Relationship between Brain Tissue Partitioning and Microemulsion Retention Factors of CNS Drugs

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Received November 15, 2008

In CNS drug discovery, knowledge of drug−tissue binding is essential for a better understanding of brain penetration by assessing unbound brain to plasma ratio as well as pharmacokinetics (PK) and pharmacodynamics (PD) relationship by relating free drug concentration to pharmacological effect in target tissues. In this work, we present a novel microemulsion based capillary electrophoresis (CE) method that enables coupling microemulsion electrokinetic chromatography (MEEKC) to mass spectrometry (MS) for prediction of biopartitioning of CNS drugs in brain tissue. Compared to LC retention based lipophilicity and calculated lipophilicity, a significantly improved correlation between the LogP values obtained from MEEKC retention factors and fraction unbound (fu) in brain tissue was observed for a training set of structurally diverse CNS drugs as well as for a test set of new chemical entities (NCEs). The current online CE/MS/MEEKC technique can also be a potential approach for lipophilicity screening amenable for highly predictive of other ADME-Tox properties of NCEs using the MEEKC partitioning coefficient as a relevant descriptor.

1. Introduction

When developing new drugs, it is essential that central nervous system (CNS) target drugs reach their site of action to obtain desired pharmacological effect, while for non-CNS targets it might be equally important to keep peripherally acting compounds out of the brain to avoid unwanted CNS side effects. Accordingly, knowledge of brain tissue binding becomes an important concern for CNS-related projects. This has been clearly demonstrated and highlighted in several recent publications.[1−6] In our previous work,[7] we presented a high-throughput method for brain homogenate binding screening and demonstrated lipophilicity as one of relevant descriptors in in silico model for brain tissue binding prediction. This observation has inspired us to further investigate alternative approaches for an accurate lipophilicity measurement that would be expected to improve the correlation with fu of CNS drugs in brain tissues. Moreover, the lipophilicity, LogPo/w (octanol−water partitioning coefficient) and LogD (distribution coefficient at physiological pH 7.4), has been used as an important parameter in many ADME[a] models. For instance, it has been shown that in vivo toxicological outcome is strongly correlated with ClogP and total polar surface area.[8] LogP value is an important factor in explaining the variation in inhibitory potency in relation with CYP2C9 and CYP2C19 enzymes.[9] Microsomal binding is shown to be best predicted using a model where LogP is used for basic compounds, whereas LogD7.4 is used for acidic and neutral compounds.[10] In contrast, it was found that LogD gave a good correlation with plasma protein binding for neutral and basic drugs and a good correlation with LogP for acidic drugs.[11] MEEKC partitioning has been used for modeling membrane phenomenon, which is considered to be more biologically relevant in in vitro models for cell membranes than traditional octanol−water partitioning for rapid screening of drug−membrane interactions.[12−16]

Up to now, several methods have been applied for lipophilicity measurements including the most widely used LogPo/w and LogD as well as reversed phase liquid chromatographic (RPLC) retention based approaches.[17−20] However, these techniques are often applied for LogD at pH 7.4 and it is not easily accessible to accurate LogP values measured at a pH where the molecule is truly neutral. An alternative technique based on MEEKC has been shown to be a potential method for accurate LogP measurement.[15,21−29] MEEKC is an electrodriven separation technique by partitioning between water and oil-in-water microemulsions consisting of surfactant (usually sodium dodecyl sulfate, SDS) coated nanometer-sized droplets of oil suspended in aqueous buffer. A cosurfactant such as a short-chain alcohol is generally employed to stabilize the microemulsion. As the microemulsion separation is considered to be rather similar to octanol−water partitioning, the capacity factor (k') of MEEKC is shown to highly correlate with the LogPo/w achieved by the conventional shake flask method, and correlates better than LogP obtained by HPLC.[27] Several groups have demonstrated that both MEEKC systems using SDS based and vesicle electrokinetic chromatography are suitable for rapid determination of LogP for neutral, weakly acidic, and weakly basic compounds with a wide range of LogP.[23,24,26−28] A long-term validation and assessment of MEEKC for high-throughput screening of LogP on a 96-capillary instrument has shown acceptable accuracies.[28]

Particular benefits of MEEKC as compared to HPLC and the shake flask method include the use of full pH range, the suitability for a diversity of structures, ease of automation, small amounts of sample consumption, and short analysis times. However, up to date, all above MEEKC based lipophilicity measurements are performed on UV detection using a single compound requiring relatively high concentration, in particular utilizing SDS as the micelles in non-volatile buffers, which is not compatible with online MS detection due to substantial ion suppression with electrospray ionization as well as ion source contamination. Alternatively, a lauric acid (LA) based micelle

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[a] Abbreviations: ADME-Tox, absorption, distribution, metabolism, excretion−toxicity; CE/MS/MEEKC, capillary electrophoresis/mass spectrometry/microemulsion electrokinetic chromatography; CNS, central nervous system; fu, fraction unbound in brain tissue; k', capacity factor; RPLC, reversed phase liquid chromatography; SDS, sodium dodecyl sulfate.

