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Research paper

# Prediction of blood–brain barrier penetration of poorly soluble drug candidates using surface activity profiling

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ABSTRACT

The aim of this study was to determine whether transepithelial transport across the blood–brain barrier (BBB) [expressed as the logarithm of blood/brain partitioning coefficient (log bb)] could be correlated to surface tension properties for a series of new chemical entities (NCEs) having extremely low solubility in aqueous media.

Surface tension data were generated by the "Du Nouy maximum pull force method" using an automated, small volume Kibron Delta 8 Multi-channel tensiometer. Using the surface pressure/concentration profiles, parameters such as the maximum surface pressure, cross-sectional area and the air–water partitioning coefficient were calculated for the individual compounds and correlated with their *in vivo* log bb values. A good linear correlation ($R^2 = 0.8669$) between log bb and cross-sectional area was observed, suggesting a morphological analogy between the molecular orientation at the air–water interface and the anisotropic cellular bilayer of the blood–brain barrier.



## 1. Introduction

The modern pharmaceutical industry is constantly in search of new, innovative chemical entities. Today, combinatorial chemistry and high throughput methods enable large numbers of drug candidates to be screened for activity. Substances identified by these methods are often characterized by high lipophilicity and low solubility, since potential drug candidates are selected primarily by their receptor affinity *in vitro*. Poor biopharmaceutical properties are a major reason for the failure of new chemical entities (NCEs) in pharmaceutical drug discovery. For example, poor aqueous solubility often results in poor bioavailability after oral dosing. But the gastrointestinal tract is often not the only barrier that the drug must pass in order to reach its site of action. For drugs that are intended to act in the central nervous system, the ability to cross the blood–brain barrier (BBB) is a further, crucial prerequisite for success. Conversely, low BBB penetration may be desirable in order to minimize CNS-related side effects for drugs that are designed to act at peripheral sites [1]. Thus, the ability to cross the BBB has emerged as an issue of primary interest, even in the early stages of drug discovery.

The BBB restricts the transport of many therapeutically important drugs from the blood stream into the brain. This barrier is formed by the endothelial cells of the cerebral capillaries, which essentially comprise the major exchange interface between the blood and the brain. Different mechanisms, such as passive transcellular diffusion, paracellular diffusion and active transport, have been proposed for uptake across this barrier [2], with the tight endothelium of brain capillaries constituting the principal permeability barrier for the passive transport of substances across the barrier.

An established method to reflect the distribution of drugs between blood and brain tissue and hence their ability to penetrate the BBB is to determine the ratio of the steady state concentration of the drug in the brain to its concentration in the blood. This ratio is usually expressed as log ($C_{brain}/C_{blood}$) and abbreviated as log bb [3]. The experimental determination of log bb is a time-consuming, expensive and difficult technique, requiring *in vivo* animal experiments. Therefore, various approaches have been taken in the literature to predict the blood–brain barrier permeability. In addition to Caco2 cells [4] and artificial membrane (PAMPA) [5] techniques, physicochemical parameters like octanol/water partition coefficients, polar surface area [6], hydrogen bond descriptors [7] or Lipinski's rules of five [8] have been applied. A correlation coefficient of $R^2 = 0.917$ was observed between the polar molecular

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surface area (PSA), defined as the area of the van der Waals surface that arises from oxygen and nitrogen atoms or hydrogen atoms attached to oxygen or nitrogen atoms and log bb [9]. Unfortunately, this and the other models mentioned earlier show poor predictions when substances outside the defined training sets are tested or the compound solubilities are very low.

Biological membranes are amphiphilic structures with a hydrophobic interior and two hydrophilic surfaces facing the aqueous environment on either side of the membrane. These membranes consist, to a large extent, of lipids [10]. Many drug substances possess amphiphilic characteristics, a property that facilitates accumulation at the interface and partitioning, at least partially, into the hydrophobic core of the membrane. In the case of drug penetration through the BBB, structural and physicochemical properties like molecular size, charge, hydrogen bonding potential and lipophilicity are further important parameters [11]. Interestingly, these parameters also combine to determine surface activity and drug behavior at the air–water interface [12]. Surface activity profiling (SAP) is a technique, which can be used to characterize the amphiphilic properties of drugs, as reflected in surface activity, which can then be correlated to their ability to interact with and penetrate through the lipid bilayer in the BBB [13].

