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Synthesis, biological evaluation and molecular docking studies of 2-piperazin-1-yl-quinazolines as platelet aggregation inhibitors and ligands of integrin $\alpha_{IIb}\beta_3$

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ABSTRACT

A series of 2-piperazin-1-yl-quinazolines were synthesized and evaluated for their antiaggregative activity. The synthesized small molecule compounds have potently inhibited platelet aggregation in vitro and blocked FITC-Fg binding to $\alpha_{IIb}\beta_3$ integrin in a suspension of washed human platelets. The key $\alpha_{IIb}\beta_3$ protein–ligand interactions were determined in docking experiments and some correlations have been observed between values of the affinity and docking scores.

Nowadays, it is known the leading role of platelet aggregation in the formation of thrombus, as a consequence emerge of dangerous cardiovascular diseases such as unstable angina, myocardial infarction, ischaemic disease, atherosclerosis, and stroke.[1] One of the rational approaches for treatment and prevention of cardiovascular diseases associated with thrombosis is the creation of drugs which inhibit platelet aggregation—antiplatelet agents. The final obligatory step in the platelet aggregation is fibrinogen binding to its receptor on the activated platelets. Over the past twenty years, the most interesting objects among antiplatelet agents are fibrinogen receptor antagonists.[2] Key domains of fibrinogen are RGD-sequences found on the α-chain (RGDF 95–98, RGDS 572–575) and the dodecapeptide sequence on the γ-chain (HHLGGAKQAGDV 400–411), by means of which the fibrinogen interacts to $\alpha_{IIb}\beta_3$ integrin on the surface of activated platelets.[3] In the majority of cases, the design of $\alpha_{IIb}\beta_3$ antagonists is based on the mimicking of RGD motif, and alternative approach represents the use of dodecapeptide sequence. These two are classic approaches. Currently three $\alpha_{IIb}\beta_3$ inhibitors exist including the small molecules eptifibatide and tirofiban (Fig. 1) which were approved for intravenous administration during percutaneous coronary intervention and in acute coronary syndromes.[4] Crystallographic analysis of 'RGD mimetics–$\alpha_{IIb}\beta_3$' complexes showed that during the binding of a ligand (RGD mimetic) a 'swing-out' motion of the β_3 subunit occurs resulting in a substantial change in conformation, namely adopting a high-affinity ligand-binding conformation.[5] It was supposed that such conformational changes are associated with thrombocytopenia that occurs in some patients after treatment with classical antagonists.[6,7] The rational approach can be used to avoid the problem of thrombocytopenia—design of antagonists that prevent receptor activation upon binding to the intracellular domain of $\alpha_{IIb}\beta_3$.[8] Recently, a number of compounds was identified which belong to the novel class of 'ion displacement ligands'. These compounds are antagonists (**RUC-1**, **RUC-2** and **RUC-4**) of $\alpha_{IIb}\beta_3$ and they limit conformational reorganization of the receptor and keep it in a low-affinity state (closed form) (Fig. 1).[9–11] The second part of our previous article was devoted to the design of potential ligands for closed form of $\alpha_{IIb}\beta_3$, we proposed three derivatives of 2-piperazin-1-yl-quinazoline based on 3D topological pharmacophore containing two positively charged centres separated by, at least, the distance of 15.8 Å.[12] In order to test this hypothesis,

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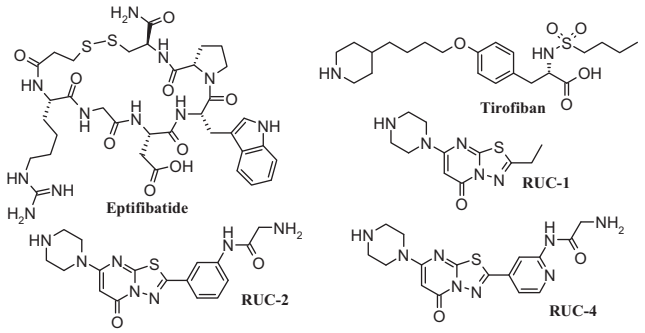


