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Reversed-Phase TLC and HPLC Retention Data in Correlation Studies with in Silico Molecular Descriptors and Druglikeness Properties of Newly Synthesized Anticonvulsant Succinimide Derivatives

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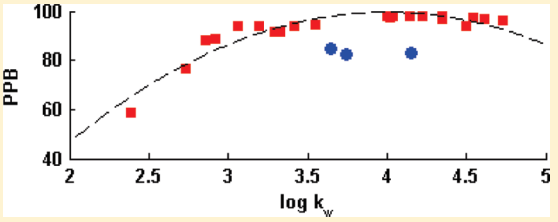
ABSTRACT: The properties relevant to pharmacokinetics of two series of newly synthesized succinimide derivatives have been studied. The properties under consideration have been either determined empirically, by reversed-phase liquid chromatography (TLC and HPLC technique), or calculated with the use of established theoretical medicinal chemistry/drug design software. Chromatographic techniques allowed determination of the retention constants R_M^0 and log k_w, which characterize lipophilicity of compounds. Considering potential pharmaceutical importance of succinimide derivatives, we (i) examined the retention behavior in the reversed-phase liquid chromatographic (RP LC) systems, in both planar and column LC, and (ii) determined the relationships between chromatographic data and selected structural features of analytes that are believed to markedly affect their processes of absorption, distribution, metabolism, excretion and toxicity (ADMETox). Significant relationships were found between the retention constants, R_M^0 and log k_w, and the in silico calculated bioactivity descriptors, in particular HIA (human intestinal absorption) and PPB (plasma protein binding) parameters. The R_M^0 and log k_w values of the investigated compounds have been recommended for description of their lipophilicity and evaluating pharmacokinetic properties. In view of results of this study the newly synthesized succinimide agents meet pharmacokinetic criteria of preselection of drug candidates and hence qualify for pharmacodynamic phase of antiepileptic drug development. Best compromising human intestinal absorption and plasma protein binding features appear to be compounds A4, A5, A10 and A11.



KEYWORDS: ADMETox prediction, anticonvulsant agents, drug design, HPLC, lipophilicity, liquid chromatography, molecular descriptors, pharmacokinetics evaluations, SAR, structure−activity relationships, retention parameters, succinimides, TLC

INTRODUCTION

Succinimide derivatives exhibit a pronounced anticonvulsant activity, combined with some antimuscarinic effects.[1] The succinimide moiety provides a pharmacophore for potential antiepileptic agents. Thus, the requested molecular pharmacology of potential new drugs from the group of succinimide derivatives seems to be structurally guaranteed. However, a safe and effective therapeutic agent must also possess proper pharmacokinetic features to attain (and maintain for some time) its effective concentrations at the site of action after administration to a living organism. In other words, a drug candidate must exhibit physicochemical properties providing its proper absorption, distribution, metabolism, excretion and toxicity (ADMETox).

Nitrogen-containing five membered rings have several applications in medicine, biology and chemistry. Succinimides and hydantoins show antimuscarinic and anticonvulsant activity, and the 3,3-diphenyl derivatives of those compounds are potent anticonvulsants.[1−3]

Presently, development of new drugs involves research on structure−activity relationships (SAR). It has been suggested[4] that two hydrophobic regions and two electron-rich centers, interconnected with similar relative orientations, represent a major molecular requirement for antiepileptic activity. Hence, having the pharmacophore system defined can allow one to concentrate on physicochemical properties of the agents which would provide their proper pharmacokinetics, which is necessary for In Vivo activity. Knowledge of physicochemical properties of drug candidates at an early phase of drug development is crucial to reduce attrition rates due to poor biopharmaceutical properties.

Lipophilicity is one of the physicochemical properties which influence a drug's permeation ability (amount and rate of intestinal absorption and/or blood−brain barrier crossing), as well as its distribution properties (e.g., degree of plasma protein binding).[5−8] Lipophilicity is usually quantified as the logarithm

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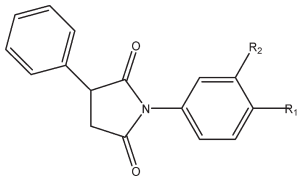
Table 1. Structure of Compounds Investigated

A



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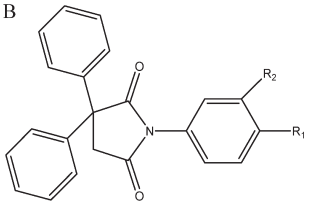

compd	R_1	R_2	compd	R_1	R_2
A1	−H	−H	B1	−H	−H
A2	$-CH_3$	−H	B2	$-CH_3$	−H
A3	$-OCH_3$	−H	B3	$-OCH_3$	−H
A4	−Cl	−H	B4	−OH	−H
A5	−Br	−H	B5	$-NO_2$	−H
A6	−OH	−H	B6	−CN	−H
A7	$-NO_2$	−H	B7	−F	−H
A8	$-OCOCH_3$	−H	B8	−Cl	−H
A9	−H	$-CH_3$	B9	−Br	−H
A10	−H	−Cl	B10	−I	−H
A11	−H	−Br	B11	$-COCH_3$	−H
			B12	−H	−CN
			B13	−H	−OH
			B14	−H	−Cl

of the water−octanol partition coefficient for partitioning of the agent between the two immiscible solvent phases. The 1-octanol−water system is a widely accepted reference system for the determination of lipophilicity expressed as log P.[4] Lipophilicity plays an important role in the transport of compounds through a biological system, and it may also influence the interaction between a drug compound and a pharmacological receptor. The key aspect of the biological activity of a majority of compounds is their ability to get through biological membranes by passive diffusion. This process involves a series of partitioning steps, in combination with diffusion through several regions, i.e., partitioning between aqueous intra- and extracellular media and phospholipid membranes.

Processes of drug absorption, distribution and excretion in the pharmacokinetic phase of drug action, as well as drug−receptor interactions in the pharmacodynamic phase, are dynamic in nature, as are the chromatographic separation processes. Therefore, chromatographic data are often used to model pharmacokinetics of drugs and other xenobiotics.[6] Owing to its simplicity, as well as efficiency, reversed-phase thin-layer chromatography (TLC) appears especially attractive for lipophilicity determination.[9,10] However, the newly proposed gradient HPLC approach[11,12] allows fast and convenient determinations of log k_w parameter (along with pK_a values), which is considered an even more reliable chromatographic lipophilicity parameter. Also, the slope, S, of the linear relationship between log k_w and volume percent of organic modifier in the aqueous eluent can readily be calculated. All that can be obtained from two gradient HPLC runs instead of 5−6 chromatographic runs required in classical isocratic mode.

