applied sciences

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Assessment of Bioavailability Parameters of Mono- and Bistriazole Derivatives of Propynoylbetulin

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Abstract: Bioavailability describes the properties that determine the passage of a compound through biological barriers. In many cases, bioavailability depends on the lipophilicity of the compound. In this study, the lipophilicity as well as other bioavailability properties of the mono- and bistriazole derivatives of betulin are presented. The lipophilicity was determined using RP-TLC and theoretical methods. The experimental lipophilicity of mono- and bistriazole derivatives is in the range from 4.39 to 7.85 and from 3.75 to 8.83, respectively. The lipophilicity of mono- and bistriazoles is similar, and the $logP_{TLC}$ depends on the type of substituent at the triazole ring. The introduction of a substituent with oxygen and nitrogen atoms decreases lipophilicity. Comparing the experimental and theoretical lipophilicity shows that the milogP and XLOGP3 programs best reproduce the experimental values. The in silico-determined pharmacokinetic parameters show that monotriazole derivatives could be used as oral drugs while bistriazoles show low availability after oral administration. Triazoles could be used as transdermal drugs. The analysis of in silico bioavailability parameters shows that the type of substituent at the triazole ring influences the pharmacokinetic properties, while the number of triazole rings slightly affects the bioavailability properties of the compound.

Keywords: betulin; triazole; lipophilicity

Citation: Kadela-Tomanek, M.; Sokal, A.; Stocerz, K.; Bębenek, E.; Chrobak, E.; Olczyk, P. Assessment of Bioavailability Parameters of Mono- and Bistriazole Derivatives of Propynoylbetulin. *Appl. Sci.* **2024**, *14*, 1695. https://doi.org/10.3390/app14051695

Academic Editors: Ivelina Desseva and Dasha Mihaylova

Received: 1 February 2024
Revised: 16 February 2024
Accepted: 19 February 2024
Published: 20 February 2024

1. Introduction

Bioavailability describes the properties of a drug which determine its absorption, distribution, metabolism, and elimination from an organism. One of the most important properties which determine bioavailability is lipophilicity, which describes the ability of a compound to cross the biological barrier. Often, with increased lipophilicity, the biological activity of a compound increases due to better permeability through biological membranes and a higher concentration of the compound inside the cell. However, too high lipophilicity may reduce the absorption of the compound due to its absorption in biological membranes [1–4]. A measure of lipophilicity is the partition coefficient between non-polar and polar phases defined as P or logP. The precursor of the determination of the P coefficient was Hansch, who designated the concentration of a compound in a composite mixture with two phases. As a non-polar and polar phase he used n-octanol and water, respectively. The method named "shake-flask" is laborious, time-consuming, and low-precision. For this reason, liquid chromatographic methods are most popular, namely,

thin-layer chromatography in a normal phase system (NP-TLC) and its variant, thin-layer chromatography in a reversed phase system (RP-TLC). Thin-layer chromatography methods have many advantages compared with the "shake-flask" method. The most important is possibility of determined the lipophilicity of many compounds in the same time, low time-consuming, high-precisions and high reproducibility [5–10].

Lipophilicity is connected to other parameters, like hydrophobicity (φ_0), permeability by Caco-2 membrane, skin, blood–brain barrier (BB), and penetration to the central nervous system (CNS). The design of a new compound is often supported by computational methods which determine the lipophilicity and permeability parameters using in silico methods [11,12]. However, most programs determining the logP parameter calculate lipophilicity using atomic methods which do not include every structural parameter, such as the spatial arrangement of atoms or the position of a heterocyclic atom in a ring. In the literature, many examples of compounds described for which the theoretical lipophilicity is very different from the experimental one. The obtained theoretical value could eliminate the compounds from further research. For this reason, determining the correlation between experimental and theoretical parameters is still an important problem [13–17].

In the past few decades, the isolation of compounds from plant material has attracted considerable attention due to pharmacologically active compounds using in natural medicine. In many cases, natural compounds have moderate biological activity and bioavailability [18–21]. One of the first compounds isolated in pure form from plant material was betulin (Figure 1). Betulin belongs to a pentacyclic triterpene alcohol with a lupane skeleton. The chemical structure of betulin contains four six-member rings and one five-member ring. Moreover, they have an isopropylidene group at the C19 position and two hydroxyl groups at the C3 and C28 positions. The compound is characterized by a broad spectrum of biological activity, like anticancer, antibacterial, antiviral, antifungal, hepatoprotective, and anti-inflammatory properties. The used of betulin in treatment is limited due to its low water solubility [22–28].

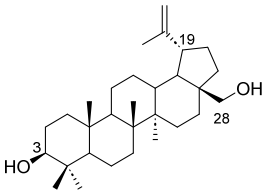


Figure 1. Chemical structure of betulin.

Chemical modifications of its structure allow new semisynthetic compounds to be obtained which have better biological properties. The replacement of one or two hydroxyl groups by an ester, amide, or carbamate group influences the activity and solubility in water of the obtained derivatives [29,30].

