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Article

Chromatographic and Computational Screening of Lipophilicity and Pharmacokinetics of Newly Synthesized Betulin-1,4-quinone Hybrids

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Abstract: Lipophilicity is one of the most important parameters determining the pharmacodynamic and pharmacokinetic properties, as well as the toxicity of many compounds. The subject of the research was to determine the lipophilicity of betulin-1,4-quinone hybrids using thin layer chromatography in reverse phase system and computer programs to calculate its theoretical models. The correlation between the experimental and theoretical values of lipophilicity was analyzed. Lipinski's and Veber's rules, as well as penetration through the blood–brain barrier were also determined using computer programs. For all of the analyzed values, a similarity analysis was performed. The dendrograms for the experimental and theoretical lipophilicity show that there is a correlation between them. However, the dendrograms for the experimental lipophilicity and pharmacokinetic parameters indicate that there is no correlation between the structure and the pharmacological properties. Hybrids exhibit a high biological activity against cancer cell lines, with a high level of NAD[P]H-quinone oxidoreductase (NQO1) protein. The enzymatic assay used has shown that these compounds are good NQO1 substrates, as evidenced by the increasing metabolic rates relative to that of streptonigrin. The similarity analysis has also shown that there is no correlation between lipophilicity and biological activity for the tested compounds.

Keywords: lipophilicity; betulin; 5,8-quinolinedione; NQO1 protein; ADMET



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1. Introduction

Lipophilicity is one of the most important parameters determining the pharmacodynamic, pharmacokinetic, and toxicity parameters of many compounds. It determines the affinity of the molecule for the organic phase, which relates to the intermolecular interactions occurring between the solute and solvent molecules [1,2]. Lipophilicity can be determined by both direct and indirect methods. One of the direct methods is the shake-flask procedure, which determines lipophilicity as the relationship of the concentration of a compound in *n*-octanol and water. This method gives encouraging results, but it has many disadvantages, such as a tedious process, being time-consuming, a large amount of compound needed, and the requirement of a compound with a high purity. The indirect methods are based on chromatographic techniques, like reversed-phase thin layer chromatography (RP-TLC), normal-phase thin layer chromatography (NP-TLC), and high-performance liquid chromatography (HPLC). The easy experimental procedure, low cost, and ability to test multiple compounds in a single run make RP-TLC the most used method to determine lipophilicity [2–8].

Betulin **1**, derived from white species of birch bark, exhibits a wide spectrum of activity, including anticancer, antimicrobial, anti-viral, and anti-inflammatory properties. The

chemical structure of betulin **1** contains four six-membered rings and one five-membered ring. Additionally, in their structure, there are two hydroxyl groups at the C-3 and C-28 positions, and an isopropenyl group at the C-19 position (Figure 1). Modification at these positions can lead to new semi-synthetic derivatives, which exhibit a better biological activity and bioavailability [9,10].

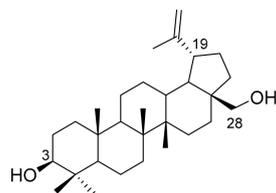


Figure 1. Structure of betulin **1**.

Many articles on new hybrids of betulin, formed by combinations with other bioactive substances, like coumarin acid, ferrocene acid, artemisinin acid, and azidothymidine, have appeared in the literature in recent years. The combination of two active substances results in new hybrids with a better activity and low toxicity [11–15].

The lipophilicity of betulin derivatives was first described by Achrem-Achremowicz. This research showed that the lipophilic parameter depends on the type of substituent at the C-3 and C-28 positions, but the pH of the mobile phase does not affect the lipophilicity [16]. According to the literature data, a relationship between the structure and lipophilicity of different mono- and di-substituted derivatives of betulin was found. This research showed that the substituent significantly affects the partition coefficient ($\log P$). Moreover, the $\log P$ value was also strongly affected by different pharmacokinetic properties, like polar surface, polarizability, molecular volume, and molecular refraction. In many cases, no correlation between the experimental lipophilicity and biological activity of the compounds was found [4,6,17,18].

The present study aimed to determine the experimental and theoretical values of lipophilicity for betulin-1,4-quinone hybrids. The cluster analyses for the lipophilicity of hybrids allowed us to determine the relationship between the structure and lipophilic properties. Moreover, the correlation between the lipophilicity and pharmacokinetic properties was also analyzed. This study also conducted an analysis of the relationship between the biological properties, like anticancer activity and NAD(P)H:quinone oxidoreductase (NQO1), and lipophilicity of hybrids.

2. Materials and Methods

2.1. Betulin-1,4-quinone Hybrids

The analyzed compounds (**2–16**) were synthesized according to the procedure described in the literature [19]. The chemical structure of the hybrids is presented in Table 1. Briefly, betulin **1** and its derivatives were combined with 1,4-quinone in the presence of potassium carbonate in tetrahydrofuran. The structures of hybrids **2–16** were confirmed by spectroscopic methods, such as ^1H NMR, ^{13}C NMR, IR, and mass spectrometric (HR-MAS). The spectral data are consistent with those published in the literature [19].

2.2. Chromatographic Analysis

The lipophilic descriptor was determined using RP-TLC. A stationary phase was obtained by impregnating a silica gel RP-18 F₂₅₄S layer with un-polar silicone oil. The (tris-hydroxymethyl)aminomethane (0.2 M, pH = 7.4) with acetone was used as the mobile phase. The percentage of acetone volume was varied within the range of 65–90% in 5% increments. Chromatographic chambers were filled with 60 mL of the mobile phase and saturated with the vapors for 1h.