Correlations of the properly determined log k_w data with the reference lipophilicity parameter log P have been firmly established.[6,9,13]

The objective of this study, apart from examination of the retention behavior in TLC and HPLC reversed-phase chromatographic systems, was to establish the relationships between the chromatographic data and the selected structural properties of succinimides that are related to the ADMETox parameters, obtained by the established computational medicinal chemistry methods. To that aim chromatographic lipophilicity parameters for the newly synthesized compounds have been determined experimentally by two independent methods. On the other hand, such druglikeness properties of the agents, like human intestinal absorption (HIA) and plasma protein binding (PPB), were evaluated theoretically.

Importance of a proper HIA value for the quality of the orally administered drugs, like succinimide derivatives, is obvious. A good absorption is the main prerequisite for reproducible plasma levels after individual equimolar drug doses.

As regards binding of drugs to plasma proteins, a high PPB (above 95%) brings a risk of a highly varying free (unbound) fraction of a drug in plasma. Small changes in that free fraction, which is active pharmacokinetically, can cause dramatic changes in bioactivity. For example, if 2 molecules of 98 previously bound to plasma protein are released (e.g., by a competing xenobiotic), then the bound to protein fraction of a drug changes minimally (from 98 to 96%), but the active unbound fraction increases by 100% (from 2 to 4 molecules per 100). In the case of a little less strongly bound drug, say 94%, releasing 2% molecules increases free fraction from 6 to 8%, i.e., for 25% only. Therefore, drugs not that strongly binding to plasma proteins (<95%) allow for an easier control of dosing.

The drugs, in particular those requiring a long-lasting therapy, like anticonvulsives, should be devoid of or have only negligible adverse effects. For that reason, drug candidates are expected not to bind markedly to irrelevant pharmacological receptors or enzymes. For the anticonvulsive succinimides studied, an affinity to GPCR, kinases, ion channels and nuclear receptors was modeled according to the established procedures based on the agents' molecular structure.

Based on the structure−activity relationships (SAR) derived, a selection of best drug candidates for bioactivity studies has been rationally guided, compromising lipophilicity affecting HIA, PPB and adverse effects.

RESULTS AND DISCUSSION

Retention Behavior of the Compounds Investigated. Two groups of newly synthesized succinimide derivatives (Table 1) were subjected to initial chemical screening of their retention behavior and evaluation of their lipophilicity and pharmacokinetic properties.

The reversed-phase HPTLC and HPLC was carried out on octadecyl-modified silica stationary phases. Aqueous mobile phases, with an organic modifier (methanol, acetone and dioxane), were used. In TLC, the R_f values employed were averages of at least three measurements. For subsequent analyses the mean R_M values were used, as calculated from the equation[14,15]

$$R_M = \log(1/R_f - 1) \quad (1)$$

In HPLC the corresponding parameter was log k, calculated as a mean of 4−5 determinations from

$$k = (t_r - t_0)/t_0 \quad (2)$$

where t_r is retention time and t_0 is the so-called dead time determined by injection of a nonretained marker.

Table 2. Data for Linear Correlation, eqs 3 and 4, between R_M and log k Values and the Volume Fraction of Organic Modifier, φ, in the Mobile Phase along with the Slopes S and S', the Correlation Coefficients, r, and the Standard Errors of Estimation, SD

	water−methanol				water−acetone				water−dioxane				water−methanol	
compd	R_M^0	S	r	SD	R_M^0	S	r	SD	R_M^0	S	r	SD	log k_w	S'
A1	1.718	−2.757	0.993	0.031	1.615	−2.828	0.993	0.034	1.273	−2.391	0.999	0.012	2.73	−4.29
A2	2.273	−3.420	0.994	0.033	1.889	−3.144	0.998	0.020	1.613	−2.834	0.986	0.044	3.19	−4.67
A3	1.647	−2.646	0.994	0.026	1.621	−2.887	0.999	0.015	1.363	−2.604	0.992	0.030	2.92	−4.57
A4	2.476	−3.537	0.993	0.037	2.143	−3.412	0.998	0.021	2.065	−3.496	0.994	0.036	3.33	−4.70
A5	2.538	−3.582	0.995	0.032	2.317	−3.651	0.995	0.038	2.256	−3.768	0.988	0.053	3.42	−4.74
A6	1.239	−2.521	0.986	0.038	1.179	−2.482	0.995	0.026	1.101	−2.621	0.988	0.038	2.39	−4.42
A7	2.162	−3.105	0.995	0.029	2.184	−3.547	0.994	0.042	1.968	−3.415	0.978	0.066	3.65	−4.94
A8	1.411	−2.393	0.996	0.022	1.508	−2.764	0.998	0.020	1.381	−2.746	0.993	0.031	2.86	−4.70
A9	2.096	−3.159	0.991	0.040	1.833	−3.100	0.998	0.021	1.695	−3.012	0.978	0.059	3.06	−4.53
A10	2.562	−3.581	0.990	0.047	2.251	−3.571	0.996	0.033	2.128	−3.590	0.999	0.016	3.29	−4.69
A11	2.643	−3.607	1.000	0.008	2.318	−3.634	0.995	0.038	2.212	−3.700	0.992	0.044	3.55	−5.02
B1	2.588	−3.465	0.992	0.045	2.546	−3.938	0.995	0.037	2.443	−3.923	0.999	0.016	nd[a]	nd
B2	3.422	−4.369	0.991	0.061	3.253	−4.927	0.992	0.058	2.729	−4.169	0.993	0.044	4.35	−5.34
B3	2.972	−3.867	0.993	0.048	3.056	−4.730	0.991	0.058	2.603	−4.105	0.999	0.018	4.14	−5.30
B4	2.595	−4.274	0.993	0.052	2.194	−4.386	0.996	0.034	1.875	−4.296	0.999	0.017	4.15	−5.49
B5	3.435	−4.217	0.996	0.040	3.374	−5.027	0.997	0.035	2.994	−4.570	0.998	0.026	4.35	−5.47
B6	3.009	−3.906	0.992	0.053	2.915	−4.461	0.997	0.030	2.836	−4.482	0.995	0.041	4.00	−5.21
B7	2.941	−3.780	0.991	0.050	2.932	−4.419	0.997	0.030	2.786	−4.273	0.997	0.030	4.22	−5.38
B8	3.516	−4.321	0.994	0.049	3.326	−4.865	0.999	0.003	2.871	−4.256	0.992	0.049	4.54	−5.52
B9	4.076	−5.086	0.999	0.018	3.368	−4.912	0.995	0.045	3.025	−4.480	0.991	0.055	4.61	−5.54
B10	3.496	−4.143	0.992	0.056	3.504	−5.070	0.994	0.050	3.110	−4.570	0.991	0.055	4.73	−5.62
B11	2.848	−3.817	0.992	0.051	2.665	−4.141	0.994	0.040	2.368	−3.855	0.993	0.041	4.04	−5.36
B12	2.931	−3.841	0.990	0.058	2.748	−4.174	0.992	0.049	2.544	−4.022	0.995	0.038	4.02	−5.34
B13	2.605	−3.743	0.995	0.040	2.292	−3.771	0.994	0.039	1.882	−3.289	0.991	0.042	3.74	−5.31
B14	4.248	−5.363	0.996	0.044	3.024	−4.423	0.995	0.041	2.820	−4.169	0.995	0.039	4.50	−5.53

