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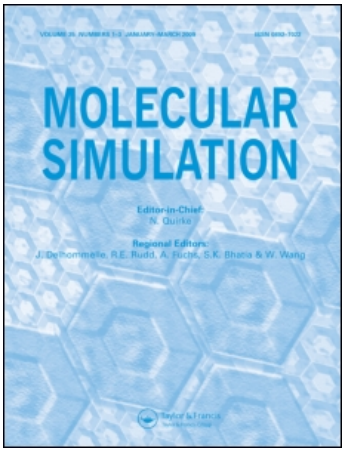

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Free Energy Calculations of Pharmaceutically Important Properties

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FREE ENERGY CALCULATIONS OF PHARMACEUTICALLY IMPORTANT PROPERTIES

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When determining the biochemical function of a proposed new drug it is useful to be able to calculate a number of properties which regulate its behaviour. By using the Free Energy Perturbation method incorporated into molecular dynamics, both on its own and in conjunction with quantum mechanical calculations, we have calculated redox potentials, pK_as, tautomer ratios and partition coefficients on model systems.

This paper reviews the techniques currently being used to perform such calculations and presents the results for a number of different systems. The accuracy with which the properties are determined is most encouraging and suggests that useful calculations on realistic systems are now possible, and will play a major role in drug-design in the future.

KEY WORDS: Free energy perturbation, partition coefficient, pK_a, redox potential, tautomer ratio.

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1. INTRODUCTION

The role of the theoretician in designing molecules that will have the required biological activity is to suggest compounds having enhanced desirable characteristics and reduced adverse ones. For this to be achieved it is necessary to know not only how the molecule acts at its receptor site but also how it behaves in the rest of the biological system. For many years Quantitative Structure Activity Relationships (QSARs) [1] have been used to correlate activity to a range of structural and physiochemical properties of the molecule, such as its partition coefficient (between an aqueous and non-aqueous phase) and its pK_a. It is evident that such properties will affect the absorption of the molecule into the system and its uptake into cells, as well as its eventual elimination from the system. If one is to be able to calculate the activity of a proposed new compound it is vital to know such physiochemical properties.

Traditionally, the discovery of new compounds in the fields of pharmacology and agrochemistry have relied upon the synthesis and testing of a vast number of molecules in the hope that a few would have the desired effects. What the theoretical chemist hopes to do is to introduce a greater degree of selectivity into the process thereby greatly reducing the time and effort required for a new drug to become available. Our efforts have therefore been targetted at using existing techniques to develop reliable methodologies for calculating those properties of a molecule which will affect its biochemical activity.

2. PARTITION COEFFICIENTS

The transport of a chemical within a biological system involves the crossing of many barriers, for example the membranes that compose body tissue, cell walls and body organs. Since these are made predominantly of lipids (but also containing some protein and carbohydrate) it seems reasonable to assume that the ability of a drug to get to its site of action from the body fluids will depend on some partition coefficient. In most QSAR studies the partitioning is measured in terms of log P, defined as follows:

$$A_{(aq)} \xrightarrow{\Delta G} A_{(organic)}$$

$$P = \frac{[A]_{(organic)}}{[A]_{(aq)}}$$

$$\log P = -\frac{\Delta G}{2.303RT} \tag{1}$$

where the organic phase is usually modelled by *n*-octanol. The problem now involves calculating a free energy difference: clearly classical mechanics is required as the system is too large to be modelled quantum mechanically. In principle the free energy difference can be calculated by using the partition function for each system, which in the isothermal–isobaric ensemble is given by

$$\Xi(A) = \frac{1}{N!}\frac{1}{h^{3N}} \int dV \int\int d\bar{p}\, d\bar{r} \exp\left[-(\mathcal{H}(\bar{p},\bar{r}) + PV)/kT\right] \tag{2}$$

$$\Delta G = -kT \ln\left[\frac{\Xi(A)_{\text{organic}}}{\Xi(A)_{\text{aq}}}\right] \tag{3}$$

