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Structural modifications of 2,3-indolobetulinic acid: Design and synthesis of highly potent α-glucosidase inhibitors

Elmira F. Khusnutdinova[a,*], Anastasiya V. Petrova[a,b], Ha Nguyen Thi Thu[c], Anh Le Thi Tu[c], Tra Nguyen Thanh[c], Cham Ba Thi[c], Denis A. Babkov[d], Oxana B. Kazakova[a]

[a] Ufa Institute of Chemistry UFRS RAS, 71 pr. Oktyabrya, Ufa 450054, Russian Federation
[b] Bashkir State University, 32 Validy Str., Ufa 450076, Russian Federation
[c] Institute of Chemistry, Vietnamese Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay Dist., Hanoi, Viet Nam
[d] Scientific Center for Innovative Drugs, Volgograd State Medical University, Novorossiyskaya st. 39, Volgograd 400087, Russian Federation

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ABSTRACT

A series of nineteen nitrogen-containing lupane triterpenoids was obtained by modification of C2, C3, C20 and C28 positions of betulonic acid and their α-glucosidase inhibiting activity was investigated. Being a leader compound from our previous study, 2,3-indolo-betulinic acid was used as the main template for different modifications at C-(28)-carboxyl group to obtain cyano-, methylcyanoethoxy-, propargyloxy- and carboxamide derivatives. 20-Oxo- and 29-hydroxy-20-oxo-30-nor-analogues of 2,3-indolo-betulinic acid were synthesized by ozonolysis of betulonic acid followed by Fischer indolization reaction. To compare the influence of the fused indole or the seven-membered A-ring on the inhibitory activity, lupane A-azepanones with different substituents at C28 were synthesized. The structure-activity relationships revealed that the enzyme inhibition activity dramatically increased (up to 4730 times) when the carboxylic group of 2,3-indolo-betulinic acid was converted to the corresponding amide. Thus, the IC_{50} values for glycine amide and *L*-phenylalanine amides were 0.04 and 0.05 μM, respectively. This study also revealed that 2,3-indolo-platanic acid is 4.5 times more active than the parent triterpenoid with IC_{50} of 0.4 μM. Molecular modeling suggested that improved potency is due to additional polar interactions formed between C28 side chain and a sub-pocket of the α-glucosidase allosteric site.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. Type 2 diabetes mellitus (T2DM) accounts for 90% of cases of diabetes and is characterized by individuals with postprandial hyperglycemia associated with low production of insulin, resistance to insulin, or both [1]. One strategy used to control T2DM is the use of inhibitors of digestive enzymes such as α-glucosidase that is present in the intestine which catalyzes the digestion of complex carbohydrates, converting them into easily absorbable monosaccharides [2]. In this regard, α-glucosidase inhibitors play a valuable role to prevent or delay the digestion or absorption of carbohydrates and suppress postprandial hyperglycemia, making such inhibitors useful in the management of type 2 diabetes. Thus, the design and development of new α-glucosidase inhibitors is a noteworthy goal for pharmacologists [3,4]. Consumption of different natural compounds is known to result in anti-diabetic effects, offering exciting possibilities for the future development of successful therapies [5].

Triterpenoids are widely distributed in both plant and animal kingdoms and possess interesting bioactivity [6,7], such as anticancer [8], antiviral [9], anti-inflammatory [10], antibacterial [11], antiparasitic [12], as well as antidiabetic [13–18]. Thus, an increasing number of publications were focused on pentacyclic triterpenoids, particularly oleanolic, ursolic and betulinic acids due to their antidiabetic properties [19,20]. Oleanolic acid is the hypoglycemic component of many traditional Chinese medicines [21]. The dammarane type triterpenoid 20(*S*)-protopanaxadiol demonstrated a regulatory function in glucose. Betulinic acid shows antidiabetic activity due to inhibition of human PTP1B activity with an IC_{50} of 3.5 μM [22]. Oleanolic and ursolic acids completely inhibited the activity of α-glucosidase in a non-competitive manner and combination of these compounds displayed a significant synergistic inhibition on α-glucosidase [23]. The combination of corosolic and oleanolic acids with acarbose showed synergistic inhibition against α-amylase. The combination of the triterpenic acids with acarbose mainly exhibited additive inhibition against α-glucosidase. These results provide valuable implications for

* Corresponding author.
E-mail address: elmaH@inbox.ru (E.F. Khusnutdinova).

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the triterpenic acids (ursolic, corosolic, and oleanolic) alone or in combination with acarbose as a therapeutic agent for the treatment of diabetes mellitus [24].

Semi-synthetic strategies based on triterpenoids are also promising in the discovery of new antidiabetic agents. Thus, methyl ester analog of betulinic acid with morpholine fragment exhibited an almost 1.4-fold increase in α-glucosidase inhibitory activity compared to betulinic acid [25]. Among the *N*-allylated/*N*-alkylated niacin and α-amyrin or lupeol hybrids tested for their α-glucosidase inhibiting activity, *N*-prenylated α-amyrine-niacin hybrid showed the highest inhibitory action with IC_{50} of 5 μM [26]. Among the different ursolic acid derivatives condensed with a suitable aldehyde at the C2 position, the analog with *p*-trifluoromethyl benzene moiety presented a great improvement of α-glucosidase inhibition activity with IC_{50} of 0.71 μM [27]. 3-Oxo-derivatives of β-boswellic acid retained good α-glucosidase inhibition compared to 3-hydroxy acids, indicating that the carboxylic group plays a key role in SAR [28]. It was shown that *in vivo* oral hypoglycemic testing, both acute and sub-acute studies demonstrated that the C2-modified oleanolic acid methyl ester derivatives containing pyridine- or morpholine fragment had high potency and long duration of action compared to the reference standards pioglitazone, acarbose and oleanolic acid [29].

Some ring A-fused triterpenoids such as 2,3-isoxazolocyclomusalenone and methyl 2,3-indolobetulinate inhibited α-glucosidase with IC_{50} of 20.4 μM and 48.5 μM being 20- and 8-fold more active than acarbose, respectively [17,18,30]. According to our previous studies, the oxidized 28-oxo-indoloallobetulone derivatives possess antiviral activity [31], and 3-indolo-betulinic acid was discovered as a new promising template for the synthesis of α-glucosidase inhibitors [32]. In this work, a series of nitrogen-containing lupane triterpenoids, mainly of 2,3-indolo-betulinic acid derivatives, was designed, synthesized and the activity of these new and some previously described lupanes against α-glucosidase was evaluated *in vitro*.

2. Results and discussion

2.1. Design

Previously we have found that 2,3-indolo-betulinic acid **1** inhibited α-glucosidase with IC_{50} of 1.8 μM being 221-fold more active than acarbose [32]. In order to get structural insight and direct further optimization efforts, a molecular modeling study was performed.

Compound **1** was docked into the allosteric site of *S. cerevisiae* α-glucosidase. Since the crystal structure of the enzyme is unavailable to date, we utilized the homology model built as described earlier [33]. It was found that **1** occupies a cleft formed between the loop region and β-sheet close to the active site (Fig. 1). Indole core interacts with Lys147 side chain *via* π-alkyl and π-cation interaction, and with Pro148 *via* H-bond with a nitrogen atom. Rings A, D, and E form multiple hydrophobic interactions with lipophilic side chains of Ile149, Pro150, Phe172, Ile415, and Lys418. Additionally, the carboxylic group of **1** forms H-bond with Ser179 backbone and Arg175 side chain. At the same time, the carboxylic group protrudes into the unexploited pocket formed by Ser161, Thr164, and Arg175. Introduction of additional hydrogen bond donors or acceptors could possibly facilitate inhibitor binding due to additional polar interactions. This observation prompted us to obtain a series of analogs comprising functionalized C28 carboxyl in order to explore the possibility of improving the potency of compound **1**.

2.2. Chemistry

The lead compound from our previous studies 2,3-indolo-betulinic acid **1** was modified at C20 and C28 positions. The synthesis of different derivatives at C28 is shown in Scheme 1. It is well known that structural modification of triterpenoids with the introduction of amine or amide fragment improve biological properties, and result also in a better bioavailability [6,34–36]. The reaction of **1** with ammonia, amino acids (glycine, *L*-alanine or *L*-phenylalanine) or imidazole *via* the intermediate acyl chloride gave the corresponding amides **2–6** with yields of 71–89%. Alkynyl derivatives **7** and **8**, were synthesized by a similar way with propargylamine or propargyl alcohol with yields of 81 and 85%. The structure of compounds was confirmed by NMR spectroscopy. For compounds **2–6** and **8**, the signals of amide function C(O)NH were detected at δ 172–179 ppm (^{13}C NMR) and at δ 5.51–6.12 ppm (^{1}H NMR). Also, for compounds **7** and **8** the signals of the acetylene fragment at δ 71.6–80.0 ppm (^{13}C NMR) and δ 2.21–2.23 ppm (^{1}H NMR) were characteristic.

Many triterpene nitriles are of interest as potential agents. For example, 2-cyano-3,12- dioxoolean-1,9(11)-dien-28-oic acid (CDDO) bearing a cyanoenone moiety in ring A exhibits 400 times greater anti-inflammatory activity than oleanolic acid [37,38]. 2,20-Dinitrile-glycyrrhetinic acid displayed 99% inhibition rate at a dose of 50 mM and exhibited stronger inhibitory activities against the LPS-induced NO production in RAW 264.7 macrophages relative to the parent compound [39]. To investigate the effect of the nitrile group on α-glucosidase inhibitory activity amide **2** was converted to compound **9** by trifluoroacetic anhydride in CH_2Cl_2.

