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Full Length Research Paper

Correlation of Lipophilicity Descriptors with Pharmacokinetic Parameters of Selected Benzodiazepines

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ABSTRACT

In early-stage drug discovery science, it is often important to reliably predict the absorption, distribution, metabolism and elimination (ADME) property of new chemical entities in pipeline, in order to filter molecules that are not drug-like. Several *in-vitro* models of lipophilicity profiling as predictor of ADME property have been developed. The validity of lipophilicity determination based on different descriptors was evaluated using 4 model compounds of the benzodiazepine class; bromazepam, clonazepam, diazepam and lorazepam. Lipophilicity descriptors describing the retention behaviours of the model compounds were obtained from three approaches, namely; planar chromatographic determination (i.e. Isocratic chromatographic hydrophobicity index), calculated log P values (i.e. clog P, ChemAxon), and octanol-water partition coefficient (i.e. log P). These descriptors were correlated with *in-vivo* pharmacokinetic parameters - maximum plasma concentration (C_{max}), time to reach peak plasma concentration (T_{max}) and area under plasma concentration – time curve (AUC), using the Pearson rank correlation. The experimental approaches ranked diazepam as most lipophilic while *in-silico* approach ranked lorazepam as most lipophilic. AUC and C_{max} correlated positively with the lipophilicity descriptors while T_{max} gave negative correlation (except for the *in-silico* method) (r = 0.60, -0.74, and -0.25) with lipophilicity descriptors clog P, ICHI and logP respectively. ICHI gave the best correlation with pharmacokinetic parameters - C_{max} (r = 1.0), AUC (r = 0.89) and T_{max} (r = -0.74) The planar chromatographic platform was shown to be a valid biomembrane model for lipophilicity profiling. The lipophilicity descriptor, ICHI is positively and strongly correlated with C_{max}, and found superior to the correlation of C_{max} with time-honoured octanol/water log P. A larger study is thus warranted to delineate the potential of ICHI for general utility.

Keywords: Lipophilicity descriptors, benzodiazepines, pharmacokinetic parameters, correlation.

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INTRODUCTION

The drug discovery process is a very rigorous, time consuming and capital intensive venture in the pharmaceutical industry, because it is usually accompanied by a high degree of attrition. As a result several molecules fail to reach the market as drugs, with failure occurring even at the last phase of clinical trials. Attrition is often attributed to poor pharmacokinetic parameters -absorption, distribution, metabolism and toxicity (ADMET) (Kennedy, 1997). Several physicochemical parameters such as lipophilicity, solubility, polar surface area, and permeability have been found to influence biopharmaceutical properties and drug likeness of a molecule (Lipinski, 2000). These properties must be accurately predicted at the early stage of the drug discovery process, to reduce incidence of attrition (Kern et al, 2003; Penzotti et al, 2004).

Lipophilicity is established as a physicochemical property with major predictive value on ADMET properties and pharmacological activity, since molecules must traverse biological membrane in order to interact with receptors and elicit drug action (Serda et al, 2012). It is reported to be closely correlated with permeability and drug solubility (Cross et al, 2003), hence it is widely used in pharmacokinetic modeling (Kaliszan et al, 2003). Although lipophilicity enhancement of molecules improves bioavailability of drug, it often leads to alteration of activity (Feng et al, 2010).

Lipophilicity refers to relative affinity of a molecule for lipophilic environment, commonly measured by its partitioning in a biphasic medium (International Union of Pure and applied Chemistry, 1997). Various approaches used in the determination include logarithm of octanol-water partition coefficient (log P), referred to as “the gold standard” (Mannhold et al, 2009); potentiometric titration techniques,

chromatographic techniques like reversed phase planar chromatography, high performance liquid chromatography, immobilized artificial membrane chromatography (IAM), immobilized liposome chromatography (ILC), micellar electrokinetic chromatography (MEKC) and Bio-partitioning Micellar Chromatography (BMC) etc.

