Lipophilicity Determination of Some Potential Photosystem II Inhibitors on Reversed-Phase High-Performance Thin-Layer Chromatography

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Abstract

The retention characteristics of 25 2-cyano-3-methylthio-3-substituted amine-acrylates are determined using reversed-phase thin-layer chromatography (RP-TLC) with methanol–water mixtures as eluents. The relationship between R_m values and partition coefficients (*C* log *P*) are established. The R_m values decrease linearly with increasing methanol concentration in the eluent. The R_m values extrapolated to zero organic modifier concentration ($R_{m\,0}$) in the eluent are highly related to *C* log *P*. The R_{m0} value can be used to evaluate the lipophilicity of this kind of compound.

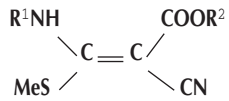
Introduction

In previous years, quantitative structure-activity relationships (QSARs) have been widely accepted and have progressed well (1,2). Lipophilicity is an important physico-chemical parameter of a compound, and it plays a vital role in QSAR studies. The traditional method of determining lipophilicity using octanol–water partitioning has some disadvantages. It is being supplanted by chromatographic procedures such as reversed-phase high-performance liquid chromatography (RP-HPLC) (3,4) and reversed-phase thin-layer chromatography (RP-TLC) (5). The TLC method has some advantages over the traditional method: it is rapid and relatively simple, it has a low cost, very small amounts of substances are required, and the compounds need not be very pure.

Using methanol–water mixtures as mobile phases and reversed-phase C_{18} as a stationary phase in HPLC, Brauman (3) found that log *k* values extrapolated to zero organic modifier concentration (R_{m0}) show a good correlation with octanol–water partition coefficients. Because the basic partitioning conditions are similar in RP-TLC and RP-HPLC, the same type of mobile and stationary phases were applied to RP-TLC.

R_m values obtained using RP-TLC have traditionally been used as lipophilicity parameters, but R_m values depend significantly on mobile phase composition (6). R_{m0} values (i.e., R_m extrapolated to 0% organic modifier concentration) are preferable

Table I. Structures of 25 2-Cyano-3-Methylthio-3-Substituted Amine-Acrylates



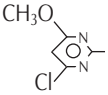
Compound	R_1	R_2
1	$C_6H_5CH_2$	C_2H_5
2	*i*-Pr	C_2H_5
3	*n*-Bu	C_2H_5
4	*p*-$NO_2C_6H_4$	C_2H_5
5	*m*-$NO_2C_6H_4$	C_2H_5
6		C_2H_5
7	*o*-$CH_3C_6H_4CH_2$	C_2H_5
8	*p*-$CH_3C_6H_4CH_2$	C_2H_5
9	*p*-$CH_3OC_6H_4CH_2$	C_2H_5
10	*o*-$CH_3OC_6H_4CH_2$	C_2H_5
11	*p*-$CH_3OC_6H_4CH_2CH_2$	C_2H_5
12	*o*-$CF_3C_6H_4CH_2$	C_2H_5
13	*p*-$CF_3C_6H_4CH_2$	C_2H_5
14	*p*-$ClC_6H_4OC_6H_4$	C_2H_5
15	*p*-$NO_2C_6H_4CO$	C_2H_5
16	*p*-$ClC_6H_4CH_2$	C_2H_5
17	$C_6H_5CH_2$	$CH_2CH_2OCH_3$
18	*p*-$CH_3C_6H_4CH_2$	$CH_2CH_2OCH_3$
19	*p*-$CH_3OC_6H_4CH_2$	$CH_2CH_2OCH_3$
20	*p*-$CF_3C_6H_4CH_2$	$CH_2CH_2OCH_3$
21	$C_6H_5CH_2$	$CH_2CH_2OCH_2CH_3$
22	*p*-$CH_3C_6H_4CH_2$	$CH_2CH_2OCH_2CH_3$
23	*p*-$CH_3OC_6H_4CH_2$	$CH_2CH_2OCH_2CH_3$
24	*p*-$CF_3C_6H_4CH_2$	$CH_2CH_2OCH_2CH_3$
25	*p*-$ClC_6H_4CH_2$	$CH_2CH_2OCH_2CH_3$

Table II. R_f Values of Twenty-five Compounds in all Experimental Conditions and Their *C* log *P* Values