The lateral packing density of the lipid bilayer membrane, $\Pi_{bi}$, can be compared to that of a lipid monolayer [14]. The penetration of a molecule into a lipid bilayer requires energy, $\Delta W$, since a cavity large enough to accommodate the drug has to be formed in the well-ordered membrane. This energy, $\Delta W$, is proportional to the surface pressure, $\Pi_M$, of the membrane and the cross-sectional area, $A_D$, of the molecule being inserted ($\Delta W = \Pi_M \times A_D$) [15]. For molecules with small cross-sectional areas, the energy requirement is low, while for molecules with large cross-sectional areas, it becomes significantly higher. It should be noted that the cross-sectional area, $A_D$, of the molecule is not necessarily proportional to the molecular weight but also depends on the conformation and orientation of the molecule at the lipid bilayer interface [16].

A wide variety of membrane-permeating drugs have amphiphilic properties and thus a tendency to accumulate at the air–water interface. If the compounds are brought in contact with the air–water interface, they organize themselves in an anisotropic manner comparable to that of detergent molecules. Since the dielectric constant of air ($\varepsilon = 1$) and the lipid core region of a biological membrane ($\varepsilon = 2$) are similar, and much lower than that of water ($\varepsilon = 80$), it is postulated that the amphiphilic orientation of the molecule will be similar at the two interfaces (air–water interface and the lipid bilayer interface). From the Gibbs adsorption isotherm, which is calculated from the surface pressure of the drug in a buffer solution as a function of concentration, the apparent air–water partition coefficient, $K_{aw}^{-1}$, the critical micelle concentration, CMC, and the surface area of the compound, $A_s$, required for its amphiphilic orientation at the interface can be calculated. If measurements are performed under conditions of minimal charge repulsion, the surface area requirement, $A_s$, corresponds to the cross-sectional area, $A_D$, of the molecule perpendicular to its axis of amphiphilicity [17].

Practically, these calculations are based on surface tension measurements, in the form of surface pressure profiles. For poorly soluble drugs, it may be difficult to make measurements over an appropriate concentration range, due to sensitivity limitations of the tensiometer at one end of the concentration scale and solubility limitations at the other end.

The purpose of this study was to analyze Gibbs adsorption isotherms obtained by surface activity profiling for development compounds with a wide array of molecular structures and pharmacological mechanisms and to develop and compare parameters to predict their log bb values. A screening method based on a small volume automatic tensiometer was used to obtain surface activity profiles, and log bb values were determined experimentally in rats.

## 2. Materials and methods

### 2.1. Chemicals

All drug compounds studied are test substances currently in the early stages of drug development and were supplied by Janssen Pharmaceutica N.V. (Beerse, Belgium). The substances were selected randomly from the development program and all are poorly soluble (<2.7 mmol/L) at pH 7.4. Physical–chemical parameters for substances are listed in Table 1. The pH 7.4 buffer solution was purchased from pION Inc. (Woburn, MA, USA) and used to determine the solubilities and to perform SAP testing for all compounds.

### 2.2. In vivo blood–brain barrier penetration studies

The blood–brain partition coefficient was generated with the tissue distribution method reported by Brewster et al. [18] in male Sprague Dawley rats using sample removed after 30 min, 1, 2, 4, 7 and 24 h. The drug was administered orally as a 20% hydroxypropyl-β-cyclodextrin solution. A UPLC/MS/MS method was used as the analytical test method [19].

### 2.3. Solubility study

Solubility and surface tension measurements were conducted in pH 7.4 buffer [20], representing physiological conditions at the blood–brain barrier. The thermodynamic solubility of each drug substance was determined in the same buffer using a standardized shake flask method at 37 °C. After shaking for 48 h, the supernatant was filtered through a 0.45 μm membrane filter, and the filtrate was assayed by the same UPLC/MS/MS method that was used for the toxicological analysis [19].

### 2.4. Physicochemical profiling

Physicochemical profiling for each drug candidate included in this study was performed *in silico* using ACD Labs PhysChem Prediction Software (Advanced Chemistry Development, Inc., Toronto, Ontario, Canada) to calculate the p*K*a, Log *P*, and Log *D* (pH 7.4) values.

