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Betulonic acid–peptide conjugates: synthesis and evaluation of anti-inflammatory activity

Anastasiya I. Govdi,[a] Sergei F. Vasilevsky,*[a] Nadezhda V. Sokolova,[b] Irina V. Sorokina,[c] Tatyana G. Tolstikova[c] and Valentine G. Nenajdenko*[b]

[a] *Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russian Federation. Fax: +7 383 330 7350; e-mail: vasilev@kinetics.nsc.ru*
[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 932 8846; e-mail: nen@acylium.chem.msu.ru*
[c] *N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russian Federation*

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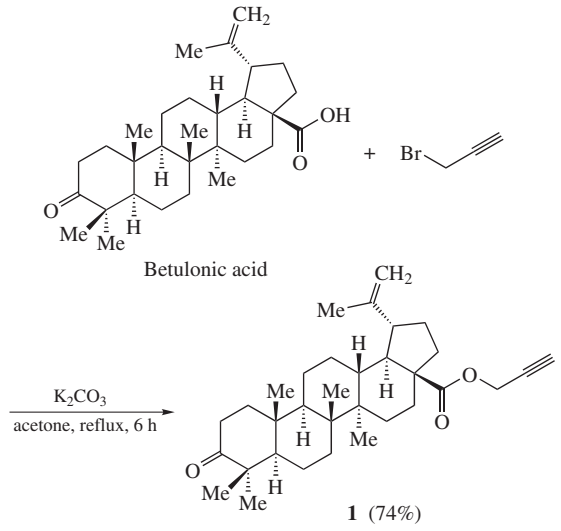
Cycloaddition of betulonic acid propargyl ester and azido-containing Boc-protected peptides afforded the corresponding 1,2,3-triazole-linked conjugates, whose anti-inflammatory properties were studied.

Betulonic acid and its derivatives can serve as attractive structural platforms for the development of new therapeutic agents[1,2] due to its high availability and a wide range of biological activities. However, the use of betulonic acid derivatives in medicine is still limited owing to their high lipophilicity and poor aqueous solubility.

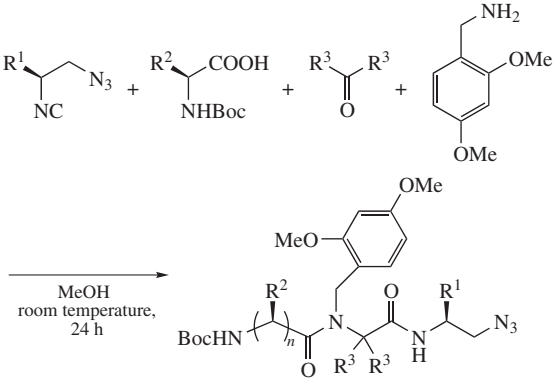
Synthesis of peptide derivatives of betulonic acid is of current interest for medicinal chemistry, since they exhibit high antiviral activity[3,4] and can act as inhibitors of the tumor cell growth.[5] We expected that incorporation of peptide fragment into betulonic acid core would reduce lipophilicity and increase aqueous solubility. Since calculated log *P* for betulonic acid is 6.8,[6] we expect the peptide conjugates after deprotection will have log *P* in range 4.5–6. Thus, collected literature data and our previous results prompted us to suggest a convenient route to the synthesis of 1,2,3-triazole-linked betulonic acid–peptide conjugates and study their pharmacological properties.

Recently, some of us reported synthesis and biological activity of betulonic acid amides containing 1,2,3-triazole moiety.[7] Along with anti-inflammatory properties, these compounds showed high antioxidant activity which exceeded the activity of the reference compound dihydroquercetin. Herein, we report the synthesis of new betulonic acid derivatives containing 1,2,3-triazole peptide fragments. For this aim, betulonic acid propargyl ester **1** (obtained by the reaction of betulonic acid with propargyl bromide in the presence of K_2CO_3) was chosen as a starting material (Scheme 1).[†]



Scheme 1

The starting azidopeptides[8] **2** are accessible by the Ugi four-component reaction of chiral isocyanoazides[9] with carbonyl compounds (formaldehyde or acetone), 2,4-dimethoxybenzylamine and Boc-protected amino acids.[10,11] Earlier, we have demonstrated that such peptides bearing azide function could be efficiently used for the modification of different biomolecules.[8,9,12] In addition, we prepared a series of new peptides **2a–d**, some of which contain hydrophilic amino acid residues such as serine and histidine (Scheme 2).