10.1021/jm801441s CCC: $40.75
Published on Web 03/03/2009

Table 1. Physicochemical Properties and Brain Tissue Binding Data of CNS Drugs

compd	no.	ClogP	ACDLogP	LogD7.4(LC)	ACDLogD	Log k'	$LogP_{MEEKC}$	fu%	pK_a	fu ref.
amantadine	**1**	2	2.22	<0	−1.14	0.84	2.82	23.3	10.8	Summerfield[5]
amoxapine	**2**	3.41	2.35	2.4	1.76	1.34	3.71	1	8.71/3.38	Summerfield
buspirone	**3**	2.19	3.43	2.7	3.36	0.79	2.74	16	7.8	Wan[7]
carbamazepine	**4**	2.38	2.67	1.77	2.67	0.54	2.29	16	neutral	Wan
carisoprodol	**5**	2.34	2.15	2.40	0.65	2.37	2.4	36.0	neutral	Wan
citalopram	**6**	3.13	2.51	1.9	0.34	1.29	3.63	3.4	9.7	Wan
clozapine	**7**	3.71	2.36	3.48	2.23	1.27	3.6	1	7.93/3.79	Wan
cyclobenzaprine	**8**	5.1	5	2.9	3.24	1.94	4.81	0.58	9.69	Wan
diazepam	**9**	2.96	2.91	3.64	2.91	1.17	3.42	4.3	3.55	Wan
doxepin	**10**	4.09	3.86	2.3	2.06	1.53	4.07	2.5	9.37	Summerfield
fluoxetine	**11**	4.57	4.09	3	1.43	1.81	4.57	0.26	10.2	Wan
haloperidol	**12**	3.85	3.01	2.49	2.11	1.23	3.53	0.82	9.02	Wan
hydroxyzine	**13**	4	2.03	3.6	2	1.5	4	1.3	7.75	Wan
lamotrigine	**14**	2.53	−0.19	<0	−0.19	0.16	1.6	25	5.19	Wan
loxapine	**15**	3.98	2.74	4.6	2.56	1.42	3.86	1.1	7.48/3.90	Summerfield
maprotiline	**16**	4.52	4.51	2.9	1.29	1.87	4.67	0.6	10.6	Summerfield
mesoridazine	**17**	4.44	3.98	1.7	1.71	1.64	4.26	1.6	9.61	Summerfield
methylphenidate	**18**	2.56	−0.61	0.4	0.83	2.67	2.67	27.0	9.24	Wan
metoclopramide	**19**	2.23	2.22	0	−0.05	0.43	2.09	36.5	9.59	Summerfield
midazolam	**20**	3.42	3.93	3.38	3.92	1.3	3.65	2.5	5.44	Wan
mirtazapine	**21**	2.81	2.75	2.3	2.1	1.21	3.48	8	7.79/3.59	Summerfield
nortriptyline	**22**	4.32	5.65	2.8	3.05	1.69	4.36	0.36	10.41	Wan
olanzapine	**23**	3.01	1.51	1.9	0.68	1.06	3.21	3.4	8.50/4.66	Summerfield
paroxetine	**24**	4.24	3.89	2.3	0.97	1.53	4.07	0.26	10.1	Wan
pergolide	**25**	4.4	4.49	3	2.09	1.62	4.22	2.7	8.78	Summerfield
perphenazine	**26**	3.81	4.34	4.1	4.21	1.4	3.83	0.4	8.04/3.72	Summerfield
propranolol	**27**	2.75	3.1	1.4	1.35	1.21	3.48	2	9.64	Wan
quetiapine	**28**	2.99	1.56	3.4	1.55	1.24	3.54	2.5	6.91/3.64	Summerfield
risperidone	**29**	2.71	2.88	1.71	2.29	0.86	2.86	8.7	8.63/3.16	Wan
selegiline	**30**	3.02	2.95	3.6	2.58	0.95	3.03	7.4	7.69	Wan
sumatriptan	**31**	0.74	0.67	<0	−1.24	0.05	1.41	72.4	9.68	Summerfield
tacrine	**32**	3.27	3.32	<0	1.68	0.52	2.26	12.4	10	Summerfield
thioridazine	**33**	6	6.13	4.4	3.85	1.91	4.75	0.1	8.9	Summerfield
trazodone	**34**	3.85	1.66	2.99	1.61	0.89	2.92	4.4	6.73	Wan
venlafaxine	**35**	3.27	2.91	1	1.04	1.05	3.2	22	9.9	Wan
zolpidem	**36**	3.03	2.91	2.3	3	0.49	2.2	17	6.02	Wan

for online CE/MS system using micellar electrokinetic chromatography (MEKC) mode for high sensitive detection of some basic drugs was demonstrated.[30] In additions, online CE/MS/MEEKC (SDS based) using APPI interface has been reported recently using nonvolatile buffers[31,32] but not for LogP determination. In this work, we present a new MEEKC system suitable for long-term online MS performance for brain tissue binding prediction as well as lipophilicity screening.

2. Methods and Materials

2.1. Sets of Compounds Used in This Study. The selection of training and test sets of compounds was based on the availability of published brain tissues binding data (Table 1) and literature LogP values (Table 2). Chemicals used include ammonia (25% in water, Merck), acetic acid (99.7%, Merck), octane (98%, Aldrich), butanol (GC ≥99.5%, Merck), LA (98%, Aldrich), methyl ammonium solution (41% in water, Fluka), 4-dodecylaniline (**4-D**) (97%, Sigma), Sudan 4 (**S-4**) (analytical grade, AG, Sigma), dodecyltrimethyl-ammonium bromide (**DT**) (99%, Sigma), dodecylbenzene (**DB**) (99%, Fluka), and dimethyl sulfoxide (DMSO) (AG). All these compounds were prepared in 10 mM stock solutions in DMSO except for (**4-D**) in 20 mM stock solution.

2.2. CE/MS. Agilent HP[3D] CE and 1100 series LC/MSD ion trap were used for CE separation and compound identification. Electrospray ionization was operated in positive mode. CE separation capillaries were untreated fused silica capillaries (50 μm × 50−60 cm) and preconditioned off-line by flushing with 0.1 sodium hydroxide for 1.5 h, followed by Milli-Q water and microemulsion for 10 min, respectively, before measurements. The sheath liquid composition was water:methanol 50:50 containing 5 mM of ammonium formate. The voltage in ion chamber was set to −4 kV. A full scan mode (from 70 to 700 m/z) was used in all experiments. Nebulizer gas was set at 5.0 psi. Drying gas and drying temperature were set at a flow of 5.0 L min^{-1} and 150 °C, respectively. Other separation conditions are denoted in respective tables and figures.

2.3. Preparation of LA Microemulsions. Microemulsions were prepared in different compositions. In briefly, LA was first dissolved in methyl ammonium. Octane was then added to the micellar solution, and the mixture was sonicated. Butanol was stepwise added in small portions to form stable microemulsions, and the solution was sonicated between the additions of butanol. When the solution became clear, it was allowed to cool and pH was measured by a pH/ion-meter from Radiometer Analytical SA, France. The pH of microemulsions was adjusted by adding a small amount of methylamine or acetic acid. The microemulsions were stable in room temperature for at least a couple of months. The microemulsions were filtered prior to analysis by a hydrophilic filter (Millex Syringe Filter Unit, manufactured by Millipore).

2.4. Sample Preparation. Samples were prepared from DMSO (which also serves as the electroosmotic flow marker) stock solutions by pooling 5 μL (10 mM) of each analyte and 10 μL (20 mM) of the microemulsion marker (**4-D**) to filtrated microemulsion to give a total volume of 1 mL and final concentrations of 50 and 200 μM for the analytes and (**4-D**), respectively. Samples were sonicated in a water bath sonicator at room temperature for 10 min to facilitate solubilization of lipophilic compounds prior to analysis.

