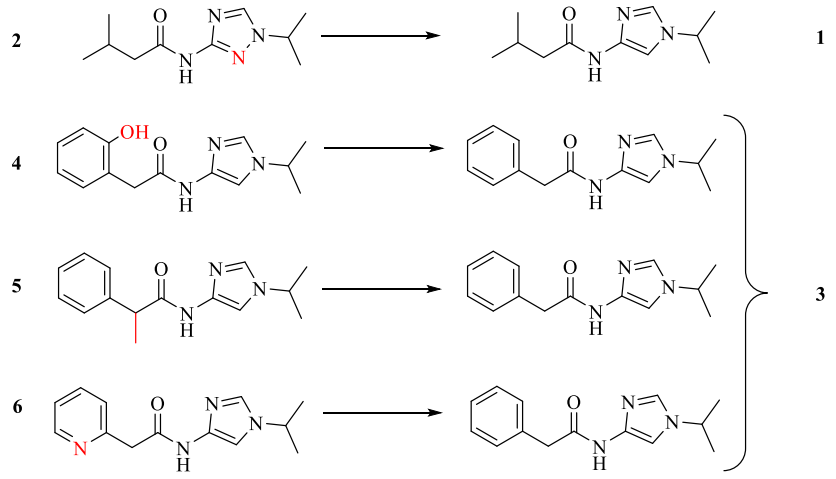
Modeling of novel CDK7 inhibitors activity by molecular dynamics and free energy perturbation methods

Polina V. Rusina, Ilya Yu. Titov, Maria V. Panova, Victor S. Stroylov, Yana R. Abdyusheva, Elizaveta Yu. Murlatova, Igor V. Svitanko and Fedor N. Novikov

1. Docking, MD and FEP/MD procedure

Molecular modeling was carried out using conventional procedures[19]. Full-atom spatial model of CDK7 (PDB: 1UA2) and molecular docking was performed using LeadFinder 1.1.15.8[20,21,22]. The best ligand conformation obtained after docking was used as a starting point for 50 ns MD simulations under conditions of explicit hydration (20 Å) and NaCl (0.15 M). The molecular dynamics simulations were performed using the GROMACS 2018.1[23] simulation package with the OPLS/AA force field parameter set. The topologies for novel inhibitors were created using LigParGen service[24].

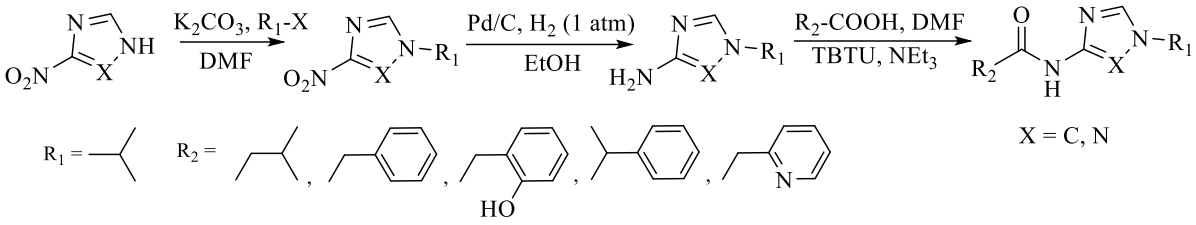
The molecules were transformed for a FEP/MD protocol according to S1, and the change in Gibbs free energy (dG) of such transitions was recorded. Relative free energies of binding (ddG) were calculated as the difference of each final and initial state and are given in Table 1. Each FEP transition was split into two steps: removing partial charges from initial molecule and conversion of Van der Waals parameters to another molecule. For alchemical FEP simulations in solution, ligands were solvated in octahedral box with TIP4P water molecules. Simulations of the ligands bound to CDK7 were performed in rectangular periodic boxes containing TIP4P water molecules and chloride ions to neutralize the total system charge. Transformations were performed in 10 separate charge ($\Delta\lambda$ = 0.1) and 11 VdW ($\Delta\lambda$ = 0.1) steps. Simulation at each distinct l value included 5000 steps of steep and l-bfgs energy minimization, 100 ps NVT equilibration, 500 (water) or 1000 (protein) ps NPT equilibration, and 2 ns NPT runs with dH/dλ collection each 10 steps. The protein side-chain during NVT and NPT simulations was set fully flexible while the backbone was fixed by position restraints of 1000 kJ·mol^{-1}·nm^{-2} to improve convergence.



Scheme S1 FEP transformation of improved derivatives.

2. Synthesis

All target compounds were obtained according to the general scheme presented below.

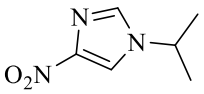


Scheme S2 Synthesis of novel CDK7 inhibitors.

