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Original Scientific Paper

Lipophilicity Parameters and Biological Activity in a Series of Compounds with Potential Cardiovascular Applications*

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The biological activity of some long hydrocarbon keto-diols (and their phosphate esters) and acids, has been correlated with their lipohilicity. IC_{50} values of the hepatocyte lipid synthesis inhibition *(in vitro)* were used to measure biological activity; lipohilicities were calculated by employing a 3D molecular size approach implemented in a QLogP software package. Although no quantitative correlation was observed, the results of the study might be significant for *in vivo* application of these compounds as potential cardiovascular agents.

Key words
cardiovascular agents
lipophilicity
log *P*

INTRODUCTION

The ability of a drug to penetrate various biological membranes, tissues and barriers is a primary factor in controlling the interaction of drugs with biological systems. In quantitative structure activity relationship models (QSAR) in which physicochemical parameters of drugs are correlated with biological activities, lipophilicity (partition coefficients, chromatographic parameters) has a major role. Other important parameters are polarizability, electronic and steric parameters, molecular weight, geometry, conformational entropies *etc*.

Lipophilicity is defined by the partitioning of a compound between an aqueous and a nonaqueous phase. The logarithm of the partition coefficient between 1-octanol and water (log *P*) is a physicochemical property widely used in medicinal chemistry. Theoretically calculated log *P* values, by using sophisticated quantum chemistry and molecular modeling procedures, are in some instances, particularly in congener series, quite well correlated with experimental partition coefficients.

Correlation of biological activity of some compounds experimented as cardiovascular agents with their ability to penetrate biological membranes, as reflected by their lipophilicities, is presented herein.

The compounds included in this study are long hydrocarbon chain keto-diols (and their phosphates) and acids. The symmetrical or unsymmetrical side chains connected to the central ketone functionality vary both in their lengths (3 to 5 carbon atoms) and in the nature of the attached geminal modifying groups (methyl, phenyl or substituted phenyl) (Chart 1, Series I). For comparison representatives of a second series of compounds that possess a central ether functionality (Chart 2, Series II), have been included in this study.

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* Dedicated to Professor Nenad Trinajstić on the occasion of his 65th birthday.

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These compounds have promising biological activity both *in vitro* and *in vivo*. Indeed, when administered orally to experiment animals models of disease, they reduced levels of low density lipoproteins (LDL) and, more importantly, also increased levels of high density lipoproteins (HDL). These are changes that are known to correlate with reducing the risk of cardiovascular disease in humans. In this series of compounds the biological activity varies with structural modifications, thus influencing lipophilicities and other physicochemical properties.

There is a strong possibility that the activity in the series of compounds can be correlated with their ability to penetrate biological membranes, which is dependent on lipophilicity factors. This study is designed to assess the correlation between the theoretically calculated lipophilicity of the compounds and their biological activity, and to assess the utility of the theoretically calculated log *P* to predict biological activity.

EXPERIMENTAL

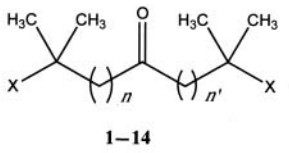
Synthesis

Compounds presented in Charts 1 and 2 were synthesized by conventional procedures as described elsewhere.[1–3]

Biological Activities

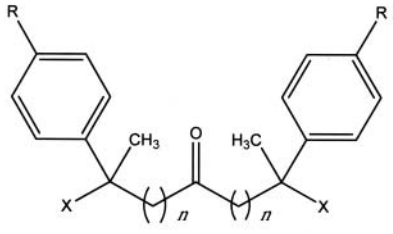
Compounds were tested for inhibition of lipid synthesis in primary cultures of rat hepatocytes. Male Sprague-Dawley rats were anesthetized with intraperitoneal injection of sodium pentobarbital. Livers were perfused and cells were isolated using a modified method of Ulrich *et al.*[4] Hepatocytes were plated in medium (DMEM supplemented to 20 % fetal bovine serum, 14 mmol dm^{-3}, 0.2 % bovine albumin, 2 mmol dm^{-3} L-glutamine, 1X MEM non-essential amino acids, 100 mmol dm^{-3} insulin, 100 μg ml^{-1} dexamethasone and 20 μg ml^{-1} gentamicin) at a density of 3.0 x 10^4 cell/well on collagen-coated 96-well plastic dishes. Cells were allowed to attach for 3–4 hours in a humidified environment (37 °C; 95 % air, 5 % CO_2) prior to exchanging media with serum and dexamethasone free-DMEM supplemented as described above. Hepatocytes were incubated in 0.2 mL/well of DMEM medium overnight prior to treatment.