However, for anything nearing the size and complexity of a realistic system the partition function cannot be calculated using a sampling scheme based on an equilibrium probability distribution function. But in a realistic drug-design situation we are only interested in relative rather than absolute partition coefficients, as we are often correlating behaviour between compounds. Thus we can consider the cycle

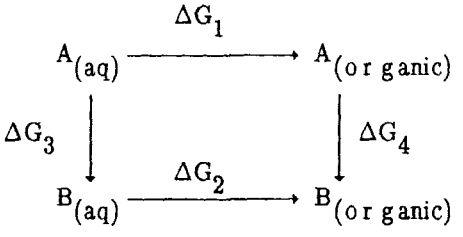

Figure 1 Thermodynamic cycle for the determination of the relative partition coefficient of two compounds, *A* and *B*, between an aqueous and non-aqueous phase.

Whereas the experimentalist might measure the difference as ΔG_2-ΔG_1 it is possible, using the Free Energy Perturbation method (FEP) [2–6], for the theoretician to calculate the difference as ΔG_4-ΔG_3. We are then left with the need to calculate free energy changes accompanying the physically non-realistic processes of perturbing one compound into another in two separate solvent systems.

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2.1 Free Energy Perturbation Method (FEP)

The methodology that enables us to perform such calculations is due to Zwanzig [7]. The hamiltonian for one system is considered to be a perturbation on that of a reference. For example, for the perturbation

$$A_{(aq)} \xrightarrow{\Delta G_{pert}} B_{(aq)} \tag{4}$$

we have

$$\mathcal{H}_B = \mathcal{H}_A + \Delta\mathcal{H} \tag{5}$$

where $\Delta\mathcal{H}$ is the difference in classical hamiltonians between our two systems. We can then obtain an expression for the free energy difference accompanying (4) by inserting (5) into the partition function for B, i.e.

$$\begin{aligned}\Delta G_{pert} &= -kT \\ &\ln\left(\frac{\int dV \int\int d\bar{r}\, d\bar{p} \exp\left[-\mathcal{H}_A(\bar{p},\bar{r}) + PV)/kT\right] \exp\left(-\Delta\mathcal{H}(\bar{p},\bar{r})/kT\right)}{\int dV \int\int d\bar{r}\, d\bar{p} \exp\left[-(\mathcal{H}_A(\bar{p},\bar{r}) + PV)/kT\right]}\right) \\ &= -kT \ln\left\{\int dV \int\int d\bar{r}\, d\bar{p}\, \mathcal{P}_A \exp\left(-\Delta\mathcal{H}(\bar{p},\bar{r})/kT\right)\right\} \\ &= -kT \ln \langle \exp\left(-\Delta\mathcal{H}(\bar{p},\bar{r})/kT\right)\rangle_A \end{aligned} \tag{6}$$

where $\mathcal{P}_A$ is the probability distribution function for configurations of system A and $\langle \ldots \rangle_A$ is just an ensemble average over the configurations generated according to this function. In essence all that is needed to evaluate the free energy difference is to use molecular dynamics (or Monte Carlo) to generate configurations according to $\mathcal{P}_A$ and then accumulate statistics to evaluate the average in equation (6).

2.2 Implementation of FEP

In practice the implementation of FEP is not so straightforward. If the configurations of A and B are not within similar regions of phase space (i.e. if the overall free energy change is not within about $2kT$) then the average will be very slow to converge or may converge on the wrong value. To circumvent this problem the perturbation is split into a series of 'windows', or smaller perturbations, each of which will have the required convergence characteristics and which can be summed to give the overall change. Computationally this is performed by making the hamiltonian of the system a smooth function of some coupling parameter, λ, such that

$$\mathcal{H} = \mathcal{H}(\lambda) = \begin{cases} \mathcal{H}_A, \lambda = 1 \\ \mathcal{H}_B, \lambda = 0 \end{cases} \tag{7}$$