Lipophilicity is a critical parameter for central nervous system agents that are active *in vivo* (Ewelina, 2013). The benzodiazepines are among the most commonly prescribed centrally acting drugs used as anxiolytics, muscle relaxants, antiepileptic and as hypnotic agents (Mihic and Harris, 2011); whose pharmacology depends on the compound's lipophilicity. Lipophilicity of benzodiazepines has been reported to influence their speed of action; and thus the rational choice of the more lipophilic members of the class e.g. diazepam in eliciting rapid effect in acute conditions (Vinkers et al, 2012). Furthermore, the absorption pharmacokinetic profile indicated that the highly lipophilic benzodiazepines penetrate the CNS rapidly with consequent tendency for high abuse (Melton and Kirkwood, 2014).

The validity of lipophilicity measurement methods is dependent on the extent of similarity of the system to the biological membrane architecture (i.e. biomimetic feature) (Smith et al., 1996). In this paper, we report a comparative evaluation of the correlation of various lipophilicity descriptors with pharmacokinetic parameters, as a validation of the strategies adopted in obtaining the descriptors, using a small chemical library of benzodiazepines (Figure 1).

MATERIALS AND METHODS

Chemicals and Equipment: Diazepam, clonazepam, lorazepam and bromazepam (Secondary reference sample, British Pharmacopoeia), pre-coated silica gel thin layer chromatographic plate (0.2mm, Merck, Germany) , *n*-hexane (Analar, British Drug House), methanol (Analar, British Drug House), liquid paraffin (Analar, British Drug House), Mettler H80 analytical balance (UK), UV lamp (254 and 365 nm, Gallenkamp, U.K.)

Preparation of reversed phase stationary phase: 5% liquid paraffin in *n*-hexane was used to coat the surface of the silica gel plates (5 x 10cm) by the ascending development method.

Preparation of the mobile phase: Various concentrations (φ) comprising of 30, 35, 40, 45, 50, 55, 60, 62.5 and 65% of methanol-water mixture was prepared in developing the chromatogram of the model compounds.

Lipophilicity profiling of the model compounds: Methanolic solutions (2 µL of 1% w/v) of the model compounds were spotted on reversed phase plates, and developed in the gradient series of mobile phase. The retardation factor (R_f) obtained was transposed into R_m values (Biagi et al, 1969). Linear plots of the R_m versus φ were made and the line of best – fit was determined by inear regression analysis (GraphPad Prism version 6.0, 2015). The *x*-intercept (φ_o) known as the ICHI, extrapolated from the graph was used in ranking the lipophilicity of the model compounds (Idowu et al, 2009).

Correlation Analysis: Secondary data obtained from the literature (www.drugbank.ca) comprising of the experimental octanol - water partition coefficient and calculated log P using the most popular and widely accepted ChemAxon clog P algorithm were pooled together with the experimentally determined chromatographic lipophilicity descriptor, ICHI. This was correlated with pharmacokinetic parameters like area under the plasma concentration-time curve (AUC), peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}) which were culled from literature. The correlation analysis of the three lipophilicity descriptors with the pharmacokinetic parameters was performed using the Pearson correlation coefficient as a measure of goodness-of-correlation between the parameters. All statistical analysis was performed by GraphPad Prism version 6.0 for Windows (Graph Pad Software, San Diego, CA, www.graphpad.com, 2015)

RESULTS

The retention behavior of the model compounds depicted by the linear regression of R_m versus the organic modifier fraction is displayed in Figure 2. The derived lipophilicity parameter (Isocratic chromatographic hydrophobicity index –ICHI), and experimental log P values and cLogP values (obtained from the literature) for the model compounds are shown in Table 1. Correlation analysis between the pharmacokinetic parameters (Table 2) revealed different levels of correlation with the lipophilicity descriptors. Figure 3 shows the correlation of the lipophilicity descriptors with the bioavailability parameter (AUC) revealing a poor correlation with experimental log P (r =0.47), negative correlation with clog P (r = -0.47) and fair correlation with chromatographic ICHI parameter (r = 0.89). Correlation between the maximum plasma concentration (C_{max}) and time for maximum plasma concentration (T_{max}) is displayed in Figure 4 and 5 respectively.

