Theoretical Study on CDK2 Inhibitors Using a Global Softness Obtained from the Density of States

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We report a theoretical study on a series of CDK2 inhibitors using a set of global reactivity indices defined in terms of the density of states. The statistical analysis was performed on the basis of two groups of 11 and 6 compounds, respectively, reported by Hardcastle et al. (*J. Med. Chem*. **2004**, *47*, 3710−3722). Both series were classified on the basis of the correlations obtained for the complete set of compounds and the sites targeted within the active site of CDK2. The comparison between the biological activity and the electronic chemical potential approached as the Fermi level yields poor results, thereby suggesting that the interaction between the hinge region (HR) of CDK2 and the ligands may have a marginal contribution from the charge transfer (CT) component. Comparison between the biological activity and global softness shows a better correlation, thereby suggesting that polarization effects outweigh the CT contribution in the HR−ligand interaction. We stress the importance to include in the evaluation of the reactivity indices all of the occupied energy states in order to assess the effects coming from the internal electronic structure involved in the HR−ligand interaction.

1. Introduction

The cyclin-dependent kinases (CDKs) are a class of enzymes involved in the eukaryotic cell-cycle regulation.[1] Particularly, as their names suggest, CDK activation partially depends on the binding of larger proteins known as cyclins. Complete activation requires phosphorylation of the CDK subunit by the CDK-activating kinase at the conserved threonine residue. Only as a complex can these proteins regulate cell growth and DNA synthesis properly.[2] The activity of the CDK−cyclin complex can be reduced by at least two major mechanisms: the phosphorylation of the CDK subunit at inhibitory sites and the binding of the specialized protein inhibitors known as CKIs or cyclin-dependent kinase inhibitors. These inhibitors compete with adenosine 5′-triphosphate (ATP) for binding to the CDK active site. However, in some cancer cells, it has been shown that the CKIs are underexpressed, and medicinal chemists have made numerous efforts to replace them with synthetic inhibitors.[3] Considerable progress has been made in the identification of pharmacologic agents targeting the CDKs.[4] Nowadays, new strategies to find more potent inhibitors have become available and they normally use structural−activity relationships derived from computational calculations.[5−7] In this study, we propose that global reactivity analysis can be used to estimate the affinity, for the hinge region (Glu81-Phe82-Leu83), of a series of CDK2 inhibitors belonging to the N^2-substituted O^6-cyclohexylmethoxypurine family.[8] (See Scheme 1.) Global descriptors of reactivity as defined in the context of density functional theory (DFT), including the electronic chemical potential (μ), global softness (S), and global electrophilicity (ω), have been shown to be useful tools to understand the mechanistic[9] and thermodynamic aspects of reacting systems[10] in their ground states. For an extensive review about the DFT-based reactivity indices, see Geerlings et al.[11] and Chermette.[12] For instance, in a seminal paper, Maynard et al.[13] used the ratio of electronegativity to hardness, χ^2/η, to explain the capacity of a series of electrophiles to promote a soft reaction (covalent) with the human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein p7 (NCp7). They found a statistically significant correlation between the rates of reaction and the ability of these agents to function as soft electrophiles. Due to the fact that the interactions between the CDK2 inhibitors and the hinge region are noncovalent in nature, and they are possibly governed by H-bond interactions, we have chosen some of the global reactivity indices to study this system. In particular, the H-bonding process is associated, to a variable extent, to a charge transfer between the acceptor and donor atoms and it would be described by the electronic chemical potential. In previous quantum mechanics/molecular mechanics studies, we have found that van der Waals energy is, to a significant extent, one of the principal components in the protein−ligand interaction,[14] so we have included in our study the global softness to check its effect on the binding force in the hinge region (HR)−ligand interaction. On the other hand, the electrophilicity index encompasses both the effect of the electronic chemical potential and the chemical softness, and for this reason, it was also included in the present study. The results obtained are expected to be useful to qualitatively describe the affinity of a series of CDK2 inhibitors by the hinge region located at the active site of CDK2. A comparison between the DOS-based reactivity indices[15] and those obtained from the frontier molecular orbital (FMO)[16−18] theory is also given.