[a] Not determined.

The relationships between the retention values (R_M or log k_w) of the compound and the volume fraction (φ) of organic modifier in the mobile phase were, as expected, linear and expressed by the well-known equations

$$R_M = R_M^0 + S\varphi \quad (3)$$

$$\log k = \log k_w + S'\varphi \quad (4)$$

where R_M^0 and log k_w (intercepts) are the extrapolated values corresponding to $\varphi = 0\%$, and S and S' are the slopes of the linear plot. Equations 1−4 served for deriving data for the further QSAR studies.

Equations 3 and 4 were applied separately for each modifier and each compound. The coefficients of the linear relationships between retention and the volume fraction of organic modifier in the mobile phase (eqs 3 and 4) are listed in Table 2.

The calculated R_M^0 and log k_w values (intercepts) were different for individual compounds due to different substituents. Comparison of the corresponding parameters for compounds of group A with those of group B, which have the same substituent attached to *N*-phenyl, had shown that the R_M^0 and log k_w data were generally higher for compounds of group B than for compounds of group A. It is reasonable as the additional phenyl group increases the ability of the compounds of the group B to participate in nonpolar attractive interactions with the stationary phase to a higher extent than with the polar molecules of the eluent. These differences become more explicit when a more polar modifier is used. Hence, it can be assumed that reversed phase liquid chromatographic retention constants, R_M^0 and log k_w, of the compounds investigated reflect their lipophilicity, the more so that increments to lipophilicity due to the phenyl substituent are by no means identical.

Correlations between Retention Constants, R_M^0 and log k_w, and log P Values. Quantitative structure−retention relationships (QSRR) are among the most extensively studied procedures by which molecular chemical structure is quantitatively correlated with a well-defined physicochemical property of analytes, such as chromatographic retention. Chromatographic retention depends on the net effect of intermolecular interactions between the analyte, the stationary phase and the mobile phase. Linear relationships between the retention factor, R_M^0 or log k_w, and the standard lipophilicity parameter, log P, can be expected because retention of compounds in reversed-phase liquid chromatography is governed by hydrophobic interactions.

In this study R_M^0 and log k_w data, determined on octadecylsilica stationary phases were correlated against log P values, calculated by the use of several softwares[16] (Table 3). The best relationships obtained are between ACD/log P and the retention constants R_M^0 and log k_w (Figure 1). Using different values of log P from computational chemistry other significant correlations were also obtained (Table 4). The present results indicate that the theoretically calculated partition coefficients can be used to predict retention constants of succinimide derivatives on

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C_{18}-silica stationary phases. That is as expected because the empirical chromatographic lipophilicity parameters are known to agree with the log P values.[6,9,13] However, such a correlation needs an empirical verification for individual sets of congeners. Having the correlation proved for the succinimides studies, instead of measuring the log P values by a tedious slow

Table 3. Different log *P* Computational Values Calculated for Investigated Compounds

compd	ACD/log P	log P_{KOWWIN}	milog P	log P X2	log P X3
A1	1.71	1.79	2.418	2.23	1.92
A2	2.17	2.34	2.867	2.66	2.76
A3	1.62	1.87	2.475	2.14	2.37
A4	2.79	2.44	3.096	2.85	3.03
A5	2.48	2.68	3.228	3.03	3.09
A6	0.97	1.31	1.939	1.82	2.04
A7	1.44	2.01	2.377	2.12	2.23
A8	1.05	1.39	1.970	2.01	2.15
A9	2.17	2.34	2.843	2.66	2.76
A10	2.64	2.44	3.072	2.85	3.03
A11	2.48	2.68	3.204	3.03	3.09
B1	3.34	3.46	3.61	4.39	3.88
B2	3.8	4.01	4.06	4.83	4.25
B3	3.26	3.54	3.67	4.31	3.85
B4	2.61	2.98	3.13	3.98	3.53
B5	3.07	3.68	3.57	4.28	3.71
B6	2.78	3.01	3.37	4.11	3.6
B7	3.4	3.66	3.77	4.55	3.98
B8	4.42	4.11	4.29	5.01	4.51
B9	4.12	4.35	4.42	5.19	4.57
B10	4.38	4.63	4.69	5.46	4.53
B11	2.79	3.14	3.51	4.23	3.57
B12	2.78	3.01	3.34	4.11	3.6
B13	2.61	2.98	3.11	3.98	3.53
B14	4.27	4.11	4.26	5.01	4.51