An interesting trend observed in chemical synthesis is the introduction of heterocyclic moieties. The insertion of moiety with nitrogen, oxygen, or/and sulfur atoms affects biological activity because heteroatoms could create a hydrogen bond with the biological target and increase water solubility [31,32]. An especially interesting moiety is the triazole scaffold which consists of two carbon and three nitrogen atoms in a five-membered unsaturated ring. The triazole ring is created between organic azide and alkyne derivatives in the reaction of 1,3-dipolar cycloaddition [33–35]. The triazole ring is found in many clinically used drugs, like Fluconazole, Bittertanol, Cyproconazole, Trazodone, Triazolam, Ribavirin, Isavuconazole, and Cefatrizine [35–40]. In the literature, the triazole moiety is often used as a linker between two compounds showing high biological activity, which allows hybrids with new biological properties to be obtained. In many cases, the modification of natural compounds by introducing a triazole substituent affects biological activities, like anticancer, antibacterial, antifungal, antiviral, antimalarial, and antioxidant [41–45].

In our scientist group, we deal with the modification of the betulin skeleton by introducing various substituents at the C3, C28, and C30 positions [46,47]. One of the important research directions was the introduction of the triazole moiety to the betulin scaffold. The obtained compounds exhibited anticancer, antibacterial, and antiviral activity [13,48–50]. Continuing research on the biological potential of triazole derivatives of betulin, we decided to designate the bioavailability parameters of mono- and bistriazole derivatives of propynoylbetulin. Lipophilicity was determined using experimental and theoretical methods. Moreover, the correlation between these two values was studied. The research was supplemented by in silico-determined bioavailability parameters.

2. Materials and Methods

2.1. General Method

Reversed phase thin-layer chromatography (RP-TLC) was carried out on silica gel RP-18 $F_{254}S$ plates (Merck, Darmstadt, Germany) using a different mixture of acetone and (tris-hydroxymethyl)aminomethane (Tris) as an eluent. The concentration of acetone in the mobile phase changed from 60% to 90%. The chromatographic spots were visualized using UV light (λ = 254 nm) and by spraying with a solution of 10% sulfuric acid for the reference compounds and compounds **1**–**18**, respectively.

2.2. Data Set

Propynoilbetulin **1**–**2** and their triazole derivatives **3**–**18** were synthesised using the methods found in the literature [48,51]. In the first stage, betulin was converted to derivatives **1**–**2** in the presence of propiolic acid, N,N′-dicyclohexylcarbodiimide (DCC), and 4-dimethylaminopyridine (DMAP) at room temperature. After purification using column chromatography, 28-propynoilbetulin **1** and 3,28-dipropynoilbetulin **2** were obtained with 52% and 15% yields, respectively. Next, compounds **1**–**2** were treated with organic azide in the presence of copper iodide and toluene at reflux. The crude products were purified using column chromatography. Triazole derivatives **3**–**18** obtained with 48-78% yield. The molecular structure of derivatives **1**–**18** is presented in Figure 2.

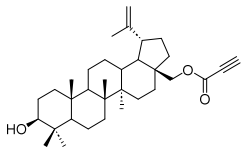
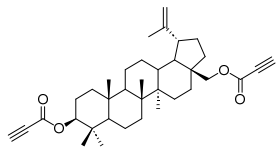
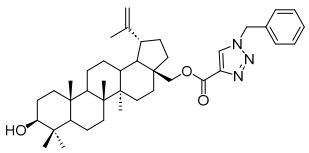
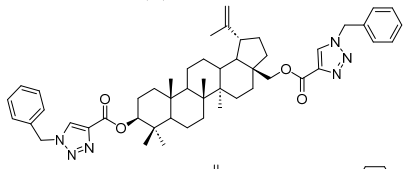
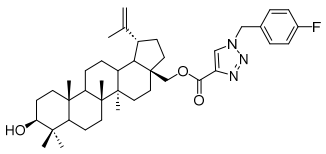
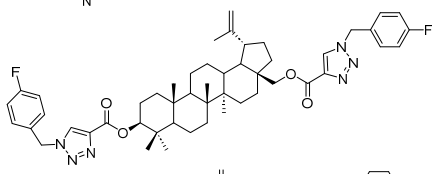
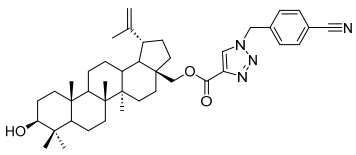
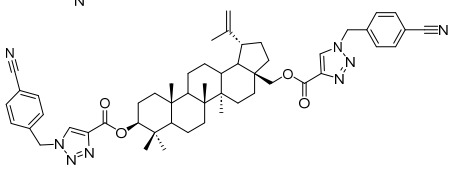
Compound	Chemical structure	Compound	Chemical structure
1		**2**	
3		**11**	
4		**12**	
5		**13**	

