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CAFCA: a Novel Tool for the Calculation of Amphiphilic Properties of Charged Drug Molecules

Holger Fischer*, Manfred Kansy, and Daniel Bur

Abstract: Amphiphilic compounds play crucial roles in biology. They represent molecules with an inherent tendency to orient themselves in a suitable environment (*e.g.* a lipid bilayer). The driving force behind this effect is probably one of the crucial reasons for structural organization in living matter. An amphiphile typically comprises a hydrophilic as well as a hydrophobic part. The prediction of amphiphilic properties of charged small molecules by means of our in-house developed program CAFCA is presented in this work. An experimentally derived quantification of amphiphilic properties of a compound, expressed in terms of free energy of amphiphilicity ($\Delta\Delta G_{AM}$), can be deduced from surface activity measurements. Amphiphilic moments are obtained by vector addition of individual atom/fragment contribution values. Calculated amphiphilic moments were subsequently calibrated with known free energies of amphiphilicity ($\Delta\Delta G_{AM}$) of a homologous series of small charged amphiphiles (n-alkylsulfonic acids). The influence of conformational effects of molecules on calculated amphiphilic moments were further investigated for a set of eight structurally diverse commercially available drugs with known free energies of amphiphilicity. It turned out that conformations with maximal distance between charged group and center of gravity of the non-charged residue of the molecule yielded best results. Our calculated data and molecular modeling studies are in good accordance with experimentally derived published values. Our program CAFCA (**CA**lculated **F**ree energy of amphiphilicity of small **C**harged **A**mphiphiles) can be used to estimate preferred conformations as well as orientations of molecules in biological membranes and to quantify amphiphilic properties of molecules.

Keywords: Amphiphilic moment · Membrane binding · Molecular properties · Pharmaceutical chemistry

Introduction

Many amphiphilic drugs are used therapeutically and cover a broad spectrum of pharmacological classes such as antidepressants, antihistaminergics, antiarrhythmics, antihypertensive and local anesthetics. Amphiphilic properties of such compounds have been shown to influence pharmacokinetics (*e.g.* transcellular passive diffusion [1][2], P-glycoprotein (P-gp) mediated cellular efflux [3]), pharmacodynamics (*e.g.* interaction with ion channels [4]) and toxicologics (*e.g.* phospholipid storage disorder [5]). Quite often therapeutic effects depend on the binding of drugs to biological membranes. On the molecular level a membrane consists of various types of amphiphilic lipids that form bilayers in aqueous solution. Lipid bilayers are highly anisotropic and act as barriers in living organisms. They separate defined compartments from each other and prevent mixing of their respective contents thereby maintaining a high degree of order.

The determination and subsequent assignment of desired physiological effects (*e.g.* passive diffusion, receptor interaction) of an amphiphilic drug from toxicologic adverse effects (*e.g.* phospholipidosis) in an early phase of drug discovery requires a precise structure-based quantification of the amphiphilic properties of drug candidates. However, this is, to the best of our knowledge, not easily possible at the moment. Here we present a new method for the quantification of amphiphilic properties of charged molecules. Obtained results are compared with recently published experimentally derived data of amphiphilic properties [2].

Materials and Methods

Generation of Conformers

Multiple 3D conformations of molecules were generated using CATALYST Version 4.5 (Molecular Simulations, San Diego, CA). Conformers were produced using the 'best' option and their number was limited to a maximum of 250 within an energy range of 20 kcal/mol.

Single conformers of each compound were generated using CORINA, a rule-based structural 3D generator, Version 2.4 [6]. All calculations were carried out on a Silicon Graphics Indigo worksta-

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Statistical Analysis and Lipophilicity Calculations

Statistical analysis was performed with STATISTICA for Windows, Version 5.1. Lipophilicities were calculated using KOWWIN v1.57 (Syracuse Research Corporation, New York, USA) based on an atom/fragment contribution method (AFC) of Meylan and Howard [7].