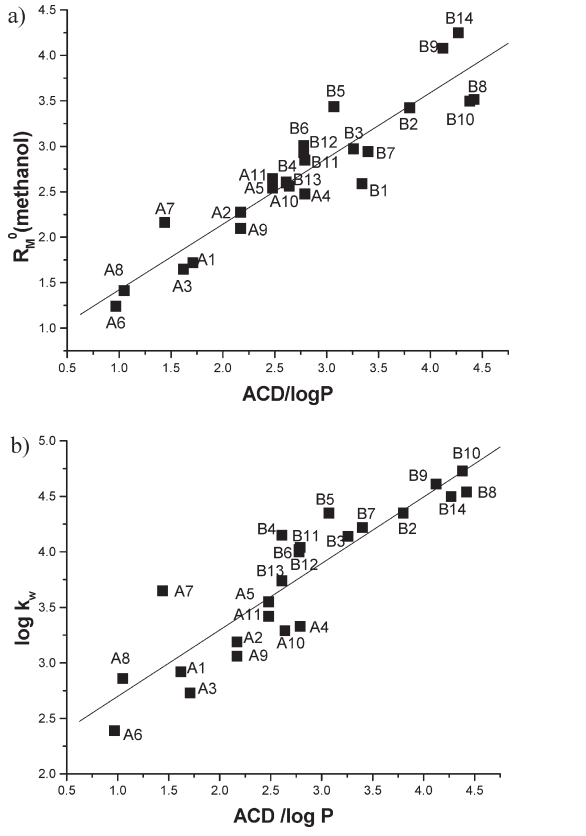

Figure 1. Relationships between retention constants, R_M^0 (a) and log k_w (b) and ACD/log P.

Table 4. Relationships between Retention Constants R_M^0 and log k_w and log P Values, Determined by Using Different Computational Programs

R_M^0 and log k_w vs log P	modifier	equation	r	SD
R_M^0−ACD/log P	methanol	$R_M^0 = 0.697 + 0.723$ ACD/log P	0.932	0.282
	acetone	$R_M^0 = 0.791 + 0.612$ ACD/log P	0.907	0.283
	dioxane	$R_M^0 = 0.725 + 0.547$ ACD/log P	0.889	0.281
R_M^0−log P_{KOWWIN}	methanol	$R_M^0 = 0.400 + 0.777$ log P_{KOWWIN}	0.940	0.263
	acetone	$R_M^0 = 0.463 + 0.652$ log P_{KOWWIN}	0.952	0.206
	dioxane	$R_M^0 = 0.453 + 0.603$ log P_{KOWWIN}	0.922	0.238
R_M^0−milog P	methanol	$R_M^0 = -0.520 + 0.978$ milog P	0.940	0.265
	acetone	$R_M^0 = -0.289 + 0.842$ milog P	0.932	0.244
	dioxane	$R_M^0 = -0.246 + 0.755$ milog P	0.915	0.247
R_M^0−log P X2	methanol	$R_M^0 = 0.475 + 0.612$ log P X2	0.911	0.319
	acetone	$R_M^0 = 0.518 + 0.540$ log P X2	0.927	0.252
	dioxane	$R_M^0 = 0.501 + 0.478$ log P X2	0.898	0.270
R_M^0−log P X3	methanol	$R_M^0 = -0.205 + 0.863$ log P X3	0.933	0.278
	acetone	$R_M^0 = -0.008 + 0.740$ log PX3	0.922	0.260
	dioxane	$R_M^0 = 0.003 + 0.259$ log P X3	0.907	0.259
log k_w−ACD/log P	methanol	log $k_w = 2.10 + 0.599$ ACD/log P	0.896	0.300
log k_w−log P_{KOWWIN}	methanol	log $k_w = 1.75 + 0.677$ log P_{KOWWIN}	0.954	0.299
log k_w−milog P	methanol	log $k_w = 1.06 + 0.818$ milog P	0.916	0.271
log k_w−log P X2	methanol	log $k_w = 1.75 + 0.552$ log P X2	0.953	0.205
log k_w−log P X3	methanol	log $k_w = 1.23 + 0.750$ log P X3	0.942	0.227

Table 5. Relationships between Slopes, S and S', and log P Values, Determined by Using Different Computational Programs

S vs log P	modifier	equation	r	SD	N
S−ACD log P	methanol	$S = -1.876 - 0.651$ ACD log P	0.898	0.319	25
	acetone	$S = -1.938 - 0.710$ ACD log P	0.886	0.370	25
	dioxane	$S = -2.066 - 0.577$ ACD log P	0.836	0.376	25
S−log P_{KOWWIN}	methanol	$S = -1.632 - 0.691$ logP_{KOWWIN}	0.896	0.321	25
	acetone	$S = -1.824 - 0.804$ logP_{KOWWIN}	0.943	0.265	25
	dioxane	$S = -1.755 - 0.644$ logP_{KOWWIN}	0.878	0.329	25
S−milog P	methanol	$S = -0.800 - 0.874$ milogP	0.899	0.319	25
	acetone	$S = -0.676 - 0.980$ milogP	0.913	0.326	25
	dioxane	$S = -1.019 - 0.803$ milogP	0.868	0.341	25
S−log P X2	methanol	$S = -1.692 - 0.546$ logPX2	0.871	0.355	25
	acetone	$S = -1.569 - 0.642$ logPX2	0.927	0.300	25
	dioxane	$S = -1.792 - 0.514$ logPX2	0.882	0.348	25
S−log P X3	methanol	$S = -1.053 - 0.780$ logPX3	0.903	0.311	25
	acetone	$S = -0.964 - 0.874$ logPX3	0.916	0.321	25
	dioxane	$S = -1.249 - 0.712$ logPX3	0.832	0.336	25
S'−ACD log P	methanol	$S = -4.138 - 0.340$ ACD log P	0.806	0.253	24
S'−log P_{KOWWIN}	methanol	$S = -3.901 - 0.398$ logP_{KOWWIN}	0.888	0.196	24
S'−milog P	methanol	$S = -3.541 - 0.466$ milogP	0.828	0.239	24
S'−log P X2	methanol	$S = -3.850 - 0.339$ logPX2	0.926	0.161	24
S'−log P X3	methanol	$S = -3.552 - 0.454$ logPX3	0.903	0.183	24

equilibrium "shake-flask" method, the chromatographic retention constants, readily determined on the RP-C_{18} stationary phases, could be successfully applied to predict lipophilicity of that class of compounds.