Compound	R_f 0.65	0.70	0.75	0.80	0.85	0.90	*C* log *P*
1	0.150	0.210	0.330	0.479	0.571	0.660	2.11
2	0.235	0.303	0.413	0.540	0.631	0.668	1.23
3	0.150	0.213	0.316	0.456	0.558	0.628	1.98
4	0.228	0.310	0.474	0.592	0.675	0.709	2.00
5	0.217	0.292	0.453	0.564	0.639	0.708	2.00
6	0.152	0.229	0.333	0.444	0.554	0.634	1.98
7	0.099	0.178	0.276	0.384	0.512	0.607	2.56
8	0.082	0.160	0.251	0.370	0.482	0.589	2.61
9	0.144	0.243	0.352	0.471	0.573	0.668	2.03
10	0.123	0.210	0.295	0.406	0.527	0.632	2.03
11	0.140	0.216	0.334	0.446	0.554	0.655	2.26
12	0.096	0.170	0.260	0.376	0.536	0.640	2.99
13	0.103	0.182	0.270	0.389	0.560	0.672	2.99
14	0.020	0.055	0.093	0.178	0.348	0.456	4.74
15	0.469	0.533	0.607	0.691	0.765	0.818	1.19
16	0.098	0.162	0.259	0.374	0.528	0.650	2.82
17	0.254	0.357	0.463	0.562	0.680	0.757	1.40
18	0.151	0.239	0.338	0.459	0.594	0.700	1.90
19	0.239	0.340	0.446	0.554	0.676	0.734	1.32
20	0.148	0.256	0.370	0.496	0.668	0.704	2.29
21	0.164	0.291	0.382	0.486	0.626	0.691	1.93
22	0.092	0.192	0.290	0.388	0.545	0.624	2.43
23	0.177	0.290	0.381	0.495	0.627	0.696	1.85
24	0.092	0.216	0.308	0.441	0.596	0.689	2.81
25	0.110	0.202	0.286	0.410	0.554	0.646	2.64

Table III. Coefficients in Eq 2

Compound	R_{m0}	*b*	*r*
1	3.564	–4.328	0.9952
2	2.766	–3.479	0.9913
3	3.407	–4.094	0.9951
4	3.015	–3.878	0.9837
5	3.059	–3.887	0.9900
6	3.320	–3.996	0.9976
7	3.886	–4.576	0.9965
8	4.078	–4.757	0.9951
9	3.499	–4.267	0.9960
10	3.611	–4.296	0.9983
11	3.548	–4.284	0.9977
12	4.153	–4.923	0.9987
13	4.182	–5.008	0.9990
14	5.817	–6.446	0.9952
15	1.956	–2.891	0.9981
16	4.182	–4.957	0.9998
17	2.956	–3.844	0.9994
18	3.641	–4.465	0.9997
19	2.985	–3.845	0.9978
20	3.764	–4.689	0.9919
21	3.360	–4.173	0.9934
22	4.028	–4.788	0.9918
23	3.296	–4.104	0.9964
24	4.298	–5.223	0.9915
25	3.855	–4.613	0.9957

as lipophilicity parameters (7–9).

2-Cyano-3-methylthio-3-substituted amine-acrylates are potential photosystem inhibitors that block electron transfer in photosystems. They show high inhibitory activities, and their inhibitory activities are related to their lipophilicity. The objectives of this work were to determine the retention of this kind of compound on precoated C_{18} high-performance TLC (HPTLC) plates using methanol–water mixtures as eluents and to find the relationship between retention characteristics and lipophilicity parameters (*C* log *P*) of the compounds.

Experimental

Materials

The structures of 25 2-cyano-3-methylthio-3-substituted amine-acrylates compounds are listed in Table I. This series of compounds was synthesized in our Organic Synthesis Laboratory, and their structures were verified using many methods: infrared, nuclear magnetic resonance, mass spectrometry, and element analysis. Approximately 1 mg/mL of each compound in methanol was used for spotting.

Apparatus

TLC was performed on precoated C_{18} RP-HPTLC plates (10 × 10 cm, F254) from Merck (Darmstadt, Germany). A Nanomat applicator (Camag, Muttenz, Switzerland) was used with a Pt–Ir pointed glass capillary. Plates were developed in a closed chamber (Camag).

An SGI Indy Workstation (Silicon Graphics Incorporated, Mountain View, CA) with Sybyl 6.22 (Tripos Company, St. Louis, MO) was used for data collecting, and a PC computer was used for data processing.

Chromatography

Methanol–water mixtures were used as mobile phases; the concentrations of methanol were 65%, 70%, 75%, 80%, 85%, and 90%. Developments were carried out in a closed chamber at room temperature, and the distance of development was approximately 5 cm. After development, the plates were dried in air and the spots were viewed under an ultraviolet lamp. The R_f values of each compound are listed in Table II. The *C* log *P* values that were obtained from the Indy workstation are also listed in Table II.

