

Design, Synthesis and Cytotoxicity Evaluation of Structural Analogues of Imatinib

Aliaksandr Faryna, Elena Kalinichenko^{*}, Olga Avdoshko, Alla Belko

Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus

Email address:

kalinichenko@iboch.bas-net.by (E. Kalinichenko)

^{*}Corresponding author

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Abstract: Several novel imatinib structure analogues were synthesized. Antiproliferative activity testing on various leukemic cell lines showed 1-2 fold less activity against K562 cells compared to imatinib. Autodock Vina's docking studies predicted no differences in binding affinity of the titled compounds while positive relationship between activity and calculated lipophilicity was found. Further imatinib structure optimization pathways were evaluated.

Keywords: Imatinib, Structural Analogues, Protein Kinase Inhibitor, Molecule Design, Anticancer

1. Introduction

Chronic myelogenous leukemia is a common type of blood cancer comprising from reciprocal chromosomal translocation between chromosome 9 and chromosome 22, which results in the formation of fusion BCR-ABL gene coding specific protein: bcr-abl tyrosinekinase [1]. High and deregulated kinase activity of the protein causes uncontrolled leukemic cell growth by altering of cell signaling pathways and apoptosis program disturbance [2].

Imatinib is the 2-phenyl amino pyrimidine derivative and a current first-line treatment for CML [3]. Imatinib is a selective inhibitor of bcr-abl tyrosinekinase and able to bind to ATP-pocket, thus blocking biological functions of the protein [4]. However, clinical use of imatinib is limited, to a certain degree, by patients' intolerance or drug resistance caused by the formation of various mutant forms of bcr-abl tyrosinkinase. Point mutations alter the structure of the active site of the protein significantly decreasing the imatinib binding ability [5].

In this study, we made an attempt to develop novel structural analogues of imatinib with enhanced inhibitory activity against CML using the modification of terminal phenyl ring as the design approach.

2. Materials and Methods

Target compounds 4a-f were obtained by the reaction of 4-Methyl-N3-[4-(3-pyridinyl)-2-pyrimidinyl]-1,3-benzenedia mine 1 with acid chlorides of corresponding benzoic acid derivatives 3a-f. Starting amine 1 was synthesized as described earlier [6]. Acid chlorides 2a-f were prepared using thionyl chloride and DMF as a catalyst. Final condensation step was carried out in chloroform. The synthetic route is summarized in Figure 1. Compounds were tested for their anti-proliferative activity against several various cell lines using MTT-test.

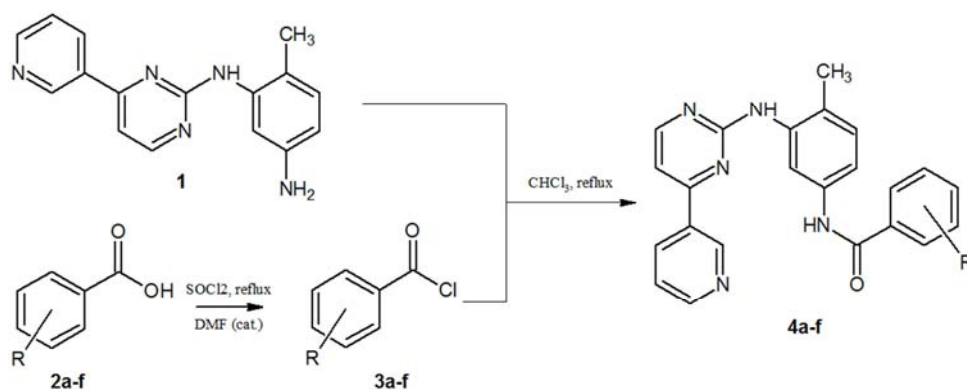


Figure 1. Synthetic route to imatinib derivatives.

3. Results and Discussion

3.1. Chemistry

Six imatinib analogues 4a-f were synthesized with the compounds 4a-e being not previously described. Structures and yields of title compounds are shown in Table 1.