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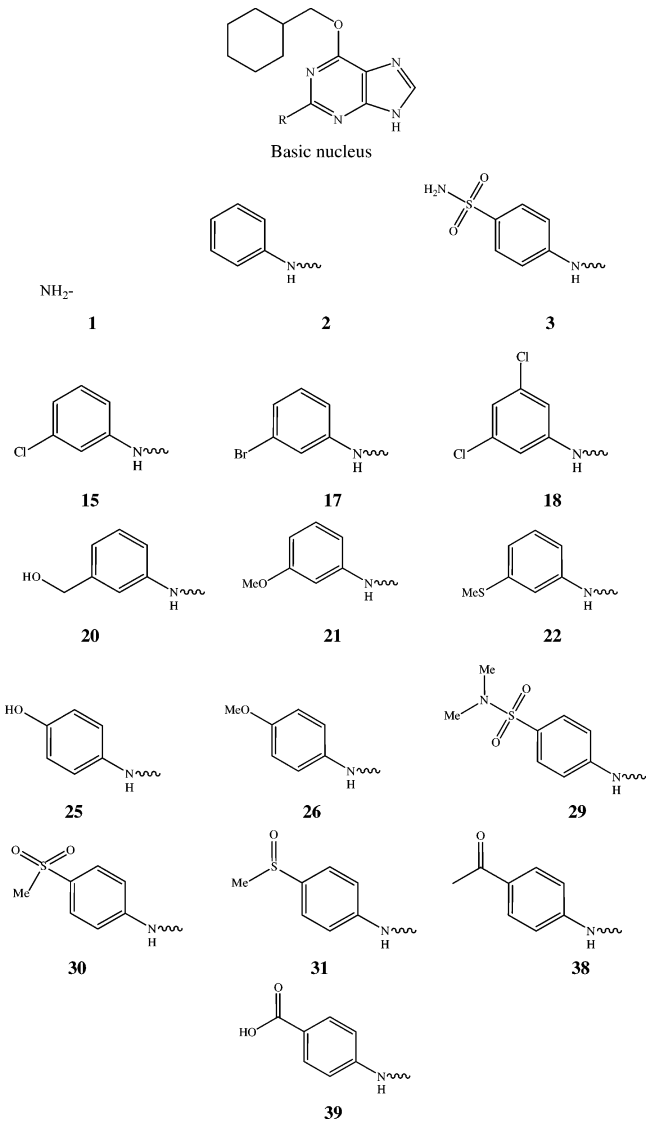
SCHEME 1: Molecular Structures for the Set of CDK2 Inhibitors Studied



2. Model Equations and Computational Details

According to Parr and Yang's approximation,[19] the global softness (S) of an extended system is given by the global density of states at the Fermi level. The global softness is defined as the change in the number of electrons (N) with respect to the electronic chemical potential. In the present approach, N may be represented by

$$N = \int^{E_{max}} g(E)\, dE \qquad (1)$$

There remains now to find a relationship between the electronic chemical potential and the last occupied Kohn−Sham orbital. In the case of a metal, the levels E_{max} and E_{max+1} (highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)) are very close to each other and therefore the electronic chemical potential is no longer discontinuous and it is equal to the Fermi level (E_F). This qualitative model is consistent with the purpose of this article, as we are interested in probing a nucleophilic response at the inhibitor moiety. We have therefore taken the Fermi level as $E_F = \mu \cong -I$, with I being the vertical ionization potential. This quantity may be further approximated with the HOMO level in the context of Koopman's theorem which mathematically entails

$$\left(\frac{\partial N}{\partial \mu}\right)_v = \left(\frac{\partial \int^{\mu} g(E)\, dE}{\partial \mu}\right)_v = g(\mu) = g(\epsilon_{max}) \qquad (2)$$