Another way to introduce a nitrile fragment to the side chain is by cyanoethylation. The synthesis of bis- and tri-cyanoethyl-triterpenoids [40], selective cytotoxicity of 28-cyanoethoxy-indolo- [41] and aminopropoxy-triterpenoids were described previously [42]. At first, the reduction of 2,3-indolobetulinic acid **1** by lithium aluminum hydride afforded 2,3-indolobetulin **10**, which gave C28-cyanoethoxy-derivative **11** after treatment with acrylonitrile. In NMR spectra of **10**, signals of the methylene group $C28H_2$ as doublets at δ 3.28–3.88 (^{1}H NMR) and δ 68–78 ppm (^{13}C NMR) were observed. In the ^{13}C NMR spectra of **9** and **11**, the typical signals of the nitrile group at δ 118–119 ppm were observed. In the ^{1}H NMR spectrum of **11**, signals of methylene protons of cyanoetoxy fragment at δ 2.59 and 3.68 ppm were characteristic.

Recently, it was shown that platanic acid derivatives are potent inhibitors of butyrylcholinesterase and anticancer agents [43–45]. At the same time, the effect of the 20-oxo-group in lupane core on the inhibition of α-glucosidase was not described. For the synthesis of platanic acid derivatives bearing an indole fragment, oxidation of betulonic acid with ozone in CH_2Cl_2 was carried out. As a result of this reaction, we obtained a mixture of 3-oxo-platanic acid **12** and 29-hydroxy-20-oxo-30-nor-lupane acid **13**, also known as messagenic acid [46], which were isolated by column chromatography with yields of 75% and 18%, respectively (Scheme 2).

We propose the following mechanism for the formation of compounds **12** and **13** formation (Fig. 2). First, 1,3-cycloaddition of ozone to C20(29)-double bond of betulonic acid led to primary ozonide **A**, followed by rearrangement to secondary ozonide **B** and formation of 3-oxo-platanic acid **12**. An alternative route based on the 1,2-cycloaddition of ozone to the double bond with formation of epoxide **C**, which rearranges to **D** with the following oxidation to compound **13** is also possible.

20-Oxo-indoles **14** and **16** were synthesized from **12** and **13** by Fischer reaction. The interaction of 2,3-indolo-platanic acid with imidazole *via* the intermediate acyl chloride gave amide **15.**

In order to compare the potency of triterpenic indoles with other types of derivatives, a series of A-azepanones and C3, C28-substituted lupanes was also synthesized (Scheme 3).

A-azapanone-17-cyano-lup-20(29)-ene **17** was synthesized through reaction of betulonic aldehyde with hydroxylamine hydrochloride to give 3,28-bisoxime with following Beckmann rearrangement which affected both A-ring and C28-position. Synthesis of azepanone betulinic acid **20** included two-step modification of betulonic acid to C3-oxime followed by Beckmann rearrangement. Amide **21** was obtained by interaction of compound **20** with imidazole *via* the intermediate acyl chloride with a yield of 65%. The formation of A ring lactam was

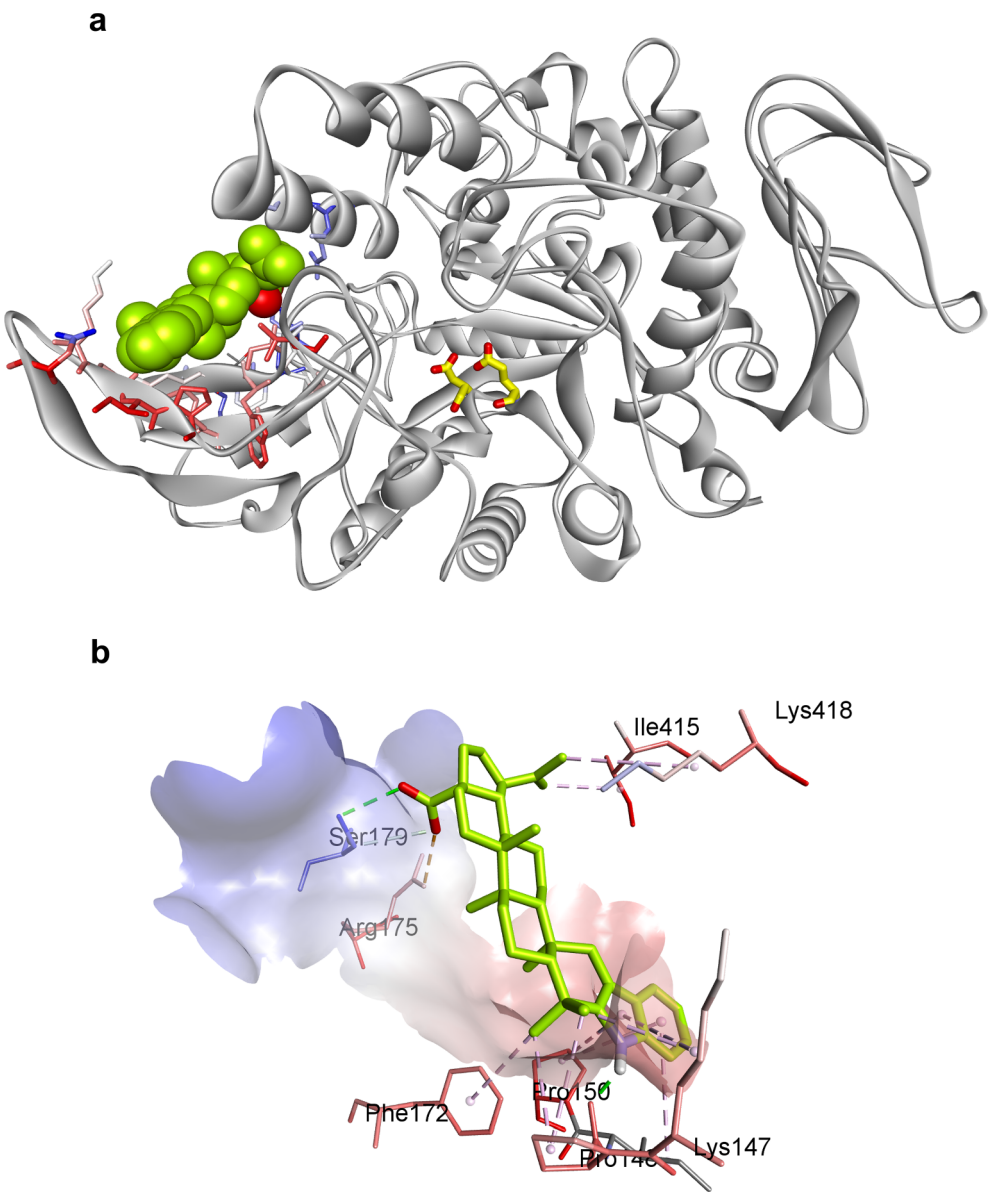


Fig. 1. CPK model of compound 1 docked into the allosteric site of *S. cerevisiae* α-glucosidase; catalytic Asp214 and Glu276 are shown with yellow carbons (A). Proposed interactions of compound **1** with amino acids of the allosteric site (B).

confirmed by the signal at δ ~ 176.0 ppm in ^{13}C NMR, that pointed the presence of C═O group. The ^{1}H NMR spectrum of the lactams **17**, **20** and **21** showed singlet at δ ~ 6 ppm typical of NH group.

Finally, compounds **18** and **19** were synthesized according to previously described methods [8,47].

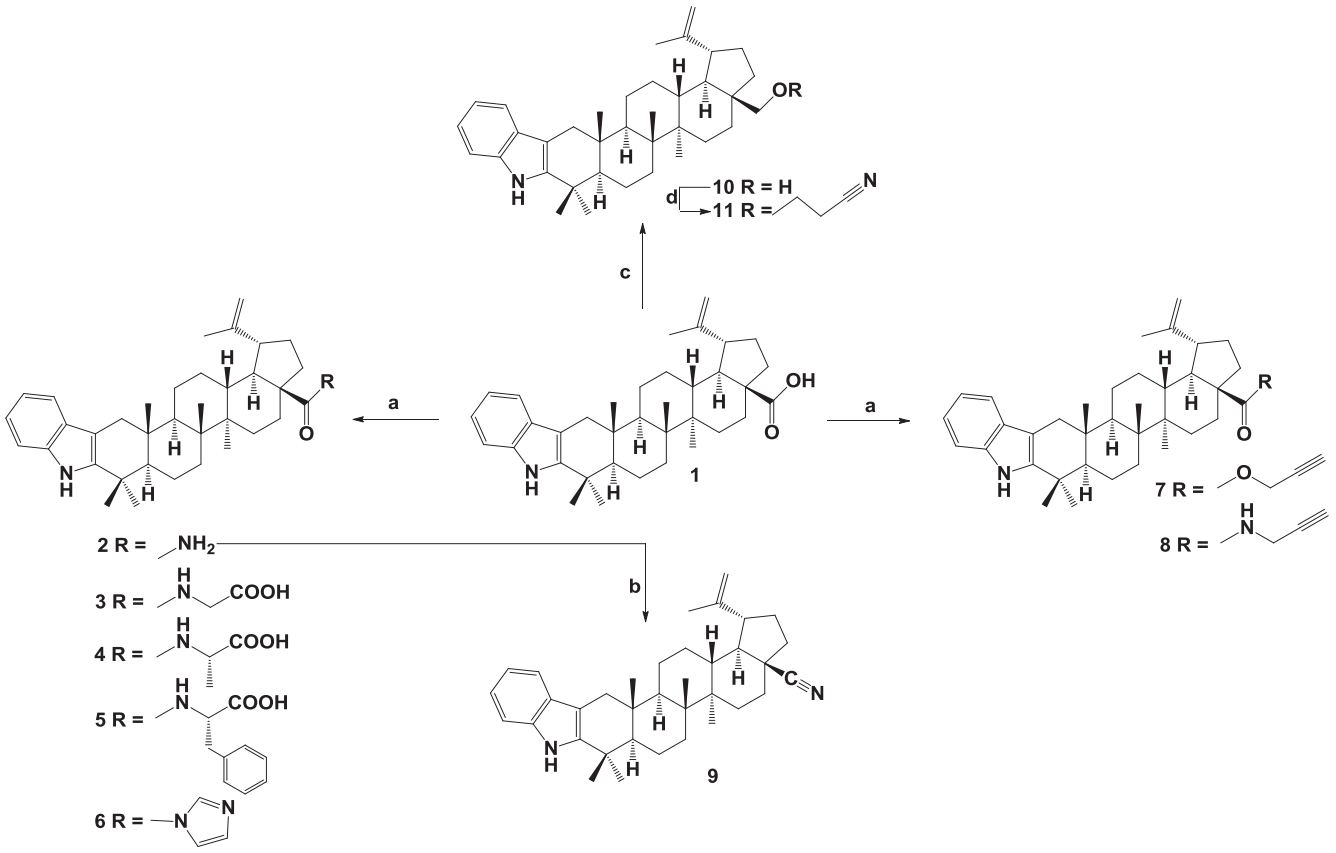
2.3. α-Glucosidase inhibition and structure-activity relationships