Figure 2. *Cont.*

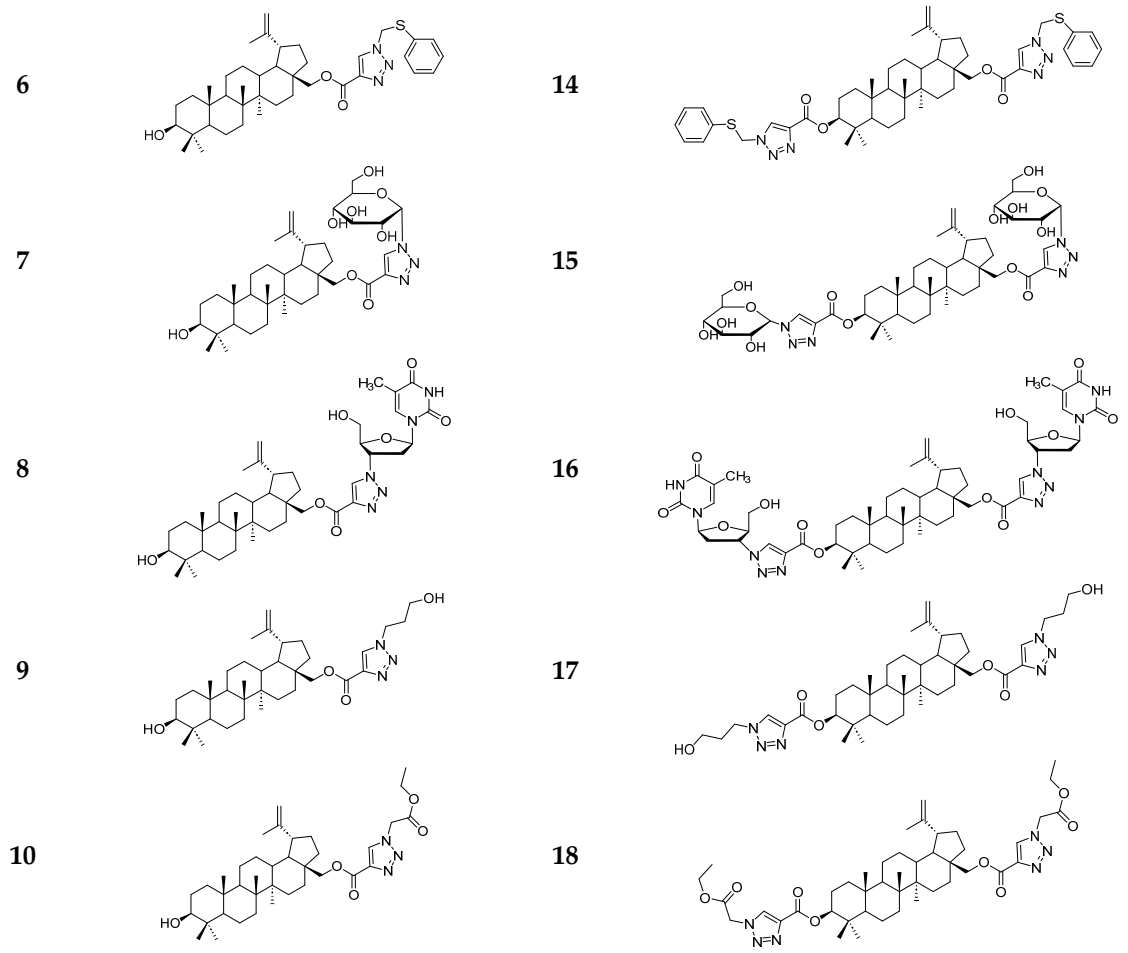


Figure 2. Molecular structure of compounds **1**–**18**.

2.3. Assessment of Experimental and Calculated Lipophilicity

The experimental lipophilicity was determined using the RP-TLC method. As a stationary phase, un-polar silicone oil placed on the silica gel layer was used, and as a mobile phase, the mixture of (tris-hydroxymethyl)aminomethane (0.2 M, pH = 7.4) with acetone was used. The concentration of acetone in the mobile phase was in the range from 60% to 90% in 5% increments.

A total of 2 µL of the ethanolic solutions of compounds **1**–**18** (1 mg/mL) and reference compounds was marked on the chromatographic plates. After visualization, the retardation factor (R_f) value for each compound was designated. The R_M parameter was calculated using Equation (1) below.

$$R_M = \log\left(\frac{1}{R_f} - 1\right), \quad (1)$$

The relative lipophilicity parameter (R_{M0}) was obtained by the extrapolation of linear regression between R_M and acetone concentration (C) to the zero value of the organic solvent (Equation (2)).

$$R_M = R_{M0} + bC, \quad (2)$$

The R_{M0} and the slope of the regression plot (b) were used to calculated the hydrophobic index (φ_0) according to Equation (3).

$$\varphi_0 = -\frac{R_{M0}}{b}, \quad (3)$$

The experimental lipophilicity study was supplemented by theoretical lipophilicity, which was determined using vcclab (2005) [52] and molinspiration (v2022.09) [53] software.