### 2.5. Sample preparation

The approximate volume of pH 7.4 buffer required to dissolve the compound was first calculated from the solubility parameter. If required, the sample was sonicated at 20 °C for 1 min to complete dissolution. Following this method, stock solutions were prepared, attaining concentrations of 0.036–2.7 mmol/L, depending on compound solubility.

For surface tension profile measurement for each drug candidate, a dilution series of 10 concentrations of the drug compound in pH 7.4 buffer was prepared in disposable 96-well plates with the wells addressed as 8 rows (from A to H) and 12 columns (from 1 to 12). (Fig. 1)

The dilution factor was 1:1 for each sample. Well A1 was filled with 200 μl of drug stock solution. Hundred microliters of buffer was pipetted into wells A2–A11. Hundred microliters was then transferred consecutively from one well to the next, from A1 through well A11. For example, if the starting concentration in A1 was 0.2 mM, then the concentration in A11 would be $1.95 \times 10^{-04}$ mM. The last column (A12) was filled with pure buffer to provide a reference value.

**Table 1**
Characterization of the compounds according to their molecular weight (MW), calculated dissociations constant (p*Ka*), water–octanol partitioning coefficient (*c*Log *P*), distribution coefficient between buffer–octanol at pH 7.4 (*c*Log *D*), blood–brain partitioning coefficient (log bb), solubility in buffer pH 7.4, maximum surface pressure (max. $\Pi$) and calculated cross-sectional area ($A_s$).

| Drug substance code | MW (g/mol) | p*Ka* (calculated) | *c*Log *P* | *c*Log *D* (pH = 7.4) | Log bb | Solubility (mmol/L) | Max. $\Pi$ (mN/m) | $A_s$ (Å$^2$) |
|---|---|---|---|---|---|---|---|---|
| 258206 | 449.51 | 8.05 (base)<br>7.34 (base) | 1.96 | 1.51 | 2.35 | 2.438 | 4.52 | 0.65 |
| 26616980 | 347.86 | 9.57 (base) | 3.53 | 1.13 | 1.37 | 2.085 | 15.42 | 22.40 |
| 39269646 | 308.31 | 7.86 (base)<br>3.22 (base) | 2.26 | 1.04 | 1.08 | 0.693 | 18.87 | 33.11 |
| 26532558 | 331.82 | 9.49 (base) | 3.59 | 2.89 | 0.87 | 1.551 | 15.38 | 46.42 |
| 31064423 | 376.93 | 10.34 (base) | 3.86 | 1.06 | 0.70 | 2.691 | 23.80 | 52.79 |
| 8024094 | 306.41 | 9.71 (acid)<br>8.31 (base) | 2.75 | 0.97 | 0.60 | 2.275 | 5.09 | 56.40 |
| 39051584 | 731.96 | 6.13 (base) | 4.81 | 4.71 | 0.30 | 0.12 | 35.55 | 40.50 |
| 27063699 | 423.52 | 8.54 (base)<br>7.41 (base)<br>1.88 (base) | 1.70 | −0.39 | 0.28 | 2.405 | 7.96 | 95.00 |
| 39546572 | 401.51 | 10.78 (base)<br>5.88 (base) | 3.75 | 0.80 | 0.11 | 2.453 | 10.12 | 73.20 |
| 16259685 | 325.41 | 9.75 (base)<br>2.33 (acid) | 4.09 | 4.09 | 0.08 | 0.261 | 14.80 | 51.20 |
| 6237608 | 331.34 | NA | 1.86 | 1.86 | −0.05 | 0.036 | 5.50 | 101.60 |
| 16527485 | 429.92 | 2.6 (base) | 4.33 | 1.82 | −0.10 | 0.070 | 3.84 | 95.97 |
| 38626926 | 434.54 | 9.54 (base)<br>1.69 (base) | 3.81 | 1.66 | −0.15 | 0.969 | 9.74 | 107.75 |
| 39130247 | 596.62 | 7.19 (base)<br>2.06 (base) | 3.12 | 2.35 | −0.30 | 1.786 | 34.90 | 80.30 |
| 38153284 | 364.51 | 4.24 (base) | 2.80 | 2.80 | −0.74 | 1.133 | 4.99 | 145.38 |
| 26041808 | 330.24 | 10.78 (base) | 2.89 | 2.89 | −0.77 | 0.601 | 11.58 | 143.17 |
| 18067712 | 392.50 | 8.30 (base) | 3.11 | 1.14 | −0.96 | 2.489 | 14.21 | 154.81 |
| 26253929 | 666.71 | 7.40 (base)<br>2.49 (base) | 3.67 | 2.89 | −1.00 | 1.114 | 33.14 | 167.55 |
| 39021203 | 408.56 | 4.49 (base) | 2.54 | 2.54 | −1.22 | 1.091 | 4.48 | 179.36 |