Synthetic procedures

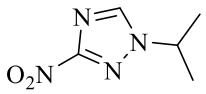
1. Azole alkylation procedure

To a solution of 50 mmol of nitroazole in 50 ml of anhydrous DMF, 9.60 g (~ 70 mmol) of freshly calcined potash was added. Then 70 mmol of alkylating agent was added and stirring at 50°C to the resulting suspension. Dimethylformamide was removed *in vacuo*, the residue was suspended in 100 ml of hot acetone and filtered. Acetone was removed in vacuum. Isolation procedure is given for each compound below.



The residue was chromatographed (silica gel column, gradient elution with mixtures of dichloromethane containing 0 to 50% ethyl acetate) to afford the product as a solid (yield 51%).;

MS $(M+H)^+$=155, 1H NMR (500 MHz,DMSO-d6.) δ 1.59 (d, *J*=6.71 Hz, 6H) 4.55-4.61 (m, 1H) 7.85 (s, 1H) 8.27 (s, 1H).



The residue was chromatographed (silica gel column, gradient elution with mixtures of dichloromethane containing 0 to 50% ethyl acetate) the desired fraction is further recrystallized from ethanol. (yield 44%).;

MS $(M+H)^+$=156, 1H NMR (500 MHz, DMSO-d6.) δ 1.50 (6H, d, *J* = 6.7, $CH(CH_3)_2$); 4.76 (1H, m, $CH(CH_3)_2$); 8.67 (1H, s, =CH)

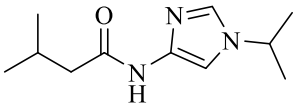
2. Nitro derivatives reduction procedure

To a mixture 20 mmole of nitroazole in isopropanol (20 mL), Pd/C (5 wt%, 0.120 g) was added. The reaction was subjected to 1 atm of hydrogen for 2-4 hours. TLC indicated that the reaction went to completion, so the reaction mixture was filtered through diatomaceous earth (Celite) and the filtrate was cooled to 0°C. The solvent is removed *in vacuo* so that the temperature does not rise above 25°C and dried in vacuum (0.5 mm Hg) to constant weight. Obtained amino derivatives were introduced into the acylation reaction without isolation and purification.

3. Acylation procedure

To 10 mmol of the aminoazole obtained in the previous step is dissolved in 10 ml of anhydrous, degassed DMF, 22 mmol (~ 2.1 ml) of Hunig base, 11 mmol of acid and 12 mmol (3.85 g) of TBTU

were added. The reaction mixture was stirred for 12 hours at room temperature, the solvents were removed *in vacuo*, the residue was dissolved in 250 ml of DCM, washed (2x50 ml) with saturated sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate, the solvents were removed, the residue was dried in an oil pump vacuum and separated by chromatography using a mixture of dichloromethane: methanol of increasing polarity. Yields are given below.



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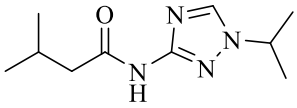

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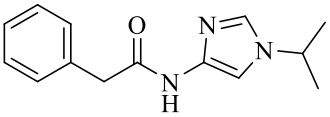

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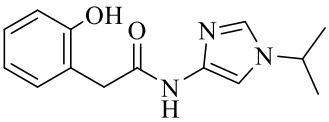
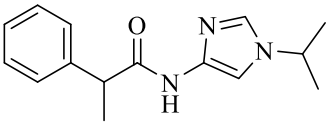
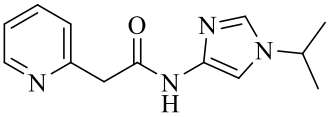


4 53%

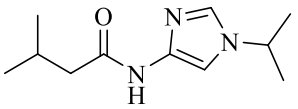
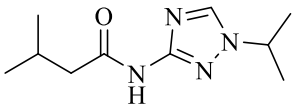
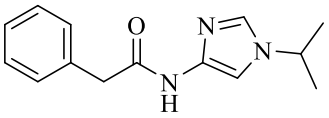
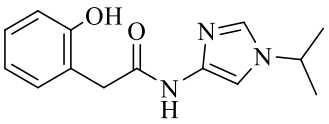
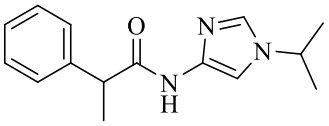
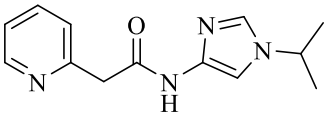