Table 1. The chemical structure of hybrids 2–16.

Chemical Structure	Chemical Structure	Chemical Structure
2	7	12
3	8	13
4	9	14
5	10	15
6	11	16

Compounds 1–16 were dissolved in chloroform (2.0 mg/mL), and a 2 μ L solution of the sample was applied on the chromatographic plates using a micropipette. The plates were placed in chromatographic chambers. The plates were developed up to a distance of 8.0 cm and then dried on a hot plate. Spots were visualized by spraying with 10% ethanol solution of sulphuric acid (VI) and then heated up to 110 $^{\circ}$ C.

The obtained values for the retardation factor (R_f) were converted to the R_M parameter according to Equation (1):

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

The R_M parameters were calculated for every concentration of acetone and were extrapolated to zero concentration of an organic component in the mobile phase. The chromatographic parameter of lipophilicity (R_{M0}) was calculated using the Soczewiński–Wachtmeister’s Equation (2), as follows:

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

where C is the concentration of acetone in the mobile phase, while b is the slope of the regression plot.

The obtained R_{M0} and b values were used to determine the chromatography hydrophobic index (φ_0) according to Equation (3):

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

2.3. Computational Analysis

The theoretical value of the lipophilicity descriptor for hybrids **2–16** and betulin **1** were determined using various online tools and available software, including ALOGPs, ACLOGP, ALOGP, miLogP, XLOGP2, XLOGP3, ACD/logP, and MolLogP. Each program uses a different calculation method, but all of them take into consideration parameters related to the chemical structure [20–23]. The pharmacokinetic parameters were calculated using the pkCMS software [24].

2.4. Enzymatic Assay

Compounds **2–16** (10 $\mu\text{mol/L}$) were tested as NQO1 substrates using a NADPH recycling assay, as described in the literature [25,26]. The recombinant NQO1 (DT-diaphorase, EC 1.6.5.5, human recombinant, Sigma-Aldrich, Saint Louis, MO, USA) was used, and the oxidation of NADPH to NADP^+ was measured at an absorption wavelength of 340 nm on a BioTek 800TS microplate reader (BioKom, Janki, Poland). Compounds were dissolved in dimethyl sulfoxide (2 μL) and then added to a 96-wall plate. NADPH (400 $\mu\text{mol/L}$) and NQO1 (1.4 $\mu\text{g/mL}$) in a 50 mmol/L potassium phosphate buffer (pH = 7.4) were added to each well (198 μL ; Nunc Thermo Fisher Scientific, Waltham, MA, USA). Once the 96-well plate was filled with the assay solutions, excluding the NADPH solution, it was placed into the instrument and left to sit for 2 min before starting the measurements. The enzyme reaction was initiated by automated dispensing of the NADPH solution into the wells, and data were recorded at 10 s intervals for 10 min at 25 $^\circ\text{C}$. The linear portion of the absorbance vs. time graphs (the first 20 s to 1 min) was fitted, and the slopes were calculated (velocity). The NADPH oxidation rates were compared with the reactions lacking compounds. The initial velocities were calculated and data were expressed as $\mu\text{mol NADPH}/\mu\text{mol NQO1}/\text{min}$. All of the reactions were carried out at least in triplicate.

2.5. Correlation and Cluster Analysis

Based on the experimental and theoretical values of lipophilicity, as well as the enzymatic activity and molecular descriptor values, correlation and cluster analyses were performed. All data used for cluster analysis were standardized, and the cluster analysis was based on the Euclidean distance, using Ward's method. The analysis was carried out using Statistica 13.1 software.

3. Results and Discussion

Hybrids **2–16** and betulin **1** were analyzed using the RP-TLC method, and the R_{M0} and b parameters were calculated based on Equations (1) and (2). The hydrophobic index (ϕ_0) for each compound was calculated according to Equation (3) (Table 2).

Table 2. The experimental values of the lipophilicity (R_{M0} and $\log P_{\text{TLC}}$) and hydrophobic index (ϕ_0) for compounds **1–16**.

Compound	R_{M0}	b	r	SD	$\log P_{\text{TLC}}$	ϕ_0
1	4.54	−0.05	0.979	0.028	5.34	90.37
2	5.49	−0.06	0.995	0.022	6.36	92.52
3	6.52	−0.07	0.994	0.023	7.46	93.10
4	6.42	−0.07	0.996	0.015	7.36	89.31
5	6.62	−0.07	0.994	0.021	7.58	93.69
6	5.86	−0.07	0.998	0.012	6.75	95.74
7	5.69	−0.06	0.992	0.023	6.58	95.74
8	6.49	−0.07	0.997	0.019	7.43	90.72
9	6.42	−0.07	0.996	0.009	7.36	90.92
10	6.80	−0.07	0.995	0.025	7.77	95.00
11	6.77	−0.08	0.994	0.011	7.74	90.71
12	6.46	−0.07	0.987	0.027	7.41	92.85

Table 2. Cont.

Compound	R _{M0}	b	r	SD	logP _{TLC}	Φ ₀
13	7.12	−0.07	0.995	0.023	8.12	93.10
14	7.13	−0.07	0.995	0.019	8.14	88.67
15	6.82	−0.08	0.982	0.014	7.19	91.54
16	6.26	−0.07	0.973	0.029	7.80	87.09

b is the slope, *r* is the correlation coefficient, and SD is the standard deviation for the linear relationship of $R_M = R_{M0} + bC$.