All synthesized compounds **1–21** were screened for *in vitro* α-glucosidase enzyme inhibition (Table 1). The 2,3-indolo-betulinic acid amides **2–6** and **8** demonstrated significant inhibitory properties with IC_{50} values ranged from 0.04 μM to 60.74 μM. Carboxamide **2** (IC_{50} 1.74 μM) was found to be 109-times more active than acarbose (IC_{50} 189.20 μM). However, the inhibition activity of the propargylamide **8** (IC_{50} 60.74 μM), as well as imidazolo-amide **6** (IC_{50} 18.54 μM), was lower than that of **2**, albeit still greater than acarbose. However, conjugation of the carboxylic group of compound **1** by amino acid residue led to a dramatic activity increase. Compounds **3** (IC_{50} 0.04 μM) and **5** (IC_{50} 0.05 μM) containing glycine and *L*-phenylalanine residues exhibited an almost 3784- and 4730-fold higher inhibitory activity compared to acarbose and 20–30-fold compared to the previously described activity of 2,3-indolobetulinic acid **1** (IC_{50} 1.8 μM) [32]. Also, the inhibitory activity of *L*-alanine amide **4** (IC_{50} 0.12 μM) was less than of compounds **3** and **5**.

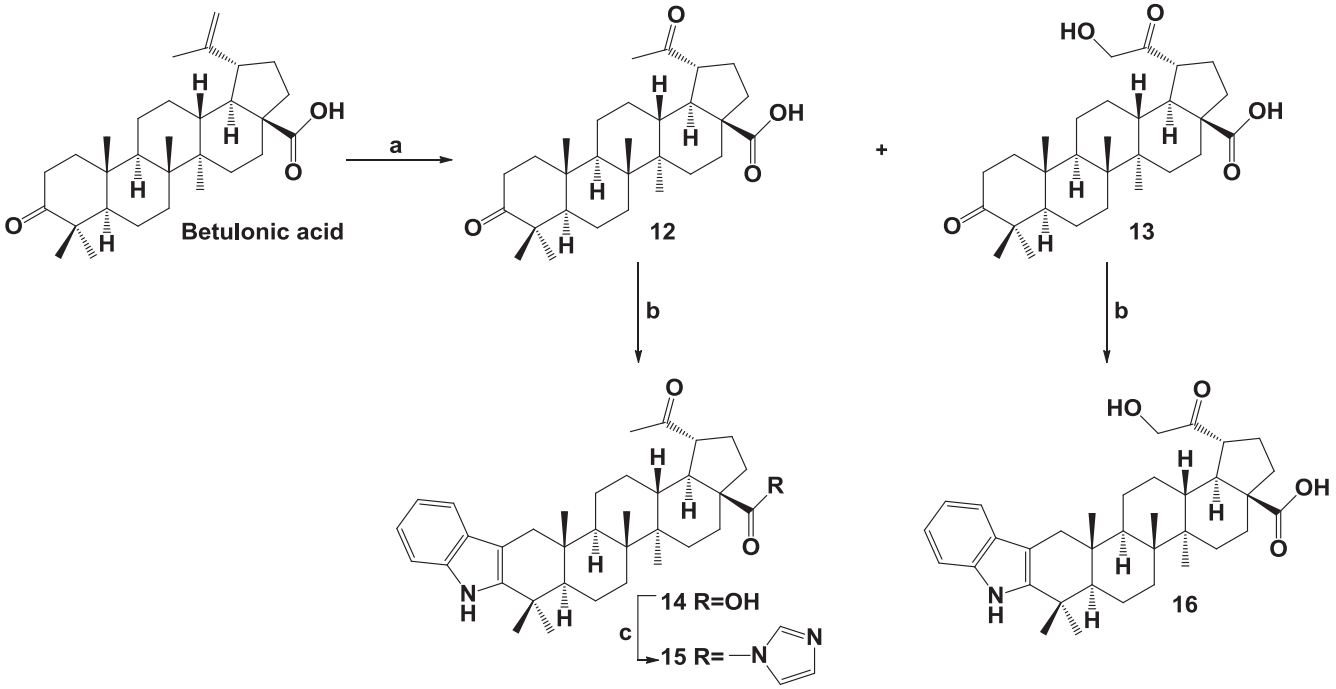
The results are in accordance with our previous study as well [18], indicating that the propargyl ester of 2,3-indolobetulinic acid **7** with IC_{50} of 183.24 μM showed almost no activity comparing with acarbose and less than methyl ester analog. On the other hand, propargylamide **8** (IC_{50} 60.74 μM) exhibited three-fold higher activity than ester **7**, indicating that the increased activity of compound **8** is indeed due to the presence of carboxamide moiety, which can provide an extra H-bond.

Reduction of the carboxyl group of compound **1** to 2,3-indolo-betulin **10** (IC_{50} 30.12 μM) led to a decreased inhibition activity. On the other hand, the cyanoethylation of the hydroxy group of **10** to compound **11** improved IC_{50} value up to 18.67 μM. Comparison of **1** and **9** (IC_{50} 76.00 μM) showed that the replacement of the carboxyl group by a nitrile group has weakened the activity against α-glucosidase.

Modification of the isopropenyl fragment of **1** had a positive effect. Thus, 2,3-indolo-platanic acid **14** showed high activity with IC_{50} 0.40 μM, being 473-fold more active than acarbose, while 29-hydroxy



Scheme 1. Synthesis of C28-derivatives of 2,3-indolo-betulinic acid. Reagents and conditions: (a) (i) $(COCl)_2$, Et_3N, CH_2Cl_2, 20 °C, 2 h, (ii) ammonia, imidazole, corresponding amino acid (OMe·HCl), propargylamine or propargyl alcohol, Et_3N, CH_2Cl_2, Δ, 3 h, (iii) 4 N NaOH, CH_3OH-THF (1 : 1), 20 °C, 6 h (for synthesis of compounds **3–5**); (b) TFAA, CH_2Cl_2, Δ, 2 h; (c) $LiAlH_4$, THF, Δ, 3 h; (d) H_2C = CHCN, 30% KOH, $BnEt_3NCl$, 1,4-dioxane, 20 °C, 2 h.



Scheme 2. Synthesis of 2,3-indoloplatanic acid derivatives. Reagents and conditions: (a) O_3, CH_2Cl_2, −70 °C; (b) $PhNHNH_2$, AcOH, Δ, 2 h; (c) (i) $(COCl)_2$, Et_3N, CH_2Cl_2, 20 °C, 2 h, (ii) imidazole, Et_3N, CH_2Cl_2, Δ, 3 h.

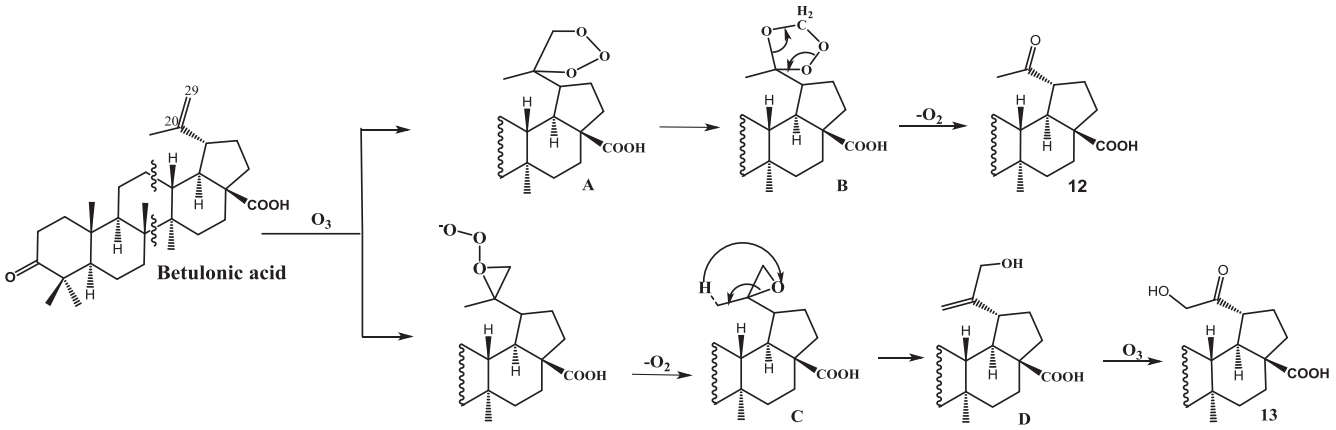


Fig. 2. Plausible way of formation of compounds **12** and **13** during oxidation of betulonic acid with ozone.