Table 1: Lipophilicity index from planar chromatographic experiment (ICHI), experimental log P (octanol/water partition) and calculated logP (ACD ChemAxon)

	Lipophilicity descriptors		
Model compound	ICHI	LogP Experimental	cLogP
Bromazepam	0.529	2.05	2.54
Clonazepam	0.531	2.41	3.15
Diazepam	0.618	2.82	3.08
Lorazepam	0.516	2.39	3.53

Table 2: Mean values of pharmacokinetic parameters of Bromazepam, Clonazepam, Diazepam and Lorazepam (Culled from literature)

	AUC (nghr/ml)	Cmax (ng/ml)	Tmax (hrs)
Bromazepam	2501 (Podilsky et al, 2009)	46 (Podilsky et al, 2009)	1.53 (Podilsky et al, 2009)
Clonazepam	561(Crevoisier et al, 2003)	50 (Sio et al, 1975)	2.5 (Crevoisier et al, 2003)
Diazepam	4430 (Guo et al, 2013)	178 (Guo et al, 2013)	1.25 (Guo et al, 2013)
Lorazepam	405 (Blin et al, 1999)	20 (www.accessdata.fda.gov)	2.37 (Blin et al, 1999)

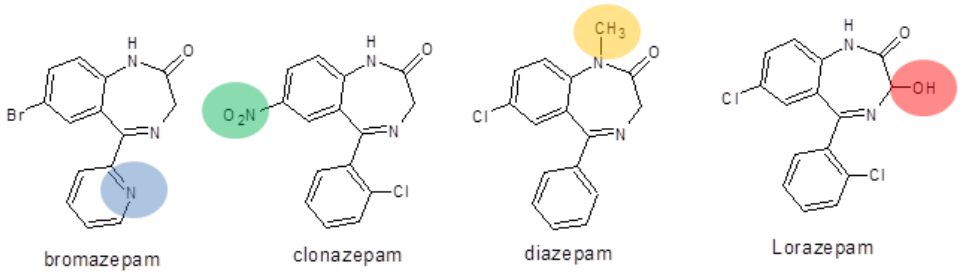

Figure 1:
Chemical structures of the study compounds

DISCUSSION

The lipophilicity parameter for the model compounds obtained from the retention behavior on the chromatographic platform displayed in Figure 2 and Table 1 revealed that lipophilicity of the model compounds follows the sequence below:

Diazepam > *clonazepam* > *bromazepam* > *lorazepam*

The ranking from the experimentally determined log P in a octanol-water partition system followed a similar order but slight disparity in the ranking of the last 2 compounds as shown below:

Diazepam > *clonazepam* > *lorazepam* > *bromazepam.*

The ranking on the computer algorithm shows:

Lorazepam > *clonazepam* > *diazepam* > *bromazepam.*

The lipophilicity of benzodiazepines homologues has been reported to vary with polarity and electronegativity of the various substituents (Brunton et al, 2008). Lipophilicity encodes different intermolecular forces, expressed as interplay of the hydrophobic and polar interactions (Liu et al, 2011). According to Van de Waterbeemd, the relationship between Lipophilicity, hydrophobicity and polarity is:

Lipophilicity = Hydrophobicity – polarity

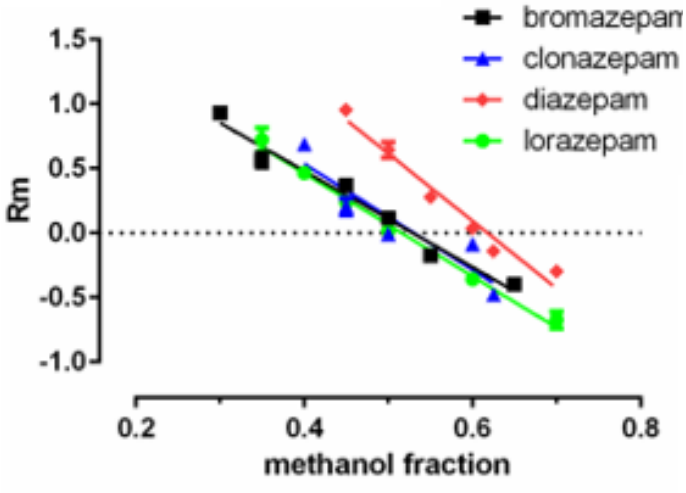

Figure 2:
Linear regression of the Rm versus the organic modifier fraction revealing the retention behavior of the model compounds