In order to obtain the logP_{TLC} value, a calibration curve used needed. As standard substances, acetanilide, 4-bromoacetophenone, benzophenone, anthracene, dibenzyl, 9-phenylanthracene, and dichlorodiphenyltrichloroethane (DDT) were used, for which the literature logP_{lit} values were in the range of 1.21–6.38 [27,28]. The R_{M0} for each standard compound was determined in the same condition as for hybrids 2–16 (Table 3).

Table 3. The experimental (R_{M0} and logP_{TLC}) and literature (logP_{lit}) values of the standard compounds.

Compound	logP _{lit}	R _{M0}	b	r	SD	logP _{TLC}
Acetanilide	1.21	0.68	−0.02	0.985	0.015	1.17
4-Bromoacetophenone	2.43	1.87	−0.02	0.981	0.018	2.46
Benzophenone	3.18	2.42	−0.03	0.992	0.013	3.05
Anthracene	4.45	4.08	−0.05	0.970	0.022	4.84
Dibenzyl	4.79	4.11	−0.04	0.972	0.023	4.87
9-Phenylanthracene	6.01	4.87	−0.06	0.985	0.019	5.69
DDT	6.38	5.50	−0.06	0.975	0.019	6.37

b is the slope and *r* is the correlation coefficient for the linear relationship for $R_M = R_{M0} + bC$.

A linear relation between the literature logP_{lit} and the experimental R_{M0} is described by Equation (4).

$$\log P_{TLC} = 1.0777 R_{M0} + 0.4407 \quad (r = 0.993; SD = 0.138) \quad (4)$$

The calibration curve was used to calculate the logP_{TLC} value of hybrids 2–16 and betulin (1) (Table 2). In the group of tested compounds, betulin 1 showed the lowest lipophilicity parameter (logP_{TLC} = 5.34). The introduction of 1,4-quinone moiety led to an increase in lipophilicity, resulting in logP_{TLC} in the range of 6.36–8.12.

In the group of betulin-5,8-quinolinedione hybrids (2–6), the lowest lipophilicity showed compound 2 containing the hydroxyl group at the C-3 position of the betulin moiety. The replacement of this hydroxyl group with an acetyl group caused an increase in lipophilicity. A comparison of logP_{TLC} for hybrids 3 and 4 showed that the elongation of the chain at the C-28 position created in an increase in lipophilicity. For hybrids 2–6, the order of the logP_{TLC} decrease was as follows: 5 > 4 = 3 > 6 > 2. The introduction of the methyl group at the C-2' position of 5,8-quinolinedione moiety, as seen for compounds 7–11, led to a slight increase in lipophilicity. In this group of compounds, the lowest value of logP_{TLC} was exhibited by hybrid 7. In the series of tested hybrids (2–16), the highest lipophilicity showed derivatives containing the 1,4-naphthoquinone moiety (12–16). For 12–16, the lowest lipophilicity was exhibited by compound 15, which contained the propynoiloxy moiety at the C-28 position of betulin. The obtained results show that lipophilicity depends on the type of 1,4-quinone, and follows the order 5,8-quinolinedione > 2-methyl-5,8-quinolinedione > 1,4-naphthoquinone (Figure 2).

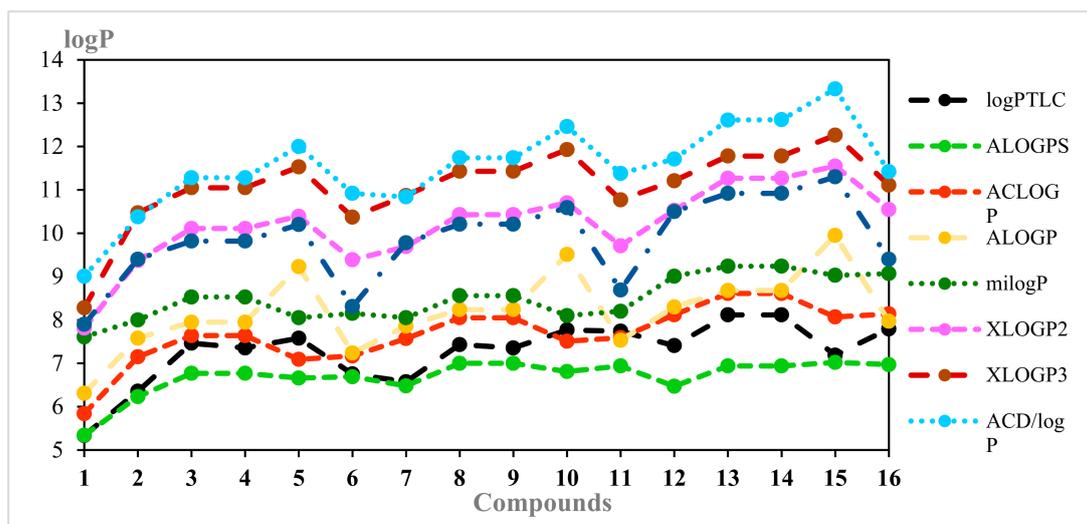


Figure 2. The profile of changes for the experimental and theoretical lipophilicity for hybrids 1–16.

Hydrophobicity is the capability of non-polar molecules or groups to form an association in water, and is measured by the hydrophobic index (ϕ_0). A higher index means that the compound is less soluble in water [3,29]. Hybrids 2–16 are characterized by a high value of ϕ_0 index, which varies in the range of 88.67–95.74. A comparison of the hydrophobicity of betulin 1 ($\phi_0 = 87.09$) and its derivatives (2–16) shows that the introduction of 1,4-quinone moiety slightly influenced their solubility in water.