Then, if we represent every energy state as a Gaussian distribution, we can express the global softness as

$$S = g(E_F) = \frac{1}{\Delta\sqrt{\pi}} \sum_i g_i \exp\left[-\left(\frac{E_F - \epsilon_i}{\Delta}\right)^2\right] \qquad (3)$$

where ϵ_i are the orbital eigenvalues, g_i is the degeneracy of state i, and Δ is the width of the Gaussian function. The parameter Δ is typically taken as 0.5 eV. The choice of this bandwidth is dictated by the need of having a smooth density of states (DOS) capable of incorporating most of the relevant contributions of different mixed states. The choice of a bandwidth narrower than this value implies the lost of information in the DOS. On the other hand, a value wider than that chosen entails that the distribution will become too flat, thereby diminishing the prevalence of the highest occupied state(s). In order to demonstrate this effect, we have calculated two additional DOS taking band widths with 0.2 and 1.0 eV, respectively. The plot of these additional DOS's has been incorporated as Supporting Information (Figure S-1). The evaluation of chemical softness using the DOS approximation has already been used in previous studies by Santos et al.[20] and Nguyen et al.[21] The electrophilicity index, $\omega = \mu^2/2\eta$, formerly introduced by Maynard et al.[13] and derived later by Parr et al. from a second-order energy model[22] was evaluated assuming that $\mu = E_{HOMO} \cong E_F$ with $\eta = 1/g(E_F)$. It is worth mentioning that the electrophilicity index defined by Parr et al.[22] differs for a constant factor of 2 with respect to the one given by Maynard et al.

The starting structures (for FMO analysis) were built up from the CDK2 crystallographic structures in interaction with the inhibitors Nu2058, Nu6094, Nu6086, and Nu6102, which were previously reported by Davies et al. (Protein Data Bank codes: 1H1P, 1H1Q, 1H1R, and 1H1S, respectively).[23] The model structures used to evaluate the global reactivity indices through the DOS for each inhibitor and its corresponding active site were built up from the final minimized structures obtained from previous quantum mechanics/molecular mechanics (QM/MM) studies performed on the system Thr160−CDK2−cyclinA complexed with five different inhibitors.[14] The model chosen to perform the global reactivity indices calculation for each set of structures (from X-ray studies and from QM/MM studies) was the amino acid Phe80-Glu81-Phe82-Leu83-His84-Gln85-Asp86 moiety and the inhibitor. Both systems were treated separately, within a static reactivity model. The dangling bonds at the ends of the polypeptide were saturated with hydrogen atoms. The geometry of the atom groups added to obtain a new inhibitor and the hydrogen added were optimized using the hybrid functional B3LYP[24,25] and the 6-31G* basis set, and keeping fixed the position of the polypeptide atoms and the remaining structure of the inhibitor.

The DOS reactivity indices calculations were implemented in the package program Mathematica5.1 for Linux,[26] using eq 3 and the complete output from an orbital energy calculation.

3. Results and Discussion

As was stated before and according to previous results obtained from QM/MM studies,[14] the van der Waals energy component is considered the driving force in the protein−ligand interaction in the CDK2/cyclinA system and remarkably it also remains the same (maximum difference of about 2.7 kcal/mol)

TABLE 1: Global Reactivity Descriptors Calculated from DOS Approximation for the Set of CDK2 Inhibitors Studied (The Electronic Chemical Potential for the Hinge Region Models (DOS-SITE) and the Experimental Biological Activity for Each Inhibitor are Also Shown)