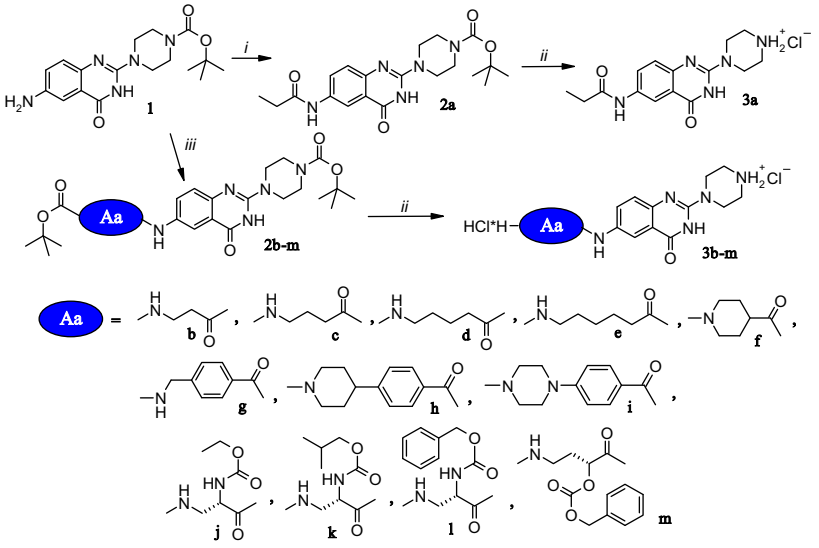
Figure 1. Structures of small molecules—antagonists of $\alpha_{IIb}\beta_3$.

compounds with varying distance between the positively charged groups have been synthesized. Also, in order to find out importance of the presence of the carbonyl group in 4-position of the quinazoline core, 4-phenyl-2-piperazin-1-yl-quinazoline derivatives have been studies. Here, we report about antiaggregative properties of the expanded set of compounds (2-piperazin-1-yl-quinazoline derivatives) and observed structure–activity relationships.

For the synthesis of a set of 2-(piperazin-1-yl)-3*H*-quinazolin-4-one derivatives (**3**), the following synthetic route was applied as outlined in Scheme 1. Description of the synthesis of 2-(4-Boc-piperazin-1-yl)-6-amino-3*H*-quinazolin-4-one (**1**) was given in the previous report.[12] Condensation of acids with amine **1** has been conducted using the HBTU or HATU. The elimination of Boc-protective groups yielded the compounds **3**.

Single crystals of di-Boc-derivative **2b** were grown from methanol, and their structure was confirmed by single crystal X-ray diffraction analysis (Fig. 2).[13] The amide fragment is about coplanar with quinazoline moiety (dihedral angle 1.63°) but almost perpendicular to the flat carbamate fragment (dihedral angle 84.27°) resulting in angular conformation of molecule. Torsion angle N6—C20—C19—C18 equals 60.8(3)°. In the crystal the classical N—H···O and N—H···N intermolecular hydrogen bonds unite molecules **2b** in well defined layer with hydrophobic *tert*-butyl groups on the surfaces of layer. The layers are stacked in parallel fashion.

The synthetic route to the target 4-phenyl-2-piperazin-1-yl-quinazolines (**9**) is summarized in Scheme 2. 6-Nitro-4-phenyl-1*H*-quinazolin-2-one (**4**) was obtained by heating 2-amino-5-nitrobenzophenone and urea. Chlorination of compound **4** using $POCl_3/PCl_5$ was carried out in order to obtain 6-nitro-2-chloro-4-phenyl-quinazoline (**5**). Then the piperazinyl ring was conveniently introduced to the 2-position of the intermediate **5** by the reaction of the latter with the 1-Boc-piperazine, which gave the compound **6**. The reduction of the nitro group of compound **6** using H_2–Pd(C) gave the amine **7** as a crucial substrate for the assembly of the target molecules. Acylation of the amine and subsequent deprotection resulted in the compounds **9**.



Scheme 1. Synthesis of compounds **3**. Reagents and conditions: (i) NEt_3, propionic acid anhydride, ACN, 50 °C, overnight, 40%; (ii) CH_2Cl_2, HCl gas, room temperature, 1 h, 90–96%; (iii) Boc-acids, NEt_3, HBTU or HATU, ACN, 50 °C, overnight, 30–58%.