Anti-proliferative activity was tested compared to substances with known inhibitory activity: imatinib and nilotinib. Imatinib derivative 5 not containing N-methylpiperazine substituent was also taken as a reference as the methylpiperazine group is known to enhance the imatinib activity by adding more protein-ligand interactions and improving solubility. Structures of references are shown in Figure 2.

Table 1. Structures and yields of title compounds.

Compound	R	Chemical formula	M.W.	Total yield, %
4a	<i>o</i> -F	C ₂₃ H ₁₈ FN ₅ O		76
4b	<i>m</i> -F	C ₂₃ H ₁₈ FN ₅ O	399.43	82
4c	<i>p</i> -F	C ₂₃ H ₁₈ FN ₅ O		74
4d	<i>p</i> -Br	C ₂₃ H ₁₈ BrN ₅ O	460.34	68
4e	<i>o</i> -OAc	C ₂₅ H ₂₁ N ₅ O ₃	439.48	56
4f	<i>o</i> -OH	C ₂₃ H ₁₉ N ₅ O ₂	397.44	62

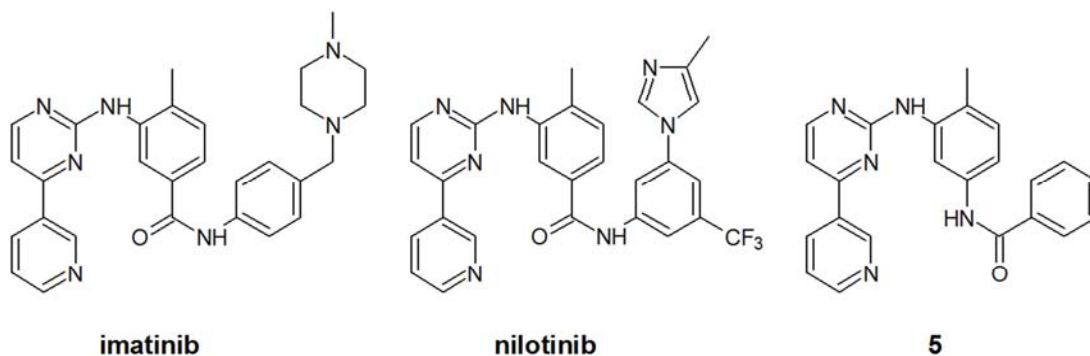


Figure 2. Reference compound structures.

3.2. Biological Activity

For K-562 human myelogenous leukemia cell line, the results of anti-proliferative studies for known inhibitors corresponded with published data [7]. Compounds 4a-f showed 1-2-fold less activity compared to imatinib. Worst results were obtained for compounds containing the

para-halogen substituent (4c-d). The most active (IC₅₀ = 6.7 μM) was the meta-fluorine derivate 4b being even more effective than compound 5. That makes promising adding the N-methylpiperazine fragment to the initial structure of 4b. Summary of biological activity testing is shown in the Table 2.

Table 2. Antiproliferative activity of 4a-f.

Compound	IC ₅₀ , μM					
	K562	MOLT-4	THP-1	MOLT-3	HL-60	KG-1
Imatinib	0.45		30	0.75	50 (6.2)	50
Nilotinib	<0.1	14.5	35	0.90	55	70
4a	40	12.2	80	65	100	100
4b	6.7	30.2				
4c	112	n.d.*				

Compound	IC ₅₀ , μ M					
	K562	MOLT-4	THP-1	MOLT-3	HL-60	KG-1
4d	n.d.	n.d.				
4e	20 (26)	95 (68)		100	85	100
4f	57.1	17.4				
5	19.3					

n.d. – not detected

3.3. Docking Studies

To investigate differences in experimental biological data we have performed docking studies of protein-ligand complexes of 4a-f with tyrosinekinase using Autodock Vina [8]. To validate docking procedure, structures of imatinib, nilotinib and compound 5 were used as references. In our case, docking results depended on the initial 3-dimentional structures of ligands. We started from the structures obtained from two sources: Cactus [9] and Molview [10]. In the case of Cactus-structures, docked pose of imatinib didn't match experimental X-ray data for the imatinib-kinase complex.