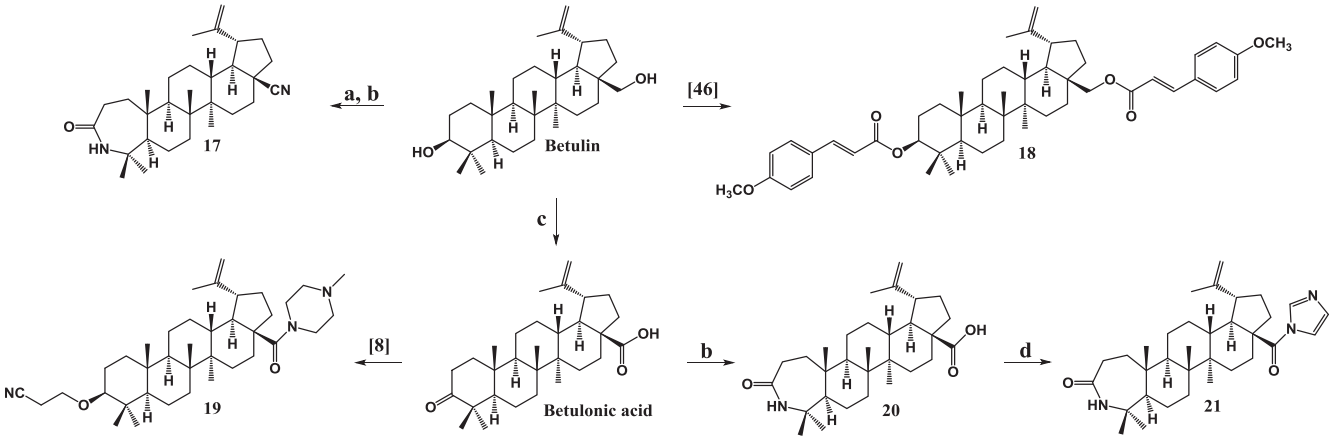
analog **16** was far less active. Imidazole amide **15** showed also moderate activity with IC_{50} 53.89 μM in comparison with compounds **1** and **6**. At the same time, analogs of compound **14**, acids **12** and **13**, are virtually inactive, hence indicating the importance of indole core presence.

Imidazole amide **21** with A-lactam cycle showed promising activity with IC_{50} value 6.19 μM which is 30-times more active than acarbose and is 3-times more active than the indole-bearing analog **6**. The replacement of imidazole amide fragment with nitrile in compound **17** resulted in a complete loss of activity. Modification of the 3-hydroxy group of lupanes with the introduction of cinnamoyloxy- **18** or cyanoethoxy- **19** fragments did not lead to positive results.

It is known that the introduction of amino acid fragments to triterpene core improve drug-like properties, particularly the poor solubility [6]. The solubility data for compounds **1**, **3–5** and **14** have been measured. For the 2,3-indolobetulinic acid **1** solubility in distilled water was determined as 0.257 μg/mL. The amino acid derivatives **3**, **4** and **5** showed a better water solubility, which was 0.450, 0.510 and 0.530 μg/mL, respectively. The solubility of compound **4** with alanine side chain is higher than the solubility of compound **3** with glycine side chain, while the inhibitory activity is 3 times lower. On the other hand, the solubility of platanic acid derivative **14** was found to be 0.263 μg/mL, which is comparable to the solubility of compound **1**, while the activity of **14** is 4.5-times higher. Thus, inhibitory properties of triterpenoid derivatives against *α*-glucosidase are predominantly influenced by the structure of lupane core and a substituent at C28.

In summary, from a structure-activity-relationship perspective, among all compounds tested for the inhibition of *α*-glucosidase 2,3-indolo-betulinic acid derivatives, we conclude that the presence of carboxamide group (*via* an amino acid spacer) at the C28 position is important. Compounds **3** and **5** with a glycine and *L*-phenylalanine amide fragments exhibited significantly better activity with sub-micromolar IC_{50} values than the parent 2,3-indolobetulinic acid (20-times higher) [32] and standard drug acarbose (3784–4730 times higher). Moreover, the *α*-glucosidase inhibition was more than 400-times effective compared with the activity of acarbose when the C20(29)-double bond of lupane indole was converted into the corresponding ketone (**14**, IC_{50} 0.4 μM). Imidazole amide of 2,3-indolo-platanic acid **15**, in comparison with the same amide of 2,3-indolo-betulinic acid **6**, was three times less active, which demonstrates a high sensitivity of activity to structural changes in the lupane core. Meanwhile, when the carbocycle A was replacement by lactam cycle, imidazole amide of A-lactame **21** exhibited three times higher activity than the indolo-lupane **6**. Reduction of carboxylic group of 2,3-betulonic acid **1** to 2,3-indole-betulin **10** and its following cyanoethylation led to a decrease of activity. Modification of C3 position by introducing ethylcyano- and *p*-methoxy-cinnamic acid fragment did not give positive results.

Chemoinformatic study of molecular characteristics associated with activity against *α*-glucosidase revealed that observed pIC_{50} values are correlated with the number of H-bond acceptors (Bravais-Pearson coefficient 0.392) and, especially, H-bond donors (Bravais-Pearson coefficient 0.648) present in the inhibitor molecule (Fig. 3). Therefore, it could be concluded that polar interactions play a key role in the binding of the studied triterpene derivatives. Simultaneously, the introduction of polar moieties has a positive impact on drug-like properties, such as water solubility.



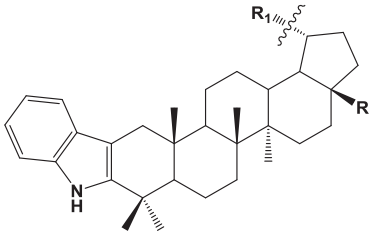
Scheme 3. Synthesis of azepanone and 3,28-modified lupane derivatives. Reagents and conditions: (a) (i) PCC, $CHCl_3$, 20 °C, 2 h; (b) (i) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, Δ, 5 h, (ii) $SOCl_2$, 1,4-dioxane, 0 °C, 30 min. (c) Jones reagent, acetone, 0 °C, 2 h; (d) (i) $(COCl)_2$, Et_3N, CH_2Cl_2, 20 °C, 2 h, (ii) imidazole, Et_3N, CH_2Cl_2, Δ, 3 h.

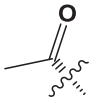
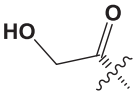
2.4. Molecular docking

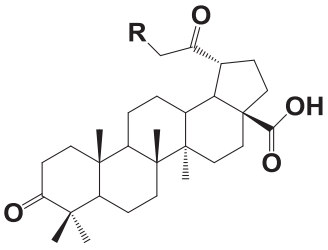
Hence, biological evaluation showed derivative **3** as a lead compound. The binding mode of **3** was investigated as described for parent compound **1** in order to elucidate the structural basis of its enhanced potency in detail. As shown in Fig. 4, extensive interaction network is formed by the fused indole core, lupane skeleton, and the C28 side chain. Specifically, the indole aromatic system maintains hydrophobic interactions with Pro150 and H-bond with Pro148 at the entrance of the pocket. The triterpene core is well fitted to establish multiple hydrophobic alkyl-alkyl interactions with Lys147, Phe165, and Phe172, which is similar to what was already observed for the parent compound **1**.

In turn, the amide side chain at C28 appears to form a hydrogen bond network, including bridged H-bonds with Arg123, Arg180, and Tyr173. These additional interactions are likely responsible for the

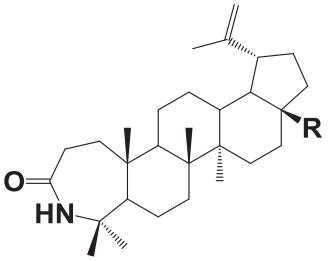
Table 1
Inhibition of *α*-glucosidase by triterpenoids **1–21**.