Correlations of Retention Parameters, R_M^0 and log k_w (intercepts), and S and S' (Slopes), of the RP LC Equations. Highly significant linear relationship between retention constants, R_M^0 and log k_w (intercepts), and S and S' (slopes), of the RP LC equations (Table 2) were obtained:

$$R_M^0(\text{methanol}) = -1.183 - 1.055S(\text{methanol}) \quad r = 0.985; \text{SD} = 0.131; n = 25 \tag{5}$$

$$R_M^0(\text{acetone}) = -0.787 - 0.838S(\text{acetone}) \quad r = 0.996; \text{SD} = 0.0063; n = 25 \tag{6}$$

$$R_M^0(\text{dioxane}) = -0.995 - 0.886S(\text{dioxane}) \quad r = 0.990; \text{SD} = 0.086; n = 25 \tag{7}$$

$$\log k_w(\text{methanol}) = -2.778 - 1.289S(\text{methanol}) \quad r = 0.961; \text{SD} = 0.223; n = 25 \tag{8}$$

Taking into account quite a satisfactory quality (in terms of statistical characteristics) of the obtained relationships between slopes, S and S', and intercepts, R_M^0 and log k_w, of the RP LC equations, the slopes can be considered alternative to intercepts lipophilicity parameters as proposed by Cserhati.[17]

Table 6. *Molinspiration* Set of Descriptors

compd	milogP[a]	TPSA	natoms	MW	nON	nOHNH	nrotb	volume
A1	2.42	37.38	19	251.28	3	0	2	228.99
A2	2.87	37.38	20	265.31	3	0	2	245.55
A3	2.47	46.61	21	281.31	4	0	3	254.536
A4	3.1	37.38	20	285.73	3	0	2	242.527
A5	3.23	37.38	20	330.18	3	0	2	246.88
A6	1.94	57.61	20	267.28	4	1	2	237.01
A7	2.38	83.204	22	296.28	6	0	3	252.32
A8	1.97	63.85	23	309.32	5	0	4	273.52
A9	2.84	37.38	20	265.31	3	0	2	245.55
A10	3.072	37.38	20	285.73	3	0	2	242.53
A11	3.2	37.38	20	330.18	3	0	2	246.88
B1	3.61	37.38	25	327.383	3	0	3	300.075
B2	4.06	37.38	26	341.41	3	0	3	316.636
B3	3.67	46.614	27	357.41	4	0	4	425.611
B4	3.13	57.608	26	343.382	4	1	3	308.093
B5	3.57	83.024	28	372.38	6	0	4	323.409
B6	3.37	61.172	27	352.393	4	0	3	316.935
B7	3.77	37.38	26	345.373	3	0	3	305.007
B8	4.29	37.38	26	361.828	3	0	3	313.611
B9	4.42	37.38	26	406.279	3	0	3	317.916
B10	4.69	37.38	26	453.279	3	0	3	324.065
B11	3.51	54.451	28	369.42	4	0	4	335.62
B12	3.34	61.172	27	252.393	4	0	3	316.935
B13	3.11	57.608	26	343.382	4	1	3	308.093
B14	4.26	37.38	26	361.828	3	0	3	313.611

[a] milogP, calculated logarithm of octanol−water partition coefficient; TPSA, total polar surface area; natoms, number of atoms; MW, molecular weight; nOH, number of hydrogen bond acceptors; nOHNH, number of hydrogen bond donors; nrotb, number of rotatable bonds; volume, molecular volume. Number of violations, nviolations, was zero in every case.

The slopes, S and S', of the RP LC equations were correlated with log P from calculation chemistry.[11] These correlations are shown in Table 5. It is evident that the slopes of RP LC equations may be applied for lipophilicity expression of the compounds investigated with similar reliability like the intercepts (Table 4). That observation well agrees with a report by Valko.[18]

Correlations of Retention Constants, R_M^0 and log k_w, and Pharmacokinetic Descriptors. Calculations of physicochemical properties of the chemical structures studied by using the *Molinspiration* online program[19] had shown that none of the compounds investigated violates Lipinski's rule of five (Table 6).

The total polar surface area (TPSA) molecular descriptor had been shown to be a good predictor of drug absorption feasibility, including intestinal absorption and general bioavailability, as well as the blood−brain barrier penetration ability. The number of rotable bonds (nrotb) parameter is considered a good descriptor of oral bioavailability of drugs. Based on the results obtained, a good intestinal absorption can be assumed for all the succinimide compounds investigated.

Plasma protein binding (PPB) and human intestinal absorption (HIA) predictors (Table 7) were estimated for the agents studied by the *ChemSilico* program.[20]

The comparison of HIA values with R_M^0 and log k_w values of the RP LC equation is shown in Figure 2. The R_M^0 and log k_w

Table 7. Predictors of Binding to Receptors Calculated by the *ChemSilico* Program[20]

compd	HIA[a]	PPB	GPCR	kinase inhibitor potency	nuclear receptor affinity	ion channel binding
1A	96.3	76.395	−0.57	−0.59	−0.67	−0.51
2A	96	93.901	−0.59	−0.58	−0.68	−0.56
3A	96.8	88.928	−0.51	−0.53	−0.52	−0.55
4A	96	91.493	−0.53	−0.55	−0.7	−0.47
5A	95.9	94.196	−0.64	−0.52	−0.84	−0.54
6A	95.4	58.765	−0.49	−0.52	−0.4	−0.44
7A	92.2	84.43	−0.65	−0.66	−0.62	−0.56
8A	96.5	88.069	−0.72	−0.75	−0.82	--
9A	96	93.87	−0.6	−0.58	−0.68	−0.58
10A	96	91.564	−0.53	−0.53	−0.89	−0.48
11A	95.9	94.332	−0.64	−0.46	−0.98	−0.53
1B	96.1	92.876	−0.2	−0.2	−0.22	−0.31
2B	95.2	96.829	−0.23	−0.22	−0.27	−0.36
3B	96.9	97.891	−0.18	−0.19	−0.18	−0.35
4B	95.8	82.949	−0.16	−0.17	−0.06	−0.27
5B	94.1	98.18	−0.3	−0.31	−0.29	−0.37
6B	94.9	97.695	−0.13	−0.14	−0.27	−0.24
7B	95.9	97.765	−0.13	−0.12	−0.16	−0.25
8B	95	97.287	−0.18	−0.19	−0.29	−0.29
9B	94.2	96.544	−0.27	−0.17	−0.39	−0.34
10B	94.1	96.241	−0.2	−0.16	−0.2	−0.45
11B	94.9	98.05	−0.2	−0.27	−0.23	−0.32
12B	94.9	97.651	−0.14	−0.14	−0.27	−0.25
13B	95.8	82.519	−0.16	−0.16	−0.2	−0.29
14B		93.935	−0.19	−0.18	−0.43	−0.29

