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Rapid access to new bioconjugates of betulonic acid via click chemistry

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ABSTRACT

Plant-derived pentacyclic triterpenoids of lupane and oleanane families provide a versatile structural platform for the discovery of new biologically active compounds. A number of semisynthetic derivatives of these molecules, possess high medical efficiency including antiviral (HIV-1), anticancer and immunomodulating activity. Even small structural changes in these triterpenoid derivatives were reported to lead to significant changes in their activity, making a convincing case for a systematic study of structure–activity relationships in this class of compounds.

Our earlier work opened synthetic access to alkynes derived from the betulonic scaffold and enabled the development of a new family of biohybrids using Click Chemistry (CC). The computer-aided prediction of several types of biological activity were performed with program PASS (Prediction Activity Spectra of Substances. Experimental studies based on mouse models verified the SAR predictions obtained by the PASS program. The observed correlation between the anti-inflammatory and antioxidant activity indicates substantial contribution of the latter in the mechanism of anti-inflammatory effect of the triazole derivatives of betulonic acid.

Innovations in organic synthesis often open new avenues for accelerated drug discovery. Cycloaddition of azides and alkynes, commonly referred to as 'click chemistry'[1] provides a particularly efficient approach to the rapid construction of complex organic molecules for the drug design.[2] This remarkably simple, selective and cost-effective process continues to find new applications in such fields as chemical biology,[3] materials science,[4] development of sensors[5] and polymer chemistry.[6]

Although 1,3-dipolar cycloaddition of organic azides and alkynes has been first reported and thoroughly studied by Huisgen,[7] the classic version of this cycloaddition required high temperatures and led to the formation of two regioisomers,[2] requiring an undesired separation step. In a significant breakthrough, two groups independently reported in 2002[8,9] that Cu(II) catalysis provides 10 million-fold acceleration to this reaction. Even more importantly, the copper catalyst directs the cycloaddition towards the formation of only one of the two regioisomers, namely the 1,4-isomer. The transformations proceed under mild conditions and are compatible with the majority of functional groups. Taken together, these factors account for the significant potential of click chemistry for the systematic search of new medicinal agents.[2,1]

Plant-derived pentacyclic triterpenoids of lupane and oleanane families provide a versatile structural platform for the discovery of new biologically active compounds. A number of semisynthetic derivatives of these molecules, possess high medical efficiency including antiviral (HIV-1), anticancer and immunomodulating activity.[10] Even small structural changes in these triterpenoid derivatives were reported to lead to significant changes in their activity,[11] making a convincing case for a systematic study of structure–activity relationships in this class of compounds. Previously, we have described the first synthesis of acetylenic derivatives of betulonic acid and our initial exploration of chemical and medicinal properties of these compounds.[12,13] Despite a limited number of examples, this approach proved itself promising since three of the reported compounds exhibited high hepatoprotective and anti-inflammatory activity.[14] Importantly, the previous work opened synthetic access to alkynes derived from the betulonic scaffold, thus lending itself to the development of a new family of biohybrids using click chemistry.

Synthesis of the alkyne starting material **1** has been described by us earlier.[12,14] The Ph-linker spatially separates the lipophilic terpene scaffold from the modification site, decreasing repulsive interactions between the alkyne and azides in the cycloaddition transition state (Fig. 1).

Gratifyingly, the click reaction of terminal acetylene **1** with alkyl and arylazides proceeded smoothly in toluene or *n*-butanol