2.4. Assessment of Bioavailability Parameters

The Lipinski and Veber parameters, such as molecular weight (M), topological polar surface (TPSA), the number of acceptors (HAs) and donors (HDs) of the hydrogen bond, and the number of rotatable bonds (RTs) were determined using SwissADMET (2022) platform [54]. Moreover, the absorption and neurotoxicity parameters, like Caco-2 permability (logPapp), human intestinal absorption (HIA), skin permeability (logKp), blood–brain barrier permeability (logBB), and central nervous system (logPS) permeability were designated using pkCSM (2015) software [55].

3. Results

In the first step of the research, the experimental lipophilicity was determined by using the RP-TLC method. As a mobile phase, the mixture of acetone and Tris buffer was used. The concentration of acetone in the mobile phase was in the range from 60% to 90%. The obtained R_f values for each compound **1–18** were converted to R_M and R_{M0} parameters using Equations (1) and (2), and are presented in Table 1.

Table 1. The experimental values of lipophilicity and hydrophobicity parameters.

Compound	R_{M0}	*b*	r	$logP_{TLC}$	φ_0	Compound	R_{M0}	*b*	r	$logP_{TLC}$	φ_0
1	5.78	−0.06	0.995	6.68	90.17	**2**	8.09	−0.09	0.992	9.20	90.09
3	6.15	−0.07	0.997	7.09	86.86	**11**	7.05	−0.08	0.992	8.07	86.29
4	6.31	−0.07	0.994	7.26	86.68	**12**	7.15	−0.08	0.993	8.17	86.04
5	6.29	−0.07	0.993	7.24	85.12	**13**	6.57	−0.08	0.995	7.54	83.38
6	6.85	−0.08	0.988	7.85	87.15	**14**	7.75	−0.09	0.994	8.83	87.18
7	3.67	−0.05	0.988	4.39	73.11	**15**	3.08	−0.05	0.950	3.75	59.69
8	4.56	−0.06	0.992	5.36	78.76	**16**	5.68	−0.08	0.998	6.58	70.65
9	4.44	−0.05	0.994	5.23	81.92	**17**	4.35	−0.06	0.990	5.13	75.65
10	5.57	−0.07	0.994	6.46	85.04	**18**	6.59	−0.08	0.987	7.57	82.89

The experimental values of R_{M0} and slope *b* were used to determine the hydrophobicity index according to Equation (3), and are presented in Table 1.

The R_{M0} parameter was converted to the lipophilicity parameter ($logP_{TLC}$) using the calibration curve. As reference compounds, 4-bromoacetophenone (**A**), benzophenone (**B**), anthracene (**C**), dibenzyl (**D**), 9-phenylanthracene (**E**), and dichlorodiphenyltrichloroethane (DDT) (**F**) were used whose lipophilicity ($logP_{lit}$) in the literature is in the rage from 2.43 to 6.38 [13,14].

The experimental lipophilicity of the reference compounds was determined similarly to the tested compounds **1–18** (Table 2). The calibration curve (Equation (4)) was obtained by linear regression between the experimental R_{M0} parameter and lipophilicity ($logP_{lit}$) in the literature.

$$LogP_{TLC} = 1.0878\ R_{M0} + 0.3972 \quad (r = 0.988; SD = 0.263), \tag{4}$$

Table 2. The $LogP_{lit}$ and $LogP_{TLC}$ values for reference compounds **A–F**.

Compound	$LogP_{lit}$	R_{M0}	*b*	r	$LogP_{TLC}$
A	2.43	1.87	−0.02	0.985	2.43
B	3.18	2.42	−0.02	0.981	3.03
C	4.45	4.08	−0.03	0.992	4.84
D	4.79	4.11	−0.05	0.970	4.87
E	6.01	4.87	−0.04	0.972	5.69
F	6.38	5.50	−0.06	0.985	6.38

According to Equation (4), the experimental lipophilicity ($logP_{TLC}$) of the reference compounds was calculated (Table 2). As seen in Table S1, the experimental results correlate well with the ones in the literature.

In the next step, the experimental lipophilicity was compared with the calculated lipophilicity which was determined using the ALOGPs, miLogP, XLOGP2, AClogP, and XLOGP3 programs. The results are shown in Table 3.

Table 3. The calculated lipophilicity of compounds **3**–**18**.