Free Energy of Amphiphilicity

Amphiphiles dissolved in water can segregate their hydrophobic portion from water either by intruding into the air-water interface or by self-association (micelle formation). Both processes are mainly entropy driven (hydrophobic effect). In contrast to the partitioning of the compound at the air-water interface, micelle formation requires the action of an additional opposing force that arises from electrostatic repulsion in case of charged molecules. Both air-water partition coefficients, K_{aw}, and critical micelle concentrations, CMC, were derived from measured surface activities plotted as a function of drug concentration. K_{aw} as well as CMC were expressed in terms of free energy according to Eqn. (1) and (2), respectively.

$$\Delta G_{AW} = -RT \ln (55.5\ K_{AW}) \quad (1)$$

$$\Delta G_{MIC} = RT \ln (CMC / 55.5) \quad (2)$$

Finally, the free energy of amphiphilicity, $\Delta\Delta G_{AM}$, was defined as the free energy of transfer of a compound from the aqueous phase to the air-water interface, ΔG_{AW}, or into a micelle ΔG_{MIC} respectively.

$$\Delta\Delta G_{AM} = \Delta G_{AW} - \Delta G_{MIC} \quad (3)$$

Free energies of amphiphilicity were taken from literature [2][8], where energy values were determined by means of surface activity measurements.

The CAFCA Program

The CAFCA (**CA**lculated **F**ree energy of amphiphilicity of small **C**harged **A**mphiphiles) program was written in C Version 7.2.1.3m (Silicon Graphics). Ionization constants were calculated with an in-house developed program by means of linear free energy relationships according to [9].

Molecular Modeling Studies

All modeling calculations were made on a Silicon Graphics Octane with a single R12000 processor using our in-house modeling package Moloc (*http://www.moloc.ch*) [1][2]. All molecules were built from scratch and optimized individually. Subsequently bis(β-diethylaminoethylether)hexestrol (DEH) was surrounded by a lipid layer of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). DEH was positioned such that charged amino groups were in the vicinity of phosphate groups of glycerides but not forming H-bond interactions. Water molecules were omitted from calculation. Optimization led to an arrangement of molecules as shown in Fig. 1.

Results

Calculation of Amphiphilic Moments

The amphiphilic moment, $\vec{A}$, of a molecule is defined as:

$$\vec{A} = \sum_i d_i \cdot \vec{\alpha}_i \quad (4)$$

where $\vec{\alpha}$ is the hydrophobic/hydrophilic contribution of an atom/fragment as described in [7] and d is the distance between the centers of gravity of the charged part of a molecule and the hydro-