To test the effect of various compounds on the synthesis rates of total lipids, the monolayer cultures were exposed to 1, 3, 10, 30, 100, or 300 μmol dm^{-3} of compound in DMEM containing 1 μCi ml^{-1} ^{14}C-acetate and 0.1 % DMSO. Control cells were exposed to the same media lacking test compounds. Metabolic labeling with ^{14}C-acetate continued for 4 h at 37 °C. After labeling, cells were washed twice with



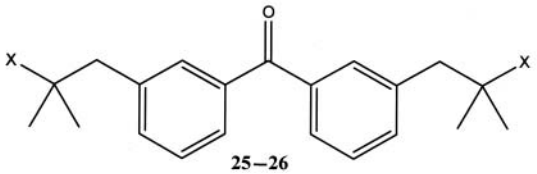
1–14

1. $n = n' = 3$; X = CH_2OH
2. $n = n' = 4$; X = CH_2OH
3. $n = n' = 4$; X = $CH_2OPO_3H_2$
4. $n = n' = 5$; X = CH_2OH
5. $n = n' = 5$; X = $CH_2OPO_3H_2$
6. $n = n' = 4$; X = COOH
7. $n = n' = 5$; X = COOH
8. $n = 3$; $n' = 4$; X = COOH
9. $n = 3$; $n' = 4$; X = CH_2OH
10. $n = 4$; $n' = 5$; X = COOH
11. $n = 4$; $n' = 5$; X = CH_2OH
12. $n = n' = 6$; X = CH_2OH
13. $n = n' = 6$; X = COOH
14. $n = n' = 7$; X = CH_2OH



15–24

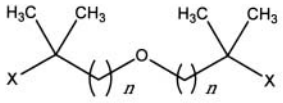
15. $n = 3$; X = COOH R = H
16. $n = 3$; X = CH_2OH; R = H
17. $n = 4$; X = COOH; R = H
18. $n = 4$; X = CH_2OH; R = H
19. $n = 4$; X = COOH; R = CH_3
20. $n = 4$; X = CH_2OH; R = CH_3
21. $n = 4$; X = COOH; R = $CH_2CH(CH_3)_2$
22. $n = 4$; X = CH_2OH; R = $CH_2CH(CH_3)_2$
23. $n = 5$; X = CH_2OH; X = H
24. Like **18** but with only one phenyl group



25–26

25. X = COOH
26. X = CH_2OH

Chart 1.



27. $n = 4$; X = CH_2OH
28. $n = 5$; X = CH_2OH
29. $n = 4$; X = $CH_2OPO_3H_2$
30. $n = 5$; X = $CH_2OPO_3H_2$

Chart 2.

ice-cold PBS and frozen. Incorporation of ^{14}C-acetate into organic soluble products was assessed using MicroScint E®) and counted on a Topcount®. The 50 % inhibitor concentration (IC_{50}) values are derived from statistical modeling of the data and are indicated in Table I.

Theoretical Lipophilicity Calculations

A 3D molecular size approach developed by Bodor and Buchwald[5] implemented in the QLogP software package has been used to calculate lipophilicities. The latest version (2.01 beta), proven to give fairly accurate results, as compared to experimental determinations, was employed. This fully computerized model employs molecular mechanics (Alchemy II; Tripos Assoc., St Louis, MO) for optimization of molecular structures. Van der Waals radii were used to calculate molecular volumes. The method has been described in detail *e.g.* as applied to predict octanol-water partition of peptides[6] or to determine QLogP as theoretical descriptor for a quantitative structure-toxicity relationship of pollutants.[7]

RESULTS AND DISCUSSION

Calculated QLogP and experimental biological activities in Series I–II are presented in Table I. Formulas and molecular weights of the examined compounds were included as well.

All calculated log *P* values are for neutral forms of compounds. While for alcohols the results should be fairly accurate, in the case of ionizable compounds (carboxylic acids, phosphates) the values describe a case in which the aqueous phase represents a buffer with a pH at which the compound is not neutralized, such as a pH < 2 for carboxylic acids. If a buffer close to the neutral as the physiological pH (= 7.4) is used, an apparent partition coefficient is calculated (log *D*). Accordingly an ionization correction should be used that is dependent on the strength (pK_a) of acids. This correction leads to lower log *P*.

As expected, lipophilicity increases with the length of the alkyl chains, as for example in the series of diols: **1** ($n = n'$ = 3; QLogP = 3.11) < **9** (n = 3; n' = 4; QLogP = 3.55) < **2** ($n = n'$ = 4; QLogP = 3.98) < **11** (n = 4; n' = 5; QLogP = 4.43) < **4** ($n = n'$ = 5; QLogP = 4.88) < **12** ($n = n'$ = 6; QLogP = 5.99) < **14** ($n = n'$ = 7; QLogP = 7.09).