Compound	ALOGPs	AClogP	miLogP	XLOGP2	XLOGP3
3	6.60	6.97	8.70	9.87	10.21
4	6.60	7.02	8.77	10.30	10.31
5	6.42	6.78	8.56	9.59	9.93
6	6.67	9.11	8.90	10.29	10.85
7	4.11	3.51	5.08	6.11	6.39
8	5.01	4.21	6.58	6.71	7.72
9	5.38	5.26	7.05	7.81	8.22
10	5.77	5.29	7.65	8.29	9.00
11	7.39	8.09	9.35	11.93	12.14
12	7.45	8.21	9.43	12.25	12.34
13	7.40	7.72	9.21	11.37	11.58
14	7.56	8.38	9.56	12.77	13.42
15	2.87	1.17	3.00	4.40	4.49
16	4.69	2.59	6.00	5.61	7.17
17	5.19	4.69	6.93	7.81	8.16
18	6.10	4.74	8.12	8.76	9.73

Lipophilicity is connected with other bioavailability parameters such as molecular mass (M), topological polar surface (TPSA), the number of acceptors (HAs) and donors (HDs) of the hydrogen bond, and the number of rotatable bonds (RTs). The research was supplemented with in silico-determined parameters responsible for the drug's permeation of the biological barrier, like Caco-2 cell (logPapp), human intestinal absorption (HIA), skin permeability (logKp), blood–brain barrier permeability (logBB), and central nervous system (logPS) penetration [56–58]. The results obtained using pkCSM software are presented in Table 4.

Table 4. The ADME parameters of compounds **3**–**18**.

Compound	M	TPSA	HA	HD	RT	logPapp	HIA	logKp	logBB	logPS
3	627.90	77.24	5	1	7	0.737	100	−2.730	−0.522	−1.288
4	648.89	77.24	6	1	7	0.808	100	−2.732	−0.741	−2.240
5	652.91	101.03	6	1	7	0.485	100	−2.733	−0.689	−1.355
6	659.95	102.54	5	1	8	0.660	99	−2.731	−0.699	−1.251
7	699.92	167.39	5	10	7	0.342	60	−2.735	−1.853	−3.671
8	761.00	152.87	9	3	8	0.250	99	−2.736	−1.314	−3.001
9	595.86	97.47	6	2	8	0.627	100	−2.780	−0.714	−2.606
10	623.87	103.54	7	1	9	0.658	100	−2.762	−0.928	−2.677
11	813.08	114.02	8	0	12	0.528	100	−2.735	−1.406	−2.289
12	849.06	114.02	10	0	12	0.484	100	−2.735	−1.845	−2.536
13	863.10	161.60	10	0	12	0.001	100	−2.735	−1.742	−2.402
14	877.21	164.62	8	0	14	0.529	100	−2.735	−1.764	−2.193
15	957.13	294.34	20	8	10	0.097	11	−2.735	−3.534	−5.467
16	1079.31	252.65	18	12	4	0.605	88	−2.735	−2.454	−4.070
17	748.99	154.48	10	2	14	0.071	100	−2.736	−1.815	−3.280
18	805.01	166.62	12	0	16	0.089	91	−2.735	−2.220	−3.420

4. Discussion

The use of new synthesised compounds in therapy depends on their biological activity and bioavailability. One of the most important properties of compounds is their lipophilicity which determines drug transport across the biological barrier. The determination of lipophilicity offers the opportunity to assess the availability and toxicity of a potential drug [1].

The propynoyl derivatives of betulin **1** and **2** are characterized by high lipophilicity which is equal to 6.68 and 9.20, respectively. Compounds **3**–**18** create two groups, the first of which includes the monotriazole derivatives of **1** and the second consists of the bistriazole derivatives of **2**. In the group of monotriazole compounds **3**–**10**, the lowest $logP_{TLC}$ value is shown by derivative **7** with the deoxy-β-D-glucopyranosyl substituent at the triazole ring. Derivatives of the benzyl group (**3**–**5**) exhibit comparable lipophilicity which is in the range from 7.09 to 7.24. The introduction of the phenyltiomethyl group at the triazole ring, compound **6**, increases lipophilicity. Comparing the $logP_{TLC}$ value of compounds **1** and **8**–**10** shows that the introduction of a substituent with oxygen and nitrogen atoms decreases lipophilicity.

The transformation of 3,28-dipropynoylbetulin **2** to bistriazole derivatives **11**–**18** decreases lipophilicity. Similar to the first group, in the group of bistriazole derivatives, the lowest value of lipophilicity is shown by compound **15** with the deoxy-β-D-glucopyranosyl substituent. In these groups of compounds (**11**–**18**), similar correlations were observed as for **3**–**10**. Comparing the lipophilicity of mono- and bistriazole compounds containing the same substituents at the triazole ring, bistriazole derivatives had higher log P_{TLC} values. The exceptions were the compounds with a β-D-glucopyranosyl substituent (**7** and **15**) and a hydroxypropyl substituent (**9** and **17**), of which derivatives with one triazole ring (**7** and **9**) had higher lipophilicity.

The hydrophobicity index describes the water solubility of a compound; if the φ_0 value is lower, then water solubility is better [59]. Comparing the φ_0 value of triazole derivatives **3**–**18** and compounds **1**–**2** shows that the introduction of a triazole ring increases water solubility. The hydrophobicity index is in the range from 73.11 to 86.86 and from 59.69 to 86.29 for mono- **3**–**10** and bistriazoles **11**–**18**, respectively. The results show that mono- and bistriazole derivatives have comparable solubility in water.