Compound	R_1	R	IC_{50}, μM	pIC_{50}
1		−COOH	1.80	5.74
2		$-CONH_2$	1.74	5.76
3		$-CONHCH_2COOH$	0.04	7.40
4		$CONHCH(CH_3)COOH$	0.12	6.92
5		−CONHCH(Bn)COOH	0.05	7.30
6		−COImd	18.54	4.73
7		$-COOCH_2C{\equiv}CH$	183.24	3.74
8		$-CONHCH_2C{\equiv}CH$	60.74	4.22
9		−CN	76.00	4.12
10		$-CH_2OH$	30.12	4.52
11		$-CHOCH_2CH_2CN$	18.67	4.73
14		−COOH	0.40	6.40
15		−COImd	53.89	4.27
16		−COOH	57.38	4.24



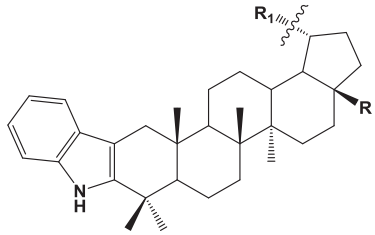
Compound	R	% Inhibition at 100 μM, m ± SEM	IC_{50}, μM	pIC_{50}
12	H	24.90 ± 4.59	> 256	< 3.59
13	−OH	14.74 ± 5.76	> 256	< 3.59



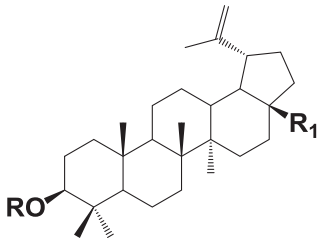
Compound	R	IC_{50}, μM	pIC_{50}
17	−CN	> 256	< 3.59
20	−COOH	34.68	4.46
21	−COImd	6.19	5.21

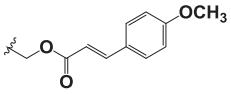
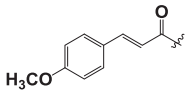
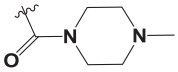
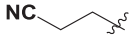
(*continued on next page*)

Table 1 (*continued*)



Compound	R_1	R	IC_{50}, μM	pIC_{50}



Compound	R_1	R	IC_{50}, μM	pIC_{50}
18			45.35	4.34
19			>256	< 3.59
Acarbose			189.20	3.72

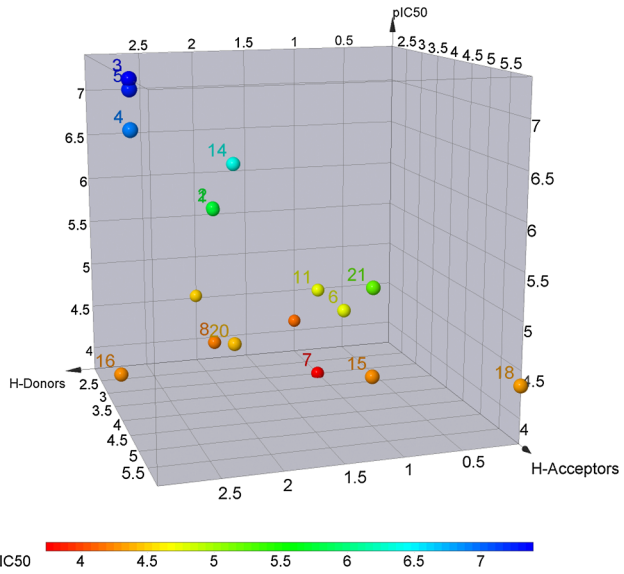


Fig. 3. The contribution of H-bond donors and acceptors to the α-glucosidase inhibitory activity of active 2,3-indolo-betulinic acid derivatives.

improved affinity of compound **3** towards α-glucosidase. Isopropyl moiety at C19 is pointing towards the solvent accessible area. This supports the improved activity of platanic acid derivative **14** since polar acetyl substituent could compensate for enthalpy penalty associated with displacement of bonded water molecules [48]. Summary of interactions of compounds **1** and **3** with the allosteric site of α-glucosidase is shown in Fig. 5. Noteworthy, unfavorable repulsive interaction of C28 hydroxyl of compound **1** is managed to be replaced with a favorable one realized *via* the amide carbonyl group of compound **3**.

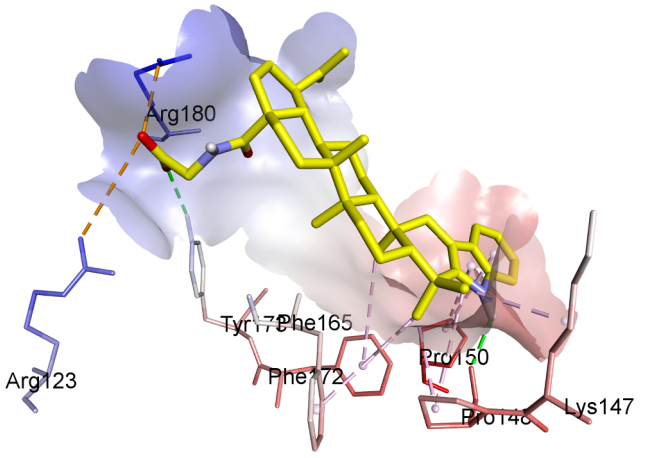


Fig. 4. Proposed binding mode and interaction of compound **3** with the allosteric site of *S. cerevisiae* α-glucosidase. Key amino acids are shown. Protein-ligand interactions are indicated with dashed lines.

3. Conclusions

Modification of previously discovered lead 2,3-indolo-betulinic acid at the C20 and C28 positions led to the identification of derivatives with up to 45-times improved *α*-glucosidase inhibiting activity. The conjugates of **1** with glycine and *L*-phenylalanine *via* an amide bond at C28 were found to be the most active with IC_{50} values of 0.04 and 0.05 μM, being 3784 and 4730-fold more active than acarbose. Most of the indole-fused modified derivatives with C28-carboxamide, nitrile, hydroxy-, cyanoethoxy-, cinnamoyloxy- and lactame fragments showed better potency than the standard drug acarbose. Structure-based activity study suggests the key role of C28 amide side chain in inhibitor binding *via* the formation of multiple H-bonds with previously unexploited polar subpocket of the allosteric site.

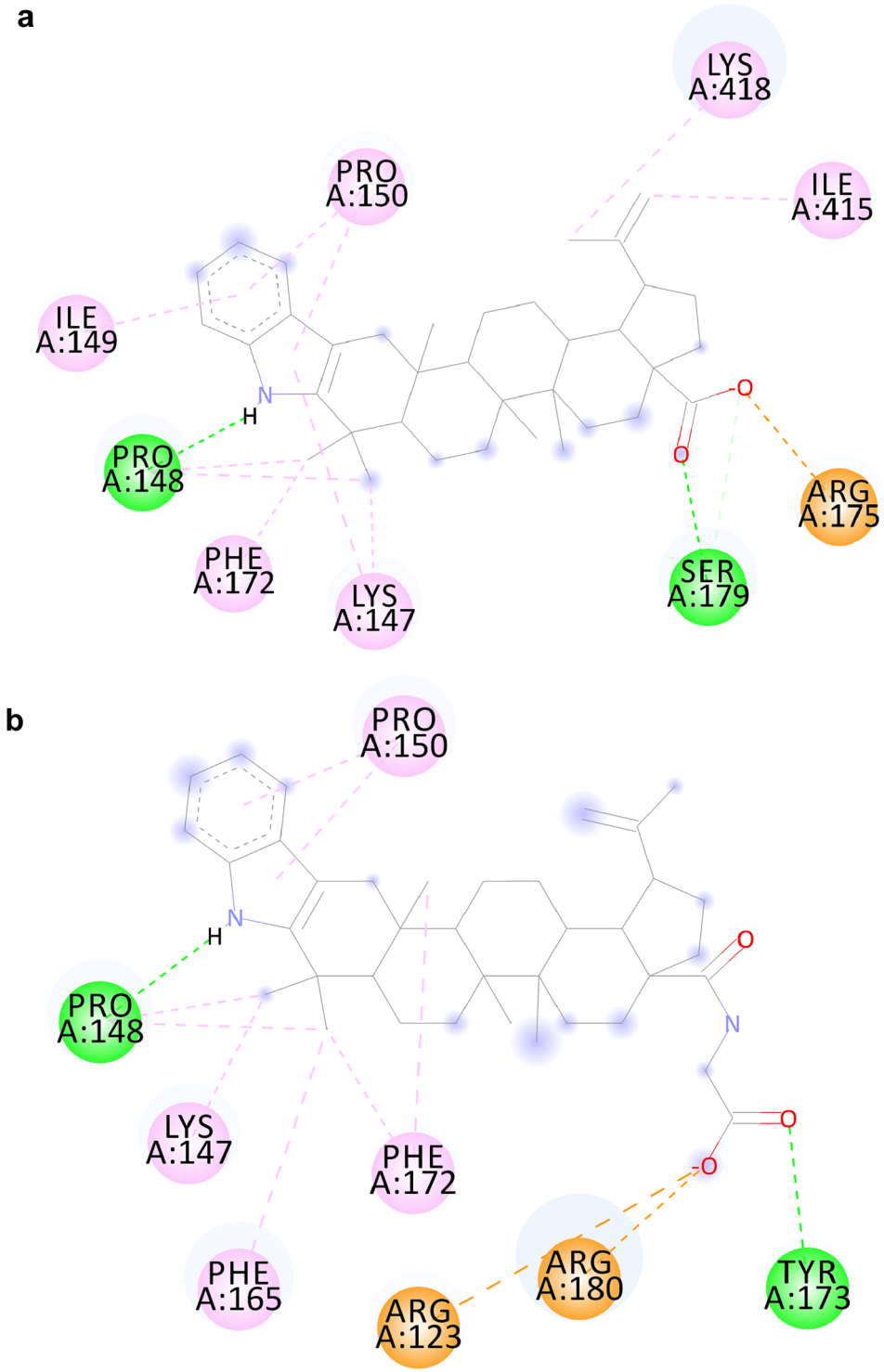


Fig. 5. Schematic 2D diagram of intermolecular interactions of compound **1** (a) and compound **3** (b) with the allosteric site of *S. cerevisiae* α-glucosidase. Key amino acids are shown. Interactions are indicated with dashed lines (purple for hydrophobic contacts, green for H-bonds, orange for charge attraction).