5 73%

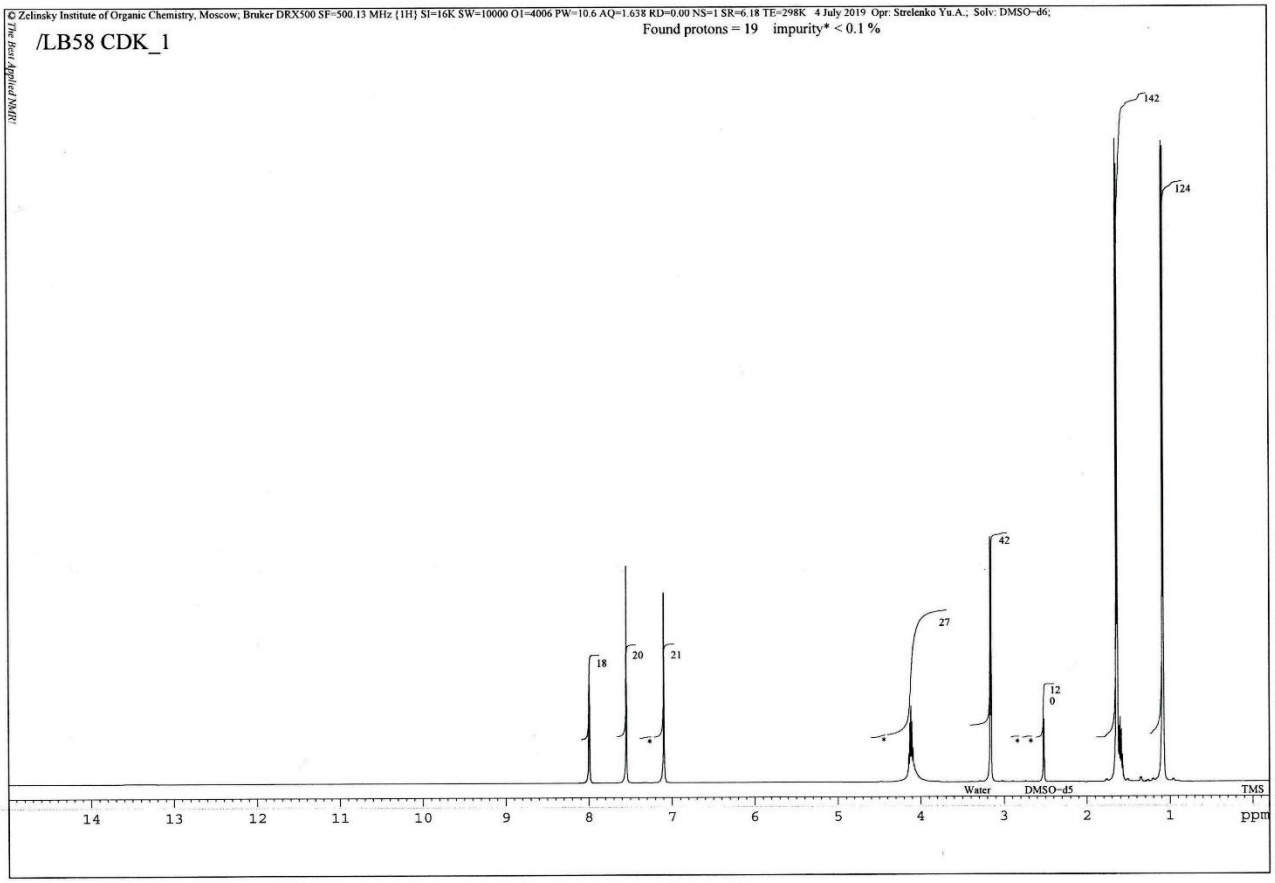


6 37%

Spectra:

№	Structure	^{1}H NMR Spectra	Yield
1		8.01 (s, 1H), 7.54 (s, 1H), 7.12 (s, 1H), 4.09 (sept., *J*=6.4Hz, 1H), 3.17 (d, *J*=6.4Hz, 2H), 1.48-1.79 (m, 7H), 1.12 (d, J=6.6Hz, 6H)	52%
2		8.76 (s, 1H), 8.21 (s, 1H), 4.18 (sept., J=6.5Hz, 1H), 3.17 (d, *J*=6.5Hz, 2H), 1.69 (d, *J*=6.5Hz, 6H), 1.58 (sept., *J*=6.5Hz, 1H), 1.16 (d, *J*=6.6Hz, 6H)	29%
3		8.24 (s, 1H), 7.77 (s, 1H), 7.48 (s, 1H), 6.88-7.15 (m, 5H), 4.09 (sept., *J*=6.3Hz, 1H), 3.56 (s, 2H), 1.13 (d, *J*=6.3Hz, 6H)	67%
4		9.12 (br.s, 1H), 8.14 (s, 1H), 7.81 (s, 1H), 7.50 (s, 1H), 7.09 (d, *J*=7.2Hz, 1H), 7.05 (t, *J*=7.5Hz, 1H), 6.78 (d, *J*=7.2Hz, 1H), 6.72 (t, *J*=7.2Hz, 1H), 4.09 (sept., *J*=6.4Hz, 1H), 3.48 (s, 2H), 1.12 (d, *J*=6.4Hz, 6H).	53%
5		8.03 (s, 1H), 7.24-7.51 (m, 6H), 7.07 (s, 1H), 3.88-3.87 (m, 1H), 3.55-3.68 (m, 1H), 1.46 (d, *J*=6.1Hz, 3H), 1.07 (d, *J*=6.2Hz, 6H)	73%
6		8.83 (d, *J*=5.6Hz, 1H), 8.51 (t, *J*=7.8Hz, 1H), 8.22 (s, 1H), 8.00 (d, *J*=7.8Hz, 1H), 7.92 (t, *J*=6.7Hz, 1H), 7.42 (s, 1H), 7.19 (s, 1H), 4.23 (s, 2H), 3.71 (sept., *J*=6.0Hz, 1H), 1.15 (d, *J*=6.0Hz, 6H)	37%