Before determining the experimental lipophilicity, it is necessary to synthesize and purify the tested compound. The experimental method is not useful during the computer design of new active compounds. For this reason, the experimental lipophilicity is replaced by theoretical methods which use a different algorithm [60–62].

The calculated lipophilicity for triazole compounds **3**–**18** was determined using theoretical methods used in software that is available online [52,53]. The obtained values were in the range from 1.17 to 13.42 (Table 3). For all programs, the theoretical lipophilicity depends on the type of substituent at the triazole ring. A relationship was observed, namely, that monotriazoles **3**–**10** have lower lipophilicity than bistriazoles **11**–**18**. The exceptions are compounds **7** and **15** containing the deoxy-β-D-glucopyranosyl substituent. Bistriazole derivative **15** has lower lipophilicity due to the larger number of hydroxyl groups which reduces the logP parameter. Comparing the experimental and theoretical lipophilicity shows that logP values obtained using the AClogP and ALOGPs programs are lower than the experimental one (Figure 3). For other programs, the calculated lipophilicity is higher than the experimental $logP_{TLC}$.

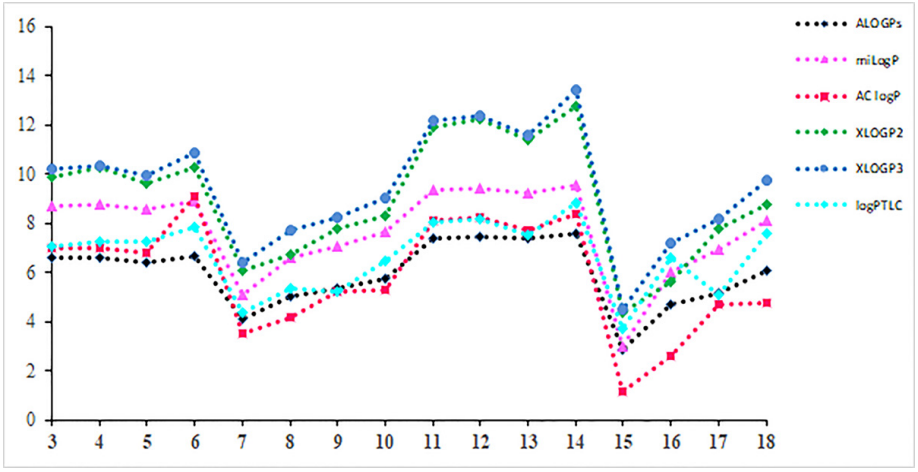


Figure 3. The profile of changes in theoretical lipophilicity for derivatives **3**–**18**.

Due to the fact that different programs use different calculating algorithms, an important aspect of the research is to determine the correlation between the experimental and theoretical lipophilicity (Table 5). The correlation equation shows that the ALOGPs and XLOGP3 programs best reproduce the experimental lipophilicity, and the correlation cofactor is equal to 0.928 and 0.937, respectively (Table 5).

Table 5. Correlation equation for the experimental (logPTLC) and theoretical (logPcalc) values of lipophilicity for derivatives **3**–**18**.

Program	Correlation Equation	r	SD
ALOGPs	$logP_{TLC}$ = 0.928 $LogP_{calc}$ + 0.580	0.928	0.670
ACLOGP	$logP_{TLC}$ = 0.841 $LogP_{calc}$ + 3.907	0.840	0.827
milogP	$logP_{TLC}$ = 0.916 $LogP_{calc}$ + 0.979	0.916	0.682
XLOGP2	$logP_{TLC}$ = 0.889 $LogP_{calc}$ + 1.926	0.889	0.675
XLOGP3	$logP_{TLC}$ = 0.937 $LogP_{calc}$ + 1.157	0.937	0.565

The lipophilicity values obtained by the correlation equation are presented in Table S1. The obtained values show that the experimental lipophilicity could be predicted using the milogP and XLOGP3 programs. The relationship between the lipophilicity and structure of the tested compounds **3**–**18** was analysed using the similarity analysis (Figure 4).

The cluster analysis shows that the compounds are localized in two main clusters. The derivatives localized in the first cluster have one or more hydroxyl groups as the substituent connected with the triazole ring. The second cluster is divided into three subclusters. In the first and third subcluster, mono- (**3**–**6**) and bistriazoles (**11**–**14**) are localized which contain a substituent with a phenyl ring. The second subcluster contains derivatives **10** and **18** which contain an ethylacetyle substituent at the triazole ring. The measure of similarity of two compounds is the Euclidean distance (ED). If the ED distance is similar, it means that the compounds exhibit similar features [63–66]. As seen in Table S2, the derivatives in different subclusters have the same ED distance, which means that the compounds show high similarity. The analysis of Euclidean distance shows that lipophilicity strongly depends on the type of substituent connected with the triazole ring. Moreover, the replacement of a hydroxyl group at the C3 position of betulin by a triazole substituent slightly affects the logP parameter.