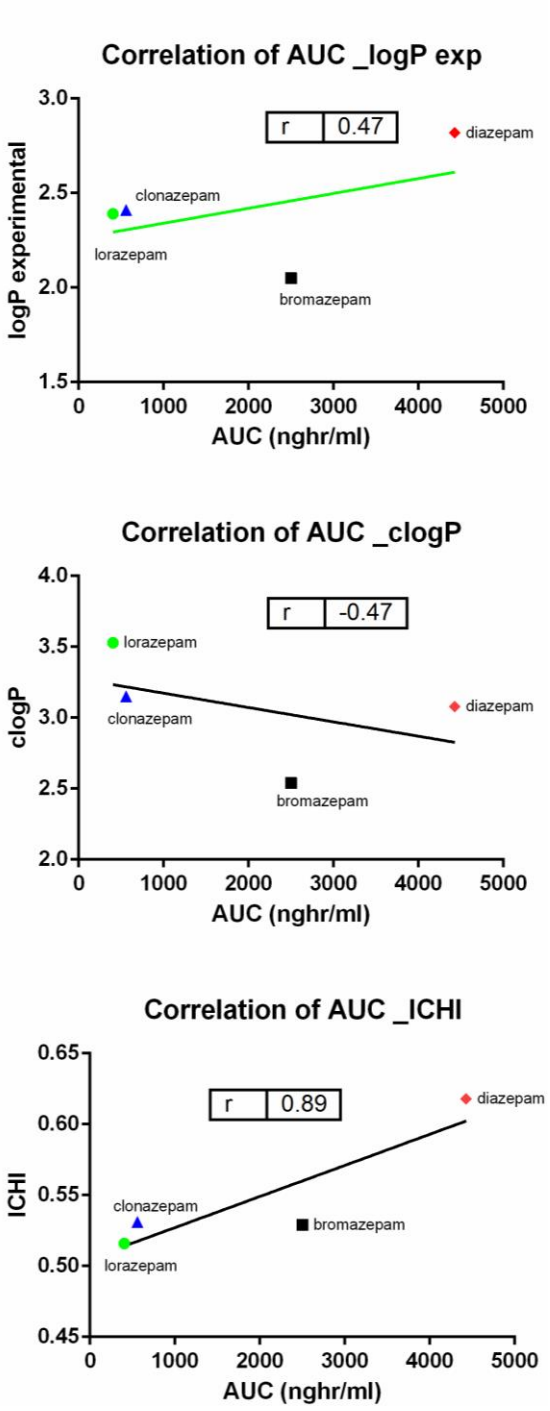

Figure 3:
Correlation analysis of the AUC with the 3 lipophilic descriptors

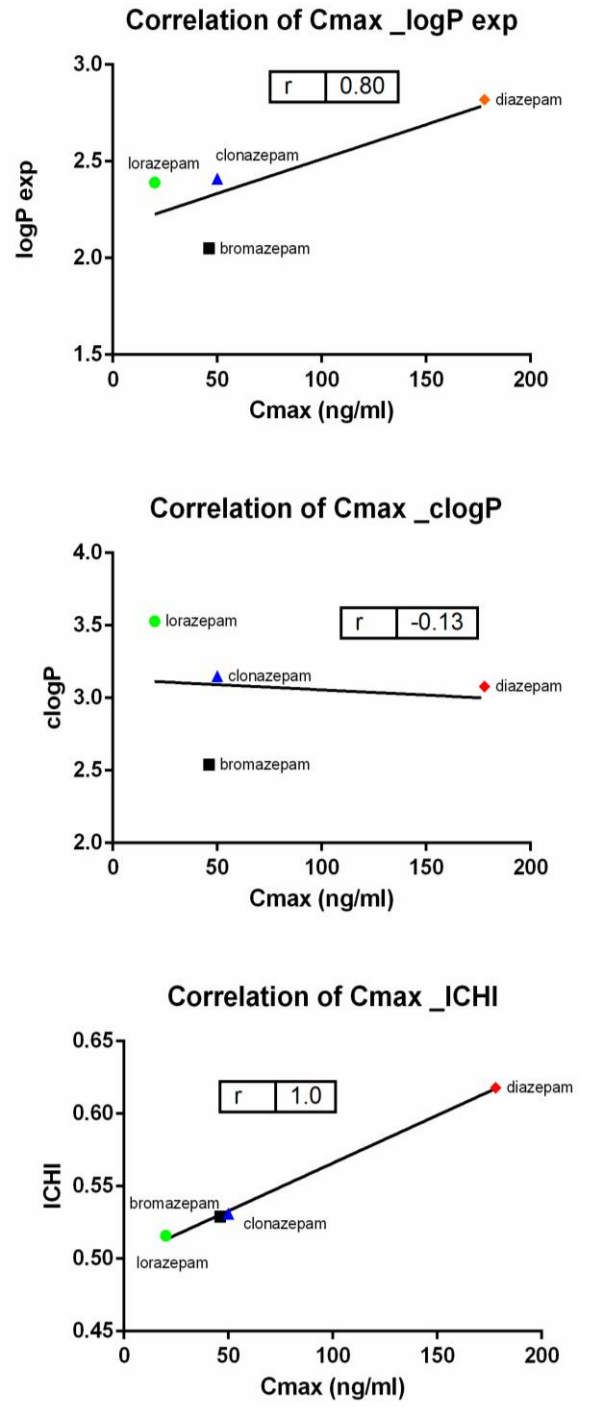

Figure 4:
Correlation analysis of C_{max} with the 3 lipophilicity descriptors

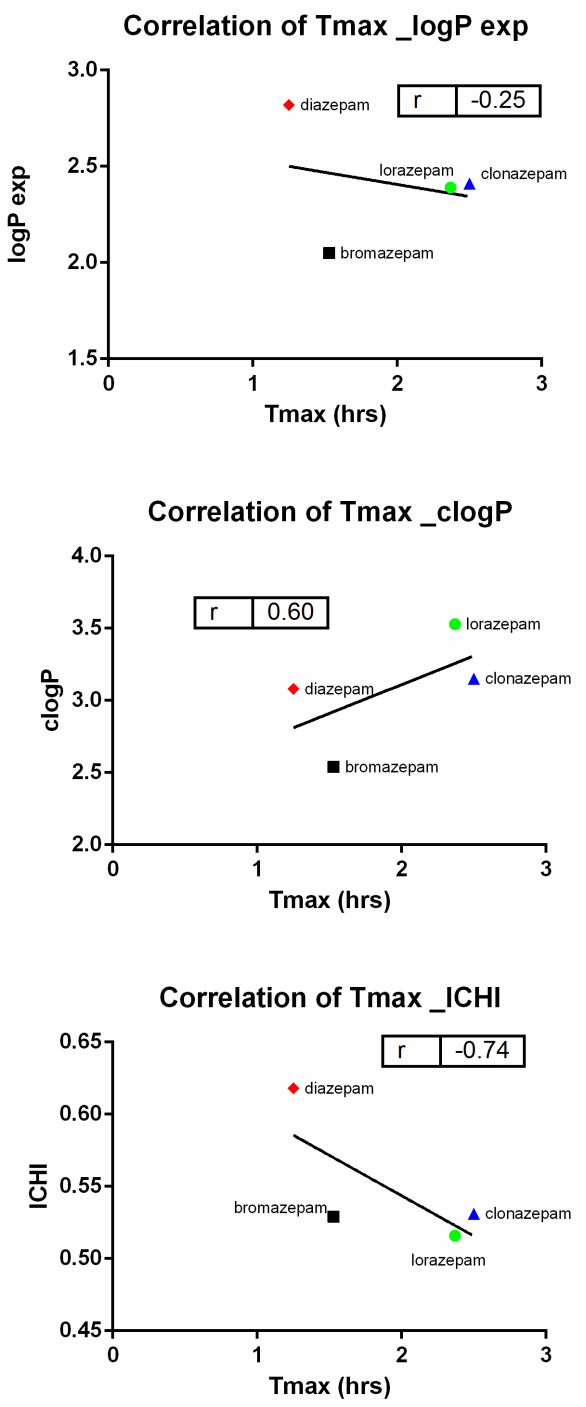

Figure 5:
Correlation analysis of Tmax with the 3 lipophilicity descriptors.