1:


© Zelinsky Institute of Organic Chemistry, Moscow; Bruker DRX500 SF=500.13 MHz {1H} SI=16K SW=10000 O1=4006 PW=10.6 AQ=1.638 RD=0.00 NS=1 SR=6.18 TE=298K 4 July 2019 Opr: Strelenko Yu.A.; Solv: DMSO-d6;
The Best Applied NMR!
/LB58 CDK_1
Found protons = 19 impurity* < 0.1 %
142
124
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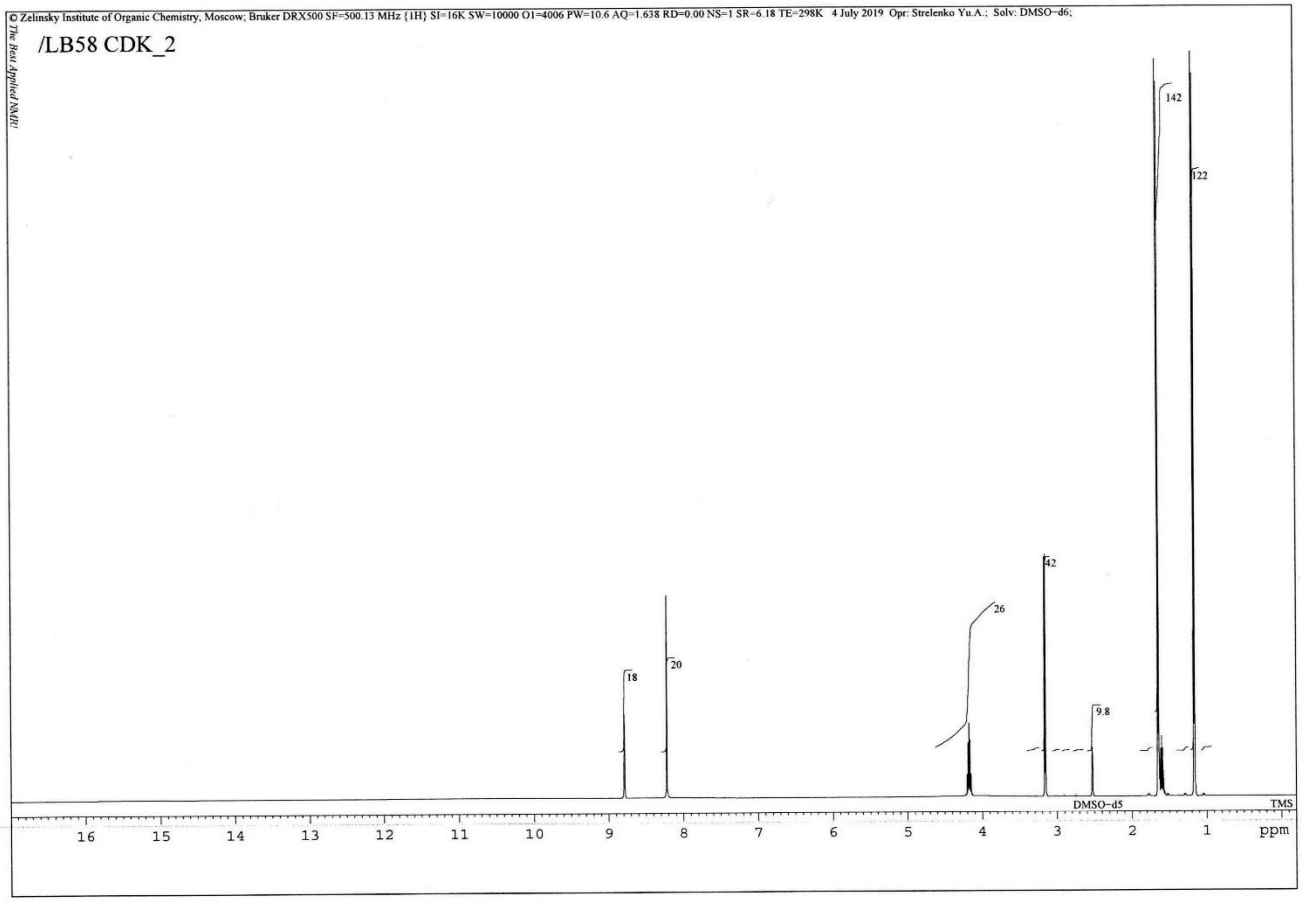
27
18
20
21
12
0
Water
DMSO-d5
TMS
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