[a] HIA, human intestinal absorption; PBP, plasma protein binding; GPCR, G-protein coupled receptor binding.

values of compounds containing nitro groups, nitrile group and acetyl group (A7, B5, B6, B11, B12) formed one parabolic correlation, while R_M^0 and log k_w values of fourteen other compounds, as seen in Figure 2, determined the other curvilinear dependence. Statistics for the relationships are given in Table 8. They can be considered satisfactory. However, to arrive at them four agents (A1, A3, A8 and B3) had to be excluded from the regression analysis as evident outliers. The maximum of the main curves in Figure 2 corresponds to a R_M^0 value between 2.25 and 2.50 and log k_w values between 3.25 and 3.50. That, in turn, according to the equations given in Table 4, would correspond to a log P value between 2 and 2.5.[21] That is a lipophilicity range which is considered optimal for drug intestinal absorption. The most commonly used anticonvulsive drugs have log P around that value range, e.g., carbamazepine 2.45, phenytoin 2.47, clonazepam 2.41, methsuximide 2.38.[22] Therefore, the agents studied appear to be promising candidates for antiepileptic drugs.

Based on data from the *ChemSilico* program, all the newly synthesized compounds are supposed to bind to plasma proteins at a relatively high degree. The relationships between PPB values and both R_M^0 and log k_w values of the RP LC equations are parabolic (Figure 3), except compounds A3, A8 and A9 in relationship PPB vs R_M^0 and compounds A7 and B13 in the relationship PPB vs log k_w. The statistical parameters of the curvilinear relationship of PPB are very good (Table 9). As can be

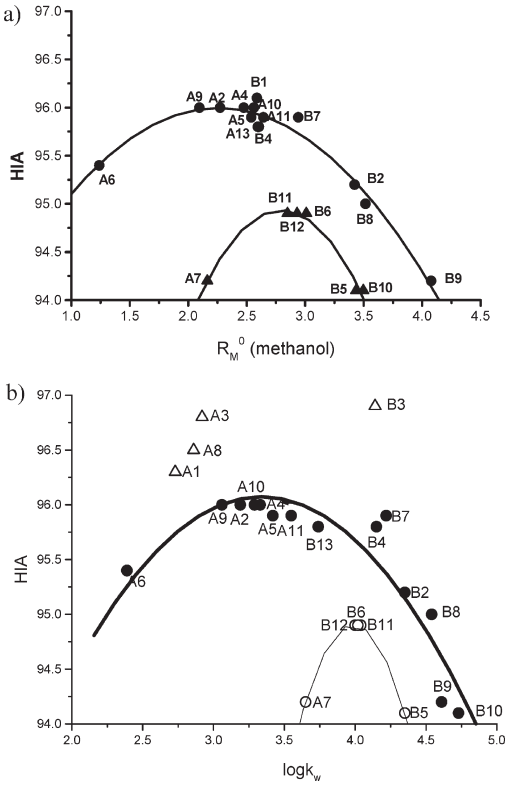

Figure 2. Relationships between retention constants R_M^0 (a) and log k_w (b) and the HIA predictor. Open triangles denote agents excluded from regression as outliers. Open circles refer to a subgroup of nitro, nitrile and acetyl derivatives.

concluded from Figure 3b, the strongest binding to plasma protein (above 95%) can be expected for agents having log k_w above 4. That would correspond to log P above 3.2. Hence, it would be significantly higher than log P optimal for intestinal absorption, namely 2−2.5 units.

Finally, the *Molinspiration* online program was used for druglikeness calculation. Compounds were analyzed as potential GPCR ligands, kinase inhibitors, ion channel modifiers and nuclear receptor ligands (Table 7). The compounds investigated were not expected to bind for GPCR, ion channels nor nuclear receptors, and they should not inhibit kinase. The results of the calculations confirmed these presumptions. Comparisons of average R_M^0 values from the systems employing methanol, acetone and dioxane as mobile phase modifiers, and log k_w from the methanol-modified eluent systems, with the parameters of succinimide binding to GPCR, ion channels and nuclear receptors, and kinase inhibition are shown in Figures 4, 5, 6, and 7. The plots are grouped in two clusters depending on the overall lipophilicity of the compounds. It can be noted that the weak activities considered are even less pronounced in the case of compounds which are less lipophilic (compounds of the group A) as compared to the compounds which are more lipophilic (compounds of the group B). One can argue that there is a jump in binding properties when moving from group A to group B of agents, despite a partial overlapping in lipophilicity parameters. That would suggest that other parameters, apart from lipophilicity, are involved. That could perhaps be true, but the binding properties are very low for all the agents studied and hence the error in their evaluation might be hardly comparable to that of lipophilicity determination. For sure, both groups of new

Table 8. Correlations between the HIA Predictor and Lipophilicity Parameters, R_M^0 and log k_w

modifier	equation	r^2	SD	N
R_M^0 methanol	HIA = 93.131 + 2.535 R_M^0 − 0.561 $(R_M^0)^2$	0.970	0.101	14
	HIA = 80.604 + 10.252 R_M^0 − 1.834 $(R_M^0)^2$	0.984	0.070	6
R_M^0 acetone	HIA = 92.194 + 3.648 R_M^0 − 0.866 $(R_M^0)^2$	0.864	0.260	15
	HIA = 78.282 + 12.053 R_M^0 − 2.182 $(R_M^0)^2$	0.998	0.026	5
R_M^0 dioxane	HIA = 91.500 + 4.759R_M^0 −1.238 $(R_M^0)^2$	0.821	0.299	15
	HIA = 76.414 + 14.904 R_M^0 − 2.989 $(R_M^0)^2$	0.856	0.221	5
log k_w methanol	HIA = 85.986 + 6.042 log k_w − 0.905 $(\log k_w)^2$	0.867	0.262	15
	HIA = −2.382 + 49.286 log k_w − 6.178 $(\log k_w)^2$	0.999	0.008	5