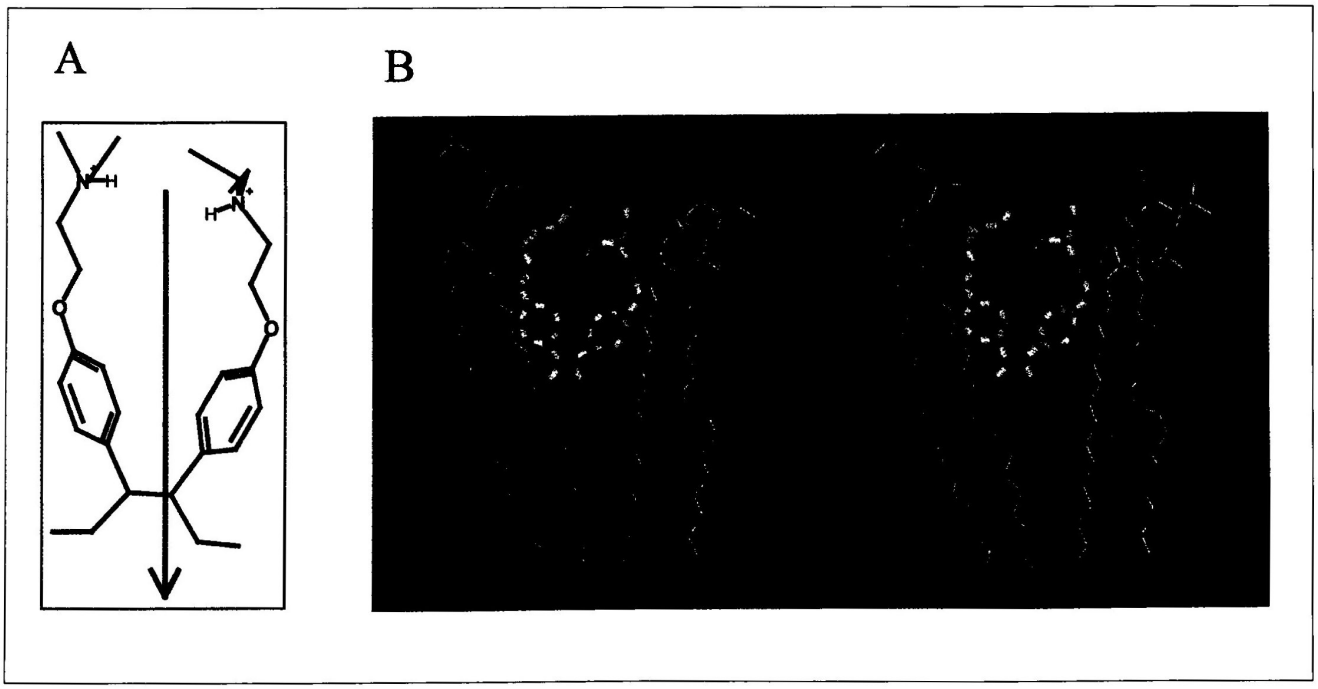

Fig. 1. (A) Calculated amphiphilic vector of bis(β-diethylaminoethylether)hexestrol (DEH). (B) Location of DEH in a 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid layer as derived from molecular modeling studies. Both basic groups of DEH were found in the vicinity of phosphocholine moieties while the aromatic ring systems were buried in the hydrocarbon core of the POPC layer. Residues at the 3,4-position of the hexane backbone of DEH preferred s-*cis*- rather than s-*trans* conformation with both basic nitrogens pointing towards the hydrophilic phosphate groups. A comparable location of DEH within a phosphatidylinositol consisting membrane was suggested previously [18] based on results of ^{31}P NMR experiments.

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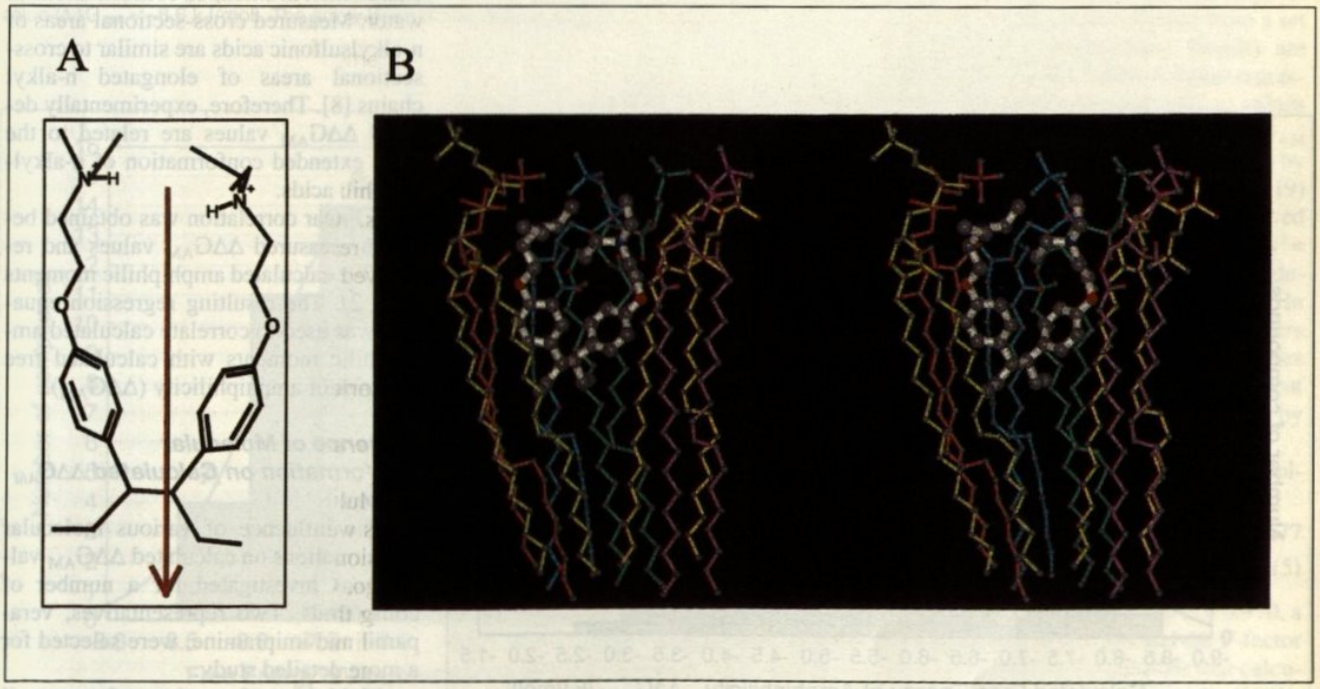