Terminal functional groups increase lipophilicity in the order phosphate < alcohol < carboxyl, for example: **3** (phosphate, QLogP = 0.55) < **2** (hydroxyl, QLogP = 3.98) < **6** (carboxyl, QLogP = 4.05). The lipophilicity of alcohols and corresponding carboxylic acid is almost identical; however considering pK_a corrections, in reality alcohols are more lipophilic.

The modifying groups increase lipophilicity in the order of their size and lipophilicity: methyl, phenyl < methylphenyl < isobutylphenyl, as illustrated by the series: **2** (bis dimethyl; QLogP = 3.98) < **21** (dimethyl, methylphenyl; QLogP = 5.42) < **15** (bis methyl, methylphenyl; QLogP = 6.87) < **17** (bis methyl, (methyl)phenyl; QLogP = 7.76) < **19** (bis methyl, (isobutyl)phenyl; QLogP = 10.46).

The central moiety does not modify significantly lipophilicity in the examined series as it only slightly increases from: C–O–C (ether) to C=O (ketone), *e.g.* **28** (ether; QLogP = 3.76) < **2** (keto; QLogP = 3.98).

Calculated QLogP values have been compared with IC_{50} values of the hepatocyte lipid synthesis inhibition. The results are indicated in Table I.

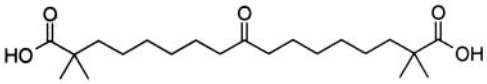
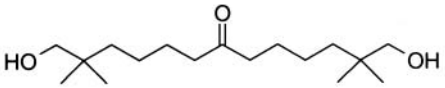
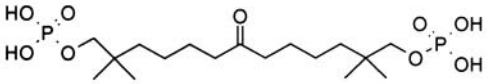
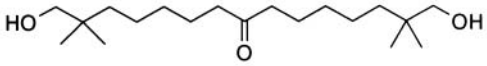
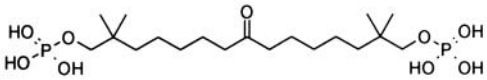
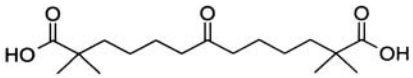
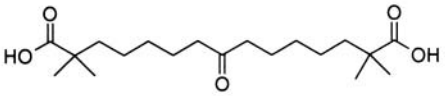
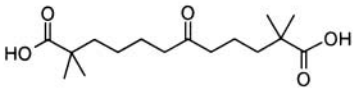
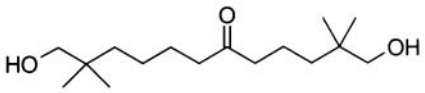
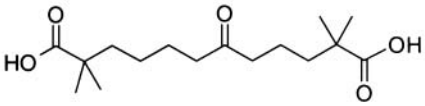
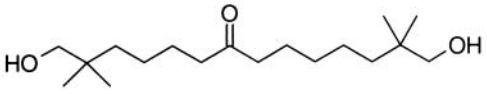
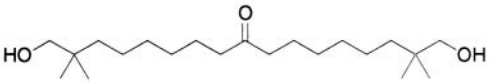
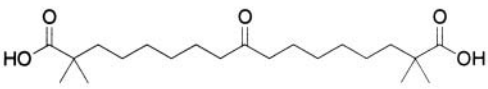
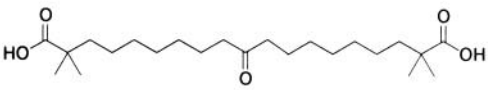
Experimentally determined biological activity indicated a certain dependence on lipophilicity, although no linear correlation was registered (Figure 1). In the series of aliphatic keto-diols **1, 2, 4, 9, 11, 12, 14**, while the least lipophilic members of the series (**1** and **9**) manifested little potency, the other compounds having calculated QLogP between 4 and 7 were quite active. The most active component of the series was the asymmetric diol **11**, having QLogP = 4.43. The series of keto-dicarboxylic acids with lipophilicities increasing in the series **8 < 6 < 7 < 13** indicated relatively modest activity, except for compound **6** (QLogP = 4.05) that proved to be quite potent. As expected, phosphates **3** and **5,** belonging to the aliphatic keto series were not biologically active.