The next step of the research was to determine the logP parameter using the available computer software [20–23]. Depending on the mathematical module of the program used, the obtained values of the calculated logP covered a wide range, from 5.34 to 13.33 (Table 4).

Table 4. The calculated value of lipophilicity for hybrids 2–16 and betulin 1.

Compound	ALOGPs	ACLOGP	ALOGP	miLogP	XLOGP2	XLOGP3	ACD/logP	MolLogP
1	5.34	5.84	6.31	7.61	7.81	8.28	9.01	7.90
2	6.23	7.15	7.58	8.00	9.37	10.47	10.38	9.40
3	6.77	7.64	7.95	8.53	10.11	11.05	11.28	9.82
4	6.77	7.64	7.95	8.53	10.11	11.05	11.28	9.82
5	6.66	7.09	9.23	8.05	10.39	11.53	12.00	10.20
6	6.69	7.17	7.24	8.15	9.39	10.37	10.92	8.31
7	6.48	7.57	7.86	8.05	9.69	10.87	10.84	9.78
8	7.00	8.05	8.24	8.56	10.43	11.43	11.74	10.21
9	7.00	8.05	8.24	8.56	10.43	11.43	11.74	10.21
10	6.81	7.51	9.51	8.10	10.70	11.93	12.46	10.59
11	6.94	7.59	7.53	8.20	9.71	10.77	11.38	8.69
12	6.47	8.12	8.30	9.01	10.53	11.21	11.71	10.50
13	6.94	8.61	8.68	9.24	11.27	11.78	12.61	10.92
14	6.94	8.61	8.68	9.24	11.27	11.78	12.62	10.92
15	7.02	8.07	9.95	9.03	11.55	12.26	13.33	11.30
16	6.97	8.14	7.97	9.07	10.55	11.11	11.42	9.40

The highest values of lipophilicity were obtained using the ACD/logP program. However, the values that were the most similar to the experimental ones ($\log P_{TLC}$ values), were found using the ACLOGP program (Figure 2). The presented calculations show that betulin 1 has the lowest lipophilicity, which is consistent with its $\log P_{TLC}$ value (Figure 2). The introduction of the 1,4-quinone moiety caused an increase in lipophilicity, which is consistent with experimental results. As shown in Figure 2, hybrids 5, 10, and 15 had the

highest calculated value of lipophilicity. In addition, the lowest values were found for compounds **2**, **6**, and **11**. These results are similar to the experimental results. In each group of compounds, there were hybrids with the same molecular formula, like **3** and **4**, **8** and **9**, and **13** and **14** (Table 4 and Figure 2). In these cases, the calculated lipophilicity was the same for both compounds, regardless of the calculation method, but the experimental lipophilicity was different. These results show that in many cases, the theoretical calculation is not enough for the evaluation of the lipophilic parameters of compounds.

Table 5 presents the parameters of the correlation equation between $\log P_{TLC}$ and the calculated $\log P$. The correlation coefficients are high ($r = 0.864 - 0.815$) for most programs. The exceptions are ALOGP, milogP, and MolLogP, for which the r value varied in the range of 0.702–0.638.

Table 5. Correlation equations for the relationship between the experimental ($\log P_{TLC}$) and theoretically ($\log P_{calc}$) values of lipophilicity for compounds 1–16.

Program	Correlation Equation	r	SD
ALOGPS	$\log PTLC = 1.454 \log P_{calc} - 2.458$	0.864	0.373
ACLOGP	$\log PTLC = 0.871 \log P_{calc} + 0.584$	0.824	0.421
ALOGP	$\log PTLC = 0.513 \log P_{calc} + 3.068$	0.638	0.571
milogP	$\log PTLC = 1.001 \log P_{calc} - 1.236$	0.702	0.528
XLOGP2	$\log PTLC = 0.666 \log P_{calc} + 0.475$	0.842	0.401
XLOGP3	$\log PTLC = 0.653 \log P_{calc} + 0.037$	0.829	0.415
ACD/ $\log P$	$\log PTLC = 0.575 \log P_{calc} + 0.633$	0.815	0.430
MolLogP	$\log PTLC = 0.482 \log P_{calc} + 2.513$	0.642	0.569

The cluster analysis (similarity analysis) was performed for all of the results, for both the experimental and theoretical values of lipophilicity. The results are presented in Figure 3.