compd no.	$\mu_{\text{DOS-SITE}}$ (eV)	μ_{DOS} (eV)	S_{DOS} (eV^{-1})	ω_{DOS} (eV)	ΔN (electrons)	IC_{50} (μM)
1	−0.60	−5.46	1.7105	25.48	−0.095	17.0
2	−0.60	−5.30	1.8156	25.46	−0.094	0.97
3	−0.60	−5.77	2.4889	41.44	−0.126	0.0054
15	−1.18	−5.44	1.8856	27.95	−0.094	2.3
17	−0.66	−5.51	2.0624	31.31	−0.106	6.8
18	−1.17	−5.59	1.9259	30.11	−0.099	12.0
20	−0.60	−5.27	1.8777	26.03	−0.096	0.4
21	−1.14	−5.30	2.5008	35.12	−0.105	1.8
22	−1.14	−5.33	3.2105	45.53	−0.121	1.7
25	−0.64	−5.18	2.0223	27.10	−0.098	0.069
26	−0.68	−5.11	1.9523	25.50	−0.093	0.65
29	−1.12	−5.70	2.0085	32.65	−0.103	0.056
30	−0.82	−5.76	2.0753	34.37	−0.111	0.063
31	−0.84	−5.46	3.3637	50.17	−0.135	0.10
38	−1.11	−5.50	2.0363	30.75	−0.102	0.30
39	−1.21	−5.57	1.8084	28.03	−0.090	0.8

for compounds which have an aniline ring in the position C2 of the purine scaffold. Thus, we have assumed (following a valuable comment addressed by one of the reviewers) that this energy term must remain constant for the set of compounds studied, in order to take into account all of the energetic data coming from the energy-minimized structures used and to have a valid starting point to make our conclusions about each global reactivity index.

The values of the global reactivity indices evaluated from the density of states (DOS) are summarized in Table 1. It is well-known that the electronic chemical potential (μ) is the natural descriptor of the direction of charge transfer (CT) during a chemical interaction.[27] We have found that the μ values obtained for each inhibitor are smaller that the corresponding values in the active site model. The electronic chemical potentials for the hinge region are given in Table 1. This result may be taken as an indication that the charge transfer takes place from the active site to the inhibitor, mainly at the points where the ligand can establish a hydrogen bond. With the μ and η values for the ligands and the hinge region model at hand, we can get an estimation of the charge transfer of the ligand (I) and HR interacting systems by using Pearson's equation, $\Delta N = (\mu_{\text{I}} - \mu_{\text{HR}})/(2*(\eta_{\text{I}} + \eta_{\text{HR}}))$.[28] It may be seen that, in general, the charge transfer between both fragments is marginal (see Table 1). Next, we plotted the μ values against the LogIC_{50} for the whole set of molecules quoted in Table 1. This comparison (not shown here but included in the Supporting Information) showed no correlation. Therefore, we may conclude that there is not a significant correlation between the μ values and the biological activity for the series of ligands considered in this study, a result that indicates that charge transfer may not be an important factor for HR−ligand interactions. As suggested by a reviewer, it is worth emphasizing here that the lack of a significant correlation between the electronic chemical potential and the experimental LogIC_{50} values may be traced to the fact that during the HR−ligand interaction no covalent bonds are formed, and therefore, a correlation similar to that reported by Maynard et al.[13] to explain the capacity of a series of electrophiles to promote a soft reaction (covalent) with the human immunodeficiency virus type 1 (HIV-1) nucleocapsid protein p7 (NCp7) should not be expected in the present case. It is also worth mentioning that Lyne et al. reported good correlations ($r = 0.71$ for a similar set of CDK2 inhibitors) between free energy of binding and the pIC_{50} values, for a more extensive set of kinase inhibitors on the basis of MM-GBSA calculations including solvent effects.[29] These authors claim that neither polarization effects nor charge transfer effects are needed to obtain good comparisons between the free energy $\Delta G_{\text{bind}} = \Delta G_{\text{solv}} + \Delta E_{\text{MM}} + \Delta G_{\text{SA}}$ and pIC_{50} values. Even though there is not an explicit description of polarization effects in this expression, polarization effects are included in the solvation term (at least the orientational contribution, and to some extent the electronic polarization as well). We do not have any argument to relate Lyne et al.'s model with ours, and the comparison is certainly not trivial. However, the electronic reactivity indices we are using encompass electrostatic contributions apart from polarization effects on binding.