The perturbation can then be performed between some hybrid states defined by $\mathcal{H}(\lambda)$ and $\mathcal{H}(\lambda + \Delta\lambda)$ (or $\mathcal{H}(\lambda - \Delta\lambda)$ for a backwards change). Thus

$$\Delta G_{\lambda} = -kT \ln \langle \exp\left(-\Delta\mathcal{H}(\bar{p},\bar{r},\lambda)/kT\right\rangle_{\lambda}$$

where

$$\Delta\mathcal{H}(\bar{p},\bar{r},\lambda) = \mathcal{H}(\bar{p},\bar{r},\lambda + \Delta\lambda) - \mathcal{H}(\bar{p},\bar{r},\lambda)$$

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and

$$\Delta G_{\text{total}} = \sum_{\lambda} \Delta G_{\lambda}$$

The functional dependence of $\mathcal{H}$ on λ is obtained by making all the terms of the classical interaction potential (see appendix) a function of λ (since kinetic terms are considered to be negligible and/or cancel out). The constants are made a linear function of λ,

$$K_{r_n} = \lambda K_{r_n}(A) + (1 - \lambda)K_{r_n}(B)$$
$$q_i = \lambda q_i(A) + (1 - \lambda)q_i(B) \qquad \text{etc.}$$

For the sake of computational simplicity the organic phase was modelled by carbon tetrachloride. In both aqueous and non-aqueous simulations, boxes of solvent containing a single solute molecule were used and periodic boundary conditions with constant temperature and pressure were applied. The AMBER force field [8] and suite of programs [9] were used to perform the molecular dynamics (MD) simulations, although alcohol and carbon tetrachloride parameters came from alternative sources [10–13]. Further details are described elsewhere [14,15].

2.3 Results and Discussion

The solutes chosen as test cases were the alcohols methanol, ethanol and propanol, and the results obtained are given in Table 1. These clearly indicate that the difference in log *Ps* is reproducible to within experimental uncertainty, although as with all simulation results they depend critically on the parameter set used to define the interaction potential function.

There are alternative methods for determining log *P* values [e.g. 16] but the method outlined here is likely to be of greater utility as electronic, conformational and dynamical effects are explicitly considered through the charge parameterisation and the phase space sampling of the simulation. In addition, the possibility exists for doing simulations of lipid bilayers and hence of obtaining a very accurate idea of how the molecules are distributed within systems having direct biological significance and for which experiments are not easy to perform. Work in this area is currently under investigation.

3. DETERMINATION OF pK'_a

The pK_a of a drug is crucial in determining how the molecule is absorbed into the biological system (e.g. whether absorption occurs from the stomach or intestine in the body), the rate at which it is transported and its rate of elimination. In addition the

Table 1 Difference in log *P* calculated for alcohols partitioning between water and carbon tetrachloride.

Solute		*log P(A)-log P(B)*	
A	*B*	*Experimental*	*Calculated*[†]
Methanol	Ethanol	−0.70	−0.52 ± 0.30
Ethanol	Propanol	−0.58	−0.70 ± 0.38

[†]Jorgensen parameter set [12,13] used for alcohols.

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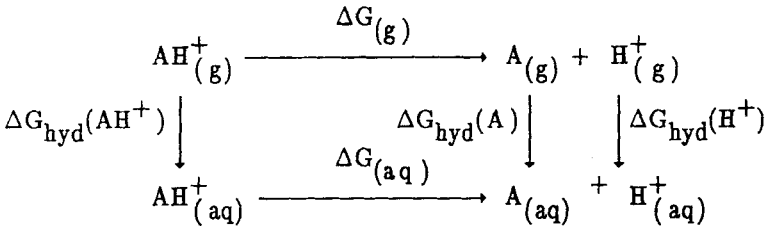

Figure 2.a Thermodynamic cycle for the determination of the absolute pK_a of a compound.