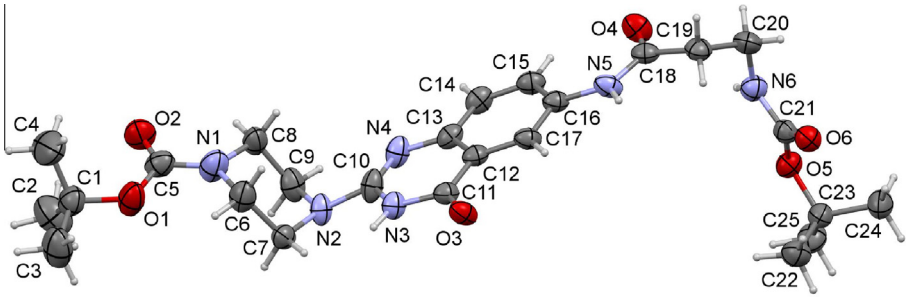
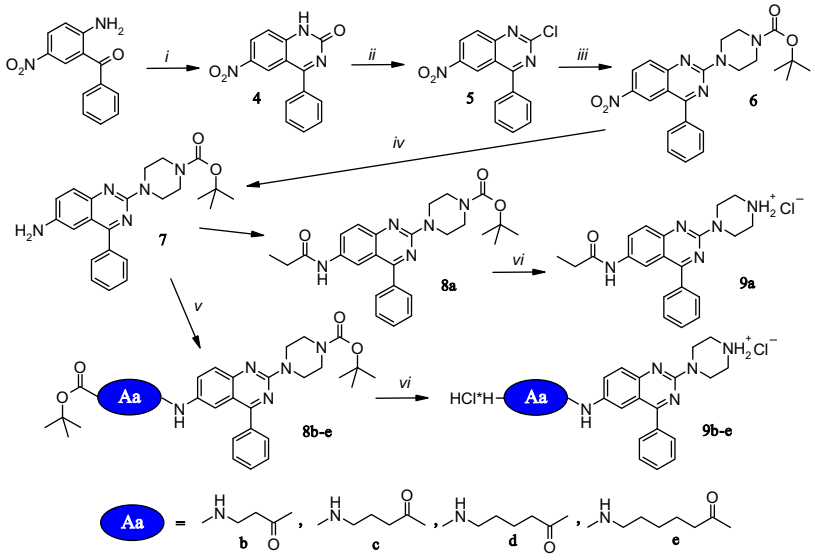


Figure 2. The X-ray crystal structure of compound **2b**.

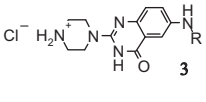


Scheme 2. Synthesis of compounds **9**. Reagents and conditions: (i) $(H_2N)_2CO$, 150 °C, 3 h, 7%; (ii) $POCl_3$, PCl_5, 100 °C, 4 h; (iii) 1-Boc-piperazine, NEt_3, ACN, 50 °C, 2 h, 87%, (iv) H_2/Pd(C), MeOH, room temperature, 3 h, 90%; (v) Boc-acids, NEt_3, HBTU, ACN, 50 °C, overnight, 43–50%; (vi) CH_2Cl_2, HCl gas, room temperature, 1 h, 93–97%.

Functional inhibitory activity of synthesized compounds **3** and **9** were evaluated by measuring the inhibition of ADP induced platelet aggregation in human platelet-rich plasma (PRP) by Born's method.[14] All results of ADP induced platelet aggregation assay are listed in Table 1.

First, we have focused on the optimization of **3** by structural modification of the amide group in 6-positions of 2-(piperazin-1-yl)-3*H*-quinazolin-4-one scaffold. The compound **3a**, that we have accepted as an analogue of **RUC-1** showed IC_{50} = 0.17 μM. For binding to β_3-subunit ligands have to bear a second amino group. On

Table 1
Biological properties of compounds **3**, **9** and tirofiban



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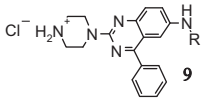
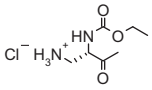
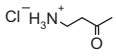
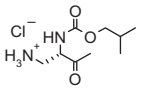
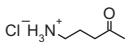
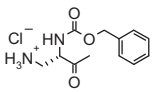
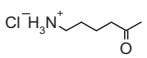
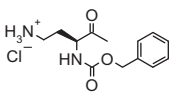
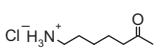
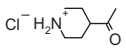
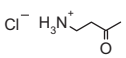
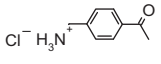
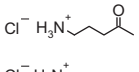
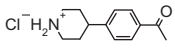
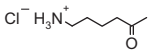
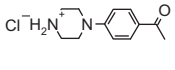
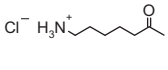