2.5. Physicochemical Properties of Test Compounds and Statistical Analysis. pK_a values of compounds were measured by a high throughput CE/MS pK_a screening method as described previously,[33] with a newly developed automated data analysis program.[19] The lipophilicity (LogD7.4) was estimated by an in-house RPLC retention based approach. Calculated lipophilicity was

Table 2. Capacity Factors of MEEKC and Lipophilicity Data of 42 Drugs

name	no.	Log k'	$LogP_{MEEKC}$	ClogP	ACDLogP	LogP literature	reference
acebtutolol	**37**	0.26	1.8	1.71	1.95	1.74	Wong 2004[28]
alprenolol	**38**	1.14	3.57	2.65	2.88	2.99	Ghasemi 2007[36]
atropine	**39**	0.68	2.64	1.3	1.53	1.89	Ghasemi 2007
benzocaine	**40**	0.26	1.79	1.92	1.95	1.89	Ghasemi 2007
bifonazole	**41**	2.14	5.56	4.74	4.84	4.77	Ghasemi 2007
bupropion	**42**	1.24	3.76	3.21	3.47	3.21	Ghasemi 2007
buspirone	**3**	0.83	2.94	2.19	3.43	2.78	Ghasemi 2007
caffeine	**43**	−0.84	−0.42	−0.04	−0.13	0.08	Wong 2004
carbamazepine	**4**	0.57	2.42	2.38	2.67	2.34	Wong 2004
chloroquine	**44**	1.66	4.61	5.06	3.71	4.69	Ghasemi 2007
chlorpheniramine	**45**	1.19	3.66	3.15	3.39	3.39	Ghasemi 2007
chlorpromazine	**46**	2.25	5.36	5.3	5.2	4.74	Wong 2004
clonidine	**47**	−0.1	1.08	1.43	1.54	1.57	Ghasemi 2007
clozapine	**7**	1.3	3.87	3.71	2.36	4.1	Ghasemi 2007
corticostereone	**48**	0.81	2.9	2.51	1.76	2.2	Donovan 2002[35]
deprenyl	**49**	0.97	3.22	3.02	2.95	2.9	Ghasemi 2007
desipramine	**50**	1.68	4.65	4.47	4.13	3.79	Ghasemi 2007
diphenhydramine	**51**	1.28	3.85	3.45	3.66	3.18	Ghasemi 2007
doxepin	**10**	1.65	4.58	4.09	3.86	4.29	Donovan 2002
fluconazole	**52**	−0.32	0.63	−0.44	0.5	0.5	Ghasemi 2007
flumazenil	**53**	−0.17	0.92	1.29	0.67	1.64	Ghasemi 2007
fluvastatin	**54**	1.32	3.92	4.05	3.62	4.17	Ghasemi 2007
haloperidol	**12**	1.19	3.66	3.85	3.01	3.67	Ghasemi 2007
hydrocortisone	**55**	0.55	2.38	1.89	1.43	1.65	Ghasemi 2007
imipramine	**56**	1.70	4.36	5.36	4.8	4.13	Wong 2004
ketorolac	**57**	0.2	1.68	1.62	2.45	1.26	Ghasemi 2007
lidocaine	**58**	0.62	2.52	1.95	2.36	2.65	Wong 2004
mebendazole	**59**	0.49	2.26	3.08	2.83	2.42	Ghasemi 2007
metoprolol	**60**	0.4	2.08	1.49	1.79	1.88	Donovan 2002
nefopam	**61**	0.96	3.2	2.91	3.44	3.02	Wong 2004
nicotine	**62**	−0.03	1.22	0.88	0.72	1.39	Wong 2004
nortriptyline	**22**	1.71	4.71	4.32	5.65	4.39	Ghasemi 2007
papaverine	**63**	0.73	2.73	3.78	3.74	2.95	Ghasemi 2007
pindolol	**64**	0.22	1.71	1.67	1.97	1.83	Ghasemi 2007
piroxicam	**65**	0.09	1.45	1.33	1.46	1.68	Ghasemi 2007
primaquine	**66**	1.26	3.8	2.6	1.53	3	Ghasemi 2007
procaine	**67**	0.45	2.18	2.54	2.36	2.2	Wong 2004
propranolol	**27**	1.17	3.62	2.75	3.1	3.48	Ghasemi 2007
pyrilamine	**68**	1.01	3.3	3.23	2.75	3.12	Wong 2004
ranitidine	**69**	−0.11	1.06	0.67	1.23	1.28	Ghasemi 2007
tetracaine	**70**	1.22	3.73	3.83	3.65	3.46	Wong 2004
verapamil	**71**	1.47	4.23	4.47	3.9	3.79	Donovan 2002

from both ClogP and ACDlabs (ACD program v10) for comparisons. Statistical analysis and correlations were made by Spotfire program (Spotfire Decision9.0 SP2). R is the overall correlation coefficient, n the number of compounds.

3. Results and Discussions

3.1. Relationship between LogP and k' in MEEKC. The relationship between LogP and k' in MEEKC can be described in eq 1.[21]

$$k' = P\frac{V_{ME}}{V_{aq}} = \frac{(t_r - t_{eo})}{(1 - t_r/t_{me})t_{eo}} \quad (1)$$

where k', P, and V_{ME}/V_{aq} are the capacity factor of the solute (i.e., the time of solute spent in the oily pseudostationary phase relative to the time spent in the aqueous mobile phase), the partitioning coefficient, the volume ratio between microemulsions, and the aqueous mobile phase; t_r, t_{eo}, and t_{me} are the migration times of solute, neutral marker, and microemulsion marker, respectively.

On the basis of eq 1, utilizing a series of standard literature LogPo/w values, LogP in a new MEEKC system can be indirectly calculated by a linear relationship between the k' and LogPo/w by eq 2.

$$\text{LogP} = b \text{ Log } k' + a \quad (2)$$

where b and a are slope and intercept of the calibration curve of a group of standards as indicated in Figure 1, which can be used to predict and calculate the lipophilicity of the compounds of interest.