The series containing bulky terminal groups (**15**–**24**) having large molecular weights and large values of calculated QLogP (from 6 to 10.5) proved to be inactive. The lack of biological activity was not unexpected. Indeed, while lipophilic compounds penetrate various membranes, including cellular membranes as well as tissues with high lipoid content, hydrophilic compounds do not penetrate lipoidal membranes and are found mainly extra cellularly. However, there is a limit for a proper lipophilicity. According to the »rule of 5« of Lipinski,[8] lipohilicities higher than 5 (as well as molecular weights higher than 500) are not advantageous, as they lead to a decrease in bioavailability. This rule although not unanimously accepted, is quite frequently applied in drug design. It is known that compounds with very high log *P* as those with very low lipophilicity do not have good bio-availability as they cannot cross hydrophilic and lipophilic barriers, respectively. Molecules with intermediary lipophilicity have better chances to arrive at the receptor site.

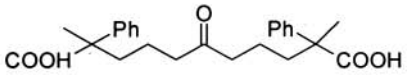
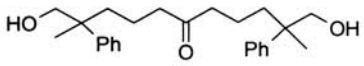
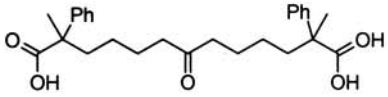
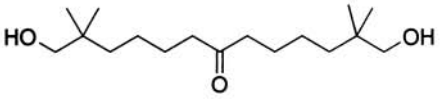
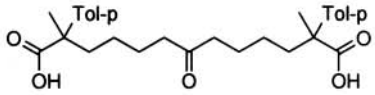
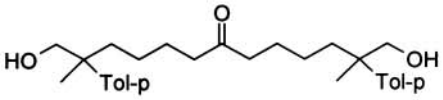
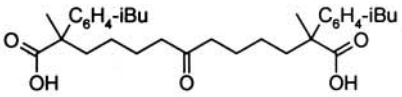
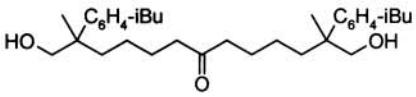
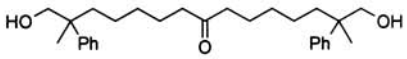
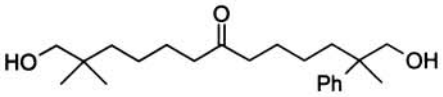
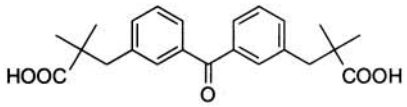
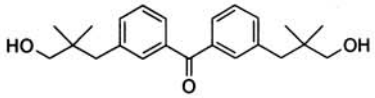
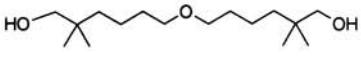
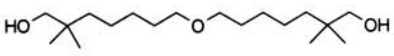
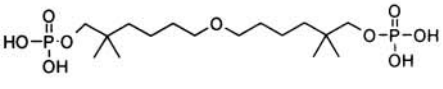
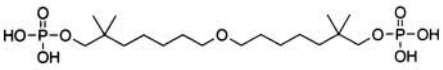
While lipophilicity of alcohols and carboxylic acids guarantee their access to the desired pharmacological targets, phosphates appear to be too hydrophilic in this purpose. Accordingly, *in vitro* studies of phosphates indicate little activity. The situation might be different *in vivo*, as phosphates can be hydrolyzed enzymatically by (alkaline or acid) phosphatases. Phosphates should act as prodrugs, the active agent being the corresponding alcohol released *in vivo*. Situated on the other extreme, compounds like **15**–**23** were not expected to be active due to their high lipophilicity and molecular weights. The experimental results confirmed these predictions. Interestingly, the asymmetric compound **24** having only one terminal phenyl group (QLogP = 5.42) proved to be the most active compound of the whole series.

Compounds belonging to series **27–30** indicated that modifying the central region (carbonyl group with ether

TABLE I. Calculated QLogP and experimental activities

Compound No.	Formula	Molecular weight	QLogP	IC_{50}	Structure
1	$C_{15}H_{30}O_3$	258.40	3.11	31	
2	$C_{17}H_{34}O_3$	286.45	3.98	4.4	
3	$C_{17}H_{36}O_9P_2$	446.41	0.55	16	
4	$C_{19}H_{38}O_3$	314.51	4.88	4.2	
5	$C_{19}H_{40}O_9P_2$	474.47	1.44	35	
6	$C_{17}H_{30}O_5$	314.42	4.05	2.8	
7	$C_{19}H_{34}O_5$	342.47	4.94	32	
8	$C_{16}H_{28}O_5$	300.39	3.61	8.3	
9	$C_{16}H_{23}O_3$	272.43	3.55	9.2	
10	$C_{18}H_{32}O_5$	328.45	4.49	3.2	
11	$C_{18}H_{32}O_3$	300.48	4.43	2.4	
12	$C_{21}H_{42}O_3$	342.56	5.99	4.1	
13	$C_{21}H_{38}O_5$	370.52	6.26	9.5	
14	$C_{21}H_{38}O_5$	370.52	7.09	4.8	

(cont.)