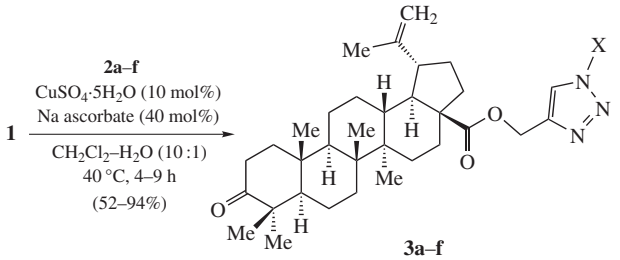
2a $R^1 = Pr^i$, $R^2 = H$, $R^3 = Me$, $n = 2$ (43%)
2b $R^1 = R^3 = H$, R^2 = (imidazol-2-yl)methyl, $n = 1$ (52%)
2c $R^1 = Bn$, $R^2 = CH_2OH$, $R^3 = Me$, $n = 1$ (50%)
2d $R^1 = Bn$, R^2 = (3-indolyl)methyl, $R^3 = Me$, $n = 1$ (72%)

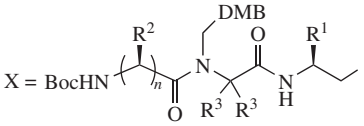
Scheme 2

Coupling between alkyne **1** and azides **2** was performed with 10% $CuSO_4 \cdot 5H_2O$–40% sodium ascorbate in a biphasic CH_2Cl_2–H_2O (10:1) mixture within 4–9 h at 40 °C and provided triazoles **3a–f** in good to excellent yields (Scheme 3).

Attempted deprotection in the obtained conjugates **3a–f** under various conditions also resulted in the cleavage of ester bond.

[†] For synthetic details and characteristics of all compounds, see Online Supplementary Materials.





3a $R^1 = Pr^i$, R^2 = H, R^3 = Me, n = 2
3b $R^1 = R^3$ = H, R^2 = (imidazol-2-yl)methyl, n = 1
3c R^1 = Bn, $R^2 = CH_2OH$, R^3 = Me, n = 1
3d R^1 = Bn, R^2 = (3-indolyl)methyl, R^3 = Me, n = 1
3e $R^1 = R^3$ = Me, $R^2 = Bu^s$, n = 1
3f $R^1 = R^3$ = Me, R^2 = Bn, n = 1

DMB = 2,4-dimethoxybenzyl

Scheme 3

Table 1 Anti-inflammatory activity of **3a–f** in the histamine-induced paw edema model.

Agent	Inflammation index (%)	Edema size relative to control (%)	Anti-inflammatory effect (%)
Control	22.7±1.1[a,d]	100	0[a,d]
3a	19.7±1.1[b,e]	86.8	13.2[b,e]
3b	17.6±2.5	77.5	22.5
3c	18.1±1.6[c,f]	79.7	20.3[c,f]
3d	20.1±1.6[b]	88.5	11.5[b]
3e	17.4±1.5[f]	76.6	23.4[f]
3f	23.0±1.0[a,e]	101.3	0[a,e]
Intraperitoneal injection of indomethacin, 50 mg kg^{-1}	13.3±1.3[g]	58.6	41.4[g]
Oral injection of indomethacin, 20 mg kg^{-1}	15.3±1.2[g]	67.4	32.6[g]

[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ compared with the group with intraperitoneal injection of indomethacin (50 mg kg^{-1}). [d] $P < 0.001$, [e] $P < 0.05$ compared with the group with oral injection of indomethacin (20 mg kg^{-1}). [f] $P < 0.05$, [g] $P < 0.001$ compared with the control group.

Nevertheless, we decided to obtain preliminary information on biological activity of the protected conjugates. The anti-inflammatory activity was evaluated using the histamine-induced paw edema model. Compounds **3e** and **3c**, which contain isoleucine and serine amino acid residues, respectively, displayed a little significant effect. The activity of compounds **3a**,**b**,**d** had no statistically significant value. The derivative **3f** showed no anti-inflammatory activity. Thus, all tested compounds appeared to be significantly inferior in activity to indomethacin given parenterally at a dose of 50 mg kg^{-1}, whereas compound **3a** is inferior to this drug given orally at a dose of 20 mg kg^{-1}.

In conclusion, we described the conjugation of betulonic acid propargyl ester **1** with azido peptides **2** *via* the Cu^I-catalyzed Huisgen 1,3-dipolar cycloaddition reaction. The anti-inflammatory activity of obtained compounds **3a–f** was evaluated using the histamine-induced paw edema model. Two compounds showed a little effect, significantly inferior in activity to indomethacin. We believe that deprotected conjugates can possess good activities, and search for synthetic access to such compounds is underway.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2013.09.007.

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