This implies that the greater the polarity of a molecule, the lower the lipophilicity. Thus, based on structural theory, lorazepam which has a hydroxyl moiety (a hydrogen bond donor and acceptor) in the benzodiazepine skeleton is expected to have the lowest lipophilicity, while diazepam which has an additional methyl group should be the most lipophilic.

Between the two extremes, bromazepam has a nitrogen heteroatom in a 6-membered heterocycle (pyridine moiety) instead of the phenyl ring in all the other compounds.

Nitrogen being a hydrogen bond acceptor, will confer water solubility, thus making bromazepam less lipophilic than clonazepam, devoid of an heterocycle albeit with a polar nitro substituent on another phenyl ring. The ranking based on the results of the chromatographic method thus corroborate structural theory. On the contrary, the *in-silico* approach (c log P) emphasized the inherent limitation of *in-silico* approach in predicting molecular properties, due to inability of computer algorithms to fully account for the 3 dimensional structural details of molecules and lack of parameterization of certain molecular fragments (Mannhold, 2008). Ranking lorazepam

as most lipophilic of the series cannot be supported by structural theory. The closeness of values used in ranking the experimentally determined parameters implies they are more accurate estimate of lipophilicity than the *in silico* predictions. (Eros et al, 2002)

The area under the plasma concentration-time curve (AUC) gave a poor correlation (r = 0.47) with experimental log P (octanol-water); fairly good correlation (r = 0.89) with the chromatographic ICHI and negative correlation (r = -0.47) with the calculated log P. The peak plasma concentration C_{max} had a better correlation (r = 0.80) with the experimental log P (octanol-water), high and positive correlation (r = 1.0) with the chromatographic ICHI and negative correlation (-0.13) with calculated log P. This underscores the more complex partition dynamics of reversed phase chromatographic system is a better simulation of the biological system. Hence, more reliable and accurate experimentally determined lipophilicity descriptor, with improved prediction of pharmacokinetic parameters, should be achievable with increasing biomimetic adaptation of reversed phase chromatographic system.

The T_{max} had a reverse correlation pattern with the lipophilicity descriptors, showing a fair correlation (r = 0.60) though positive correlation with calculated logP , and negative correlation (r = -0.74 and -0.25) with chromatographic ICHI and the experimental logP (octanol-water). AUC is a measure of bioavailability of the drugs, and it is clearly shown that ICHI gave the highest correlation with bioavailability of the drugs. Negative correlation with T_{max} is also consistent with the correlation pattern with the two other pharmacokinetic parameters. The more lipophilic member, which has a high C_{max} and high AUC achieves the C_{max} the fastest (i.e a short T_{max}). On the contrary, a positive correlation found between c log P and T_{max} further indicates the inaccuracy of the *in-silico* method.

In conclusion, Lipophilicity measurement is one of the most crucial physicochemical parameters in predicting *in-vivo* pharmacokinetic (ADME) properties. Experimentally determined lipophilicity descriptors i.e. logP (octanol-water) and chromatographic ICHI gave similar, though slightly different ranking of the lipophilicity of the drug molecules, underscoring the significance of disparity in the complexity of partition process involved in the conventional ‘‘shake-flask” and partition (reversed-phase) chromatography models. The result of the correlation analysis revealed that the C_{max} correlated best with the experimentally determined lipophilicity data, implying a good *in-vivo-in vitro* correlation (IVIVC) that could be used for biopharmaceutical prediction of a new chemical entity. The *in-silico* parameter, c log P generally gave a poor correlation. The chromatographic ICHI was also revealed as the better predictor (relative to log $P_{\text{octanol-water}}$) of the pharmacokinetic parameters - AUC, C_{max}, and T_{max}, . Since the current study investigated a small chemical library of benzodiazepines, a much larger study is warranted for pharmacological classes of compounds that have shown profound influence of compound lipophilicity on bioactivity profile. The findings will also delineate the potential of planar chromatographic parameter (ICHI) for general utility in predicting drug pharamacokinetic parameters in early-stage drug discovery science. Such a larger study is ongoing in our laboratory.

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