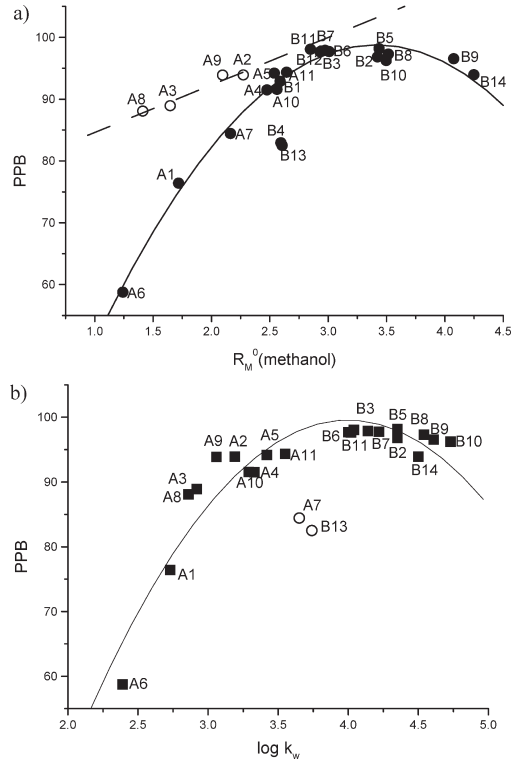

Figure 3. Relationships between retention constants R_M^0 (a) and log k_w (b) and PPB predictor. Open symbols denote agents excluded from regression as outliers.

succinimide derivatives are devoid of significant receptor and enzyme activity which would interfere with their main anticonvulsant mechanism of action.

EXPERIMENTAL SECTION

Synthesis of Succinimide Derivatives. All of the investigated 1-(4-substituted-phenyl)-3-phenylpyrrolidine-2,5-diones and 1-(4-substituted-phenyl)-3,3-diphenylpyrrolidine-2,5-diones were synthesized from the corresponding succinic anhydrides using a modified literature procedure.[2,23] The succinic anhydride was treated with the corresponding substituted aniline in refluxing acetone for 2 h and concentrated, and the succinimic acid was taken in acetic anhydride with sodium acetate. The mixture was heated at 70 °C for 2 h. Acetic anhydride was removed under vacuum, and the crude material was purified by crystallization. The chemical structures and the purities of the synthesized succinimides were confirmed by melting points and the ^{1}H NMR, ^{13}C NMR, FT-IR and UV spectra.

Chromatography. *TLC.* Precoated RP-18W/UV$_{254}$ plates (Machery-Nagel GmbH and Co., Düren, Germany) were used for TLC analysis. The following mobile phases were used:

$$\text{water} - \text{methanol}\ (\varphi_{\text{methanol}} = 0.55 - 0.75\%\ \text{v/v})$$

$$\text{water} - \text{acetone}\ (\varphi_{\text{acetone}} = 0.45 - 0.7\%\ \text{v/v})$$

$$\text{water} - \text{acetonitrile}\ (\varphi_{\text{acetonitrile}} = 0.45 - 0.75\%\ \text{v/v})$$

The plates were developed by ascending technique without previous saturation of the chromatographic chambers with the solvent. All measurements were carried out at ambient temperature. The investigated compounds were dissolved in acetone (2 mg/mL) and 0.2 μL solutions were separately spotted onto the plates. Following development and drying of the plates, the spots were observed under a UV light at λ = 254 nm. All the other reagents used were of analytical grade purity.

HPLC. Experiments were done using a Merck-Hitachi La-Chrome (Darmstadt, Germany; San Jose, CA, USA) apparatus equipped with a diode array detector, autosampler and thermostat. Chromatographic data were collected using a D-7000 HPLC System Manager, version 3.1 (Merck-Hitachi). The system dwell volume V_d equaled 1.74 mL. The extra column volume equaled 0.59 mL. It served to find the extra column time that has been subtracted from all the retention times prior to any calculation.

XTerra MS C-18 column, 150 × 4.6 mm, particle size 5 μm (Waters Corporation, Milford, MA, USA) was used. 1% thiourea was injected to determine the column dead volume V_o, which was 1.44 mL.

Phosphate buffer of pH = 7.4 at concentration 0.01 M was used.

Chromatographic measurements were done at 25 °C with eluent flow-rate of 1.0 mL/min.

Two chromatographic runs were conducted for each analyte with different gradient development times, tg_1 = 20 min and, tg_1 = 60 min, and with a wide methanol concentration range from 5% to 100%. The retention time for organic modifier gradients is given by[11,12]

$$t_R = \begin{cases} t_e + t_0 + t_0 k_0 \text{ for } t_R' \le t_d \\ t_e + t_0 + t_d + \dfrac{t_0}{b}\log\left(2.303 b k_0\left(1 - \dfrac{t_d}{t_0 k_0}\right) + 1\right) \\ \quad \text{for } t_d < t_R' \le t_G + t_d \\ t_e + t_0 + t_d + t_G - \dfrac{t_0}{2.303 b} + \left(t_0 k_0 - t_d + \dfrac{t_0}{2.303 b}\right) \\ \quad \times 10^{-b t_G / t_0} \text{ for } t_R' > t_G + t_d \end{cases} \quad (9)$$

Table 9. Correlations between the PPB Predictor and Lipophilicity Parameters, R_M^0 and log k_w

modifier	equation	r^2	SD	N
R_M^0 methanol	PPB = 2.507 + 56.408 R_M^0 − 8.262 $(R_M^0)^2$	0.982	1.373	21
	PPB = 76.929 + 7.683 R_M^0		0.855	4
R_M^0 acetone	PPB = −4.496 + 67.379 R_M^0 − 11.084 $(R_M^0)^2$	0.957	2.149	21
	PPB = 61.798 + 17.164 R_M^0		0.743	4
R_M^0 dioxane	PPB = 6.831 + 62.659R_M^0 −10.850 $(R_M^0)^2$	0.919	2.915	21
	PPB = 63.679 + 18.184 R_M^0		0.980	4
log k_w methanol	PPB = −111.646 + 105.592 log k_w − 13.197 $(\log k_w)^2$	0.939	3.327	20