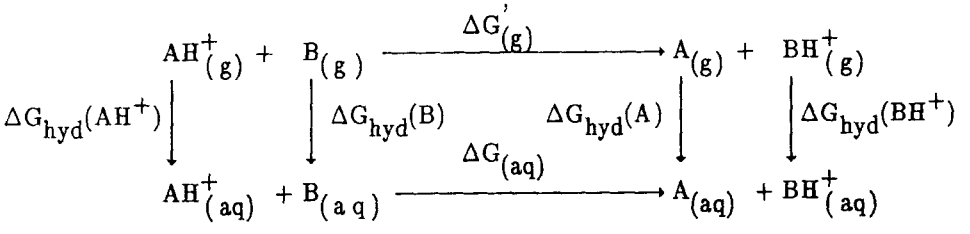

Figure 2.b Thermodynamic cycle for the determination of the relative pK_a between two compounds *A* and *B*.

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protonation state of a molecule often determines whether or not binding will occur at the receptor site.

One can consider the reaction

$$AH^+_{(aq)} \xrightarrow{\Delta G_{(aq)}} A_{(aq)} + H^+_{(aq)}$$

as representing the acid/base nature of the molecule, and if this is to be established one requires the accompanying free energy change. Once again a classical determination based on partition functions is not feasible, but either absolute or relative pK_a's can in principle be calculated by considering the cycles in Figure 2, from which it can be seen

$$\Delta G_{(aq)}(\text{absolute}) = \Delta G_{(g)} + \{\Delta G_{hyd}(A) - \Delta G_{hyd}(AH^+)\} + \Delta G_{hyd}(H^+) \quad (8)$$

$$\Delta G_{(aq)}(\text{relative}) = \Delta G'_{(g)} + \{\Delta G_{hyd}(A) - \Delta G_{hyd}(B)\} - \{\Delta G_{hyd}(AH^+) - \Delta G_{hyd}(BH^+)\} \quad (9)$$

The role of FEP is now in determining the free energy differences required in

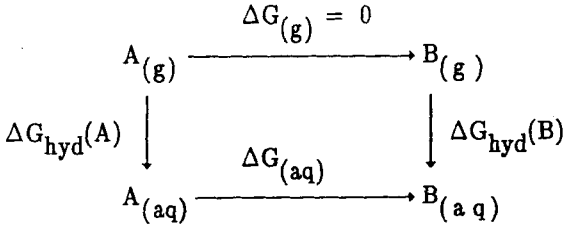

Figure 3 Thermodynamic cycle for the determination of differences in free energy of hydration between two molecules *A* and *B*.

equations (8) and (9). Considering the cycle in Figure 3 we can see that perturbing A to B in solution and evaluating only intermolecular interactions is equivalent to calculating the difference in hydration free energies, i.e.

$$\Delta G_{(\text{pert})}(A \longrightarrow B) = \Delta G_{\text{hyd}}(B) - \Delta G_{\text{hyd}}(A) \tag{10}$$

Thus

$$\Delta G_{(\text{aq})}(\text{absolute}) = \Delta G_{(\text{g})} + \Delta G_{\text{pert}}(AH^+ \longrightarrow A) + \Delta G_{\text{hyd}}(H^+) \tag{11}$$

$$\Delta G_{(\text{aq})}(\text{relative}) = \Delta G'_{(\text{g})} + \Delta G_{\text{pert}}(B \longrightarrow A) - \Delta G_{\text{pert}}(BH^+ \longrightarrow AH^+) \tag{12}$$

In practice relative basicities are calculated rather than absolute ones for the following reason: as the interaction potential is truncated to make the simulations computationally feasible, any simulation involving a formally charged molecule neglects a significant proportion of the long-range coulombic $1/r$ terms. Thus a perturbation which involves the creation or annihiliation of a formal charge will have disproportionate truncation effects at each end of the simulation giving unreliable results. However, where the perturbation is between solutes having the same formal charge the error introduced through potential truncation should cancel out to a large degree, giving more reliable results. Thus relative pK'_as were chosen for calculation.