2:


© Zelinsky Institute of Organic Chemistry, Moscow; Bruker DRX500 SF=500.13 MHz {1H} SI=16K SW=10000 O1=4006 PW=10.6 AQ=1.638 RD=0.00 NS=1 SR=6.18 TE=298K 4 July 2019 Opr: Strelenko Yu.A.; Solv: DMSO-d6;
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42
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TMS
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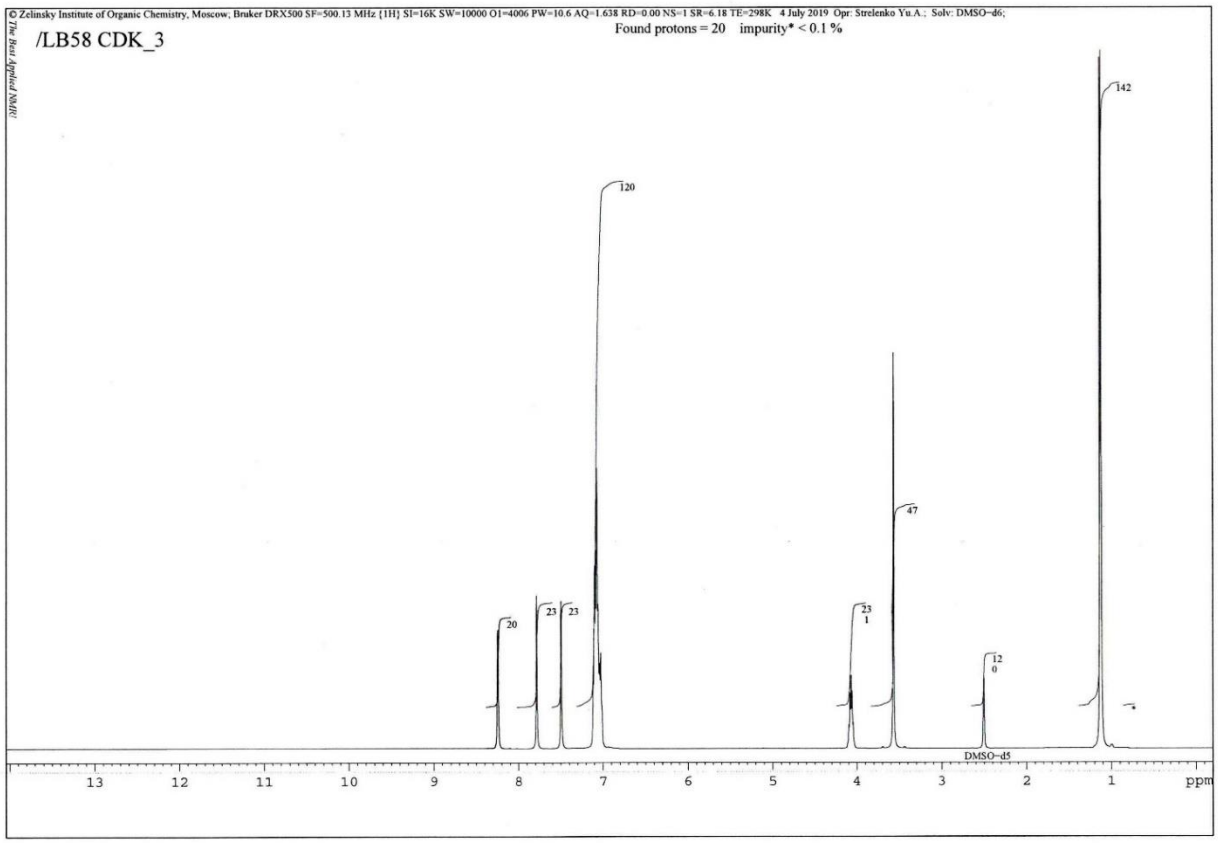