On the other hand, the literature suggests that the chemical softness of hydrogen bond interactions may contribute a second-order energy term (relative to electrostatic interaction) in influencing the hydrogen bond strength[30] that may be related to the polarizability of the system. Therefore, we performed two additional statistical analyses, one incorporating the global softness (S) and the other incorporating the global electrophilicity index (ω). The separated analyses, including the whole series of compounds in Table 1, suggested the classification of the whole series into two groups of ligands that are in qualitative agreement with the classification made by Hardcastle et al.,[8] which are based on the sites targeted at the CDK2 active site (see Supporting Information Figures S-4 to S-7). It is worth emphasizing that the global softness correlation coefficients obtained for the whole series was not statistically significant. However, this analysis suggested the separation of the whole series into two groups. The first, group 1, is formed by compounds **17**, **18**, **21**, and **22** belonging to 3′-substituted C^2-anilino-O^6-cyclohexylmethyl-purines studied. This group was augmented with compound **31** because in our model it is targeting the same region in the CDK2 active site and also with the lead compound **1**. They were designed to mimic a favorable reported interaction between the 3′-chloro group in purvanalol B and the enzyme.[31] The second, group 2, includes compounds **2**, **3**, **15**, **20**, **25**, **26**, **29**, **30**, **38**, and **39** plus the lead compound **1**. They were designed to target a hydrogen bond acceptor in the region of the protein accessed by the 2-anilino group, presumably the residue Asp86.[23] We must mention that compound **15** has two conformers at the 3′ and 5′ positions, with partially occupied conformations according to Davies et al.[23] Therefore, we have included the conformer II (see Davies et al.), substituted at position 5′, in the group 2 because this species can target the Asp86 residue at the active site.

The values of the global softness for groups 1 and 2 of compounds quoted in Table 1 show a more significant correlation with LogIC_{50} compared to that performed with the electronic chemical potential. The result of this comparison yield correlation coefficients of $R^2 = 0.82$ and 0.80 for groups 1 and 2, respectively (see Figures 1 and 2). Since the global softness is related to the electronic polarizability of the system,[32] we may conclude that polarization effects are more important than CT effects for these interactions and we have confirmed that those noncovalent interactions, which are closely related to the van der Waals energy term reported before, are very important in the protein−ligand interaction. We also have observed that global softness can predict the selectivity of the inhibitors to target some aminoacids in the CDK2 active site and this selectivity is related to the H-bond strength and the compound's categorization made in this study. We can conclude that this selectivity is probably given by the second-order energy term of the global softness. However, we could not quantitatively

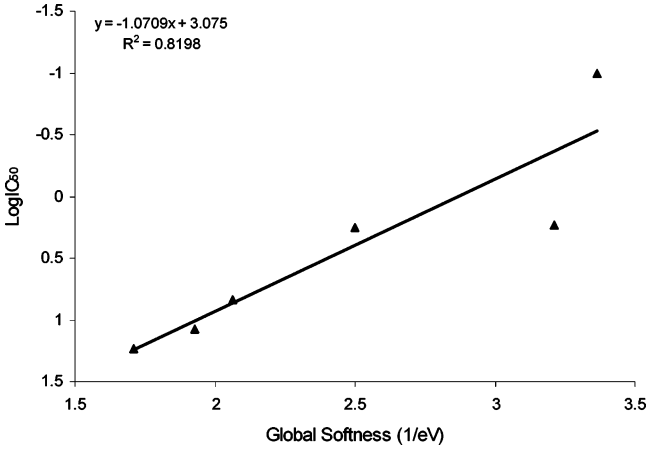


Figure 1. Graph of $LogIC_{50}$ vs global softness (S_{DOS}) (group I).