4. Experimental

4.1. Materials and methods

The spectra were recorded at the Center for the Collective Use 'Chemistry' of the Ufa Institute of Chemistry UFRS RAS. ^{1}H and ^{13}C NMR spectra were recorded on a "Bruker AM-500" (Germany, 500 and 125.5 MHz respectively, δ, ppm, Hz) in $CDCl_3$, internal standard– tetramethylsilane. Mass spectra were obtained on a liquid chromatograph–mass spectrometer LCMS-2010 EV (Shimadzu). Melting points were detected on a micro table "Boetius". Optical rotations were measured on a polarimeter "Perkin-Elmer 241 MC" (Germany) in a tube length of 1 dm. Elemental analysis was performed on a Euro EA-3000 CHNS analyzer, the main standard is acetanilide. IR spectra were recorded on a spectrometer IRPrestige-21 Shimadzu (Japan) in a paste with vaseline oil. Thin-layer chromatography analyses were performed on Sorbfil plates (Sorbpolimer, Russian Federation), using the solvent system chloroform-ethyl acetate, 40:1. Substances were detected by a 10% solution of sulfuric acid solution

with subsequent heating at 100–120 °C for 2–3 min. The ozone generator used was Ozone–4K (Russian Federation). Compounds **1** [35], **8** [49], **10** and **11** [41], **18** [47], **19** [8] and **20** [50] were obtained according to the methods described previously.

4.2. Chemistry

4.2.1. General procedure for the synthesis of compounds 2–7, 15 and 21

To a solution of compound **1** (1 mmol; 0.53 g), **14** (1 mmol; 0.53 g) or **20** (1 mmol; 0.47 g) in CH_2Cl_2 (20 mL) $(COCl)_2$ (3 mmol; 0.26 mL) was added and stirred at room temperature during 2 h. The mixture was concentrated to dryness under reduced pressure and resulting acid chlorides were dissolved in CH_2Cl_2 (10 mL), 3 drops of Et_3N and following reagents were added:

a) 1.5 mmol of conc. the solution of NH_3 (for the synthesis of compound **2**);
b) 1.5 mmol of corresponding amino acid (OMe·HCl). After isolation and purification (as shown below), the deprotection was performed according to [51];
c) 1.5 mmol of propargyl alcohol (for synthesis of compound **7**);
d) 1.5 mmol of imidazole (for synthesis of compounds **6**, **15** or **21**);

After completion of the reactions (TLC control) the organic layers were treated with H_2O (3 × 50 mL), separated, washed with 5% HCl, H_2O (3 × 50 mL) until neutral pH, dried over $CaCl_2$, and evaporated under reduced pressure. The residue was purified by column chromatography on Al_2O_3 using petroleum ether/$CHCl_3$ (1:1) as eluent for **2–7**, **15** and $CHCl_3/CH_3OH$ (50:1) for **21**.

*4.2.1.1. N-[3,2b]indolo-lup-20(29)-en-28-amide **(2)***. Beige powder; yield (89%); m.p.: 183 °C; $[\alpha]_D^{20}$ = +15° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1641 (C=C), 1656 (CONH), 3398 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.81, 1.00, 1.05, 1.15, 1.20, 1.65 (s, 18H, $6CH_3$), 1.62–3.15 (m, 23H, CH and CH_2), 4.61 and 4.70 (both s, ^2J = 2.0 Hz, 2H, H-29), 5.51 (br. s, 2H, NH_2), 7.02–7.58 (m, 4H, Ar-*H*), 7.64 (1H, br. s, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.82, 15.98, 16.36, 19.09, 19.27, 21.41, 23.16, 25.38, 27.16, 29.15, 29.74, 30.86, 33.47, 34.00, 34.13, 37.26, 37.52, 38.27, 41.01, 42.79, 47.82, 48.76, 49.26, 53.23, 106.99 (C-2), 109.80 (C-arom), 110.34 (C-29), 117.87 (C-arom), 118.89 (C-arom), 120.94 (C-arom), 128.34 (C-arom), 136.15 (C-arom), 140.89 (C-3), 150.47 (C-20) 179.56 (C-28) ppm; MS: *m/z* 526. $[M + H]^+$. Anal. calcd for $C_{36}H_{50}N_2O$: C, 82.08; H, 9.57; N, 5.32. Found: C, 82.09; H, 9.55; N, 5.31.

*4.2.1.2. N-glycine-[3,2b]indolo-lup-20(29)-en-28-amide **(3)***. Beige solid; yield (75%); m.p.: 202 °C; $[\alpha]_D^{20}$ = +7° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1643 (C=C), 1661 (CONH), 3386 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89, 1.05, 1.21, 1.27, 1.58, 1.70 (s, 18H, $6CH_3$), 1.68–3.22 (m, 23H, CH and CH_2), 4.05 (m, 2H, H-37), 4.62 and 4.78 (both s, ^2J = 2.0 Hz, 2H, H-29), 6.10 (br. s, 1H, NH), 7.0–7.48 (m, 4H, Ar-*H*), 7.76 (1H, br. s, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.70, 15.89, 16.36, 19.27, 19.49, 21.50, 22.54, 23.14, 25.80, 29.51, 30.77, 30.85, 33.64, 34.13, 37.28, 37.90, 38.32, 40.84, 41.11, 42.58, 46.71, 49.51, 50.06, 52.28, 53.33, 55.81, 107.05 (C-2), 109.52 (C-arom), 110.31 (C-29), 117.90 (C-arom), 118.86 (C-arom), 120.90 (C-arom), 128.34 (C-arom), 136.15 (C-arom), 140.90 (C-3), 150.84 (C-20), 174.91 (C-28), 179.62 (C-38) ppm; MS: *m/z* 584 $[M + H]^+$. Anal. calcd for $C_{38}H_{52}N_2O_3$: C, 78.04; H, 8.96; N, 4.79. Found: C, 78.05; H, 8.94; N, 4.77.

*4.2.1.3. N-L-alanine-[3,2b]indolo-lup-20(29)-en-28-amide **(4)***. White solid; yield (71%); m.p.: 206 °C; $[\alpha]_D^{20}$ = +34° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1641 (C=C), 1644 (CONH), 3446 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75, 1.02, 1.18, 1.27, 1.43, 1.42, 1.69 (s, 21H, $7CH_3$), 1.70–3.20 (m, 23H, CH and CH_2), 4.58 and 4.72 (both s, ^2J = 2.0 Hz, 2H, H-29), 4.55 (m, 1H, H-37), 6.12 (br. s, 1H, NH), 7.0–7.42 (m, 4H, H-arom), 7.62 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.13, 15.87, 16.36, 18.44, 19.51, 21.50, 22.69, 23.14, 25.79, 29.36, 29.70, 30.84, 31.93, 33.65, 34.12, 37.27, 38.31, 40.87, 41.48, 42.52, 46.74, 47.82, 49.49, 50.12, 52.42, 53.31 (CH-37), 55.65, 107. 07 (C-2), 109.43 (C-arom), 110.29 (C-20), 117.91 (C-arom), 118.88 (C-arom), 120.92 (C-arom), 128.33 (C-arom), 136.13 (C-arom), 140.85 (C-3), 150.93 (C-20), 174.00 (C-28), 178.80 (C-39) ppm; MS: *m/z* 598 $[M + H]^+$. Anal. calcd for $C_{39}H_{54}N_2O_3$: C, 78.22; H, 9.09; N, 4.68. Found: C, 78.23; H, 9.07; N, 4.66.

*4.2.1.4. N-L-phenylalanine-[3,2b]indolo-lup-20(29)-en-28-amide **(5)***. Beige solid; yield (75%); m.p.: 134 °C; $[\alpha]_D^{20}$ = +13° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1642 (C=C), 1665 (CONH), 3453 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85, 0.90, 0.97, 1.19, 1.28, 1.68 (s, 18H, $6CH_3$), 1.06–3.16 (m, 26H, CH and CH_2), 4.60 and 4.73 (both d, ^2J = 2.5, 2H, H-29), 4.85 (m, 1H, H-37), 5.92 (br. s, 1H, NH), 7.04–7.38 (m, 8H, H-arom), 7.84 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.62, 15.89, 16.33, 19.25, 19.44, 21.43, 23.11, 25.72, 29.34, 30.68, 30.81, 33. 54, 33.56, 34.11, 37.25, 37.77, 38.04, 38.05, 38.27, 40.75, 42.46, 46.58, 49.44, 49.86, 52.76, 53.29, 55.77, 106.94 (C-2), 109.47 (C-29), 110.34 (C-arom), 117.86 (C-arom), 118.80 (C-arom), 120.84 (C-arom), 128.30 (C-arom), 128.72 (C-41), 128.72 (C-43), 128.98 (C-40), 128.98 (C-44), 136.15 (C-arom), 136.37 (C-41), 140.87 (C-3), 150.79 (C-20), 175.99 (C, C-28), 178.57 (C-45) ppm; ^{15}N NMR ($CDCl_3$): δ 110.85 (N); 116.56 (*N* Ind) ppm; MS: *m/z* 674 $[M + H]^+$. Anal. calcd for $C_{45}H_{58}N_2O_3$: C, 80.08; H, 8.66; N, 4.15. Found: C, 80.07; H, 8.65; N, 4.14.