© Zelinsky Institute of Organic Chemistry, Moscow; Bruker DRX500 SF=500.13 MHz {1H} SI=16K SW=10000 O1=4006 PW=10.6 AQ=1.638 RD=0.00 NS=1 SR=6.18 TE=298K 4 July 2019 Opr: Strelenko Yu.A.; Solv: DMSO-d6;
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47
20
23
23
23
1
12
0
DMSO-d5
13
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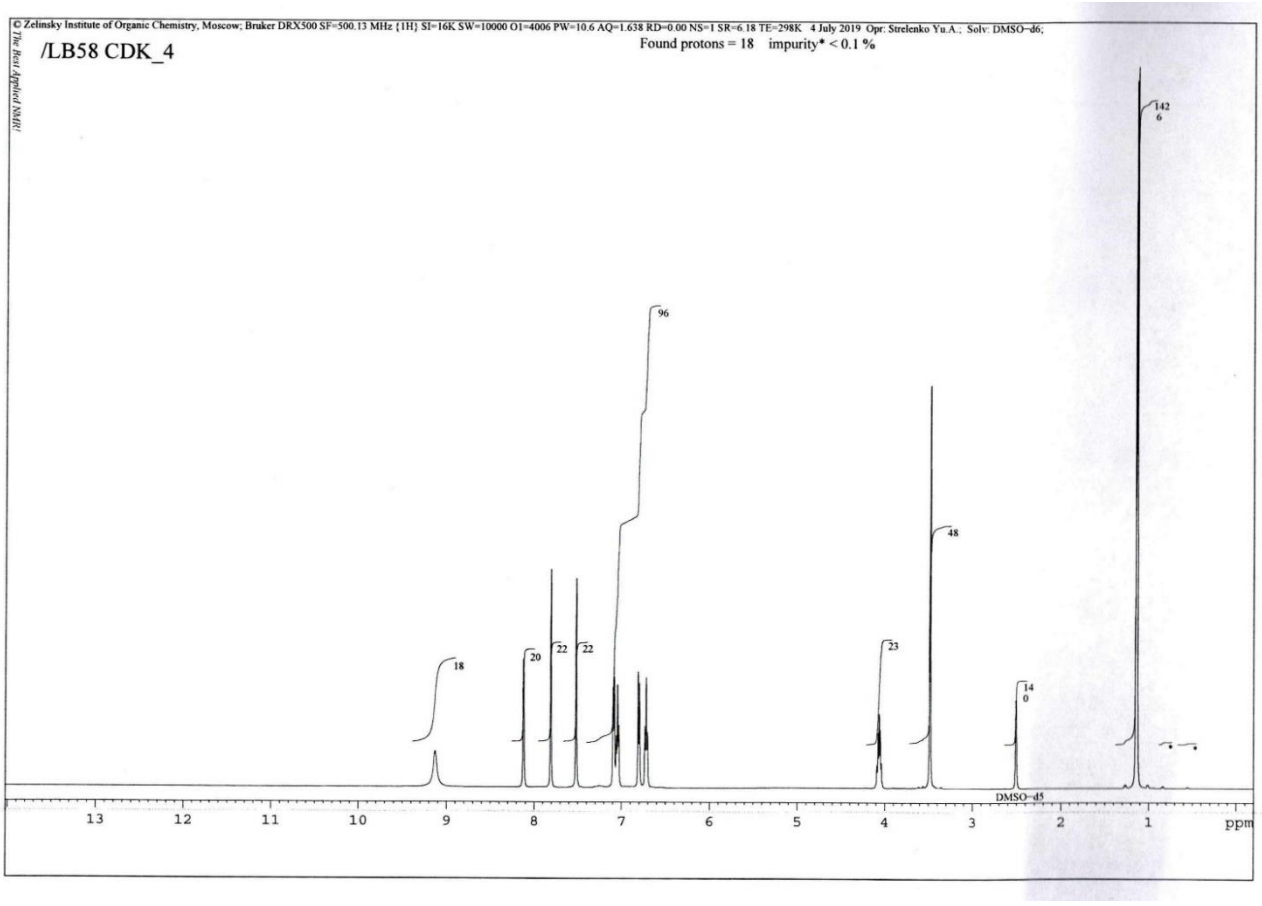
11
10
9
8
7
6
5
4
3
2
1
ppm

3:


© Zelinsky Institute of Organic Chemistry, Moscow; Bruker DRX500 SF=500.13 MHz {1H} SI=16K SW=10000 O1=4006 PW=10.6 AQ=1.638 RD=0.00 NS=1 SR=6.18 TE=298K 4 July 2019 Opr: Strelenko Yu.A.; Solv: DMSO-d6;
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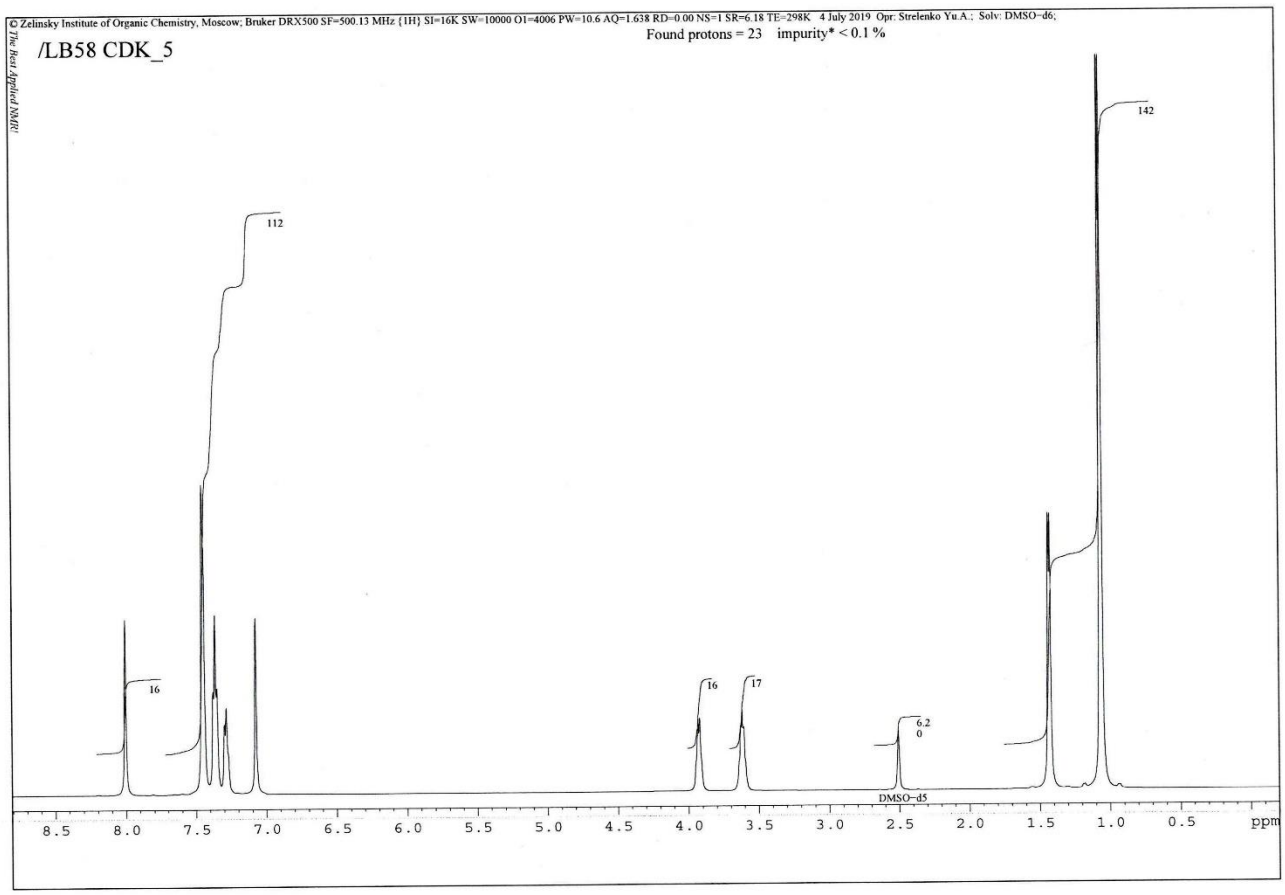
7
6
5
4
3
2
1
ppm

4:

5:


© Zelinsky Institute of Organic Chemistry, Moscow; Bruker DRX500 SF=500.13 MHz {1H} SI=16K SW=10000 O1=4006 PW=10.6 AQ=1.638 RD=0.00 NS=1 SR=6.18 TE=298K 4 July 2019 Opr: Strelenko Yu.A.; Solv: DMSO-d6;
The Best Applied NMR!
/LB58 CDK_5
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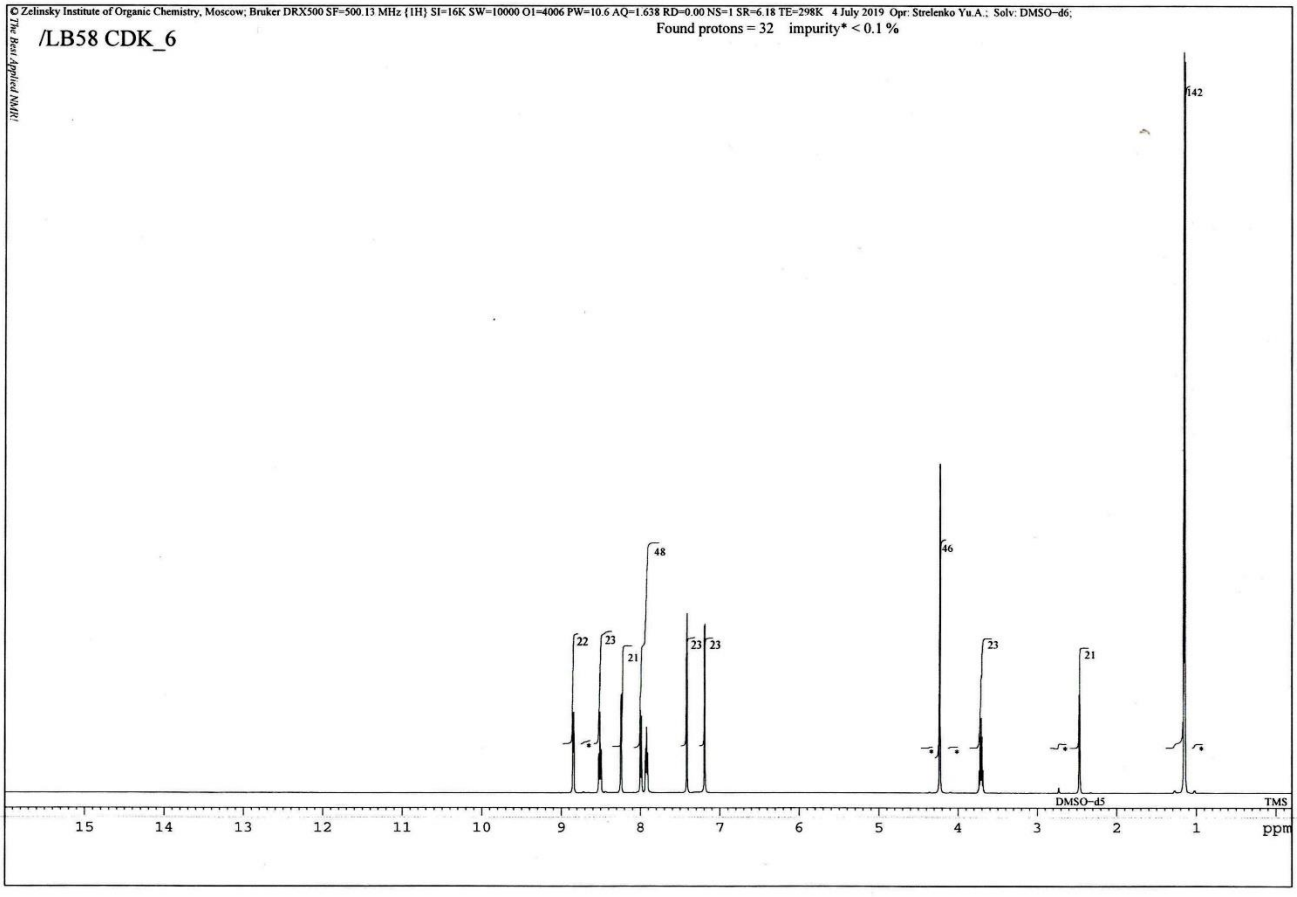
112
16
16
17
6.2
0
DMSO-d5
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

6:


© Zelinsky Institute of Organic Chemistry, Moscow; Bruker DRX500 SF=500.13 MHz {1H} SI=16K SW=10000 O1=4006 PW=10.6 AQ=1.638 RD=0.00 NS=1 SR=6.18 TE=298K 4 July 2019 Opr: Strelenko Yu.A.; Solv: DMSO-d6;
The Best Applied NMR!
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48
46
22
23
21
23
23
23
21
DMSO-d5
TMS
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

Kinase inhibition assay

K_i determination assays were performed at Reaction Biology Corporation[25]. Briefly, compounds were tested in single dose duplicate mode at a concentration of 0.5 μM. Control compound, Staurosporine, was tested in 10-dose IC_{50} mode with 4-fold serial dilution starting at 20 μM. Reactions were carried out at 1 μM ATP. IC_{50} values were calculated from % of Enzyme Activity (EA) after inhibition using the formula: $IC_{50} = 50*0.5/(100 – EA)$. IC_{50} values were recalculated into inhibition constants K_i based on the concurrent inhibition equation: $K_i = IC_{50}/(1 + [ATP]/K_m)$, where [ATP] is ATP concentration used in the experiment, and Km is a Michaelis constant for enzymatic reaction (according to the published data[26], $K_m = 59$ μM).