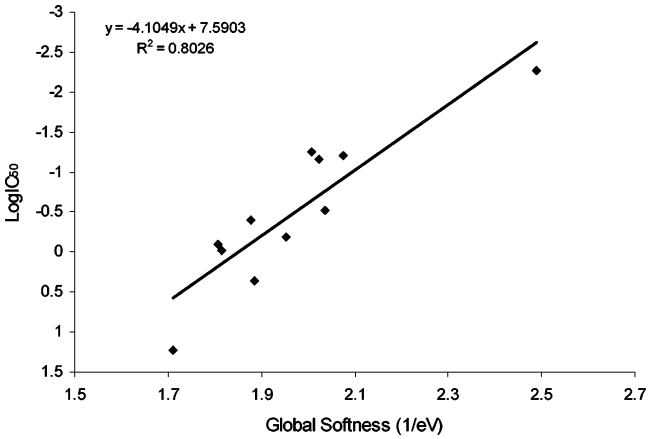


Figure 2. Graph of $LogIC_{50}$ vs global softness (S_{DOS}) (group II).

describe this behavior, but actually, we are performing some additional calculations to tackle this issue.

Finally, and prompted by the results reported by Maynard et al.,[13] we decided to perform a final comparison between a similar reactivity index which is again defined in terms of the DOS, ω_{DOS} hereafter, and $LogIC_{50}$. In principle, this index should give a better result as compared to the $LogIC_{50}$−global softness correlation, because it contains more information about the electronic structure of ligands, namely, the square of electronegativity and the global softness. The values calculated for the global electrophilicity index are also reported in Table 1. The correlation coefficients are $R^2 = 0.85$ and 0.65 for groups 1 and 2, respectively (see Supporting Information Figures S-10 and S-11). Despite the fact that for group 1 the $LogIC_{50}$−electrophilicity correlation is slightly better, it is evident that when both groups are considered, the $LogIC_{50}$−global softness correlation is more stable. The failure of the global electrophilicity to qualitatively correlate with the $LogIC_{50}$ values may probably be traced to the fact that, in opposition to Maynard's results, the HR−ligand interaction has a marginal covalent component, a result that was already anticipated from the $LogIC_{50}$−electronic chemical potential correlation.

A similar analysis performed with the same reactivity indices defined in the context of the frontier molecular orbital theory yields poorer results. The statistical analysis based on this molecular approach is given as Supporting Information. This result may be traced to the fact that the additional electronic states that are close in energy to the Fermi level were not included, which may make relevant contributions to the reactivity pattern displayed by these systems.

4. Concluding Remarks

In this paper, we have presented a theoretical study to describe the affinity of a set of CDK2 inhibitors by a model of its active site, namely, the hinge region (HR). We have used the global reactivity descriptors obtained from the DOS to deal with the reactivity pattern expected for the set of compounds studied. We have shown that reactivity indices obtained from the DOS approach, especially the global softness and electrophilicity, describe in a reasonable way the affinity of the CDK2 inhibitors by the hinge region. Comparison between the biological activity and global softness shows a better correlation, thereby suggesting that polarization effects outweigh the CT contribution in the HR−ligand interaction. Improvements in the description of the ligand affinity using DOS-based reactivity indices can be explained by the inclusion of electronic states closer to the frontier molecular orbitals. They may contain more useful and complete information about the local and global reactivity pattern than the frontier molecular orbitals alone. This is particularly useful for biological or condensed systems where the representation of the frontier molecular orbital is replaced by the band representation due to the many electronic states that can participate in a chemical interaction. Additional studies are in progress with the purpose of further validating this computational approach in an expanded set of CDK−ligand systems and to explain more in depth the role of the second-order energy term of the global softness in the categorization of the whole set of compounds.

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Supporting Information Available: The correlation figures obtained for the comparison between each reactivity index, obtained from the DOS and FMO approaches, and $LogIC_{50}$ values and tables including the global reactivity indices obtained from the FMO approach and the atomic coordinates of the CDK2 hinge region−inhibitor model complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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