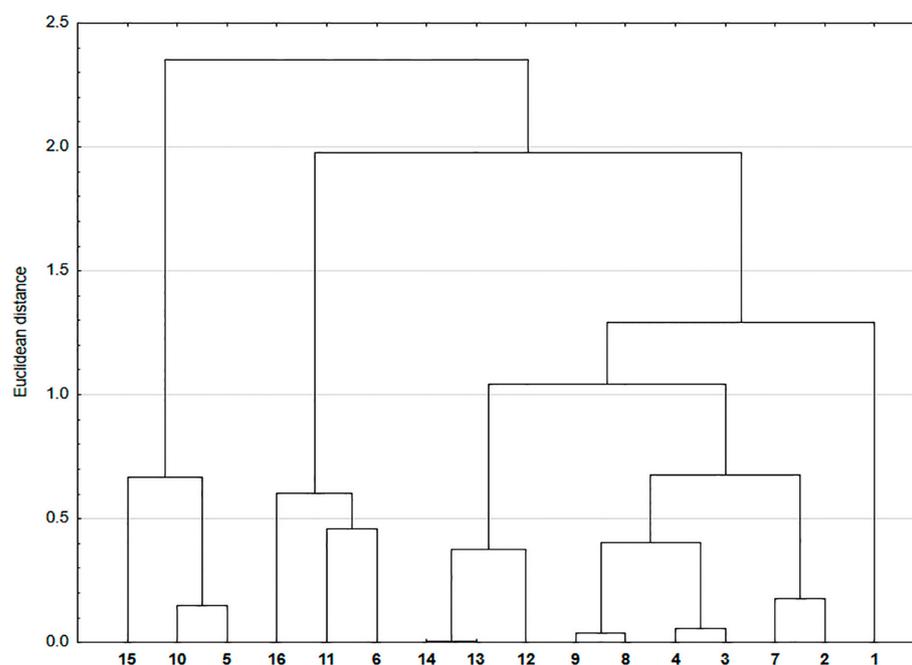


Figure 3. Similarity analysis of the experimental and theoretical values of lipophilicity for hybrids 2–16 and betulin 1.

The similarity analysis shows three main clusters. The first consists of compounds **5**, **10**, and **15**; the second **6**, **11**, and **16**; and the third **1–4**, **7–9**, and **12–14**. In the first and second clusters, hybrids are arranged according to the type of betulinyl oxy fragment, which means

barrier, if $\log BB < -1$, there is a poor distribution to the brain [35,36]. The $\log BB$ values for hybrids 2–16 are lower than 0.3, which suggests that these compounds could have a low permeability through BBB. Moreover, two of them, 6 and 11, show $\log BB < -1$, which means they do not penetrate the central nervous system.

The correlation between $\log P_{TLC}$ and the calculated parameters is characterized by low regression coefficients ($r = 0.066$ – 0.624). The results indicate that there is no significant relationship between the lipophilicity and pharmacokinetic parameters. As an additional description of the analyzed hybrids (2–16), cluster analysis was performed, taking into account the experimental lipophilicity values and the physicochemical (M, nHA, nHD, nRT, and TPSA) and pharmacokinetics properties ($\log BB$). The results are presented in Figure 4.

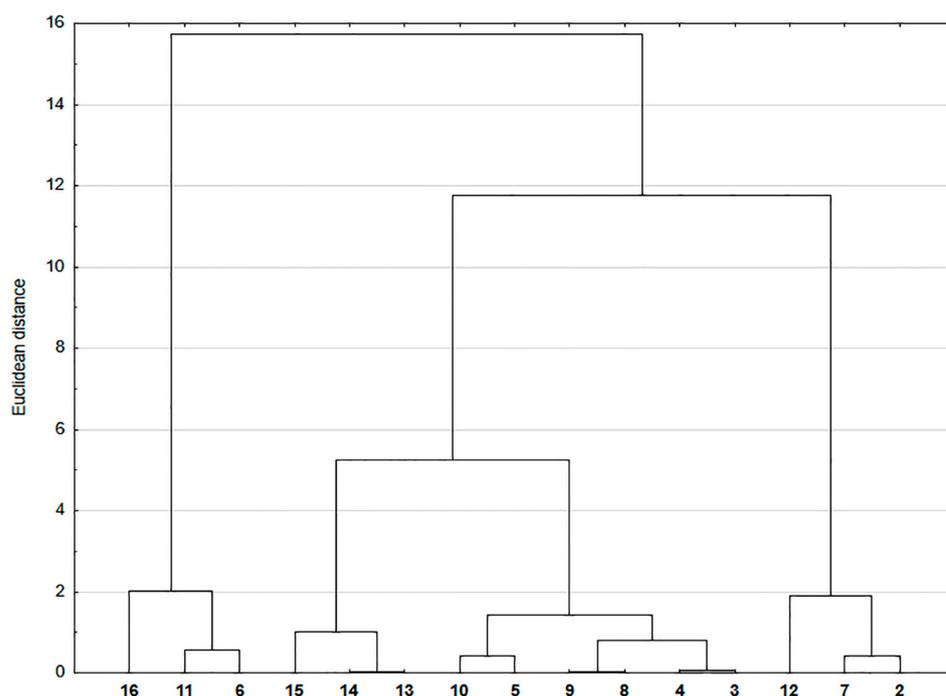


Figure 4. Similarity analysis for hybrids 2–16 investigated based on the experimental lipophilicity and the physicochemical (M, nHD, nHA, nRT, and TPSA) and pharmacokinetic properties ($\log BB$).

As can be seen in Figure 4, there are several clusters. Compounds 6, 11, and 16, containing the 1,4-quinone moiety at the C-30 of 3,24-diacetyl-30-betulinoyloxy, form the first cluster. Hybrids 13, 14, and 15 with the 1,4-naphthoquinone fragment at different positions of the betulinoyloxy moiety, form another cluster. Derivatives 3–5 and 8–10 containing the 5,8-quinolinedione or 2-methyl-5,8-quinolinedione moiety in their structure, are the next cluster. The last cluster is formed by compounds with the 28-betulinoyloxy moiety. The obtained results show that there is no correlation between the lipophilicity and pharmacokinetics parameters for the tested hybrids (2–16).

The next step of the research was the analysis of the biological activity of hybrids 2–16. It was found that derivatives 2–16, which exhibit a high anticancer activity against the lung cancer cell line (A549), also have a high level of the NOQ1 protein (Table 7) [19]. For this reason, all compounds were tested as a substrate of NAD[P]H-quinone oxidoreductase 1 (NQO1). In this assay, hybrids at a concentration of 10 $\mu\text{mol/L}$ were incubated with human NQO1 and NADPH. Streptonigrin was used as a reference compound. The results of the quinone reduction are expressed in units $\mu\text{mol}\cdot\text{L}^{-1}$ NADPH oxidized $\cdot\text{min}^{-1}\cdot\mu\text{mol}^{-1}$ NQO1 in Table 7.