3.1 Relative pK_a Calculations

The system chosen for study was the homologous amine series ammonia, methyl-, dimethyl- and trimethylamine. This is a very useful test case as the amino moiety is common to many biologically active molecules and is much studied since the order of basicity is different in the gas phase compared to that in aqueous solution.

The gas phase free energy difference required in equation 12 is obtained quantum mechanically: energies at 0 K are given by *ab initio* calculations using standard programs [17] and these are converted to absolute free energies at 298 K using statistical mechanics to obtain single molecule partition functions. In order to establish how critical the quantum mechanical part of the calculation was to the nature of the wavefunction, a series of geometry optimisations and single point energy calculations were performed using different basis sets. As before the perturbation was done using molecular dynamics.

3.2 Results and Discussion

The results using the highest quality basis sets for the gas phase part of the calculation, both with and without the second-order Møller-Plesset perturbation correction [18], are given in Table 2. (Individual results can be better using lower quality basis sets, although those quoted provide the most consistant set of values).

It can be seen that the results are within 2.5 pK_a units of the experimental ones, but the trend is not reproduced with significant accuracy. Here the errors from the quantum and classical mechanical parts of the calculation combine, and it should be noted that an error of 2.5 units corresponds to an error of only 3.4 kcal/mol in the calculated free energy. Thus we can conclude that while the relative pK_a of a compound can be determined using this combined *ab initio*/FEP approach to a reasonable accuracy it is not useful where there are small and subtle differences in the observed values. Fuller details of the calculations can be found in reference [19].

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Table 2 Difference in pK_a relative to the lower homologue, for the series ammonia, methyl-, dimethyl- and trimethyl ammonia, with the highest quality basis sets used for the determination of the gas phase free energy differences†.

	NH_3	$MeNH_2$	Me_2NH	Me_3N
RHF/6-31G**//RHF/6-31G	0.0	1.31	2.54	1.84
MP2/6-31G**//RHF/6-31G	0.0	0.68	2.12	1.45
EXPERIMENT	0.0	1.41	0.08	−0.92

†RHF implies a restricted Hartree-Fock calculation was performed and MP2 means that electron correlation was incorporated by performing a second-order Møller-plesset perturbation calculation. The notation corresponds to (level of calculation for energy)//(level of calculation for geometry).

4. DETERMINATION OF REDOX POTENTIALS

There is much interest in the redox properties of certain compounds where the potential exists for bioreductive anti-cancer activity. Solid tumour cells have a reduced blood supply compared to normal cells since the cancer tends to destroy blood vessels while invading surrounding tissue. Thus a route to designing a more selective chemotherapeutic agent is to synthesize a molecule with a redox potential in the correct range so that it is in its active form (i.e. reduced) in the tumour cell, and in its inactive form (i.e. oxidized) in the normal cell [20].

One postulated goal is to use a quinone type molecule to block the enzyme dihydrofolate reductase [21], thereby preventing the cell synthesizing certain metabolites required for proliferation. In addition, dihydrofolate reductase has an active site perfectly suited to bioreductive activity. A 2-electron reduction potential is required in which the quinone form of a molecule is converted into its quinol form:

$$A_{(aq)} + 2e^-_{(aq)} + 2H^+_{(aq)} \xrightarrow{\Delta G} AH_{2(aq)}$$

and again the determination of the free energy change accompanying this reaction would not be feasible. Instead, as with the pK_a case, the difference in redox potential between two quinones A and B can be found from the cycle in Figure 4, from which we have

$$-nF(E^0_B - E^0_A) = \Delta G_{(aq)} = \Delta G_{(g)} + \Delta G_{pert}(B \longrightarrow BH_2) + \Delta G_{pert}(A \longrightarrow AH_2) \tag{11}$$

As before, $\Delta G_{(g)}$ can be calculated using quantum mechanics and determining the correction terms by evaluating partition functions for the individual species. The perturbations themselves can be performed on molecules in periodic boxes of water using molecular dynamics. Full details are reported elsewhere [22–24].