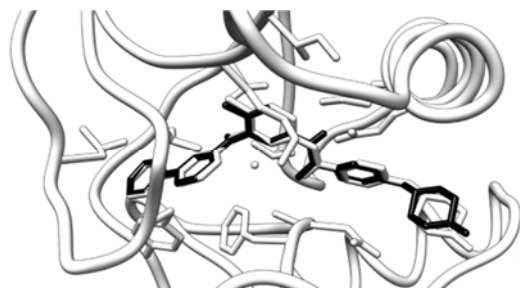
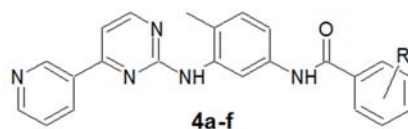


Figure 3. Docked pose of imatinib (black) compared to experimental data (white) of imatinib-kinase complex.

Table 3. Autodock Vina docking results.



Compound	R	Autodock Vina Score*
4a	<i>o</i> -F	-12.6
4b	<i>m</i> -F	-12.7
4c	<i>p</i> -F	-12.6
4d	<i>p</i> -Br	-12.7
4e	<i>o</i> -OAc	-12.7
4f	<i>o</i> -OH	-12.6
nilotinib	-	-13.9
5	-	-12.7
imatinib	-	-13.3

* - dimensionless value, less value means better binding affinity

Using Molview-generated structures the uncommon docked position of pyrimidine system for structures 4a-c was observed. Due to these facts, we applied energy minimization on the initial ligand structures with GAFF and MMFF94s force fields using OpenBabel [11]. Docked poses of GAFF-minimized Molview structures had no visual problems and were considered as valid results (Figure 3). Final docked poses of imatinib and nilotinib were identical to experimental data. There also was a correlation between estimated and experimental binding affinities of reference structures with nilotinib predicted to be more active than imatinib and imatinib to be more active than compound 5. The other fact is that there was no significant difference in predicted binding affinities for title compounds 4a-f (Table 3). It may be due to the changings in protein structure or more likely the distinction of ADME properties between the compounds.

To check this assumption partition coefficients (logP) of tested compounds were predicted using Molinspiration [12] (Table 4). The results showed good linear correlation with experimental IC₅₀ values ($R^2=0.69$). Least lipophilic 4-bromo

imatinib derivative 4d has showed no inhibitory activity in biological tests. On the contrary, imatinib was an excellent inhibitor with the greatest calculated lipophilicity. This correlation was more significant when the structures of 4b and 4f were excluded ($R^2 = 0.91$) that may be explained by additional protein-ligand interactions of the terminal phenyl ring region of 4a and 4f compared to imatinib.

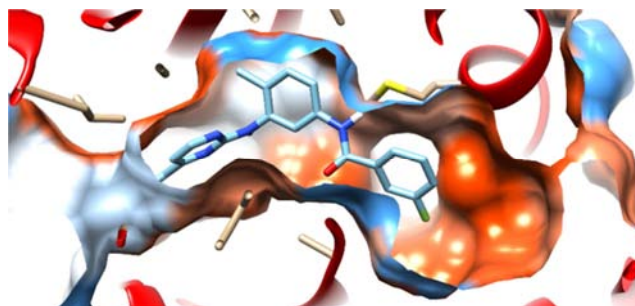


Figure 4. The fluorine atom of 4b resting in hydrophobic pocket of the allosteric site of abl-kinase.

For 4a it is likely the fluorine atom at the meta position is resting in the nearby hydrophobic pocket thus increasing binding affinity (Figure 3). In the case of 4f interactions of the hydroxyl group cannot be explained straightforward. Having

relatively poor lipophilicity 4f showed comparable to compound 5 activity. Probably interactions of hydroxyl interfere with some sort of steric hindrance.

Table 4. Predicted partition coefficient values (logP) for the compounds.

Compound	4a	4b	4c	4d	4e	4f	5	imatinib
predicted logP	4.31	4.33	4.36	5	4.25	4.68	4.19	3.89
IC ₅₀ , μ m	40	6.7	112	300*	26	57.1	19.3	0.4

* - IC₅₀ value was assumed

Summarizing, one may point out the most promising directions for imatinib structure modifications to enhance its inhibitory activity. Firstly, terminal phenyl ring of imatinib structure may be expanded by the introduction of meta-substituents of different nature. Secondly, the phenyl ring can be replaced with 5-membered aromatic system with the hydrophilic group in second position relative to the amide bond.