Figure 1 exemplifies a linear relationship between LogPo/w and k' for a group of standards with known LogP ranging from −1 to 5. This calibration standard was used in present work to examine the relationship with brain tissue binding and correlations with lipophilicity measured by other approaches. It should be addressed that in order to obtain accurate k' values, it is essential to choose an electroosmotic flow marker (neutral) that has no interaction with the microemulsion and a microemulsion marker (most hydrophobic compound) that is totally retained in the microemulsion. In addition, both markers must be detected by MS. In the present work, the DMSO acts as an electroosmotic flow marker with good MS response. **DB** is often used as the microemulsion marker in MEEKC-UV application but is not ionizable and thus unsuitable for MS detection. Therefore, three new microemulsion markers were compared, including **4-D**, **S-4**, and **DT**. It was found that all three compounds exhibited the same retention times as compared with **DB** in CE/UV conditions, which indicates that they are all totally retained in the microemulsions and can serve as the microemulsion marker. As a result of relatively better MS response, the **4-D** (in Figure 1) was utilized as the microemulsion markers for all k' calculations in this work. Resulting lipophilicity in MEEKC

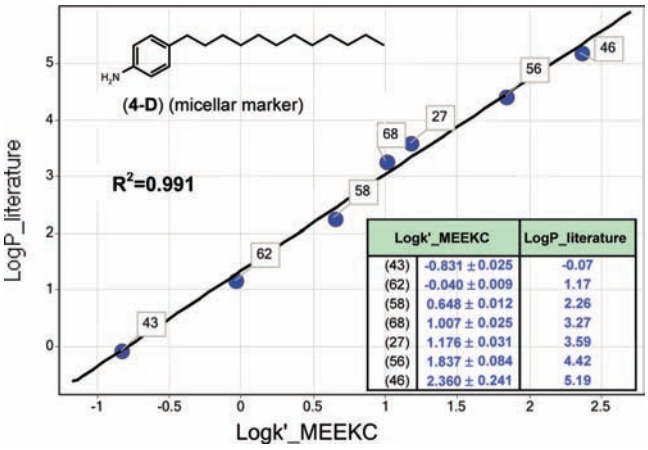

Figure 1. Calibration curve used for LogP determination in MEEKC system: the LogP_literature values of reference standards in this calibration are from published literature.[20,28,35−37] Microemulsion composition consists of 80 mM LA, 37 mM octane (0.6% v/v), and 765 mM butanol (7% v/v) in 200 mM methyl ammonium, apparent pH = 11. Compounds were identified by MS (M + 1). k' was calculated by eq 1 using the DMSO (78 + 1) and (**4-D**) (261 + 1) as the neutral and microemulsion markers, respectively. Capillary was conditioned with the microemulsions for 3 min before injection. Injection volume: 50 mbar × 5 s. A pressure (25 mbar) was applied during the MEEKC separation; temperature: 25 °C. Separation performed within 10 min, and the migration times were recorded by extracted chromatograms.

system can be calculated by $\text{LogP}_{\text{MEEKC}}$ = slope × log k' + intercept, where the mean values of slope (1.695 ± 0.11) and intercept (1.347 ± 0.02) from 11 measurements of calibrations were used for $\text{LogP}_{\text{MEEKC}}$ calculations. The standard deviations of k' measurements are given in Figure 1. It appears that the variability of slope and intercept are small, with relative standard deviation (RSTDEV or CV) less than 6.5% and 1.5%, respectively, although the highest CV of k' values for two compounds, **46** with CV 10.7% and **62** with CV 22.4%, were observed.

3.2. Relationship between fu and Lipophilicity. For QSAR study and new prediction model validation, it is always preferable to use a set of structurally diverse compounds with validated data in this purpose. In this work, most of brain tissue binding data used have been validated, e.g.: (1) comparing measurements between single and pooled compounds, (2) at different conditions (homogenate 1:2 and 1:3 dilution), (3) various compound concentration used, which shows consistent fu data.[3,5,7] On the basis of cross-validation, it can be further concluded that the drug−tissue binding occurs primarily by partitioning (drug concentration independent)[7] rather than binding site mechanism (like drug plasma protein binding, fu might be concentration dependent in some circumstances).[34] A training set of 36 CNS drugs (Table 1) and test set of 26 in-house compounds were measured for their k' values on the same MEEKC system according to eq 1. Correlations between MEEKC lipophilicity and fu in brain tissue were examined and compared with other lipophilicity. Figure 2 depicts the correlations between fu and measured lipophilicity $\text{LogP}_{\text{MEEKC}}$ and calculated lipophilicity. The quantitative relationships between (1) fu and measured MEEKC lipophilicity (eq 3), (2) fu and calculated lipophilicity (eqs 4−6), (3) fu and LogD7.4 (LC) measured by a LC retention approach are described in eqs 3−7, respectively.

$$\text{Log fu\%} = -0.73\ \text{LogP}_{\text{MEEKC}} + 2.99 \qquad R^2 = 0.79 \quad (n = 36\ \text{CNS drugs}) \quad (3)$$

$$\text{Log fu\%} = -0.62\ \text{CLogP} + 2.63 \qquad R^2 = 0.74 \quad (n = 36\ \text{CNS drugs}) \quad (4)$$

$$\text{Log fu\%} = -0.34\ \text{ACDLogP} + 1.54 \qquad R^2 = 0.46 \quad (n = 36\ \text{CNS drugs}) \quad (5)$$

$$\text{Log fu\%} = -0.28\ \text{ACDLogD} + 1.03 \qquad R^2 = 0.26 \quad (n = 36\ \text{CNS drugs}) \quad (6)$$

$$\text{Log fu\%} = -0.38\ \text{LogD7.4 (LC)} + 1.40 \qquad R^2 = 0.34 \quad (n = 36\ \text{CNS drugs}) \quad (7)$$

It is apparent that the correlation of fu with measured $\text{ClogP}_{\text{MEEKC}}$ is better than for both LogD7.4 (LC) and calculated lipophilicty such as ClogP, ACDLogP, and ACDLogD, albeit a relatively better prediction was observed for these CNS drugs by CLogP than other calculated ACDLogP and ACDLogD (Figure 2A−C). Among these, the lowest correlation was observed between fu and ACDLogD7.4 (Figure 2D). It is important to note that although the correlation of fu with CLogP looks promising for the training set of CNS compounds, this does not necessarily mean fairly good predictions by the ClogP as the experimental data of these marked drugs might have been collected in the databases. Oftentimes, it is not surprising to see poor predictions of fu based merely on ClogP for the test set of the NCEs, particularly with conjugated structures, which is consistent with our previous observation.[7]

Furthermore, a comparison of partitioning for a group of 23 structurally unrelated CNS drugs was made at two apparent pHs, i.e., 10.4 and 7.4 (many of them having a basic pK_a above 8.5 are partially positively charged at pH 7.4). It was quite interesting to observe that electrostatic interaction between negatively charged LA (microemulsions) and positively drugs seems to be negligible as illustrated in Figures 3 and 4. More specifically, although the electronic property of microemulsions and the charge state of molecules has attributions to the partitioning (slightly increased k' values when the apparent pH of microemulsion was lowered from 10.4 to 7.4), this does not affect the actual correlations with fu. Indeed, an equally good correlation was obtained at apparent pH 7.4 as compared to the apparent pH 10.4 for this group of CNS drugs using current MEEKC system (Figure 3A and Figure 4). However, a poor correlation was obtained between fu and lipophilicity measured at apparent pH 7.4 on a RPLC C-18 column (Figure 3B). As demonstrated in Figure 3A, **11** is slightly an outlier, which might not result from the charge contribution at pH 7.4 as the compounds **22** and **24** have a basic pK_a above 10 as well.