9

Compound	R	IC_{50}, μM (PRP)[a]	IC_{50}, μM (FITC-Fg/$\alpha_{IIb}\beta_3$)[b]	Compound	R	IC_{50}, μM (PRP)[a]	IC_{50}, μM (FITC-Fg/$\alpha_{IIb}\beta_3$)[b]
3a		0.17 ± 0.02	0.055 ± 0.004	**3j**		0.06 ± 0.003	–
3b		0.15 ± 0.025[12]	0.0050 ± 0.0008[12]	**3k**		0.040 ± 0.006	0.0018 ± 0.0002
3c		0.011 ± 0.001[12]	0.0022 ± 0.0003[12]	**3l**		0.019 ± 0.002	0.00153 ± 0.00006
3d		1.40 ± 0.17	–	**3m**		0.0100 ± 0.0008	0.00157 ± 0.00011
3e		1.30 ± 0.17	–	**9a**		0.72 ± 0.07	–
3f		0.100 ± 0.015[12]	0.0038 ± 0.0003[12]	**9b**		0.57 ± 0.071	–
3g		1.9 ± 0.3	–	**9c**		0.049 ± 0.006	–
3h		0.57 ± 0.08	–	**9d**		48.0 ± 6.0	–
3i		1.3 ± 0.1	0.020 ± 0.005	**9e**		58.0 ± 6.0	–
Tirofiban		0.030 ± 0.003	0.0019 ± 0.0005				

[a] Concentration required to reduce ADP-induced human platelet aggregation response by 50%. The IC_{50} values are expressed as the average of at least three determinations.
[b] Concentration required to reduce binding of FITC-Fg to $\alpha_{IIb}\beta_3$ on the suspension of washed human platelets by 50%. The IC_{50} values are expressed as the average of at least three determinations.

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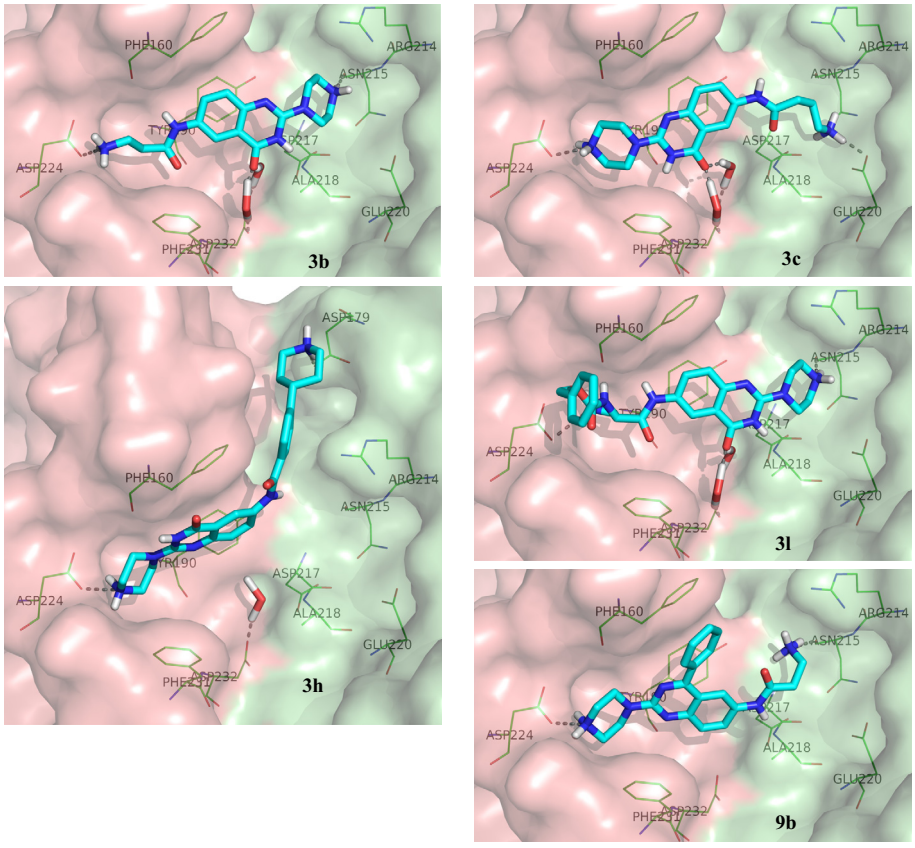