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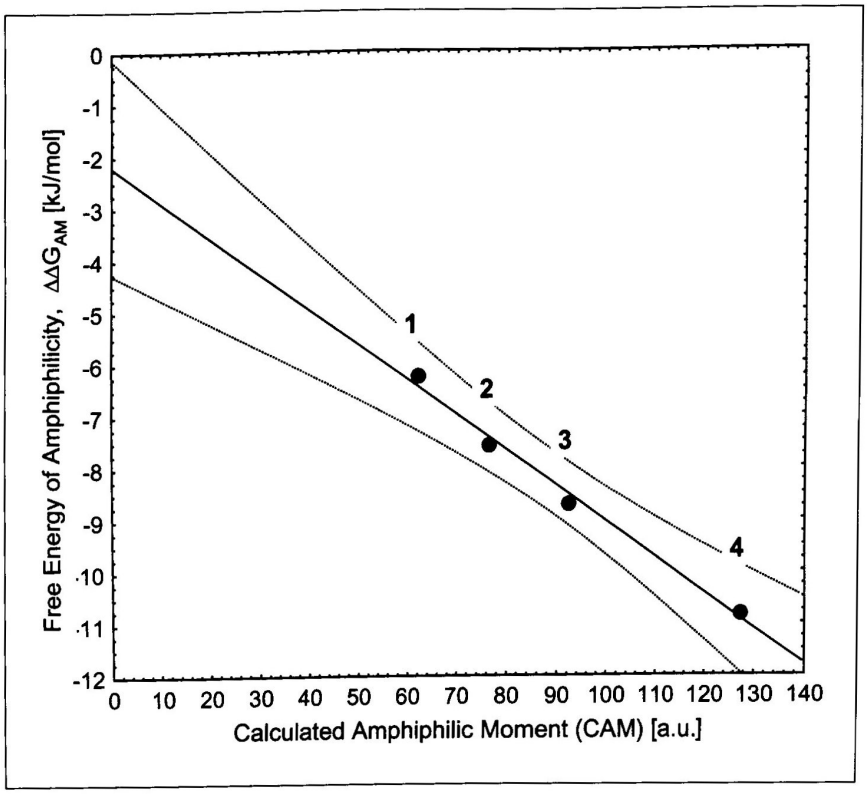

Fig. 2. Correlation between measured free energies of amphiphilicity ($\Delta\Delta G_{AM}$) of a series of n-alkylsulfonic acids [8] and amphiphilic moments (CAM) calculated according to Eqn. (1). The compounds measured are: (1) n-octylsulfonic acid, (2) n-nonylsulfonic acid, (3) n-decylsulfonic acid and (4) n-dodecylsulfonic acid. Linear regression analysis yields the following equation: $\Delta\Delta G_{AM} = -2.20(\pm0.47)-0.069(\pm0.05)CAM$. $r^2 = 0.983$; sd = 0.251; F = 178. Dashed line depicts the 95% confidence interval.