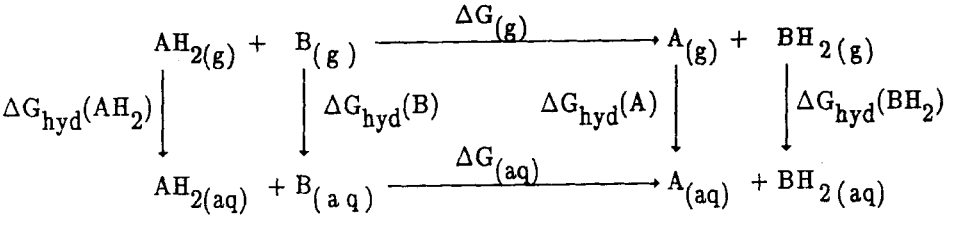

Figure 4 Thermodynamic cycle for the calculation of relative 2-electron redox potential between two quinone-type molecules A and B.

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Table 3 Calculated redox potentials using the literature value for the reference para-benzoquinone.

Compound	*Experiment (V)*	*Calculated (V)*	*Difference (mV)*
orthobenzoquinone	0.813	0.792	21
parabenzoquinones:			
2-methyl	0.644	0.681	37
2,4-dimethyl	0.590	0.571	19
2-chloro	0.712	0.685	27
2,3-dicyano	0.971	0.940	31
2-hydroxy	0.594	0.569	25
1,4-diaminobenzene	0.812	0.837	25
Naphthaquinone	0.470	0.446	24

4.1 Results and Discussion

Table 3 lists results for a whole series of model quinone compounds, chosen to see whether substitutional effects could be reproduced. Clearly there is remarkable agreement between the calculated and experimental results, the difference typically being 30 mV or less which is little more than the uncertainty in the experimental results. Having reached the size of naphthaquinone the application to prospective drugs is only limited by computer time, but clearly the potential exists for performing calculations of high accuracy which will have direct relevance to biological chemistry and drug design.

5. DETERMINATION OF TAUTOMER RATIOS

Tautomerism is implicated in the mechansism of many biological processes such as the action of histamine at the so called H2 receptor site [25]. If one could understand the tautomeric dependence one could gain an insight into the structural and mechanistic requirements for activity and so allow workers to design agonists or antagonists.

For our test case we decided to study methyl imidazole, as a model for histamine, and then histamine itself. The required cycle is shown in Figure 5, from which we have

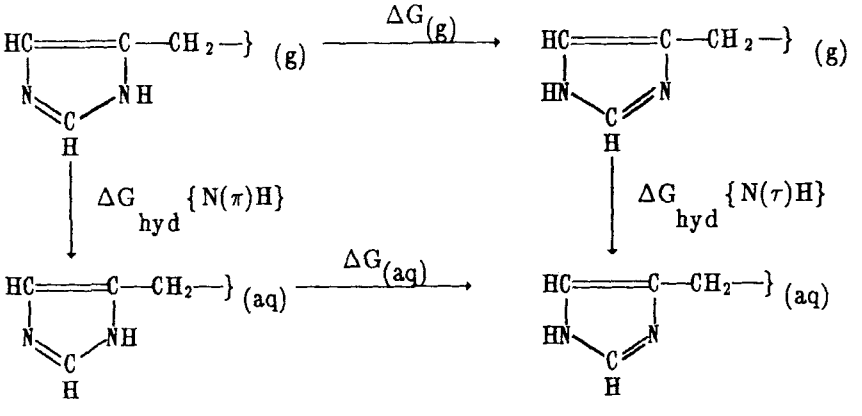

Figure 5 Thermodynamic cycle for the determination of the free energy of tautomerisation of a compound.