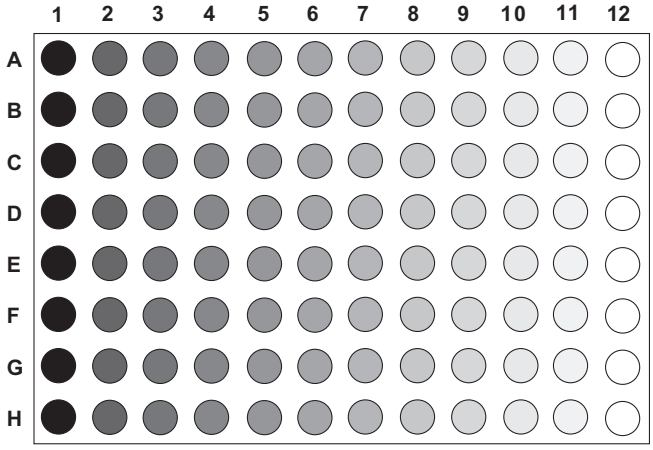


**Fig. 1.** 96-well sample plate for surface tension measurements (Column 1: stock solution, Columns 2–11 dilutions, Column 12 blank buffer as control).

The entire dilution series of dilutions was repeated in three further rows (row B/C/D) to enable a quadruplicate measurement.

### 2.6. Surface activity profiling (SAP)

Surface pressure profiles represent the change in the surface activity (mN/m) as a function of drug compound concentration at the air–water interface. All substance measurements were performed over a range of concentrations, with the upper concentration limit being the solubility limit of the compound in the buffer solution. Surface tension measurements were carried out using a multi-channel microtensiometer (Delta 8, Kibron Inc., Helsinki, Finland). This instrument is designed for medium throughput screening using a sample volume of 50 μl [21]. The system is a multi-channel tensiometer in which eight parallel microbalances are aligned in a configuration compatible with standard 96-polyethylene-well plates. The Delta 8 setup allows for the measurement of 96 samples within 5 min. The measurements are based on the Du Nuoy ring method, but instead of a ring, small metal rods are employed to record the maximum pull force exerted by the surface tension [22]. (Fig. 2) The entire analysis is computer controlled (Delta 8 Manager Vers. 2.72 (Kibron Inc., Helsinki, Finland [23]), and the metal rods are automatically cleaned by exposing them briefly to very high temperatures between measurements.

Prior to performing the surface tension measurements, the microtensiometer was calibrated with Milli Q® water to 72.8 mN/m. After calibration, a validation test (water loop test) was run to monitor intra- and inter-channel variations, using a plate containing 50 μl Milli Q water in each well. All obtained validation test data were within the vendor specified surface tension range of 72.8 ± 0.5 mN/m for Milli Q water.

### 2.7. Profile analysis

Surface activity characteristics were calculated from plots of surface tension as a function of drug concentration, and the

**Fig. 2.** The metal rods used to make the surface pressure measurements (courtesy of Kibron Inc., Helsinki, Finland).

isotherms were analyzed by Gibbs adsorption thermodynamics. The method specifies the partitioning of dissolved molecules at the air–water interfaces in order to obtain a relationship between molecular concentration in the bulk and at the interface. It further defines the molecular cross-sectional area and thus indicates the orientation of molecules. Fig. 3 shows the surface tension profile for drug 31064423.

The characteristics of surface activity can be described by classical Gibbs' thermodynamics for air–water interfaces

$$\Gamma = -\frac{d\gamma}{d\mu} \tag{1}$$

The surface excess, $\Gamma$, is the concentration of surface active compound at the surface in excess of the bulk concentration [13], described as the change in surface tension, $d\gamma$, as a function of the change in the chemical potential $d\mu$.