4. Conclusions

Several novel structural analogues of imatinib were synthesized. Although better inhibitory activity of the title compounds compared to imatinib was not achieved, docking studies and calculated lipophilicity along with IC₅₀ values revealed possible imatinib structure optimization pathways.

5. Experimental

All reagents were purchased from commercial sources and used without any additional purification. ¹H NMR spectra were recorded on Bruker NMR 400 MHz spectrometer. The chemical shifts, δ , were recorded relative to tetramethylsilane as an internal standard. MS were recorded on HPLC/MS Accela with LCQ Fleet detector with Thermo Electron 3D quadrupole ion trap using ESP ionization. The reactions were monitored by TLC using silica gel plates Merk «DC-Plasticfolien Kieselgel 60 F 254» with the chloroform-methanol mixture.

5.1. Docking

Crystal structure of C-Abl kinase domain in complex with imatinib was taken from PDB entry 1IEPB. Chimera [13] was used for the visualization of the results as well as for the preparation of the receptor and ligands. Autodock Vina's exhaustiveness of search was set to 24.

5.2. Antiproliferative Activity

The effects of the compounds on cell growth were evaluated using the MTT assay. Cell lines obtained from the Institute of Cytology RAS were cultivated in RPMI 1640 media with 10% (20% for MOLT-3) Hyclone fetal bovine serum. Cells were plated in 96-well plates at a concentration of 1×10^4 cells/well, and incubated for 24 h at 37°C followed by addition of target compounds at various concentrations for 48 h. Then, 20 μ L MTT (5 mg/mL) was added to each well and incubated for 4 h

at 37°C. The plates were centrifuged and the supernatant was removed. 150 μ L DMSO was added to each well to dissolve MTT crystals, and the absorbance values for each well were read by a microplate reader at 490 nm. For cell viability data 4 parameter logistics regression was applied and IC₅₀ was estimated.

5.3. Synthesis

2-fluoro-N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)amino)phenyl)benzamide (4a).

To 200 mg (0.5 mmol) of 2-fluoro benzoic acid 1 ml of thionyl chloride and one drop of DMF were added. Reaction mixture was heated to 50°C and was maintained at this temperature for 8 hours. Excess of thionyl chloride was removed by reduced pressure distillation. To the residue 138.7 mg (0.5 mmol) of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidineamine 1 in 5 ml of chloroform was added and the mixture was refluxed for 12 h. The solid was filtered and washed with chloroform. The crude product was purified by column chromatography (methanol-chloroform 1:20), giving 148 mg of yellowish solid (yield 74%).

MS (m/z): 400,1[MH⁺]; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 10.38 (s, 1 H) 9.27 (s, 1 H) 9.00 (s, 1 H) 8.64 - 8.74 (m, 1 H) 8.51 (d, J = 5.13 Hz, 1 H) 8.47 (dd, J = 8.01, 1.60 Hz, 1 H) 8.01 - 8.12 (m, 1 H) 7.66 (t, J = 7.05 Hz, 1 H) 7.54 - 7.60 (m, 1 H) 7.52 (dd, J = 8.01, 4.81 Hz, 1 H) 7.40 - 7.46 (m, 2 H) 7.29 - 7.39 (m, 2 H) 7.21 (d, J = 8.33 Hz, 1 H) 2.23 (s, 3 H).

Compounds 4b-f were prepared as described above.

3-fluoro-N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)amino)phenyl)benzamide (4b).

MS (m/z): 400,1[MH⁺]; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 10.28 (s, 1 H) 9.22 - 9.36 (m, 1 H) 8.95 - 9.04 (m, 1 H) 8.64 - 8.77 (m, 1 H) 8.53 - 8.59 (m, 1 H) 8.47 - 8.53 (m, 1 H) 8.05 - 8.15 (m, 1 H) 7.80 (d, J = 7.69 Hz, 1 H) 7.73 - 7.78 (m, 1 H) 7.52 - 7.61 (m, 1 H) 7.38 - 7.49 (m, 1 H) 7.21 (d, J = 8.01 Hz, 1 H) 2.22 (s, 1 H).