Table 7. The NAD[P]H-quinone oxidoreductase 1 (NQO1) activity of hybrids 2–16 monitored at an absorbance at A340 nm.

Compound	A549/IC ₅₀ (μM) [19]	NQO1 Activity (μmolNADPH/μmolNQO1/min)
1	Neg.	NO
2	8.58 ± 1.70	1685 ± 12
3	14.34 ± 0.31	1451 ± 9
4	13.46 ± 1.45	1419 ± 6
5	0.45 ± 0.20	2192 ± 21
6	3.30 ± 0.48	1505 ± 15
7	1.62 ± 0.91	1705 ± 19
8	0.64 ± 0.04	2008 ± 19
9	0.59 ± 0.13	1744 ± 14
10	18.41 ± 1.91	1784 ± 7
11	0.84 ± 0.01	1839 ± 23
12	10.63 ± 1.45	1459 ± 18
13	1.15 ± 0.10	1784 ± 6
14	0.77 ± 0.12	2009 ± 17
15	1.28 ± 0.06	1870 ± 13
16	53.9 ± 8.02	1040 ± 9
Streptonigrin	NO	621 ± 32

Neg.—IC₅₀ > 30 μM; NO—not test.

Hybrids 2–16 show good reduction rates using NQO1, indicating that these compounds are good substrates for this protein. They show also higher metabolic rates (1040–2192 μmolNADPH/μmolNQO1/min) in comparison with streptonigrin (621 μmolNADPH/μmolNQO1/min). In the group of 5,8-quinolinedione derivatives (2–6), hybrid 5 exhibits the highest enzymatic conversion rates, which are 3.5-time higher than for streptonigrin. Moreover, this compound exhibits a high activity against A549 cells. The introduction of the ethanoyl group at the C-28 position of botulin, instead of the propynoyl group (4 and 5), causes a decrease in the enzymatic conversion rates. A comparison of the 5,8-quinolinedione (2–6) and 2-methyl-5,8-quinolinedione (7–11) hybrids suggests that the introduction of the methyl group at the 5,8-quinolinedione moiety causes an increase in enzymatic conversion rates. The exceptions are compounds 5 and 10, for which the introduction of the 5,8-quinolinedione moiety gives more active derivatives than those with the 2-methyl-5,8-quinolinedione moiety. In the group of the 1,4-naphthoquinone compound (12–16), derivative 14 shows the highest enzymatic conversion rates. The correlation of the NQO1 activity with IC₅₀ values against A549 cells shows high regression coefficients ($r = 0.773$), suggesting that for this group of analogs, the anticancer activity refers to its interaction with the NQO1 protein.

The next step in the research was the analysis of the correlation between the experimental lipophilicity ($\log P_{TLC}$), cytotoxic activity, and NQO1 activity. The calculated regression coefficient was low ($r = 0.140$ – 0.174), which means there is no significant relationship between lipophilicity and activity. The next similarity analysis was done for the experimental lipophilicity and biological activity (IC₅₀ and NQO1 activity; Figure 5).

Figure 5 shows that there are two main clusters. The first consists of compounds with a low value of NQO1 activity, like 3, 4, 6, 12, and 16. The second consists of hybrids 2, 5, 7–11, and 13–15; however, it is difficult to find the similarity in the structure of these derivatives. This result shows that there is no relationship between lipophilicity and the biological activity of hybrids 2–16.

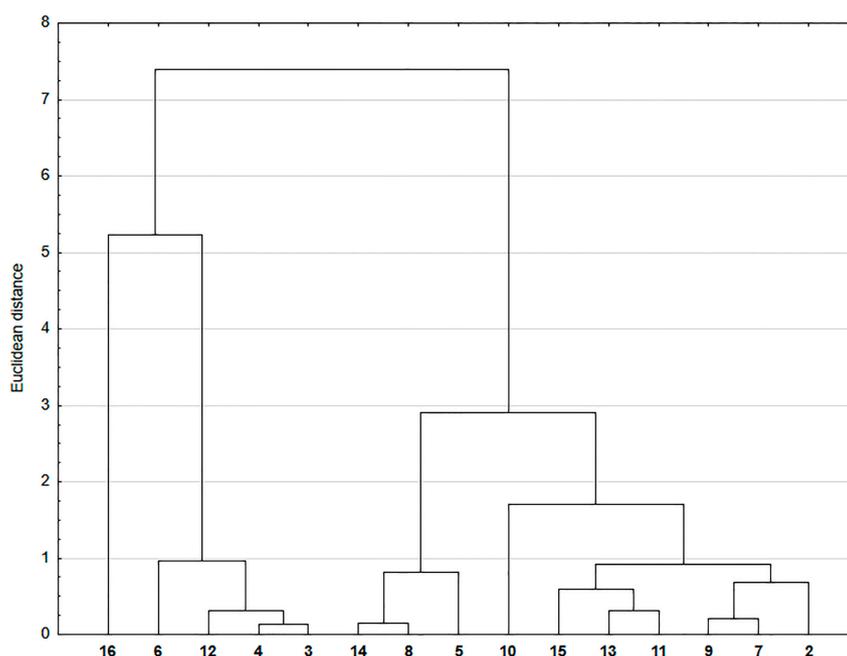


Figure 5. Similarity analysis for hybrids 2–16 investigated based on the experimental lipophilicity and biological activity (IC_{50} and NQO1 activity).