In addition, a tight correlation (R^2 = 0.991) of k' values between two different temperatures 25 and 37 °C was obtained. It should be noted that fu data were measured at physiologically relevant temperature 37 °C while the lipophilicity in MEEKC was measured at 25 °C. However, the k' values between two different temperatures were highly correlated for the same group of CNS compounds except for that fact that the migration times and k' at 37 °C are relatively smaller than these measured at corresponding 25 °C (data not shown). This result also confirms that the MEEKC partitioning data at 25 °C is relevant to correlate brain tissue binding measured at 37 °C.

3.3. Suitability of Online CE/MS/MEEKC for LogP Screening. In addition to an improved prediction of brain tissue binding, the same MEEKC system has been explored to examine the correlation of lipophilicity between MEEKC partitioning and lipophilicity by other approaches (Table 2). The k' values of 42

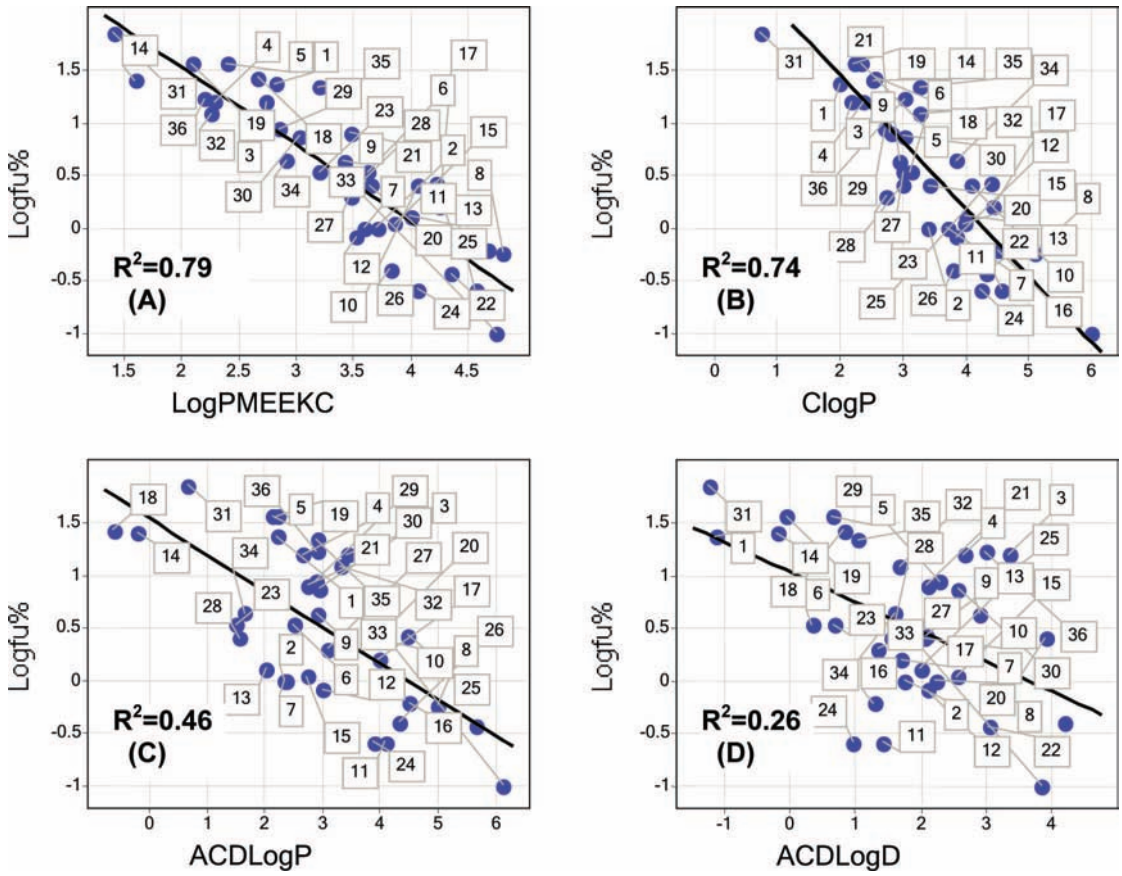

Figure 2. Improved correlation of brain tissue binding with MEEKC lipophilicity; fu brain binding data of 36 CNS drugs (training set) in Table 1. $LogP_{MEEKC}$ measured in MEEKC at apparent pH 11. Other conditions same as in Figure 1.