For dilute solutions $d\mu$ becomes $RT^{*}\ d\ln c$, where $c$ is the molar concentration of the surface active compound and $RT$ is the thermal energy per mole. Eq. (1) can thus be re-expressed as:

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln c} \tag{2}$$

Particularly for drugs with low solubility in physiological media, the concentration range over which the surface tension profiles are obtained may not be extensive enough to calculate the value of $d\gamma/d\ln c$. Thus, an alternative mathematical method is needed for calculating surface activity profiles of poorly soluble drugs.

The surface excess can alternatively be expressed as the inverse product of the Avogadro constant, $N_A$, and the area requirement of the surface active molecule at the interface, $A_s$ [24]:

$$\Gamma = (N_A A_s)^{-1} \tag{3}$$

The cross-sectional area of a surface active substance can be calculated from the slope of its Gibbs' absorption isotherm [25]. Since the area available per molecule at the air–water interface $A_s$ is inversely proportional to the surface excess at the air–water interface, $A_s$ can be expressed as:

$$A_s = \frac{-RT}{N_A}\cdot\frac{d\ln c}{d\gamma} \tag{4}$$

In the experiments, surface pressure ($\Pi$) rather than surface tension ($\gamma$) is measured. In general, the surface tension of a solution decreases with increasing concentration at low concentrations and tends to plateau at higher concentrations (see Fig. 3). Fig. 4 gives the corresponding plot of surface pressure versus drug concentration. These parameters are related through:

$$\Pi = \gamma^0 - \gamma \tag{5}$$

where $\gamma^0$ is the surface tension of the blank solvent and $\gamma$ is the surface tension in presence of a surface active compound [26].

Thus, $A_s$ can be calculated from the experimentally determined $\Pi$ by

$$A_s = \frac{-RT}{N_A}\cdot\frac{d\ln c}{d\Pi} \tag{6}$$

For poorly soluble compounds, the ratio of $c_{max}$ to $\Pi$ can be used as an approximation to the slope of the surface pressure curve, resulting in:

$$A_s = \frac{RT}{N_A}\cdot\frac{\ln c_{max}}{\Pi_{max}} \tag{7}$$

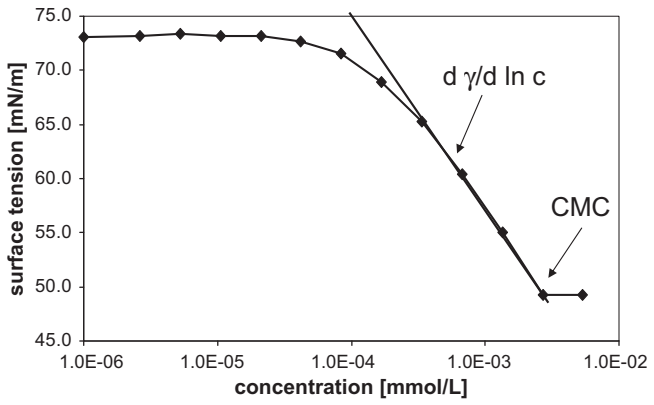


**Fig. 3.** Sample surface tension profile for substance 31064423, indicating how the thermodynamic parameters are derived from the profile.

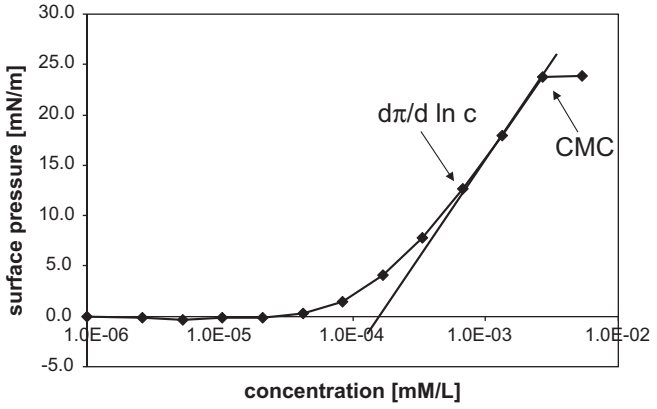


**Fig. 4.** Sample surface pressure profile for substance 31064423, indicating how the thermodynamic parameters are derived from the profile.

where $c_{max}$ is maximum concentration of the drug candidate at which a measurement was obtained and $\Pi_{max}$ is the surface pressure at this concentration.