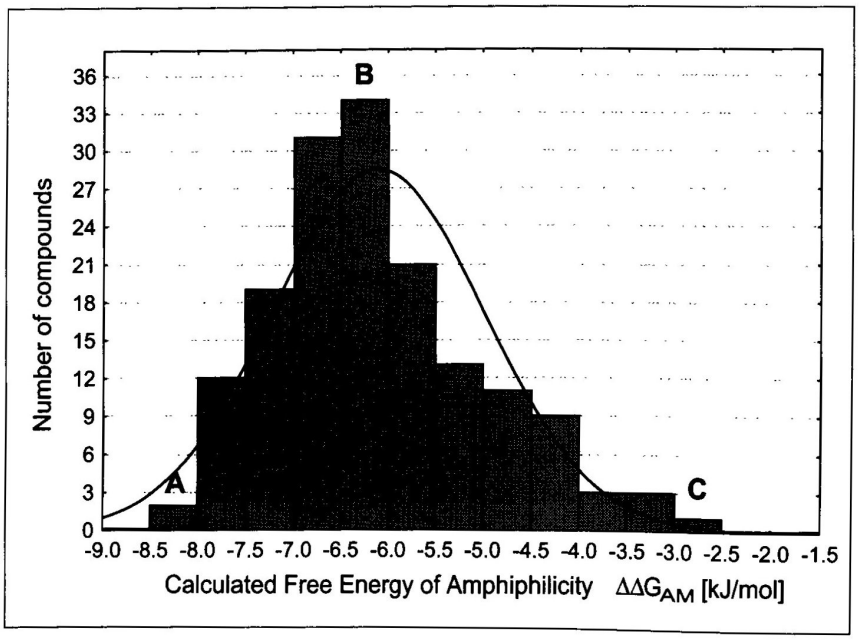

Fig. 3: Histogram of the calculated free energy of amphiphilicity ($\Delta\Delta G_{AM}$) of verapamil for 159 conformers generated by means of CATALYST (see methods). Gaussian regression analysis (solid line) yields a maximum at $\Delta\Delta G_{AM} = -6.1$ kJ/mol. Capital letters refer to tree conformations

phobic/hydrophilic remnant of the molecule respectively. In case of diprotic compounds the midpoint between the charged groups was taken as initial point for calculations. Ionization constants were taken into account if the estimated basic pK_a value was higher than 8.5 and the estimated acid pK_a value was lower than 6.5 respectively. In both cases at least 90% of the molecules are protonated/deprotonated at physiological pH (pH = 7.4). For reasons of simplicity, all molecules were considered to be charged under these conditions. Individual hydrophobic/hydrophilic contributions of each atom/fragment were weighted by means of the atom/fragment contribution method (AFC) [7].

Relationship Between Calculated Amphiphilic Moments and Calculated Free Energies of Amphiphilicity

Calculated amphiphilic moments obtained as the sum of the individual vectors (Eqn. (4)) were initially determined for a homologous series of n-alkylsulfonic acids. Single conformations of each molecule were generated with CORINA [6] and subsequently used for calculations of amphiphilic moments. By determining Krafft points and critical micelle concentrations of n-alkyl sulfonates, Saito *et al.* [10] could show that their alkyl chains exist in extended conformations in water. Measured cross-sectional areas of n-alkylsulfonic acids are similar to cross-sectional areas of elongated n-alkyl chains [8]. Therefore, experimentally derived $\Delta\Delta G_{AM}$ values are related to the most extended conformation of n-alkylsulfonic acids.

A linear correlation was obtained between measured $\Delta\Delta G_{AM}$ values and respective calculated amphiphilic moments (Fig. 2). The resulting regression equation was used to correlate calculated amphiphilic moments with calculated free energies of amphiphilicity ($\Delta\Delta G_{AM}$).

Influence of Molecular Conformation on Calculated $\Delta\Delta G_{AM}$ Values

The influence of various molecular conformations on calculated $\Delta\Delta G_{AM}$ values was investigated for a number of compounds. Two representatives, verapamil and imipramine, were selected for a more detailed study.

Multiple conformations of verapamil were generated and free energies of amphiphilicity were determined for each of the 159 conformers. The histogram in Fig. 3 illustrates the distribution of calcu-

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$\Delta\Delta G_{AM}$ values cover a range from –2.5 kJ/mol for conformers with almost no amphiphilic properties (Fig. 4C) to –8.2 kJ/mol for highly amphiphilic conformers (Fig. 4A).