4. Conclusions

In the presented study, the lipophilicity of betulin-1,4-quinone hybrids was determined and analyzed in terms of their structure, pharmacokinetic parameters, and anticancer and enzymatic activity. The introduction of 1,4-quinone moiety to the betulin structure caused an increase in lipophilicity. The cluster analysis showed that there is a strong relationship between the structure of compounds and both their experimental and calculated lipophilicity, which can serve to predict these values when designing new compounds. Additionally, Lipinski's and Veber's rules, supplemented by blood–brain penetration properties, were used to determine the bioavailability of hybrids 2–16. The obtained parameters suggest that the oral application could have some limitations because of the high value of the lipophilicity and molecular mass. The similarity analyses showed that lipophilicity does not correlate with the pharmacokinetic parameters.

The enzymatic study showed that hybrids 2–16 can be characterized as efficient substrates for the NQO1 protein. The correlation between the anticancer activity against lung cancer (A549) and the NQO1 activity shows that anticancer activity refers to its interaction with the NQO1 protein. Based on the similarity analysis, it has been found that there is no correlation between the biological activity and lipophilicity of the tested compounds.

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References

1. Arnott, J.; Planey, S. The influence of lipophilicity in drug discovery and design. *Expert Opin. Drug Discov.* **2012**, *7*, 863–875. [CrossRef]
2. Bhatt, N.; Chavada, V.D.; Sanyal, M.; Shrivastav, P. Influence of organic modifier and separation modes for lipophilicity assessment of drugs using thin layer chromatography indices. *J. Chromatogr. A* **2018**, *1571*, 223–230. [CrossRef]
3. Rutkowska, E.; Pajak, K.; Jóźwiak, K. Lipophilicity-methods of determination and its role in medicinal chemistry. *Acta Pol. Pharm.* **2013**, *70*, 3–18.
4. Bębenek, E.; Bober-Majnus, K.; Siudak, S.; Chrobak, E.; Kadela-Tomanek, M.; Wietrzyk, J.; Boryczka, S. Application of TLC to evaluate the lipophilicity of newly synthesized betulin derivatives. *J. Chromatogr. Sci.* **2020**, *15*, 117–126. [CrossRef] [PubMed]
5. Kadela-Tomanek, M.; Bober, K.; Bębenek, E.; Chrobak, E.; Boryczka, S. Application of thin-layer chromatography to evaluate the lipophilicity of 5,8-quinolinedione compounds. *J. Planar Chromatogr.-Mod. TLC* **2017**, *30*, 219–224. [CrossRef]
6. Bober, K.; Bębenek, E.; Boryczka, S. Application of TLC for evaluation of the lipophilicity of newly synthesized esters: Betulin derivatives. *J. Anal. Methods Chem.* **2019**, *3*, 1297659–1297667. [CrossRef] [PubMed]
7. Pyka, A. Evaluation of the lipophilicity of fat-soluble vitamins. *J. Planar Chromatogr.-Med. TLC* **2009**, *22*, 211–215. [CrossRef]
8. Šegan, S.; Andrić, F.; Radoičić, A.; Opsenica, D.; Šolaja, B.; Zlatović, M.; Milojković-Opsenica, D. Correlation between structure, retention and activity of cholic acid derived cis-trans isomeric bis-steroidal tetraoxanes. *J. Sep. Sci.* **2011**, *34*, 2659–2667. [CrossRef] [PubMed]
9. Hodon, J.; Borkova, L.; Pokorny, J.; Kazakova, A.; Urban, M. Design and synthesis of pentacyclic triterpene conjugates and their use in medicinal research. *Eur. J. Med. Chem.* **2019**, *182*, 111653–111678. [CrossRef]
10. Amiri, S.; Dastghaib, S.; Ahmadi, M.; Mehrbod, P.; Khadem, F.; Behrouj, H.; Aghanoori, M.R.; Machaj, F.; Ghamsari, M.; Rosik, J.; et al. Betulin and its derivatives as novel compounds with different pharmacological effects. *Biotechnol. Adv.* **2020**, *38*, 107409–107448. [CrossRef]
11. Gao, F.; Sun, Z.; Kong, F.; Xiao, J. Artemisinin-derived hybrids and their anticancer activity. *Eur. J. Med. Chem.* **2020**, *188*, 112044–112058. [CrossRef]
12. Xiao, J.; Sun, Z.; Kong, F.; Gao, F. Current scenario of ferrocene-containing hybrids for antimalarial activity. *Eur. J. Med. Chem.* **2020**, *185*, 111791–111802. [CrossRef] [PubMed]
13. Wang, G.Y.; Jin, L.; Piao, F.; Han, R. Betulin esters with coumarin-3-carboxylic and 3,4,5-trimethoxybenzoic acids. *Mendeleev Commun.* **2017**, *27*, 93–94. [CrossRef]
14. Bori, I.; Hung, H.; Qian, K.; Chen, C.; Morris-Natschke, S.; Lee, K. Anti-AIDS agents 88. Anti-HIV conjugates of betulin and betulinic acid with AZT prepared via click chemistry. *Tetrahedron Lett.* **2012**, *53*, 1987–1989. [CrossRef] [PubMed]
15. Xiong, J.; Kashiwada, Y.; Chen, C.; Qian, K.; Morris-Natschke, S.; Lee, K.; Takaishi, Y. Conjugates of betulin derivatives with AZT as potent anti-HIV agents. *Bioorg. Med. Chem.* **2010**, *18*, 6451–6469. [CrossRef]
16. Achrem-Achremowicz, J.; Kepczyńska, E.; Zylewski, M.; Janeczko, Z. Synthesis of betulin derivatives and the determination of their relative lipophilicities using reversed-phase thin-layer chromatography. *Biomed. Chromatogr.* **2010**, *24*, 261–267. [CrossRef]
17. Bildziukevich, U.; Vida, N.; Rárová, L.; Kolář, M.; Šaman, D.; Havlíček, L.; Drašar, P.; Wimmer, Z. Polyamine derivatives of betulinic acid and β -sitosterol: A comparative investigation. *Steroids* **2015**, *100*, 27–35. [CrossRef]
18. Thibeault, D.; Gauthier, C.; Legault, J.; Bouchard, J.; Dufour, P.; Pichette, A. Synthesis and structure-activity relationship study of cytotoxic germanicane- and lupane-type 3β -O-monodesmosidic saponins starting from betulin. *Bioorg. Med. Chem.* **2007**, *15*, 6144–6157. [CrossRef] [PubMed]
19. Kadela-Tomanek, M.; Bębenek, E.; Chrobak, E.; Marciniec, K.; Latocha, M.; Kuśmierz, D.; Jastrzębska, M.; Boryczka, S. Betulin-1,4-quinone hybrids: Synthesis, anticancer activity and molecular docking study with NQO1 enzyme. *Eur. J. Med. Chem.* **2019**, *177*, 302–315. [CrossRef]
20. Virtual Computational Chemistry Laboratory. Available online: <http://www.vcclab.org/lab/alogps/> (accessed on 2 January 2021).
21. Molsoft, L.L.C. Available online: <http://www.molsoft.com/> (accessed on 2 January 2021).
22. ACD/I-Lab. Available online: <https://ilab.acdlabs.com/iLab2/> (accessed on 2 January 2021).
23. Molinspiration Cheminformatics. Available online: <https://www.molinspiration.com/> (accessed on 2 January 2021).
24. pkCSM. Available online: http://biosig.unimelb.edu.au/pkcsm/prediction_single/ (accessed on 2 January 2021).
25. Li, X.; Bian, J.; Wang, N.; Qian, X.; Gu, J.; Mu, T.; Fan, J.; Yang, X.; Li, S.; Yang, T.; et al. Novel naphtho[2,1-d]oxazole-4,5-diones as NQO1 substrates with improved aqueous solubility: Design, synthesis, and in vivo antitumor evaluation. *Bioorg. Med. Chem.* **2016**, *24*, 1006–1013. [CrossRef]
26. Bian, J.; Deng, B.; Xu, L.; Xu, X.; Wang, N.; Hu, T.; Yao, Z.; Du, J.; Yang, L.; Lei, Y.; et al. 2-Substituted 3-methylnaphtho[1,2-b]furan-4,5-diones as novel L-shaped ortho-quinone substrates for NAD(P)H:quinone oxidoreductase (NQO1). *Eur. J. Med. Chem.* **2014**, *82*, 56–67. [CrossRef]
27. Mannhold, R.; Cruciani, G.; Dross, K.; Rekker, R. Multivariate analysis of experimental and computational descriptors of molecular lipophilicity. *J. Comput. Aided Mol. Des.* **1998**, *12*, 573–581. [CrossRef] [PubMed]
28. Bodor, N.; Gabanyi, Z.; Wong, C.K. A new method for the estimation of partition coefficient. *J. Am. Chem. Soc.* **1989**, *111*, 3783–3786. [CrossRef]