Results and Discussion

The R_m values of each compound were obtained using the following equation:

$$R_m = \log (1/R_f - 1) \quad \text{Eq 1}$$

Linear correlation between R_m values and the concentration of organic modifier in the eluents was calculated separately for each compound according to the following equation:

$$R_m = R_{m0} + bc \quad \text{Eq 2}$$

where c is the concentration of methanol in the eluent. The R_{m0} and b values are listed in Table III.

All of the compounds showed normal retention behavior; that is, their R_m values decreased linearly with the increasing concentration of methanol in the mobile phase, as can be seen in Figure 1.

The relationship between R_m values and C log P under all experimental conditions was determined, and the results are listed in Table IV. Note that the R_m values are related to the lipophilicity of these compounds. The regression coefficient in the relationship between R_m and C log P increased with decreasing concentration of methanol in the mobile phase.

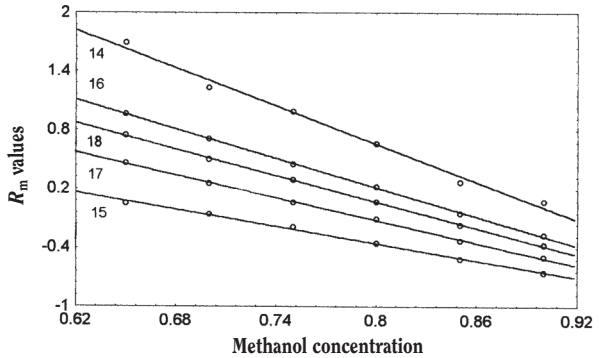


Figure 1. Relationship between R_m values and methanol concentration in the mobile phase for some selected compounds.

Table IV. Relationships Between R_m and C log P in all Experimental Conditions (n = 25, P < 0.0001)

CH_3OH (%)	Regression equations	r	s	F
65	$R_m = -0.453 + 0.370C \log P$	0.9275	0.112	142
70	$R_m = -0.114 + 0.284C \log P$	0.9118	0.0957	113
75	$R_m = -0.299 + 0.266C \log P$	0.9071	0.0924	107
80	$R_m = -0.438 + 0.230C \log P$	0.8965	0.0850	94.2
85	$R_m = -0.529 + 0.169C \log P$	0.8190	0.0886	46.8
90	$R_m = -0.618 + 0.140C \log P$	0.7792	0.0842	35.5

Table V. Results of Factor Analysis

Variable	Factor 1
C log P	0.9778
R_{m0}	0.9936
b	–0.9924
Eigen values	2.928
% Total variance	97.61

Factor analysis was carried out for the three parameters C log P, R_{m0}, and b. The results are listed in Table V. The results show that the three parameters are highly correlated. Using linear regression analysis, the following equations were obtained:

$$R_{m0} = 1.542 + 0.922C \log P$$
(n = 25, r = 0.9512, s = 0.224, F = 218.8, P < 0.00001) Eq 3

$$b = -2.429 - 0.875C \log P$$
(n = 25, r = 0.9476, s = 0.221, F = 202, P < 0.00001) Eq 4

The correlation coefficients (r) of the two equations are very high, which indicates that R_{m0} and b values are highly related to C log P in a linear relationship. Therefore, R_{m0} or b values can be used to evaluate the lipophilicity of these kinds of compounds, and R_{m0} is better than b.

It can be seen that the retention data or C log P values of these compounds are related to their structures. First, the more CH_2 in the R_1 group, the higher the R_m values and the higher the C log P values (for example, compounds 9 and 11). This indicates that CH_2 is a lipophilic group. Second, although the regioisomers may have the same C log P values, their R_m values are different, which may result from the different position of the substitute (for example, compounds 4 and 5, 9 and 10, or 12 and 13). There is an exception in compounds 7 and 8; they have different C log P values as well as different R_m values. It is thought that the position of CH_3 may have a relatively greater influence on C log P value. Finally, when the compounds that have the same R_1 group and a different R_2 group (for example, compounds 1, 17, and 21; 8, 18, and 22; or 13, 20, and 24) are considered, it is discovered that their R_f values are in the order of C_2H_5 < $CH_2CH_2OCH_2CH_3$ < $CH_2CH_2OCH_3$; their R_m values are C_2H_5 > $CH_2CH_2OCH_2CH_3$ > $CH_2CH_2OCH_3$; and their C log P values are C_2H_5 > $CH_2CH_2OCH_2CH_3$ > $CH_2CH_2OCH_3$.

Conclusion

It has been shown that R_{m0} values obtained using RP-TLC are a good alternative for lipophilicity determination. This method is low in cost, rapid, and requires minute amounts of samples that need not necessarily be very pure. RP-TLC can be extensively applied to determine lipophilicity.

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