The majority of conformers have $\Delta\Delta G_{AM}$ values ranging from –6.0 kJ/mol to –6.5 kJ/mol (Fig. 4B). However, the measured $\Delta\Delta G_{AM}$ of verapamil is –8.6 kJ/mol which fits best to the lowest calculated free energy of amphiphilicity ($\Delta\Delta G_{AM}$ = –8.2 kJ/mol). Fig. 5 demonstrates that similar results are obtained for imipramine.

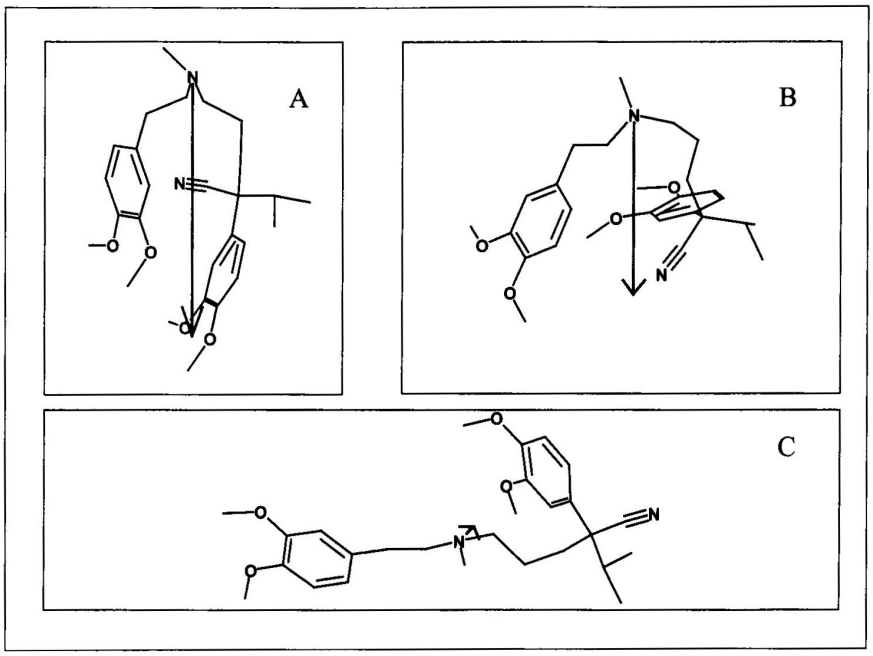

Fig. 4: Three conformations of verapamil with low (A), medium (B) and high (C) calculated free energies of amphiphilicity ($\Delta\Delta G_{AM}$). (A) $\Delta\Delta G_{AM}$ (calculated) = –8.2 kJ/mol, (B) $\Delta\Delta G_{AM}$ (calculated) = –6.3 kJ/mol, (C) $\Delta\Delta G_{AM}$ (calculated) = –2.5 kJ/mol. The measured value of $\Delta\Delta G_{AM}$ of verapamil was determined to be –8.6 kJ/mol. The most extended conformation that refers the lowest calculated free energy of amphiphilicity ($\Delta\Delta G_{AM}$ = –8.2 kJ/mol) come closest to the measured value ($\Delta\Delta G_{AM}$ = –8.6 kJ/mol). The arrow depicts the calculated amphiphilic vector.