Figure 3. Binding of **3b**, **3c**, **3h**, **3l** and **9b** to $\alpha_{IIb}\beta_3$ integrin estimated by molecular docking (α_{IIb} chain is marked by pink surface, β_3 by green surface).

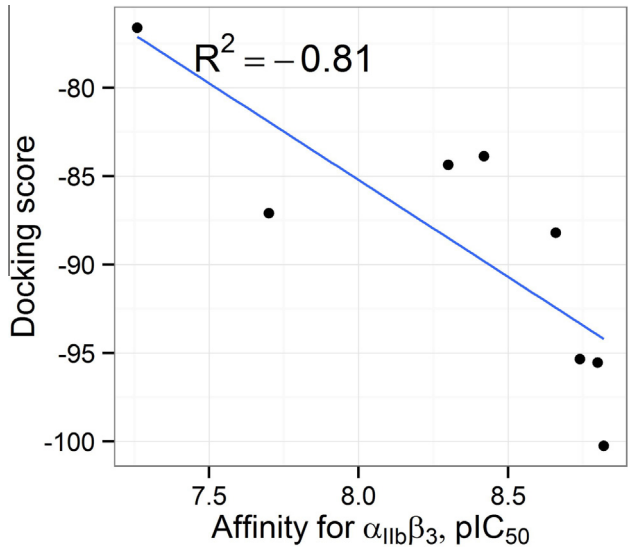

Figure 4. Plot of docking score vs. measured values of affinity for $\alpha_{IIb}\beta_3$ integrin of compounds **3a–c**,**f**,**i**,**k**,**l**.

the base of this concept, compounds **3a**–**m** have been synthesized and studied. The compound **3b**, containing a residue of β-alanine, showed an insignificant increase of antiaggregative activity compared to the compound **3a**. Change from β-alanine residue to the γ-aminobutyric acid residue leads to the more active compound **3c**. Interestingly, the compounds **3d** and **3e**, containing residues of δ-aminovaleric or ε-aminocaproic acid, respectively, exhibit significantly lower antiaggregative activity. Replacement of β-alanine fragment with a residue of isonipecotic acid has led to the compound **3f**. However, antiaggregation activity of the compound **3f** is comparable to **3b** and is an order of magnitude less than that for the compound **3c**. Use of 4-aminomethylbenzoyl group provides a negative effect on antiaggregative activity, so IC_{50} value for **3g** is the smallest one in a series of 2-(piperazin-1-yl)-3*H*-quinazolin-4-ones. Two compounds **3h** and **3i**, containing residues of 4-(piperidin-4-yl)benzoic and 4-(piperazine-4-yl)benzoic acids, respectively, showed low in vitro antiaggregative activity. It should be noted that we observed a significant increase in in vitro activity upon introduction of a substituent in α-position of β-alanine fragment.

Addition of ethoxycarbonylamino group to the β-alanine residue led to the 2.5 times increase in the activity of analogue **3j** in comparison with the compound **3b**. Changing from ethyl to *iso*-butyl in **3j** produced the analogue **3k** with equivalent activity, and replacement with benzyl group (analogue **3l**) gave an increase in the activity of about 2 times. Homologation of compound **3l** resulted in increased antiaggregative activity for analogue **3m** containing a residue of 4-amino-2-benzyloxycarbonylaminobutyric acid. In addition, the value for antiaggregation activity of compound **3m** (IC_{50} = 0.01 μM) was slightly higher than the value for compound **3c**. Finally, the replacement of an oxo group with phenyl in the heterocyclic scaffold has been done.