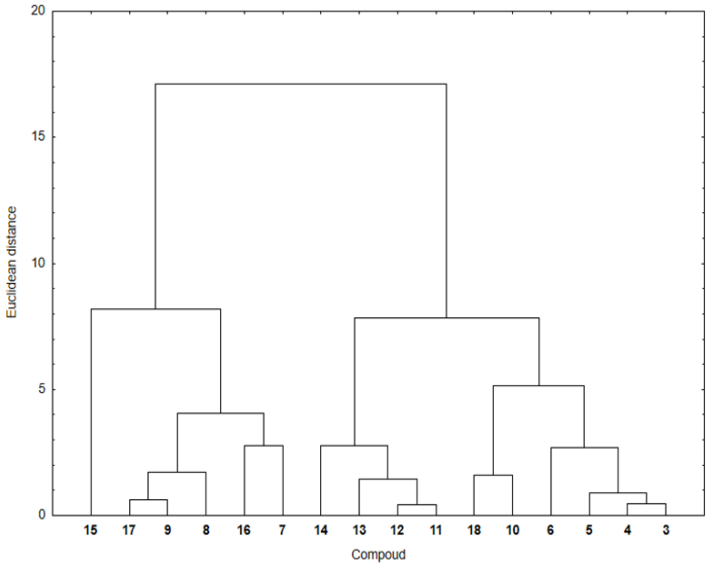


Figure 4. The cluster analysis of the experimental and theoretical lipophilicity for compounds **3**–**18**.

Lipophilicity is connected with other parameters which determine bioavailability. The first rules defining oral availability were described by Lipinski and Veber [67–69]. According to Lipinski's rule, lipophilicity should be less than 5. Only the experimental lipophilicity of derivatives **7** and **15** meets this condition. However, the $\log P_{TLC}$ of compounds **8**, **9**, and **17** is slightly greater than 5 (Table 1). Almost all derivatives meet the criteria that the number of donors (HDs) and acceptors (HAs) of the hydrogen bond are less than 5 and 10, respectively. Only compounds **15**–**16** do not meet both rules. The molecular weight (M) of derivatives **3**–**18** is higher than 500, which means that none of tested compounds meets the mass criterion. Veber's rules connect good oral bioavailability with topological polar surface (TPSA) and the number of rotatable bonds (RTs). Most of the tested monotriazoles **3**–**10** meet the Veber criteria, except for compounds **7**–**8** for which the TPSA value is more than 140 Å. None of the bistriazole derivatives **11**–**18** meets the Veber criteria. In summary, monotriazole derivatives **3**–**10** show moderate oral availability, and bistriazole derivatives **11**–**18** are characterized by low oral availability.

Multilinear regression (MLR) was used to determine the correlation equation between the experimental lipophilicity and the calculated parameters. The best correlation was obtained for the relationship between experimental lipophilicity, molecular mass (M), and topological polar surface (TPSA) (Equation (5)), for which the correlation cofactor is equal to 0.860. The lipophilicity ($\log P_{calc}$) values calculated using Equation (5) are presented in Table S1.

$$\log P_{calc} = 1.31M - 1.50\ TPSA + 1.120$$
$$(r = 0.860;\ SD = 1.465;\ VIP = 2.13;\ F = 18.45), \quad (5)$$

Comparing the lipophilicity obtained by different methods shows that Equation (5) reproduces the experimental lipophilicity better than the theoretical methods.

The physicochemical parameters were used to determine the in silico pharmacokinetic properties determining the absorption of the compound [70]. The intestinal mucosa absorption was described using Caco-2 permeability (logPapp) and human intestinal absorption (HIA) [56,57]. The logPapp values calculated for mono- and bistriazoles are in the range from 0.342 to 0.808 and from 0.001 to 0.605, respectively (Table 4). The tested compounds show moderate Caco-2 permeability, because the logPaap values are lower than 0.9. Most monotriazoles (**3**–**10**) were characterized by better Caco-2 permeability than bistriazoles (**11**–**18**). The higher HIA index shows that most derivatives could be absorbed from the gastrointestinal system into the bloodstream, except from triazole **15** which could be poorly absorbed. The tested derivatives **3**–**18** could be used as transdermal drugs because the calculated logKp for all compounds is lower than −2.5 [55]. The neurotoxicity of com-

pounds depends on the passage through the blood–brain barrier and the penetration of the central nervous system (CNS), which was determined by logBB and logPS parameters, respectively [58]. The in silico values of logBB and logPS were determined using pkCSM software [55]. The logBB value for the tested compounds is in the range from −2.220 to −0.522, which means that triazole was poorly distributed to the brain (Table 4). Comparing the logBB parameter of mono- (**3**–**10**) and bistriazoles (**11**–**18**) shows that the introduction of triazole substituent at C3 position of betulin scaffold reduces passage through blood–brain barrier. Most monotriazoles could penetrate the central nervous system because the logPS value is higher than −3, except for triazoles with a sugar moiety (**7** and **8**), which were unable to penetrate the central nervous system. In the series of bistriazoles (**11**–**18**), only compounds with a phenyl substituent in the triazole moiety (**11**–**14**) could penetrate the CNS. Based on the obtained results, it can be concluded that the introduction of a phenyl substituent to the triazole ring increases the neurotoxicity of the obtained compounds.