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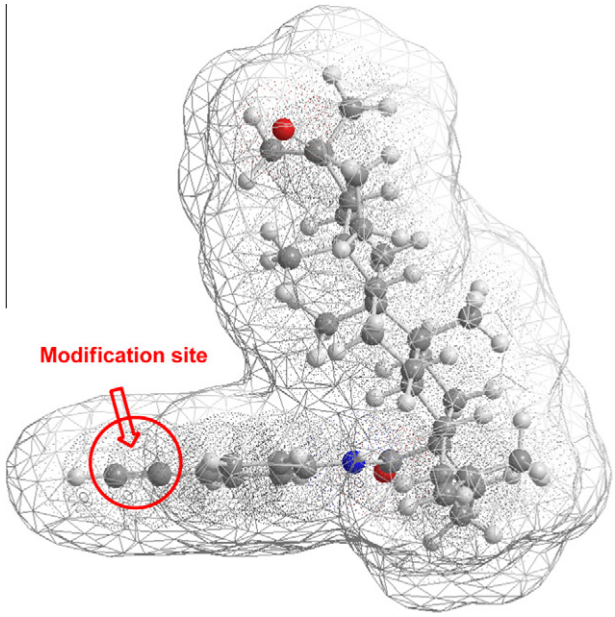
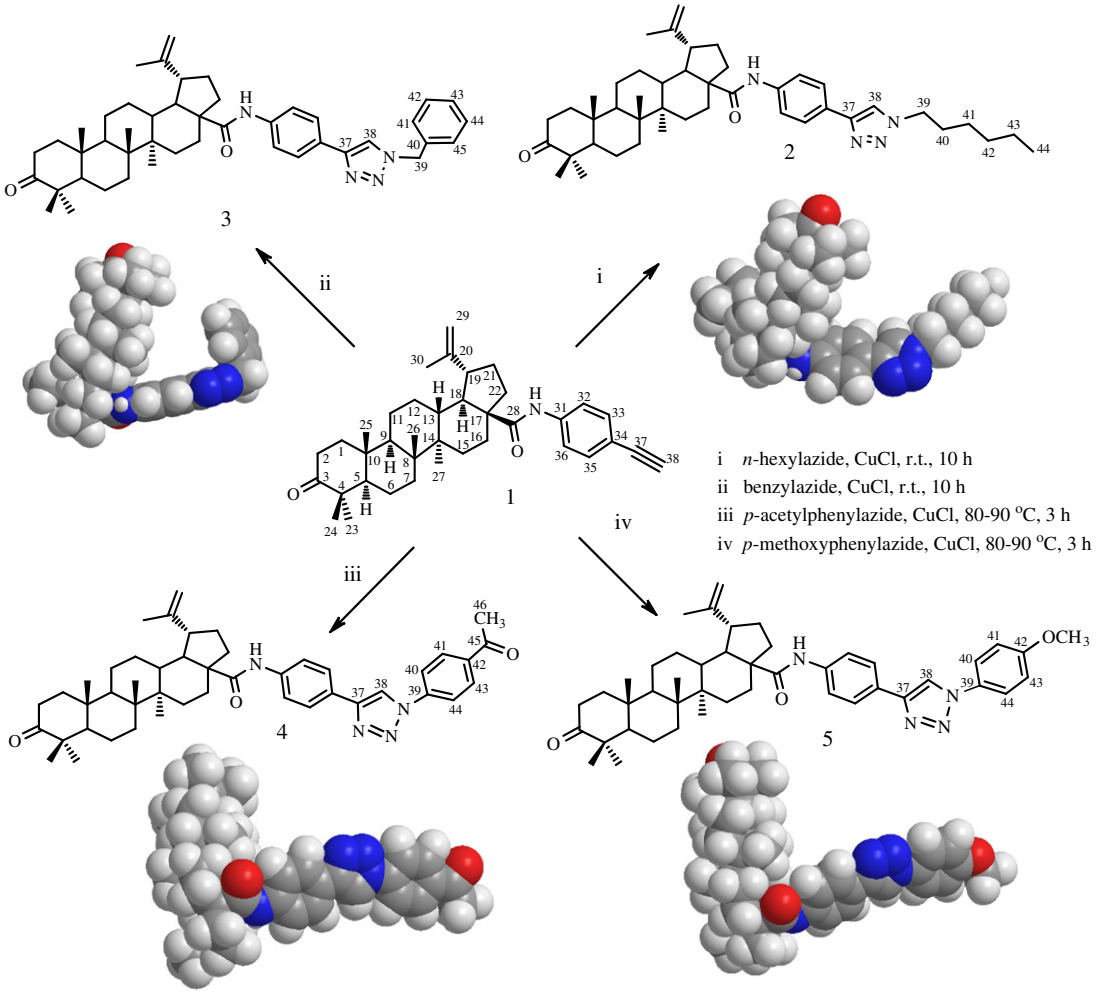


Figure 1. Molecular shape of compound **1** (MM2 optimization) with solvent accessible surface.

in the presence of CuCl and afforded 4-substituted 1,2,3-triazoles **2–5** in 67–88% yields. Interestingly, alkylazides react at the room temperature whereas the more stable aryl azides require heating up to 90 °C (Scheme 1, see Supplementary data for full experimental details).

^{1}H- and ^{13}C-assignments for the aromatic part of the NMR spectra of compounds **3** and **4** was accomplished on the basis of 2D-heteronuclear correlation techniques which provided both direct (hxcobiqf) and remote (coloc) spin–spin coupling constants. Spectral assignments for the triterpenoid part were based on analogy with Ref.[15] In particular, in the 2D spectra with remote correlation for compound **3**, protons of 39-CH_2-group correlate with carbons C-37 and C-38 as well as *ipso*-C-40 and *ortho*-C-41-45 aromatic carbons. On the other hand, proton from the 38-CH moiety interacts with C-37 and *ipso*-C-34 carbons of the *para*-substituted ring which confirms this compound being a 4-isomer.

For compound **4**, protons from the CH group correlate with C-37 and *ipso*-C-34 carbons of the *para*-substituted ring but the correlation with C-37 from the acetyl-substituted benzene ring could not be observed. However, this information should not be taken as an evidence for the 1,5-isomer structure and can be attributed, instead, to the weak spin–spin interaction through the nitrogen atom. One should also note the similarity in chemical shifts of C-37 and C-38 carbons in compounds **3** and **4**. Final experimental confirmation has been obtained from the Overhauser effect



Scheme 1. Synthesis and MM2-optimized geometries of betulonic acid/triazole hybrids.