All 2-(piperazin-1-yl)-4-phenylquinazoline derivatives (**9**) showed lower antiaggregative activity as compared to the subgroup of compounds **3** containing a 2-(piperazin-1-yl)-3*H*-quinazolin-4-one scaffold. However it is important to note, that influence of the structure of substituents in the 6-position on the activity remained the same. Although activity of the compound **9c** is in the desired range, but the value is less than that of its direct analogue **3c**.

Mode of action for randomly selected representatives of compounds **3** was subsequently determined in vitro by assessment of their ability to inhibit the binding of fluorescein isothiocyanate-labelled fibrinogen (FITC-Fg) to $\alpha_{IIb}\beta_3$ (in a suspension of human washed platelets). Tirofiban was used as a positive control.

Molecular docking of synthesized compounds in the pocket of $\alpha_{IIb}\beta_3$ integrin (PDB code: 3T3M) was performed with PLANTS docking software.[15,16] The SPORES utility was used for protonation of the protein and all ligands. In our previous study it was shown that water molecules near Asp232 can be important for proper orientation of ligands inside the binding pocket.[12] Therefore these two water molecules were remained. In the docking setup these water molecules kept only rotational degrees of freedom and were replaceable. Self-docking of X-ray ligand **RUC-2** showed good reproducibility of its pose (RMSD = 1.01 Å).

Docking of the investigated compounds **3a**–**m** and **9a**–**e** revealed their most probable binding poses and corresponding ligand–protein interactions. The main anchor residues in the pocket of $\alpha_{IIb}\beta_3$ integrin in its closed from are α_{IIb}Asp224 and β_3Glu220 to which ligands bind with positively charged groups. All investigated compounds except **9d** bind to the former residue and just seven of them bind to the latter one mainly due to the too short distance between two positively charged groups in ligands or conformational constraints. However, almost all compounds bind near the β_3Glu220 residue and thus probably may compete with Mg^{2+} ion for binding with β_3 chain of the integrin. Only two compounds cannot adopt the binding pocket due to their size and conformational rigidity, **3h** and **3i**. The docking pose of the compound **3h** is shown in Figure 3. Other residues mainly involved in ligand–protein interactions are Phe160, Ser161, Tyr190, Leu192, Phe231 of the α_{IIb} chain and Asn215, Arg216, Asp217 of the β_3 chain.

Selected water molecules participate in binding of almost all ligands **3** and coordinate mainly carbonyl group of quinazoline ring. However, the compounds **9** are not coordinated with the receptor with the participation of two water molecules. This may explain low antiaggregative values for the subgroup **9**. As it was found in our previous study[12] ligands can adopt either orientation relative to the binding pocket, cf. poses of **3b** and **3c** on Figure 3 (piperazine group can bind either α_{IIb} or β_3 chain of the integrin). It should be noted that one of the most active compounds, **3l**, is oriented in the 'opposite' direction and its benzyl group is outside the binding pocket (Fig. 3).

Compounds bearing a phenyl substituent in quinazoline ring (**9a**–**e**) have similar docking poses relatively to compounds with carbonyl group at the same position (**3a**–**e**) (Fig. 3). The size of the binding pocket is quite large and contains hydrophobic residues α_{IIb}Phe160 and α_{IIb}Phe231 involved in the ligand–protein interactions that probably could explain the similarity of ligands of these two groups in binding modes.

For compounds with measured affinity for $\alpha_{IIb}\beta_3$ integrin the reasonable correlation between those values and docking scores is observed with values of Pearson correlation coefficient −0.81 and Kendall correlation coefficient −0.79 (Fig. 4).

In summary, analysis of the structure–activity relationship of the series of studied $\alpha_{IIb}\beta_3$ antagonists demonstrated the importance of the optimal distance between the two positively charged centres of ligands. The γ-aminobutyric derivative **3c** is more potent than β-aminopropionic derivative **3b** and much more potent than δ-aminovaleric or ε-aminocaproic derivatives **3d** and **3e**, respectively. Introduction of substituent in α-position of β-aminopropionic or γ-aminobutyric fragment has a positive impact on the antiaggregative activity and affinity for $\alpha_{IIb}\beta_3$. Docking studies revealed that ligand–protein interactions of studied compounds mainly correspond to those observed in the crystal structure of the complex **RUC-2**–$\alpha_{IIb}\beta_3$ and water molecules may participate in these interactions.

Acknowledgments

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