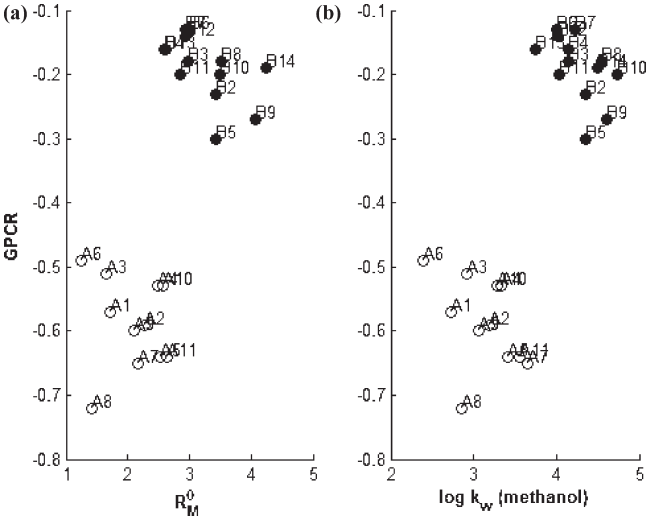

Figure 4. Relationship between retention constants R_M^0 (a) and log k_w (b) and GPCR binding predictor.

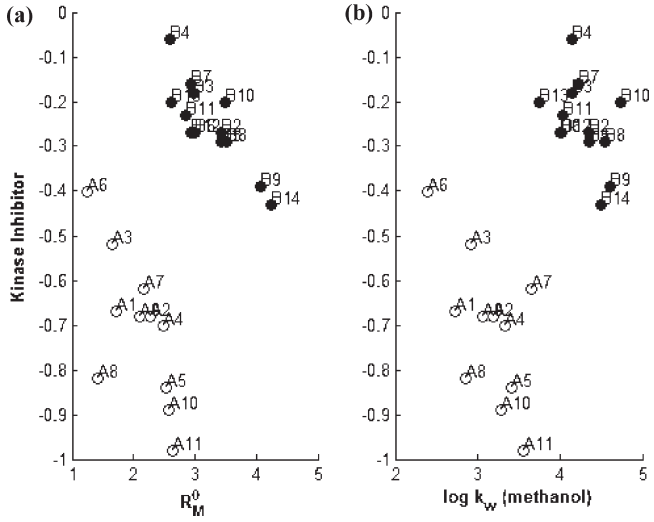

Figure 6. Relationship between retention constants R_M^0 (a) and log k_w (b) and kinase inhibitor predictor.

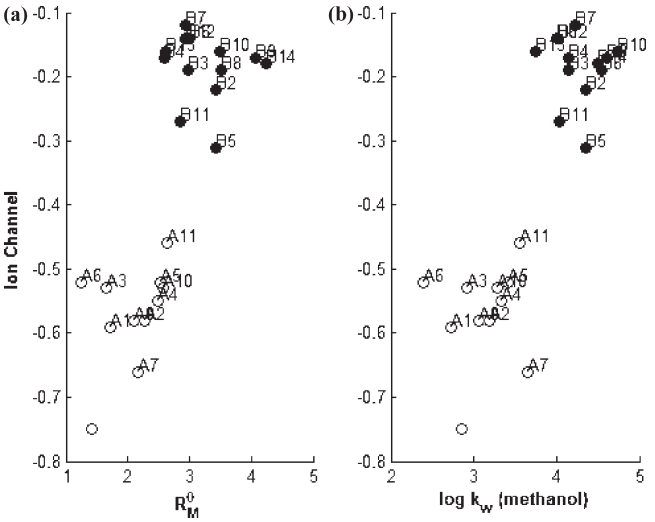

Figure 5. Relationship between retention constants R_M^0 (a) and log k_w (b) and ion channel binding predictor.

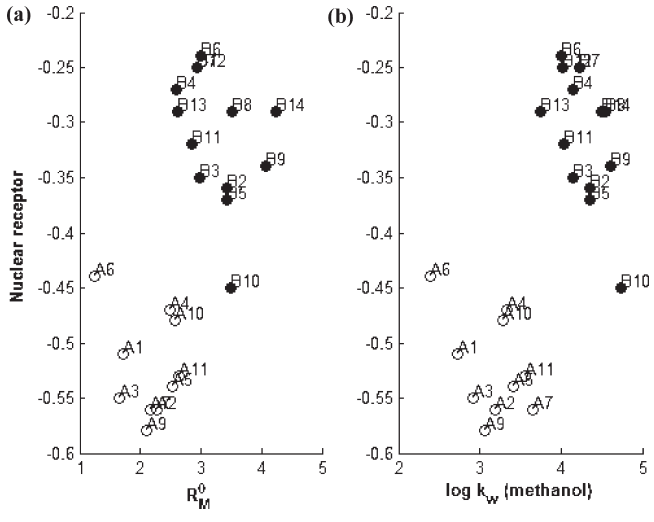

Figure 7. Relationship between retention constants R_M^0 (a) and log k_w (b) and the nuclear receptor binding predictor.

where t_e is an extra column time; t_0 is a hold-up time; t_d is dwell time, b is a steepness parameter equal to $b = S\beta t_0$; β is the steepness of the gradient equal $\beta = (\varphi_f - \varphi_0)/t_G$; φ_0 and φ_f are the initial and final organic modifier contents in the mobile phase, and k_0 is a retention factor corresponding to the initial mobile phase composition. The log k_w is given by $\log k_w = \log k_0 + S\varphi_0$.

Two unknowns (log k_w and S) were determined by solving a system of two equations of the type of eq 9 using Matlab.

CONCLUSION

A series of 25 new succinimide derivatives synthesized in the project are potential anticonvulsant drug candidates. The

compounds possess a characteristic for antiepileptic drug structural feature (pharmacophore). Their lipophilicity, both determined experimentally by two high performance reversed-phase chromatographic techniques (HPLC and TLC) and calculated from structural formula by the established drug design softwares, is within the range providing efficient intestinal absorption. At the same time, the plasma protein binding ability of the agents has been evaluated as moderate and the side effects due to interactions with selected receptors and enzymes can be expected negligible. Certainly, the TLC method of determination of lipophilicity of drug candidate is simple and inexpensive. However, HPLC provides the data which correlate better with the pharmacokinetically relevant compound descriptors, in particular, as regards the plasma protein binding, PPB.

Of the set of 25 compounds considered for further drug development studies the agents A4, A5, A10 and A11 are proposed as the best compromising the human intestinal absorption and plasma protein binding properties.

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