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Table 4 Calculated free energy differences accompanying the ring tautomerism N(π)H → N(τ)H

Compound	*Calculated (kJ/mol)*	*Experimental (kJ/mol)*
Methyl Imidazole	−0.63	−1.00 to 1.98
Histamine Base	−4.34	−3.43
Histamine (+)	−9.62	−5.44 to 2.06

$$\Delta G_{(aq)} = \Delta G_{(g)} + \Delta G\{N(\tau)H\} - \Delta G_{hyd}\{N(\pi)H\}$$
$$= \Delta G_{(g)} + \Delta G_{pert}\{N(\pi)H \longrightarrow N(\tau)H\} \quad (12)$$

Thus the free energy difference accompanying the tautomeric shift of a proton from $N(\pi)$ to $N(\tau)$ on the imidazole ring can be calculated from a gas phase free energy difference and a single perturbation calculation in which one tautomer is converted into the other. The method of calculation is as used in the previous cases, and is fully described in references [26–28].

5.1 Results and Discussion

The calculated tautomerisation free energies for methyl imidazole and histamine are given in Table 4. It can be seen that there is a significant range of uncertainty in the reported experimental values, and the calculated results typically have an error associated with them of < 5kJ/mol. What is important to note is that the agreement is very good and the correct tautomer preference is reproduced. In studies where the tautomer equilibrium is implicated in the mechanism of action of a compound it should thus prove possible to predict whether a given molecule should have a required activity, and also distinguish between molecules having similar behaviour.

6. CONCLUSIONS

We have shown that the combination of greatly enhanced computers and the novel theoretical methods which have come to light over the past few years have allowed a number of properties to be calculated which are critical in determining the activity of a small molecule in a biological system. In addition to the work described here much work has been performed using FEP to calculate differences in free energy of binding for compounds to macromolecular hosts with remarkable success [4,29,30]. Not only is there renewed optimism in the search for effective drugs, but also in the search for fungicides, pesticides and herbicidal agents. We are now able to calculate properties which are crucial to understanding fully the action of biologically important molecules, which a few years ago would have been impossible.

The improvement is tied to the increase in available computer resources. More reliable results could be obtained from more thorough phase space sampling, which will become more feasible with the more widespread use of parallel computer architechture.

The discontinuous change in drug-design at a theoretical level has been very marked, yet we are still at a relatively early stage in the development of accurate potentials, consistent methodologies and the types of problem that can be tackled. A

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useful and realistic start has been made at studying a number of fundamental problems, and the groundwork has been laid for further progress.

Acknowledgement

We would like to thank the National Foundation for Cancer Research and the SERC under whose funding the work described here was performed.

APPENDIX

The AMBER interaction potential function [31].

$$V_{\text{total}} = \sum_{n=1}^{N_r} k_{r_n}(r_n - r_{eq_n})^2 + \sum_{n=1}^{N_\theta} k_{\theta_n}(\theta_n - \theta_{eq_n})^2$$

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$$+ \sum_{n=1}^{N_\phi} \sum_{m=1}^{N_\eta} \frac{V_{mn}}{2}[1 + \cos(\eta_{mn}\phi_{mn} - \gamma_{mn})]$$

DIHEDRALS

$$+ \sum_{j=1}^{N_A} \sum_{i>j}^{N_A} \varepsilon^*_{ij}\left[\left[\frac{R^*_{ij}}{R_{ij}}\right]^{12} - \left[\frac{R^*_{ij}}{R_{ij}}\right]^{6}\right]$$

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$$+ \sum_{j=1}^{N_{hb}} \sum_{i>j}^{N_{hb}} \left[\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{10}}\right] + \sum_{j=1}^{N_A} \sum_{i>j}^{N_A} \frac{q_i q_j}{\varepsilon R_{ij}}$$

HYDROGEN BONDING COULOMBIC

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