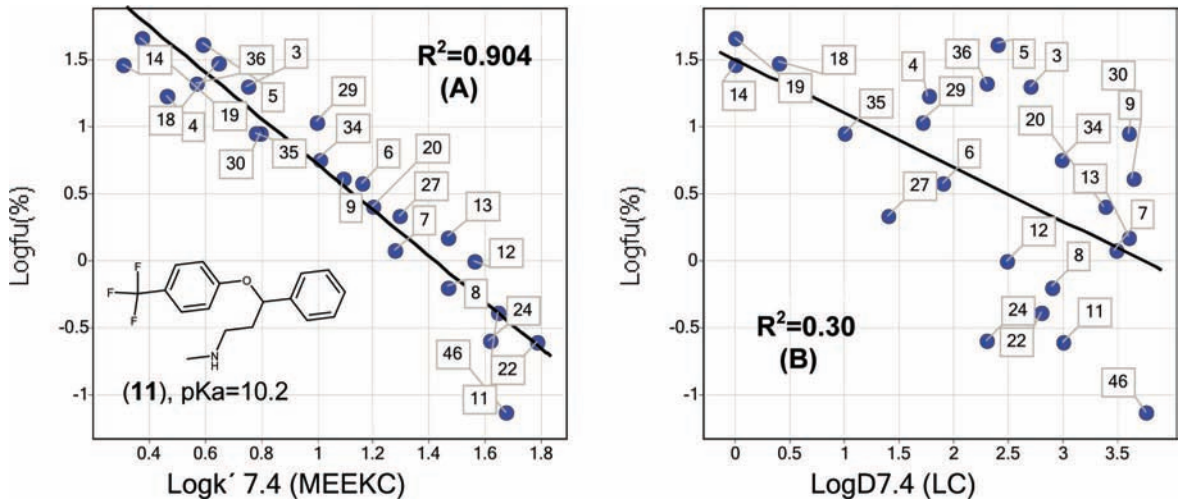

Figure 3. Comparison of MEEKC partitioning and retention in LC at pH 7.4; for this set of 23 CNS drugs, fu data used was from in-house measured under consistent conditions.[7] Microemulsion composition consists of 80 mM LA, 37 mM octane (0.6% v/v), and 874 mM butanol (8% v/v) in 85 mM methyl ammonium, apparent pH = 7.4. Other conditions same as in Figure 1. LC retentions were performed at apparent pH7.4 on a RPLC C-18 column with a fast gradient from 5% acetronitrile (ACN) to 95% ACN in 4 min.

marked drug compounds were thus measured using a sample pooling approach. The same calibration curve as shown in Figure 1 was used for lipophilicity calculation. A significant correlation was observed between published literature LogP and measured LogP by current MEEKC technique (Figure 5A, eq 8).

$$LogP_{MEEKC} = 1.12\ LogP_{literature} + 0.16 \qquad R^2 = 0.928 \quad (n = 42, \text{drugs}) \quad (8)$$

It should be addressed that LogP literature data used in the this work are taken from three different publications or different techniques[28,35,36] without further validation. For instance, **46** has a variety of LogP values (4.74, 5.19, 5.20, 5.40) reported by different techniques.[28,36,37] As a result, **46** seems to be slightly an outlier in Figure 5A but not the outlier in Figure 5B. As shown in the Figure 5B, there is an excellent correlation (R^2 = 0.995) between reported MEEKC (based on single compound measurement and UV detection[28]) and our new CE/MS/MEEKC

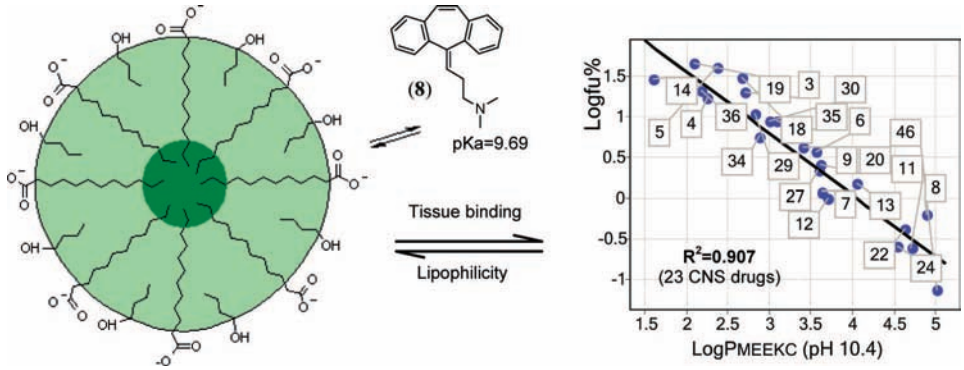

Figure 4. MEEKC partitioning for brain tissue binding prediction and lipophilicity screening; microemulsion composition consists of 80 mM LA, 37 mM octane (0.6%v/v), and 765 mM butanol (7% v/v) in 120 mM methyl ammonium, apparent pH = 10.4. Other conditions same as in Figure 1.

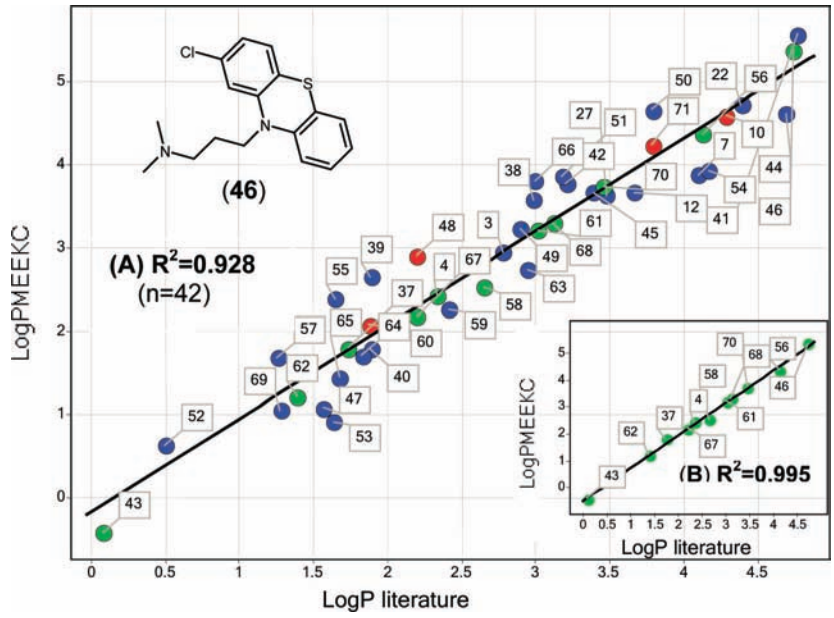

Figure 5. Correlations of MEEKC lipophilicity and literature lipophilicity (A); literature LogP data in blue,[36] green,[28] and red[35] measured with different techniques (Table 2). Green data (B) shows a tight correlation ($LogP_{MEEKC} = 1.20\ LogP_{literature} - 0.46$) between published MEEKC (SDS based microemulsion with single compound measurement and UV detection in nonvolatile buffer) and our current MEEKC system with pooled compounds by MS detection. Microemulsion composition consists of 80 mM LA, 37 mM octane (0.6%v/v), and 765 mM butanol (7% v/v) in 200 mM methyl ammonium, apparent pH = 11. Other conditions same as in Figure 1.

method. The result suggests that our new CE/MS/MEEKC system is very similar to the commonly used SDS based MEEKC system and suitable for lipophilicity screening. Differently from the reported CE/UV based MEEKC LogP methods,[15,26] our CE/MS/MEEKC method offers several advantages such as the suitability of analyzing pooled compounds (10−25 or more compounds in each sample) and correct identification of compounds by highly sensitive and selective online MS detection, small sample consumption, and rapid and reproducible LogP screening at much shorter time in a single run experiment without any apparent change of background electrolyte. In addition, like our automated CE/MS pK_a screening method,[29,33] the CE/MS/MEEKC approach can provide a better solution for automated data analysis by readily tracking its molecular mass (M + 1) of compound, which should be another benefit over CE/UV based method in term of data flow for high throughput screening.