15	$C_{25}H_{30}O_5$	410.51	6.08	Not active	
16	$C_{25}H_{34}O_3$	382.54	5.97	227	
17	$C_{27}H_{34}O_5$	438.56	6.92	Not active	
18	$C_{27}H_{38}O_3$	410.60	6.87	145	
19	$C_{29}H_{38}O_5$	466.62	7.84	195	
20	$C_{29}H_{42}O_3$	438.65	7.76	130	
21	$C_{35}H_{50}O_5$	550.78	10.47	106	
22	$C_{35}H_{54}O_3$	522.81	10.46	Not active	
23	$C_{29}H_{42}O_3$	438.65	7.77	103	
24	$C_{22}H_{36}O_3$	348.52	5.42	1.1	
25	$C_{23}H_{26}O_5$	382.46	5.89	Not reported	
26	$C_{23}H_{30}O_3$	30	5.85	52	
27	$C_{16}H_{34}O_3$	274.44	3.76	10	
28	$C_{18}H_{38}O_3$	302.50	4.64	3.8	
29	$C_{16}H_{36}O_9P_2$	434.40	0.32	40	
30	$C_{18}H_{40}O_9P_2$	462.46	1.20	25.5	

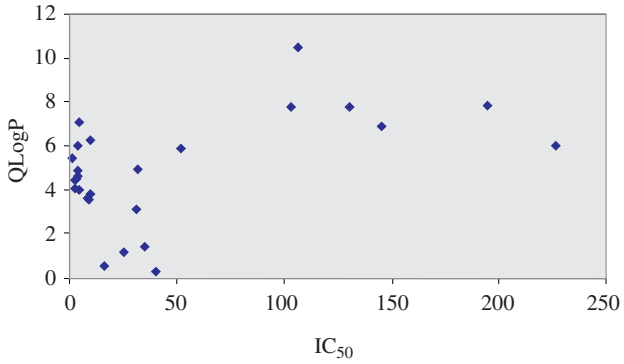


Figure 1. IC_{50} values *versus* QLogP values.

moiety) does not influence dramatically the activity. Indeed, while phosphates are less active, the two diols, particularly compound **28** having QLogP = 4.64 proved to be biopotent.

In conclusion, calculated QLogP values have been correlated with IC_{50} values of the hepatocyte lipid synthesis inhibition. Compounds with the best *in vitro* biological activity had QLogP values in the range 4–7. However, compounds with QLogP higher than 5 might not be appropriate candidates for *in vivo* experiments. Structural features are important for activity. It appears that in order to be active, both side chains connected to the central atom(s) of a compound should consist of at least four carbon atoms. While the environment at the center of molecules does not seem to have much influence over activity, at least in the examined structures, bulky linkers and terminal groups tend to reduce biological activity. The activity of compound **24** is of interest and will be better examined. While alcohols and carboxylic acids having the proper structures and required lipophilicity are generally potent, phosphates do not have much *in vitro* activity; however, they might be experimented as water soluble prodrugs of the alcohol type combinations. In the present study, no quantitative correlation between calculated lipophilicity and experimental biological activity was observed. The situation might be different *in vivo*, where lipophilicity becomes more important. Indeed, while *in vitro* the only existing barrier is the cell membrane, *in vivo* a drug has to surpass several biological barriers to reach its target and to assure a proper bioavailability.

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SAŽETAK

Lipofilni parametri i bioaktivnost niza spojeva s mogućom kardiovaskularnom primjenom

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Korelirana je bioaktivnost nekih acikličkih dugolančanih keto-diola (i njihovih fosfatnih estera) i kiselina s lipofilnošću. IC_{50} vrijednosti inhibicije hepatocitne lipidne sinteze (*in vitro*) upotrebljene su kao mjera bioaktivnosti razmatranih spojeva, a lipofilnost je izračunana pomoću programskoga paketa QlogP u koji je implementiran računski postupak koji uzima u obzir 3D strukturu molekula. Premda autori nisu dobili kvantitativnu korelaciju između bioaktivnosti i lipofilnosti razmatranih spojeva, njihovi rezultati mogu se upotrijebiti u *in vivo* aplikaciji tih spojeva kao mogući kardiovaskularni agensi.