4-fluoro-N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)amino)phenyl)benzamide (4c).

MS (m/z): 400,2[MH⁺]; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 10.23 (s, 1 H) 9.27 (s, 1 H) 8.98 (s, 1 H) 8.68 (d, J = 3.53 Hz, 1 H) 8.51 (d, J = 5.13 Hz, 1 H) 8.47 (d, J = 8.01 Hz, 1 H) 8.06 - 8.11 (m, 1 H) 8.03 (dd, J = 8.49, 5.61 Hz, 2 H) 7.52 (dd, J = 7.69, 4.81 Hz, 1 H) 7.47 (d, J = 8.01 Hz, 1 H) 7.42 (d, J = 4.81 Hz, 1 H) 7.33 - 7.39 (m, 2 H) 7.21 (d, J = 8.01 Hz, 1 H) 2.22 (s, 3 H).

3-bromo-N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)

(amino)phenyl)benzamide (4d).

MS (m/z): 460,1 and 462,1[MH⁺]; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 10.31 (s, 1 H) 9.29 (s, 1 H) 8.99 (s, 1 H) 8.65 - 8.73 (m, 1 H) 8.52 (d, *J* = 5.13 Hz, 1 H) 8.46 - 8.51 (m, 1 H) 8.07 - 8.14 (m, 1 H) 7.92 (d, *J* = 8.66 Hz, 2 H) 7.75 (d, *J* = 8.66 Hz, 2 H) 7.51 - 7.56 (m, 1 H) 7.46 - 7.50 (m, 1 H) 7.44 (d, *J* = 5.13 Hz, 1 H) 7.24 (s, 1 H) 2.24 (s, 3 H).

3-O-acetyl-N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)amino)phenyl)benzamide (4e).

MS (m/z): 564,0[MH⁺]; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 10.36 (s, 1 H) 9.51 (d, *J* = 1.60 Hz, 1 H) 9.37 (br. s., 1 H) 9.18 (d, *J* = 8.33 Hz, 1 H) 9.02 (d, *J* = 4.81 Hz, 1 H) 8.65 (d, *J* = 5.13 Hz, 1 H) 8.35 (s, 1 H) 8.13 (dd, *J* = 8.17, 5.61 Hz, 1 H) 8.05 (br. s., 1 H) 7.72 (dd, *J* = 7.69, 1.28 Hz, 1 H) 7.65 (d, *J* = 5.13 Hz, 1 H) 7.56 (td, *J* = 7.85, 1.60 Hz, 1 H) 7.36 - 7.42 (m, 2 H) 7.24 (d, *J* = 8.01 Hz, 1 H) 7.21 (d, *J* = 8.33 Hz, 1 H) 2.22 (s, 3 H) 2.15 (s, 3 H).

3-hydroxy-N-(4-methyl-3-((4-(pyridine-3-yl)pyrimidine-2-yl)amino)phenyl)benzamide (4f).

MS (m/z): 398,1[MH⁺]; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 10.47 (s, 1 H) 9.51 (d, *J* = 1.60 Hz, 1 H) 9.32 (s, 1 H) 9.16 (dt, *J* = 8.33, 1.60 Hz, 1 H) 9.00 (dd, *J* = 5.45, 1.28 Hz, 1 H) 8.65 (d, *J* = 5.13 Hz, 1 H) 8.34 (s, 1 H) 8.07 - 8.12 (m, 2 H) 8.04 (dd, *J* = 7.85, 1.44 Hz, 1 H) 7.64 (d, *J* = 5.13 Hz, 1 H) 7.37 - 7.45 (m, 2 H) 7.24 (d, *J* = 8.33 Hz, 1 H) 7.03 (dd, *J* = 8.33, 0.96 Hz, 1 H) 6.92 - 6.98 (m, 1 H) 2.24 (s, 3 H).

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