It can be concluded that the LA based microemulsions are well compatible with CE/MS/MEEKC system without apparent ion suppression and/or ion source contamination after being extensively performed for more than six months. The current new development is also expected to open other drug discovery applications based on CE/MS techniques, following our previously successful development of pK_a screening,[29,33,38] providing an accurate lipophilicity screening useful for ADME modeling and predictions. As the selectivity of MEEKC is largely dependent on the type of surfactants,[39,40] it would be interesting to explore other types of surfactants like phospholipids, which are the main components in the brain tissue.[41] It can be speculated that the utility of such naturally occurring phospholipids as microemulsions instead of LA might further improve the correlation with brain tissue binding or membrane biopartitioning as it more mimics the key components of the brain tissue.

4. Conclusions

We have developed a novel microemulsion based CE/MS method applicable for brain tissue binding prediction and lipophilicity screening. To the best of our knowledge, this is the first LA based CE/MS/MEEKC that has been successfully utilized in drug discovery application. Current MEEKC approach

also provides a new insight into drug−tissue partitioning of CNS drugs (phospholipids may function as the microemulsions driving drug partitioning by hydrophobic interaction in the brain tissue), which further corroborates our previous conclusion that drug−brain tissue binding is mainly driven by the partitioning mechanism rather than the binding site.[7] Apparently, the partitioning from MEEKC better predicst brain−tissue binding and conventional octanol−water partitioning (LogPo/w) than calculated lipophilicity (ClogP, ACDLogP and ACDLogD). This approach can be useful to evaluate the tissue binding by a single easily measurable parameter during the lead optimization (IC_{50}/fu)[42] for CNS penetration assessment as well as compound selection for PK/PD studies. In addition, this CE/MS/MEEKC technique can also be used as an alternative approach for accurate lipophilicity measurement amenable for highly predictive of other ADME-Tox parameters, where the partitioning coefficient (LogP) is a more relevant descriptor than the distribution coefficient (LogD7.4), as demonstrated in many examples.

Acknowledgment. Dr. Antionio Llinas is acknowledged for proofreading and revision comments.

References

(1) Kalvass, J. C.; Maurer, T. S.; Pollack, G. M. Use of plasma and brain unbound fractions to assess the extent of brain distribution of 34 drugs: comparison of unbound concentration ratios to in vivo P-glycoprotein efflux ratios. *Drug Metab. Dispos.* **2007**, *35* (4), 660–666.

(2) Liu, X. R.; Chen, C. P. Strategies to optimize brain penetration in drug discovery. *Curr. Opin. Drug Discovery Dev.* **2005**, *8* (4), 505–512.

(3) Maurer, T. S.; DeBartolo, D. B.; Tess, D. A.; Scott, D. O. Relationship between exposure and nonspecific binding of 33 central nervous system drugs in mice. *Drug Metab. Dispos.* **2005**, *33* (1), 175–181.

(4) Summerfield, S. G.; Stevens, A. J.; Cutler, L.; Osuna, M. D.; Hammond, B.; Tang, S. P.; Hersey, A.; Spalding, D. J.; Jeffrey, P. Improving the in vitro prediction of in vivo central nervous system penetration: integrating permeability, P-glycoprotein efflux, and free fractions in blood and brain. *J. Pharmacol. Exp. Ther.* **2006**, *316* (3), 1282–1290.

(5) Summerfield, S. G.; Read, K.; Begley, D. J.; Obradovic, T.; Hidalgo, I. J.; Coggon, S.; Lewis, A. V.; Porter, R. A.; Jeffrey, P. Central nervous system drug disposition: The relationship between in situ brain permeability and brain free fraction. *J. Pharmacol. Exp. Ther.* **2007**, *322* (1), 205–213.

(6) Friden, M.; Gupta, A.; Antonsson, M.; Bredberg, U.; Hammarlund-Udenaes, M. In vitro methods for estimating unbound drug concentrations in the brain interstitial and intracellular fluids. *Drug Metab. Dispos.* **2007**, *35* (9), 1711–1719.

(7) Wan, H.; Rehngren, M.; Giordanetto, F.; Bergstrom, F.; Tunek, A. High-throughput screening of drug−brain tissue binding and in silico prediction for assessment of central nervous system drug delivery. *J. Med. Chem.* **2007**, *50* (19), 4606–4615.

(8) Hughes, J. D.; Blagg, J.; Price, D. A.; Bailey, S.; Decrescenzo, G. A.; Devraj, R. V.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Greene, N.; Huang, E.; Krieger-Burke, T.; Loesel, J.; Wager, T.; Whiteley, L.; Zhang, Y. Physiochemical drug properties associated with in vivo toxicological outcomes. *Bioorg. Med. Chem. Lett.* **2008**, *18* (17), 4872–5.

(9) Lewis, D. F. V.; Lake, B. G.; Ito, Y.; Dickins, M. Lipophilicity relationships in inhibitors of CYP2C9 and CYP2C19 enzymes. *J. Enzyme Inhib. Med. Chem.* **2006**, *21* (4), 385–389.

(10) Austin, R. P.; Barton, P.; Cockroft, S. L.; Wenlock, M. C.; Riley, R. J. The influence of nonspecific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties. *Drug Metab. Dispos.* **2002**, *30* (12), 1497–1503.

(11) Yamazaki, K.; Kanaoka, M. Computational prediction of the plasma protein-binding percent of diverse pharmaceutical compounds. *J. Pharm. Sci.* **2004**, *93* (6), 1480–1494.

(12) Carrozzino, J. M.; Khaledi, M. G. Interaction of basic drugs with lipid bilayers using liposome electrokinetic chromatography. *Pharm. Res.* **2004**, *21* (12), 2327–2335.

(13) Pascoe, R. J.; Masucci, J. A.; Foley, J. P. Investigation of vesicle electrokinetic chromatography as an in vitro assay for the estimation of intestinal permeability of pharmaceutical drug candidates. *Electrophoresis* **2006**, *27* (4 Special Issue SI), 793–804.

(14) Ornskov, E.; Gottfries, J.; Erickson, M.; Folestad, S. Experimental modelling of drug membrane permeability by capillary electrophoresis using liposomes, micelles and microemulsions. *J. Pharm. Pharmacol.* **2005**, *57* (4), 435–442.