Table 1
The computer-aided prediction of biological activity for triazole derivatives of betulonic acid

Mode of biological activity	Probabilities of biological activity of agents **2**–**5**							
	2		**3**		**4**		**5**	
	Pa	*Pi*	*Pa*	*Pi*	*Pa*	*Pi*	*Pa*	*Pi*
Anti-inflammatory	0.552	0.046	0.561	0.043	0.626	0.027	0.589	0.035
Antinephritic	0.504	0.243	0.552	0.150	<0.500	0.370	0.526	0.199
Antineoplastic	0.532	0.074	<0.500	0.087	0.515	0.079	0.531	0.074

Pa—probability 'to be active'; *Pi*—probability 'to be inactive'.

Table 2
Anti-inflammatory activity of betulonic triazoles in the histamine-induced paw edema model

Agent	Dose (mg/kg)	Inflammation index (%)	Edema size relative to control (%)	Anti-inflammatory effect (%)
Control	—	32.6 ± 3.1	100	0
2	20	29.0 ± 2.6[a]	89.0	11
	50	28.4 ± 2.1[a]	87.1	13
3	20	34.1 ± 4.4[a]	104.6	0
	50	25.6 ± 2.6[a]	78.5	21
4	20	35.4 ± 4.0[a]	108.6	0
	50	24.2 ± 1.9[a,b]	74.2	26
5	20	25.6 ± 3.1[a]	78.5	21
	50	25.4 ± 2.6[a]	77.9	22
Indomethacin	20	12.2 ± 1.6[c]	37.4	63

[a] P <0.001 relative to the reference agent.
[b] P <0.05.
[c] P <0.001 relative to the control.

Table 3
Compound effect at the concentration of thiobarbituric acid-reactive substances (TBARS) in the blood serum of mice with chronic hepatitis

Agent	Concentration of TBARS		Activity (%)
	(μmol/l)	(%)	
Control	2.33 ± 0.33	100	0
2	1.54 ± 0.28	66	34
3	1.36 ± 0.23[a]	58	42
4	0.93 ± 0.14[b]	40	60
5	0.96 ± 0.08[c]	41	59
Dihydroquercetin	1.34 ± 0.28[a]	57	43

[a] P <0.05.
[b] P <0.01.
[c] P <0.001 relative to the control.

between hydrogens of 38-CH-moiety and *ortho*-hydrogen atoms of both phenyl rings. Spectral assignments for compound **5** have been based on assignments for **4** and the correction for the introduction of OCH_3-group.

The triazole derivatives of lupane family represent a poorly studied class of triterpene compounds and, thus, provide an interesting target for pharmacological screening. Due to the lack of literature data regarding biological activity of such triazoles, we investigated the 'structure–activity' relations for the new triazole derivatives of betulonic acid using program PASS (Prediction Activity Spectra of Substances).[16] Our goal was to provide experimental verification for the computer-aided predictions for the biological activity of compounds **2**–**5**.

According to the computational predictions, all synthesized substances have the drug-likeness probability of more than 0.7. Table 1 shows the results of biological activity prediction at any values of *Pa* (no *Pa* limit). The main types of activity, with probability of more than 0.500, are anti-inflammatory, antinephritic and antineoplastic (Table 1).

Table 1 illustrates that the principal pharmacological effect of betulonic triazoles is the anti-inflammatory activity which is derived from both the triterpenoid scaffold and the side chain structure. The probability of anti-inflammatory effect in triazoles increases upon changing of hexyl substituent at the triazole moiety into a benzyl (**3**) and, to an even larger extent, acetylphenyl (**4**) and metoxyphenyl (**5**) group. The antinephritic activity of these compounds is likely to stem from their immunosuppressive action, in which they behave as interleukin 5 antagonists.

For experimental verification of SAR predictions, we carried out animal studies of anti-inflammatory activity of the new set of betulonic conjugates using histamine-induced paw edema model. In addition, antioxidant properties of the triazoles were tested using the toxic hepatitis model, which allowed us to ascertain their effect through the manifestation of oxidative stress accompanying inflammation.

We established that, in the doze range of 20–50 mg/kg these compounds show relatively low anti-inflammatory activity (Table 2). Only one conjugate **4** caused statistically significant effect when introduced at the highest dose, its activity being half of that of indomethacin. Table 2 illustrates that anti-inflammatory activity increase for the triazole derivatives of betulonic acid in the following order **2** < **3** < **5** < **4**.

In the model of CCl_4-induced hepatitis, all compounds administrated orally in the dose of 20 mg/kg were shown to significantly decrease the concentration of secondary oxidation products in blood. Notably, compounds **2** and **3** are comparable with the reference antioxidant dihydroquercetin whereas compounds **4** and **5** exceed this activity by the factor of 1.4 (Table 3).

Table 3 illustrates that antioxidant effect increases in the order of **2** < **3** < **5** < **4** which correlates with the observed anti-inflammatory activity. Therefore, experimental results with the mouse models verified the SAR predictions obtained by the PASS. The observed correlation between the anti-inflammatory and antioxidant activity indicates substantial contribution of the latter in the mechanism of anti-inflammatory effect of the triazole derivatives of betulonic acid.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.072.

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