*4.2.1.5. N-(1H-imidazol-1-yl)-[3,2b]indolo-lup-20(29)-en-28-amide **(6)***. Yellow powder; yield (74%); m.p.: 171 °C; $[\alpha]_D^{20}$ = +3° (c 0.10, CHCl3); IR (ύ max, cm^{-1}): 1641 (C=C), 1668 (CONH), 3430 (NH); ^{1}H NMR (75 MHz, $CDCl_3$): δ 0.88, 1.02, 1.05, 1.20, 1.30, 1.70 (s, 18H, $6CH_3$), 1.72–3.01 (m, 22H, CH and CH_2), 4.68 and 4.82 (both s, ^2J = 2.0 Hz, 2H, H-29), 7.01–7.41 (m, 4H, H-arom), 7.25 (s, 1H, Ar-*H* Imd) (s, 1H, H-38), 7.59 (s, 1H, H-37), 7.95 (br. s, 1H, NH), 8.32 (s, 1H, H-39) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.79, 15.76, 16.43, 19.45, 21.57, 23.14, 25.66, 29.77, 29.86, 30.85, 33.16, 33.23, 33.54, 34.16, 36.99, 37.19, 37.35, 38.34, 40.77, 42.21, 45.28, 49.56, 51.50, 53.35, 57.79, 106.85 (C-2), 110.31 (C-29), 110.39 (C-arom), 117.55 (C-arom), 117.87 (CH, C-37), 118.86 (C-arom), 120.92 (C-arom), 128.35 (CH, C-38), 129.34 (C-arom), 136.19 (CH, C-39), 137.28 (C-arom), 140.94 (C-3), 149.78 (C-20), 172.88 (C-28) ppm; MS: *m/z* 577 $[M + H]^+$. Anal. calcd for $C_{39}H_{51}N_3O$: C, 81.06; H, 8.90; N, 7.27; Found: C, 81.05; H, 8.93; N, 7.25.

*4.2.1.6. Prop-2-yn-1-yl-[3,2b]indolo-lup-20(29)-en-28-oat **(7)***. Yellow solid; yield (85%); m.p.: 207 °C; $[\alpha]_D^{20}$ = +51° (c 0.10, CHCl3); IR (ύ max, cm^{-1}): 1642 (C=C), 1710 (C=O); 2210 (C≡CH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.73, 1.00, 1.02, 1.20, 1.27, 1.68 (s, 18H, $6CH_3$), 1.32–3.10 (m, 23H, CH and CH_2), 2.21 (t, J = 2.4 Hz, 1H, C≡C<u>H</u>), 4.65–4.75 (m, 2H, CH_2), 4.60 and 4.68 (both s, ^2J = 2.0 Hz, 2H, H-29), 6.59–7.40 (m, 4H, H-arom), 7.70 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.79, 15.84, 16.38, 19.28, 19.41, 21.49, 23.16, 25.75, 29.78, 30.54, 30.87, 31.97, 33.63, 34.15, 36.84, 37.32, 38.34, 38.51, 40.93, 42.50, 46.92, 49.48, 49.55, 51.36, 53.34, 56.67, 74.33 (C-39), 78.18 (C-38), 107.07 (C-2), 109.78 (C-arom), 110.33 (C-20), 117.91 (C-arom), 118.91 (C-arom), 120.96 (C-arom), 128.39 (C-arom), 136.18 (C-arom), 140.89 (C-3), 150.44 (C-20), 175.25 (C-28) ppm; MS: *m/z* 565 $[M + H]^+$. Anal. calcd for $C_{39}H_{51}NO_2$: C, 82.78; H, 9.09; N, 2.48. Found: C, 82.77; H, 9.10; N, 2.46.

*4.2.1.7. N-(1H-imidazol-1-yl)-[3,2b]indolo-20-oxo-29-norlupan-28-amide **(15)***. Yellow solid; yield (78%); m.p.: 200 °C; $[\alpha]_D^{20}$ = +56° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1642 (C=C), 1663 (CONH), 1733 (C=O), 3419 (NH); ^{1}H NMR (75 MHz, $CDCl_3$): δ 0.87, 1.00, 1.09, 1.22, 1.29,

2.23 (s, 18H, 6CH_3), 1.32–3.28 (m, 23H, CH and CH_2), 7.03–7.40 (m, 4H, H-arom), 7.19 (s, 1H, H-38), 7.55 (s, 1H, H-37), 7.94 (br. s, 1H, NH), 8.41 (s, 1H, H-39) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.76, 15.66, 16.39, 19.19, 21.57, 23.13, 27.56, 28.20, 29.89, 30.37, 30.83, 32.60, 33.4, 34.15, 36.19, 36.62, 37.27, 38.34, 40.65, 42.09, 49.23, 49.40, 51.31, 53.29, 57.12, 106.76 (C-2), 110.37 (C-arom), 117.49 (C-37), 117.89 (C-arom), 118.85 (C-arom), 120.93 (C-arom), 128.28 (C-38), 128.93 (C-arom), 136.18 (C-39), 137.06(C-arom), 140.88 (C-3), 172.80 (C-28), 211.39 (C-20) ppm; MS: *m/z* 580 $[M + H]^+$. Anal. calcd for $C_{38}H_{49}N_3O_2$: C, 78.72; H, 8.52; N, 7.25. Found: C, 78.73; H, 8.51; N, 7.27.

4.2.1.8. N-(1H-imidazol-1-yl)-3-oxo-3-aza-3-homo-lup-20(29)-en-28-amide ***(21)***. Beige solid; yield (65%); m.p.: 201 °C; $[\alpha]_D^{20}$ = +10° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1680 (C═O), 1659 (CONH), 3230 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80, 0.85, 0.94, 1.03, 1.07, 1.62 (6 s,18H, 6CH_3), 1.70–3.00 (m, 25H, CH and CH_2), 4.62 and 4.76 (both d, ^2J = 2.0 Hz, 2H, H-29), 5.82 (br. s, 1H, NH), 7.21 (s, 1H, H-32), 7.51 (s, 1H, H-33), 8.29 (s, 1H, H-31) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.43, 15.75, 18.19, 19.36, 22.05, 22.41, 25.73, 27.14, 29.57, 30.56, 31.88, 33.00, 33.26, 33.53, 36.91, 37.06, 39.21, 40.22, 40.62, 42.18, 45.08, 51.05, 51.26, 53.03, 56.28, 57.62, 110.25 (C-29), 117.38 (C-33), 129.45 (C-32), 138.64 (C-31), 149.54 (C-20), 172.82 (C-28), 176.56 (C-3) ppm; MS: *m/z* 519 $[M + H]^+$. Anal. calcd for $C_{33}H_{49}N_3O_2$: C, 76.26; H, 9.50; N, 8.08. Found: C, 76.24; H, 9.51; N, 8.09.

4.2.2. Synthesis of [3,2b]indolo-lup-20(29)-en-17-nitrile ***(9)***

To a solution of amide **2** (0.53 g; 1 mmol) in anhydrous CH_2Cl_2 (20 mL) $(CF_3CO)_2O$ (1 mmol; 0.14 mL) was added and refluxed for 2 h. Then poured into H_2O (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). Organic layers were washed with 5% HCl and H_2O (3 × 50 mL) until neutral pH, dried over $CaCl_2$, and evaporated under reduced pressure. The crude product was purified by column chromatography (SiO_2; $CHCl_3$/petroleum ether (1 : 1)). Yellow powder; yield (89%); m.p.: 164 °C; $[\alpha]_D^{20}$ = −15° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1641 (C═C), 2251 (C═N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90, 1.02, 1.14, 1.15, 1.28, 1.72 (s, 18H, 6CH_3), 1.80–2.88 (m, 23H, CH and CH_2), 4.70 and 4.82 (both s, ^2J = 2.0 Hz, 2H, H-29), 7.05–7.48 (m, 4H, H-arom), 8.12 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.94, 15.81, 16.42, 17.45, 19.41, 21.29, 23.16, 25.17, 29.53, 30.88, 31.06, 33.67, 34.15, 35.87, 37.33, 38.32, 41.43, 42.40, 48.63, 49.12, 49.17, 49.35, 53.28, 53.28, 53.46, 106.85 (C-2), 110.41 (C-20), 111.03 (C-arom), 117.86 (C-arom), 118.89 (C-arom), 120.96 (C-arom), 123.56 (C-17), 128.36 (C-arom), 136.17 (C-arom), 140.89 (C-3), 148.17 (C-29) ppm; MS: *m/z* 508 $[M + H]^+$. Anal. calcd for $C_{36}H_{48}N_2$: C, 84.98; H, 9.51; N, 5.51. Found: C, 84.97; H, 9.53; N, 5.50.

4.2.3. Oxidation of betulonic acid with ozone

A rapid stream of ozone was passed through a solution of betulonic acid (2 mmol; 0.908 g) in CH_2Cl_2 (30 mL) at −40 °C until the starting compound disappeared (TLC control). The solvent was removed under reduced pressure, and the residue was purified by column chromatography on SiO_2 eluting with $CHCl_3$ and CH_3OH (from 10:0 to 1:1) giving compounds **12** and **13**.