## 3. Results and discussion

Table 1 summarizes the drug substances tested in pH 7.4 buffer, their calculated physicochemical parameters, their experimentally determined solubilities and their blood–brain partitioning coefficients, log bb. The experimentally determined solubilities ranged between 0.03 mM and 2.7 mM. Log bb values were found to lie within the range of 2.35 to −1.22, representing virtually the entire spectrum of drug permeability through the blood–brain barrier. Positive log bb values imply a significant tendency to penetrate the blood–brain barrier, while negative values reflect little or no access of the drug molecule to the brain. The molecular weight of the tested drug substances ranged from 300 to 730 gram per mole, the typical molecular weight range for APIs.

Surface activity profiles (SAP) for 19 randomly selected drug candidates were experimentally determined using an automated, small volume tensiometer. The calculated SAP parameters for predicting log bb are summarized along with the experimental log bb data in Table 1.

All tested compounds generated a maximum surface pressure greater than 3 mN/m enabling reliable data evaluation and interpretation. As reported by Boguslavsky et al. [15], the charge of the molecules at the surface, and likewise their surface area, $A_s$, depends on the pH of the solution. Thus, it is important that the surface activity profiles of the drug substances are generated in a biorelevant medium. The pION buffer pH 7.4 was selected for this study as it has the same pH as blood and has been previously identified as a buffer system enabling prediction of blood–brain permeability by the PAMPA method [27].

Data evaluation showed that the apparent air–water partitioning coefficient, $K_{AW}^{-1}$, did not correlate with blood–brain barrier penetration. $K_{AW}^{-1}$ was calculated by determining the intersection between the slope of surface pressure curve and the $X$-axis, log $c$ (see Fig. 3). A prerequisite for an accurate calculation of $K_{aW}^{-1}$ is a completely defined surface pressure curve that ideally includes the critical micelle concentration (CMC), the point at which surface pressure no longer increases with increasing concentration. With a well-defined surface pressure curve, a precise determination of the slope and hence its intercept with the $X$-axis ($K_{AW}^{-1}$) can be established. For the compounds selected for this study, the CMC could only be attained in one case (drug substance 31064423) due to the generally poor API solubility of the test set in the pH 7.4 buffer. As a result, it was not possible to establish a good correlation between log bb and $K_{aW}^{-1}$.

By contrast, the calculation of surface area ($A_s$) is not affected by poor solubility of the compound in the buffer system. As Eq. (6) could not be applied to the data set (since the profiles could not be characterized well enough to calculate the slope of the linear portion), Eq. (7) was used to calculate the area of the molecules at the air–water interface ($A_s$).

Eq. (7) consists of two terms. The first term is a constant at a given temperature since it comprises the molar gas constant and Avogadro's number. The second term is only influenced by the drug candidate's maximum concentration ($c_{max}$) and its corresponding surface pressure ($\Pi$) at this concentration. Eq. (7) is particularly suitable for poorly soluble drugs, for which neither a CMC nor a reliable estimate of the slope is possible. Due to the low surface pressure generated, a very sensitive tensiometer is needed to obtain reliable data. The tensiometer used in these studies was able to measure surface pressure as low as 3 mN/m consistently.

For the compounds included in this study, the experimentally determined cross-sectional surface area, $A_s$, ranged from 0.65 to 180 Å$^2$. Fig. 5 shows the relationship between the *in vivo* log bb values obtained from testing in rats and the corresponding cross-sectional area data calculated from the *in vitro* surface activity profiling measurements. A strong correlation is observed between the experimental steady state log bb values and the calculated cross-sectional areas, as indicated by the high linear regression coefficient of $R^2 = 0.8669$. This correlation coefficient clearly indicates that brain penetration decreases as the cross-sectional area of the drug candidate increases.