(15) Wang, Y. J.; Sun, J.; Liu, H. Z.; He, Z. G. Prediction of human drug absorption using liposome electrokinetic chromatography. *Chromatographia* **2007**, *65* (3−4), 173–177.

(16) Holland, L. A.; Leigh, A. M. Bilayered phospholipid micelles and capillary electrophoresis: a new additive for electrokinetic chromatography. *Electrophoresis* **2003**, *24* (17), 2935–2939.

(17) Avdeef, A.; Testa, B. Physicochemical profiling in drug research: a brief survey of the state-of-the-art of experimental techniques. *Cell. Mol. Life Sci.* **2002**, *59* (10), 1681–1689.

(18) Giaginis, C.; Tsantili-Kakoulidou, A. Current state of the art in HPLC methodology for lipophilicity assessment of basic drugs. A review. *J. Liq. Chromatogr. Relat. Technol.* **2008**, *31* (1), 79–96.

(19) Wan, H.; Holmen, A., High throughput screening of physicochemical properties and ADME profiling in drug discovery. *Comb. Chem. High Troughput Screening* **2009**, *12*, (3), (an invited review, in press).

(20) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F.; Abraham, M. H. ElogP(oct): A tool for lipophilicity determination in drug discovery. *J. Med. Chem.* **2000**, *43* (15), 2922–2928.

(21) Ishihama, Y.; Oda, Y.; Uchikawa, K.; Asakawa, N. Evaluation of solute hydrophobicity by microemulsion electrokinetic chromatography. *Anal. Chem.* **1995**, *67* (9), 1588–1595.

(22) Altria, K. D.; Mahuzier, P. E.; Clark, B. J. Background and operating parameters in microemulsion electrokinetic chromatography. *Electrophoresis* **2003**, *24* (3), 315–324.

(23) Klotz, W. L.; Schure, M. R.; Foley, J. P. Determination of octanol−water partition coefficients of pesticides by microemulsion electrokinetic chromatography. *J. Chromatogr.* **2001**, *930* (1−2), 145–154.

(24) Klotz, W. L.; Schure, M. R.; Foley, J. P. Rapid estimation of octanol−water partition coefficients using synthesized vesicles in electrokinetic chromatography. *J. Chromatogr., A* **2002**, *962* (1−2), 207–219.

(25) Hansen, S. H. Recent applications of microemulsion electrokinetic chromatography. *Electrophoresis* **2003**, *24* (22−23), 3900–3907.

(26) Poole, S. K.; Durham, D.; Kibbey, C. Rapid method for estimating the octanol−water partition coefficient (log Po/w) by microemulsion electrokinetic chromatography. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2000**, *745* (1), 117–126.

(27) Poole, S. K.; Poole, C. F. Separation methods for estimating octanol−water partition coefficients. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2003**, *797* (1−2), 3–19.

(28) Wong, K. S.; Kenseth, J.; Strasburg, R. Validation and long-term assessment of an approach for the high throughput determination of lipophilicity (log Po/w) values using multiplexed, absorbance-based capillary electrophoresis. *J. Pharm. Sci.* **2004**, *93* (4), 916–931.

(29) Wan, H.; Thompson, R. A. Capillary electrophoresis technologies for drug screening. *Drug Discovery Today: Technol.* **2005**, *2* (2), 171–178, an invited review.

(30) Goetzinger, W. K.; Cai, H. Buffer system for the separation of neutral and charged small molecules using micellar electrokinetic chromatography with mass spectrometric detection. *J. Chromatogr.* **2005**, *1079* (1−2), 372–381.

(31) Himmelsbach, M.; Haunschmidt, M.; Buchberger, W.; Klampfl, C. W. Microemulsion electrokinetic chromatography with on-line atmospheric pressure photoionization mass spectrometric detection. *Anal. Chem.* **2007**, *79* (4), 1564–1568.

(32) Schappler, J.; Guillarme, D.; Rudaz, S.; Veuthey, J. L. Microemulsion electrokinetic chromatography hyphenated to atmospheric pressure photoionization mass spectrometry. *Electrophoresis* **2008**, *29* (1), 11–19.

(33) Wan, H.; Holmen, A. G.; Wang, Y. D.; Lindberg, W.; Englund, M.; Nagard, M. B.; Thompson, R. A. High-throughput screening of pK_a values of pharmaceuticals by pressure-assisted capillary electrophoresis and mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17* (23), 2639–2648.

(34) Wan, H.; Bergström, F. High-throughput screening of drug−protein binding in drug discovery. *J. Liq. Chromatogr. Rel. Technol.* **2007**, *30*, 681–700.

(35) Donovan, S. F.; Pescatore, M. C. Method for measuring the logarithm of the octanol−water partition coefficient by using short octadecyl-poly(vinyl alcohol) high-performance liquid chromatography columns. *J. Chromatogr., A* **2002**, *952* (1−2), 47–61.

(36) Ghasemi, J.; Saaidpour, S. Quantitative structure−property relationship study of *n*-octanol−water partition coefficients of some of diverse drugs using multiple linear regression. *Anal. Chim. Acta* **2007**, *604* (2), 99–106.

(37) *ACD/LogP Database*; Advanced Chemistry Developments, Inc.: Toronto, 2007.

(38) Wan, H.; Ulander, J. High-throughput pK_a screening and prediction amenable for ADME profiling. *Exp. Opin. Drug Metab. Toxicol.* **2006**, *2* (2), 139–155, an invited review.

(39) Yang, S.; Bumgarner, J. G.; Kruk, L. F. R.; Khaledi, M. G. Quantitative structure−activity relationships studies with micellar electrokinetic chromatography Influence of surfactant type and mixed micelles on estimation of hydrophobicity and bioavailability. *J. Chromatogr., A* **1996**, *721*, 323–335.

(40) Gabel-Jensen, C.; Hansen, H. S.; Stig, P.-B. Separation of neutral compounds by microemulsion electrokinetic chromatography: fundamental studies on selectivity. *Electrophoresis* **2001**, *22*, 1330–1336.

(41) Poulin, P.; Theil, F. P. A priori prediction of tissue: plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. *J. Pharm. Sci.* **2000**, *89* (1), 16–35.

(42) Trainor, G. L. The importance of plasma protein binding in drug discovery. *Exp. Opin. Drug Discovery* **2007**, *2* (1), 51–64.

JM801441S