The ADME in silico-determined parameters were compared with the experimental lipophilicity ($logP_{TLC}$) using similarity analysis (Figure 5).

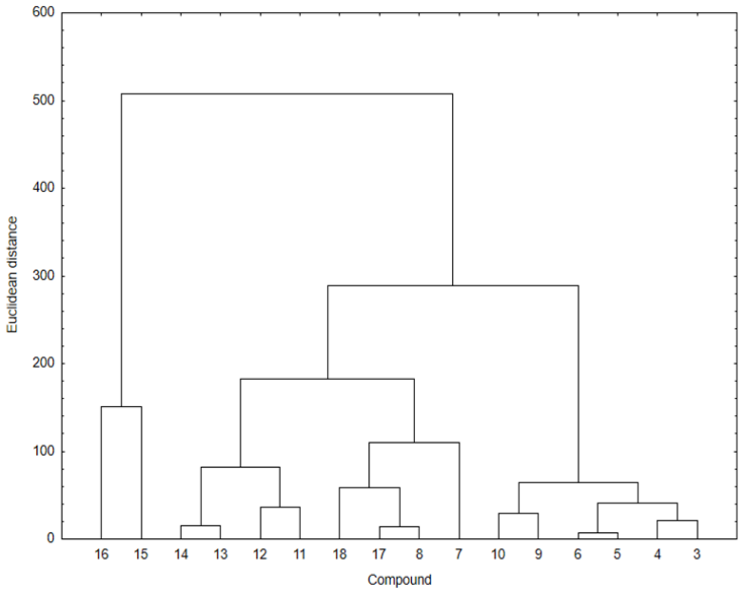


Figure 5. The similarity analyses of ADME parameters and experimental lipophilicity for compounds **3**–**18**.

As seen on Figure 5, compounds are localized in two main clusters. The first cluster includes bistriazoles with a sugar moiety (**15**–**16**). The second cluster is divided into three subclusters which contain bistriazoles **11**–**14**, compounds **7**–**8** and **17**–**18**, and monotriazoles **3**–**6** and **9**–**10**, respectively. The analysis of the Euclidean distance between subclusters shows that the type of substituent at the triazole ring influences the ADME (adsorption, distribution, metabolite, and extraction) properties, while the number of triazole rings slightly affects the bioavailability properties of the compound.

5. Conclusions

This research shows that triazole derivatives of betulin are characterized by high values of lipophilicity ($logP_{TLC}$) which is in the range from 3.75 to 8.83. Mono- and bistriazoles have similar $logP_{TLC}$ values. The lowest values were obtained for compounds with a hydroxyl group as the triazole substituent (**7**, **9**, **15**, and **17**). This research shows that lipophilicity depends on the type of substituent at the triazole moiety. The introduction of a triazole ring influences the water solubility of the compound. Comparing the hydrophobicity index of propynoyl betulin and its triazole derivatives shows that triazole compounds have better solubility. Moreover, bistriazoles have slightly better water solubil-

ity than monotriazoles. The experimental lipophilicity was compared with the lipophilicity determined using computer programs. The milogP and XLOGP3 programs best reproduce the experimental lipophilicity.

The bioavailability of compounds was determined using in silico-calculated Lipinski and Veber parameters. The analysis of parameters shows that monotriazoles **3–10** could be used as orally administered drugs while bistriazoles **11–18** have low absorption after oral administration. The molecular weight and topological polar surface could be used to determine lipophilicity, and the obtained $\log P_{calc}$ better reproduces the experimental value than the theoretical logP.

The last part of the study was the determination of in silico pharmacokinetic parameters responsible for the permeability of the gastrointestinal system, skin, and blood–brain barrier. The parameters determining the absorption from the gastrointestinal system (log-Papp and HIA index) show that monotriazole derivatives (**3–10**) were characterized by better Caco-2 membrane penetration than bistriazoles (**11–18**). Triazoles could be used as transdermal drugs because the logKp is lower than −2.5. The tested compounds were poorly distributed in the central nervous system. The similarity analysis showed that the ADME parameters depend on the type of substituent in the triazole moiety. Mono- and bistriazoles have similar properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14051695/s1, Table S1: The theoretical value of lipophilicity of compounds **3–18**. Table S2: The similarity parameter (ED) for the theoretical and experimental lipophilicity for compounds **3–18**.

Author Contributions: Conceptualization, methodology, and writing—original draft preparation, M.K.-T.; methodology, A.S. and K.S.; validation and formal analysis, E.B. and E.C.; writing—review and editing, P.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Medical University of Silesia, grant number BNW-1-013/N/3/F.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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