It has previously been reported that BBB penetrating drug substances (positive log bb values) tend to generate a surface area ranging from 0 to 80 Å$^2$ and BBB non-penetrating substances (negative log bb values) tend to generate a surface area between 80 and 180 Å$^2$ [28]. Based on *in silico* calculations, Kelder et al. reported that CNS active drugs, which penetrate the brain by passive absorption

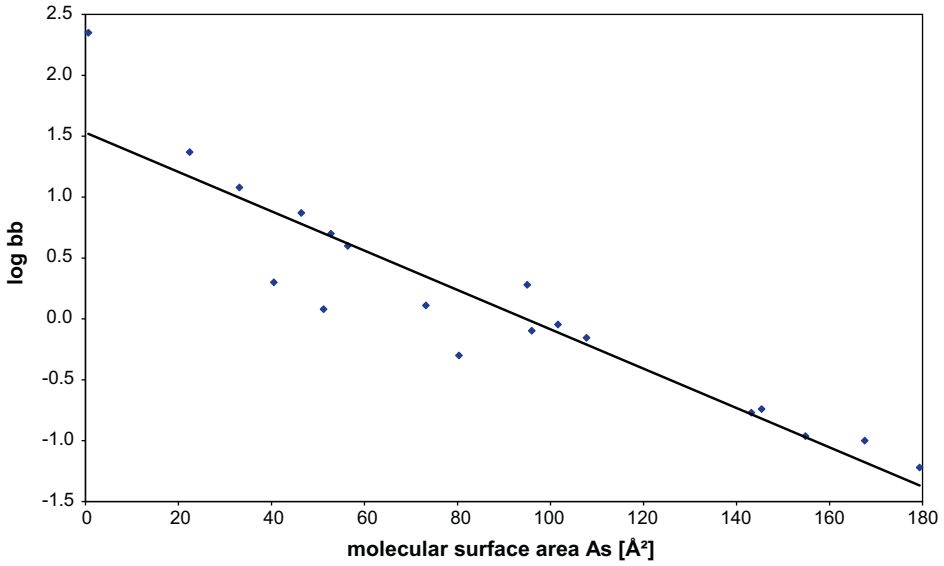


**Fig. 5.** Linear correlation of the steady state log blood–brain penetration and the cross-sectional area $A_s$ (Å$^2$) of all compounds in the test series. The linear regression equation is $y = -0.0162x + 1.5294$, with a linear regression coefficient of $R^2 = 0.8669$.

should have a polar surface area below 70 Å$^2$. The work of Kelder et al. was further substantiated by the work of Fischer et al., who showed that passive diffusion across the BBB of freely soluble compounds with cross-sectional area $A_D$ > 80 Å$^2$ is strongly reduced [16]. Thus, the results of this study, which suggests that compounds having a cross-sectional area <90 Å$^2$ will penetrate the BBB, are in concordance with previous observations.

An important mechanism of passive blood–brain barrier penetration is the partitioning of molecules into the lipid bilayer of the cell membrane. SAP testing is intended to predict blood–brain barrier penetration via passive, transcellular transport. Thus, the good correlation between cross-sectional area and log bb indicates that most substances in the set are likely transported by transcellular diffusion. For substances, which are subject to exotransport by P-Glycoprotein, e.g. loperamide, both SAP results and affinity for P-Glycoprotein would have to be considered to arrive at a satisfactory prediction of net BBB transport.

Additionally, the excellent correlation between log bb and the cross-sectional area, $A_s$, suggests that crossing the blood–brain barrier by passive transport is not determined primarily by the functional groups of the molecule, but rather by its physicochemical properties. These determine the concentration and orientation of the dissolved molecule at the interface, as well as its ability to interact with the biological membrane. Molecules providing low cross-sectional areas at the air–water interface appear to penetrate the barrier better than those which exhibit by a high cross-sectional area. This observation is likely related to the higher intermolecular forces necessary for accommodation of molecules with greater cross-sectional area in the membrane.

## 4. Conclusion

Blood–brain barrier uptake is important to the clinical success of central nervous system acting drugs and of equal importance in predicting the potential for central nervous system side effects of peripherally acting compounds. Small volume (μL) SAP screening in physiological media combined with simple mathematical modeling based on thermodynamic principles enables the *in vitro* prediction of blood–brain barrier penetration early in the discovery phase of drug development. SAP is applicable for the evaluation of poorly soluble drug candidates, an important attribute considering that most contemporary pipelines are largely comprised of compounds exhibiting poor aqueous solubility. Thus, the use of the SAP automated, *in vitro* screening protocol may help to further increase the efficiency of NCE development.

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