4.2.3.1. 3-Oxo-platanic acid ***(12)***. White solid; yield (75%); m.p.: 231 °C; $[\alpha]_D^{20}$ = +17° (c 0.01, $CHCl_3$); lit.: m.p. 231 °C; $[\alpha]_D$ = +5.4° (c 0.39, $CHCl_3$) [43]; IR (ύ max, cm^{-1}): 1687 (CO-OH), 1705 (C═O), 1733 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90, 0.94, 1.00, 1.01, 1.06 (5 s, 15H, 5CH_3), 2.18 (s, 3H, CH_3), 1.02–3.24 (m, 26H, CH and CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.60, 15.25, 15.66, 15.98, 19.57, 20.94, 21.34, 26.70, 27.15, 28.22, 29.64, 30.12, 31.35, 33.38, 34.01, 36.65, 37.50, 39.46, 40.44, 42.23, 47.24, 48.97, 49.60, 51.10, 54.64, 56.14, 181.68 (C-28), 212.41 (C-20), 218.52 (C-3). MS: *m/z* 457 $[M + H]^+$. Anal. calcd for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.25; H, 9.70.

4.2.3.2. 29-Hydroxy-20-oxo-30-norlupan-28-oic acid ***(13)***. Yellow powder; yield (18%); m.p.: 147 °C; $[\alpha]_D^{20}$ = +37° (c 0.01, $CHCl_3$); IR (ύ max, cm^{-1}): 1699 (CO-OH), 1713 (C═O), 1727 (C═O), 3450 (OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90, 0.95, 1.00, 1.00, 1.06 (5 s, 15H, 5CH_3), 1.02–2.48 (m, 26H, CH and CH_2), 3.20 (br. s, 1H, OH), 4.22–4.44 (m, 2H, H-30); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.54, 15.65, 15.94, 17.21, 19.56, 20.97, 21.26, 26.67, 27.29, 28.93, 29.58, 31.24, 33.38, 34.01, 36.77, 36.85, 37.43, 39.46, 40.46, 42.26, 46.48, 47.28, 49.42, 49.56, 54.70, 56.11, 56.61, 68.07 (C-30), 181.76 (C-28), 213.21 (C-20), 218.41 (C-3). MS: *m/z* 473 $[M + H]^+$. Anal. calcd for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.67; H, 9.37.

4.2.4. Synthesis of compounds ***14*** *and* ***16***

A mixture of compound **12** (1 mmol; 0.46 g) or **13** (1 mmol; 0.47 g) and $PhNHNH_2$ (3.5 mmol; 0.42 mL) in glacial AcOH (20 mL) was refluxed for 15 h. Then poured into water (50 mL) and the precipitate was filtered off and washed. The residue was purified by flash chromatography (SiO_2, petroleum ether/Et_2O (1 : 1))

4.2.4.1. [3,2b]Indolo-20-oxo-30-norlupan-28-oic acid ***(14)***. Yellow solid; yield (91%); m.p.: 147 °C; $[\alpha]_D^{20}$ = −8° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1638 (C═C), 1700 (CO—OH), 1735 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79, 0.92, 1.00, 1.09, 1.21 (s, 15H, 5CH_3), 1.22–2.08 (m, 14H, CH and CH_2), 2.21 (s, 3H, CH_3), 2.20–3.29 (m, 9H, CH and CH_2), 6.98–7.46 (m, 4H, H-arom), 7.68 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.78, 15.78, 16.32, 19.19, 21.40, 23.13, 24.55, 27.39, 28.34, 29.86, 30.20, 30.83, 31.43, 33.42, 34.11, 36.74, 37.76, 38.30, 40.64, 42.30, 49.19, 49.30, 51.23, 53.18, 56.32, 106.88 (C-2), 110.34 (C-arom), 119.96 (C-arom), 120.95 (C-arom), 124.44 (C-arom), 129.01 (C-arom), 136.13 (C-arom), 140.85 (C-3), 181.86 (C-28), 212.32 (C-20) ppm; MS: *m/z* 529 $[M + H]^+$. Anal. calcd for $C_{35}H_{47}NO_3$: C, 79.35; H, 8.94; N, 2.64. Found: C, 79.34; H, 8.92; N, 2.65.

4.2.4.2. [3,2b]Indolo-29-hydroxy-20-oxo-30-norlupan-28-oic acid ***(16)***. Beige solid; yield (89%); m.p.: 151 °C; $[\alpha]_D^{20}$ = -8° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1642 (C═C), 1794 (CO—OH), 1725 (C═O), 3450 (OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82, 0.89, 0.95, 1.00, 1.06 (5 s, 15H, 5CH_3), 1.02–2.48 (m, 24H, CH and CH_2), 3.20 (br. s, 1H, OH), 4.20–4.35 (m, 2H, H-30), 7.08–7.39 (m, 4H, H-arom), 7.48 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.54, 15.65, 17.21, 19.56, 20.97, 21.26, 26.67, 27.29, 28.93, 29.58, 31.24, 33.38, 34.01, 36.77, 37.43, 39.46, 40.46, 42.26, 46.48, 47.28, 49.42, 54.70, 56.11, 56.61, 68.44 (C-30), 106.88 (C-arom); 110.34 (C-arom), 117.92 (C-arom), 118.88 (C-arom), 124.44 (C-arom), 128.35 (C-arom), 136.13 (C-arom), 140.85 (C-arom), 181.27 (C-28), 213.13 (C-20); MS: *m/z* 546 $[M + H]^+$. Anal. calcd for $C_{35}H_{47}NO_4$: C, 77.03; H, 8.68, N, 2.57. Found: C, 77.05; H, 8.67, N, 2.56.

4.2.5. Synthesis of 3-oxo-3-homo-3-aza-lup-20(29)-en-17-nitrile ***(17)***

To a solution of 3,28-dioximino-lup-20(29)-en (1 mmol; 0.47 g), obtained according [52] in dried 1,4-dioxane (15 mL), $SOCl_2$ (3 mmol; 0.22 mL) was added drop wise with cooling. The obtained mixture was stirred at room temperature for 30 min and then poured into H_2O (50 mL). The precipitate was filtered off, washed with water until neutral pH. The product was purified by column chromatography, using $CHCl_3$ for elution. Yellow solid; yield (69%); m.p.: 195 °C; $[\alpha]_D^{20}$ = +27° (c 0.10, $CHCl_3$); IR (ύ max, cm^{-1}): 1680 (C═O), 3230 (NH), 2246 (C═N); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82, 1.03, 1.04, 1.21, 1.30, 1.62 (6 s, 18H, 6CH_3), 1.70–2.68 (m, 25H, CH and CH_2), 4.61 and 4.73 (both d, ^2J = 2.0 Hz, 2H, H-29), 5.92 (br. s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.65, 15.88, 18.21, 19.36, 21.78, 22.39, 25.25, 27.13, 28.94, 29.46, 30.93, 31.62, 33.38, 33.70, 35.75, 39.07, 40.29, 40.63, 41.35, 42.41, 48.48, 48.96, 49.06, 50.82, 53.09, 56.70, 111.04 (C-29), 123.40 (C-17), 147.94 (C-20), 176.61 (C-3) ppm; MS: *m/z* 450 $[M + H]^+$. Anal. calcd for $C_{30}H_{46}N_2O$: C, 79.95; H, 10.29; N, 6.22. Found: C, 79.93; H, 10.28; N, 6.21.

4.3. Solubility assay

The solubility of compounds **1**, **3–5** and **14** was examined simultaneously in distilled water and different 10 μM sodium phosphate solutions of pH 7.1 to 11.8. Each triterpene derivatives was dissolved in the aqueous media (100 mg/mL) and heated to 100 °C for one hour under reflux. The sample was cooled to room temperature. Filtered and analyzed daily, until saturation according [53].

4.4. Pharmacological studies

4.4.1. α-Glucosidase inhibition assay method

The α-glucosidase inhibition assay was performed according to the method of [54,55] with slight modification. Compounds were dissolved in DMSO at a concentration of 40 mg/ml. A series of dilutions for each compound were prepared in 100 mM phosphate buffer (pH 6.8) to final concentrations of 256, 64, 16, 4 and 1 μg/mL. In a 96-well plate, a reaction mixture containing 10 μL of compounds of varying concentrations, 40 μL of 100 mM phosphate buffer (pH 6.8), 25 μL of α-glucosidase (0.4 U/mL, Sigma G0660) were pre-incubated for 10 min at 37 °C. DMSO was added to the respective control wells to a 0.64% final concentration. Then 25 μL of a 2.5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG, Sigma N1377) solution was added to the mixture as a substrate. After further incubation at 37C for 30 min, the reaction was stopped by addition of 100 μL of 0.2 M sodium carbonate. The amount of released p-nitrophenol from pNPG was measured at 410 nm with microplate reader equipped spectrophotometer. Acarbose was used as a positive control, and all assays were carried out in triplicate. The % inhibition was obtained using the formula:

$$\%\text{Inhibition} = 100\% \times (OD_{control} - OD_{sample})/OD_{control}$$

IC_{50} value was defined as the concentration of compound exhibiting 50% inhibition of α-glucosidase activity under the assay conditions.

4.4.2. Chemoinformatic methods

Library preparation, calculation of molecular descriptors and compound properties were conveniently performed with OSIRIS DataWarrior 4.7.2 [56].

4.4.3. Molecular docking

Input structures were prepared with MarvinSketch 18.8.0 (ChemAxon Ltd.) [57]. Ligands were hydrogenized and conformations with minimal potential energy were obtained using the MMFF94 force field. Protein and ligand structures followed standard preparation procedure using AutoDocTools 1.5.6. We used AutoDock Vina 1.1.2 [58] to perform all docking runs. Grid box with 70 × 80 × 80 Å dimensions was centered on the enzyme molecule to include the entire 3D-space around the protein. The top-score binding poses were used in subsequent analysis. Protein-ligand interactions were analyzed with Discovery Studio Visualizer 17.2.0.16349 (Dassault Systemes Biovia Corp.) [59].

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.102957.

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