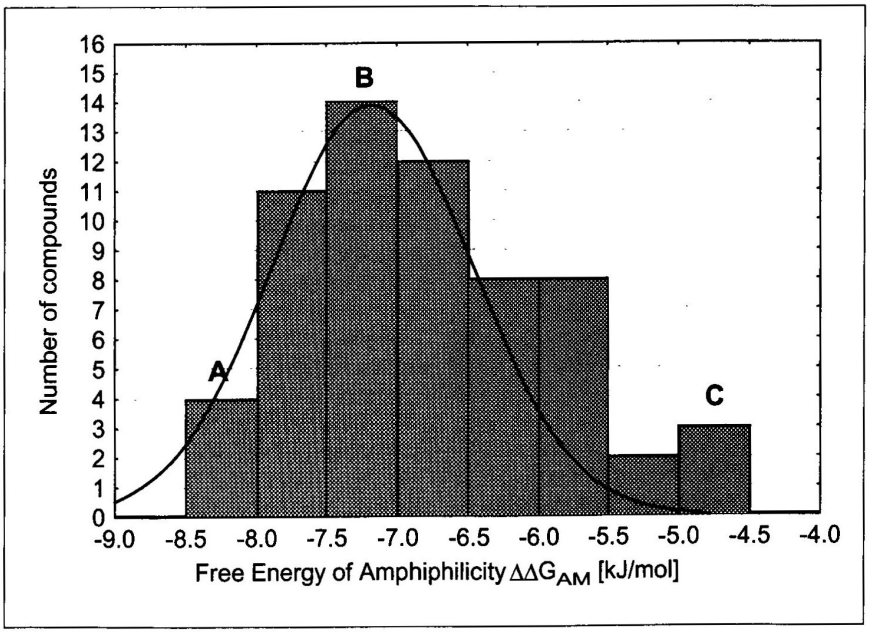

Fig. 5: Histogram of calculated free energies of amphiphilicity ($\Delta\Delta G_{AM}$) of imipramine for 69 conformers generated by means of CATALYST (see methods). Gaussian regression analysis (solid line) yields a maximum at $\Delta\Delta G_{AM}$ = –7.2 kJ/mol. Capital letters refer to tree conformations

The distribution of calculated $\Delta\Delta G_{AM}$ for all 69 conformers of imipramine is more compact than for verapamil. $\Delta\Delta G_{AM}$ values range from –8.3 kJ/mol (Fig. 6C) to –4.5 kJ/mol (Fig. 6A).

The measured $\Delta\Delta G_{AM}$ value of imipramine is determined to be –8.8 kJ/mol. Although the majority of conformers have calculated $\Delta\Delta G_{AM}$ values between –7.0 kJ/mol and –7.5 kJ/mol (Fig. 6B), the lowest calculated $\Delta\Delta G_{AM}$ value of –8.3 kJ/mol comes closest to the measured value. However, since calculated $\Delta\Delta G_{AM}$ values critically depend on the conformation of the molecule, a more detailed investigation was performed to shed light on conformational influences on $\Delta\Delta G_{AM}$ values.

Comparison Between Measured and Calculated $\Delta\Delta G_{AM}$ Values

An extended conformational analysis was subsequently performed for a set of eight structurally diverse drugs with known measured $\Delta\Delta G_{AM}$ values ranging from –2.2 kJ/mol to –8.8 kJ/mol (Table). $\Delta\Delta G_{AM}$ values were calculated for (i) conformers with lowest energies *in vacuo*, (ii) CORINA derived conformers and finally (iii) conformers with the highest amphiphilic moments selected from a set of multiple conformations. Results are summarized in the Table. A linear regression analysis of measured $\Delta\Delta G_{AM}$ values revealed that neither calculated $\Delta\Delta G_{AM}$ values of conformations generated by CORINA (r^2 = 0.711; sd = 0.985; F = 19) nor calculated $\Delta\Delta G_{AM}$ values derived from minimal energy conformations (r^2 = 0.617; sd = 1.19; F = 14) are suited to describe the measured $\Delta\Delta G_{AM}$ values. In contrast, $\Delta\Delta G_{AM}$ values of conformers with the lowest calculated free energies of amphiphilicity are in good agreement with measured values as illustrated by Fig. 7.

The regression analysis yields the following Eqn. (5):

$$\Delta\Delta G_{AM(measured)} = -2.31(\pm 0.38) - 0.77(\pm 0.06)\,\Delta\Delta G_{AM\,(calculated)} \quad (5)$$

with a correlation coefficient r^2 = 0.970, a standard error sd = 0.384 and the F-factor of 191. It is interesting to note that calculated lipophilicities derived by the KOWWIN program do not correlate with measured $\Delta\Delta G_{AM}$ values (r^2 = 0.501) although both KOWWIN as well as CAFCA derived calculations use the AFC

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