29. Constantinescu, T.; Lungu, C.; Lung, I. Lipophilicity as a central component of drug-like properties of chalcones and flavonoid derivatives. *Molecules* **2019**, *24*, 1505. [[CrossRef](#)] [[PubMed](#)]
30. Mälkiä, A.; Murtomäki, L.; Urtti, A.; Kontturi, K. Drug permeation in biomembranes: In vitro and in silico prediction and influence of physicochemical properties. *Eur. J. Pharm. Sci.* **2004**, *23*, 13–47. [[CrossRef](#)]
31. Alavijeh, M.; Chishty, M.; Qaiser, M.; Palmer, A. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *NeuroRx* **2005**, *5*, 554–571. [[CrossRef](#)] [[PubMed](#)]
32. Duchowicz, P.; Castro, E. QSPR studies on aqueous solubilities of drug-like compounds. *Int. J. Mol. Sci.* **2009**, *10*, 2558–2577. [[CrossRef](#)] [[PubMed](#)]
33. Lipinski, C.; Lombardo, F.; Dominy, B.; Feeney, P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–26. [[CrossRef](#)]
34. Veber, D.F.; Johnson, S.R.; Cheng, H.Y.; Smith, B.R.; Ward, K.W.; Kopple, K.D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **2002**, *45*, 2615–2623. [[CrossRef](#)] [[PubMed](#)]
35. Kadela-Tomanek, M.; Bębenek, E.; Chrobak, E.; Latocha, M.; Boryczka, S. Alkoxy and enediyne derivatives containing 1,4-benzoquinone subunits-synthesis and antitumor activity. *Molecules* **2017**, *22*, 447. [[CrossRef](#)]
36. Hutter, M.C. Prediction of blood-brain barrier permeation using quantum chemically derived information. *J. Comput. Aided Mol. Des.* **2003**, *17*, 415–433. [[CrossRef](#)] [[PubMed](#)]