Structure–reactivity relationships for electrophilic sugars in interaction with nucleophilic biological targets

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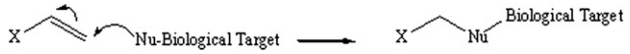
Abstract—The global electrophilicity index that incorporates electrostatic and polarizability contributions shows a quantitative correlation with antiviral and cytotoxic activities of electrophilic sugars. The model is applied to a series of compounds that behave as Michael acceptors in interaction with biological nucleophilic targets.

1. Introduction

The interaction of reactive organic chemicals with biological macromolecules often involves covalent binding at nucleophilic cellular sites susceptible to attack by an electrophilic substrate through a Michael reaction.[1–4] These reactions are nucleophilic additions to α or β unsaturated compounds classified as Michael acceptors. The basic criterion to classify a compound as a Michael acceptor is the presence of a double or triple bond bearing an electron-withdrawing substituent X. The role of the X group is related to its ability to stabilize a negative charge on the carbon atom to which it is bound. Therefore, electron-donating substituents reduce reactivity; whereas electron-attracting substituents increase reactivity. Substitution at α- or β-carbon atoms may have strong influences on the reactivity of the interacting electrophile/nucleophile partners[4] (see Scheme 1).

The nature of these compounds and the magnitude of their biological activities depend on the following main factors[3]: (i) Reactivity between compounds (electrophiles) and biological target (nucleophiles). (ii) The pair electrophile/nucleophile determines the chemical mechanism. (iii) The chemical mechanism determines the biological mechanism of action. (iv) The biological mechanism determines the mode of action between drugs and biological targets. The reactions of electrophiles with biological nucleophiles have been reviewed in the seminal work by Coles.[5] This author persuasively argued in favor of a molecular model that related the toxicity of some chemicals through the irreversible (covalent) binding of their electrophilic metabolites to nucleophilic sites within biological macromolecules.[6] He went on to propose that the structure of the electrophile including both the chemical nature of the electrophilic center and the physicochemical properties of the electrophile as a whole (i.e., global electrophilicity) is relevant to covalent binding. The electrophiles discussed by Coles included polarizable double bonds and formally charged species. Both quantities polarizability and electrostatic interactions are formal reactivity indices defined in the context of the conceptual density functional theory.[7] For instance while polarizability is associated with the chemical softness,[8] the electrostatic term may be related to the electronic chemical potential (the negative of electronegativity). Coles[5] has given a general classification of nucleophilic sites in macromolecules and hard/soft electrophiles presenting a wide variety in structure and bonding properties.



Scheme 1.

Sugar derivatives constitute a class of soft organic electrophiles which may be classified as Michael acceptors,

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whose toxicity depends on their ability to react with soft biological nucleophiles. The formation of covalent binding between these series of sugars with the oncogenic Polyoma virus has led to its use as useful therapeutic agents.[9,10]

The series of sugar electrophiles studied here are: (**1**) 4-cyanovinyl, (**2**) nitroenose, (**3**) 4-cyanochromen-2-yl, and (**4**) 3-nitrochromen-2-yl derivatives (see Scheme 2). These compounds markedly differ in their cytotoxic and antiviral properties against the oncogenic Polyoma virus which bears both, lytic and oncogenic activities and it is capable of inducing the formation of tumors, normally present in host as latent infections potentially oncogenic.[9] This series of electrophilic sugars has been theoretically studied using reactivity descriptors based on the molecular electrostatic potential.[10] However, a closer scrutiny of Coles classification into hard/soft electrophilic/nucleophilic interactions prompted us to revisit the chemistry of these electrophilic sugars with a more general model that incorporates polarization effects. Polarization effects have been proposed as one of the main factors on which electrophilicity and nucleophilicity depend from the down of quantitative experimental scales reported by Swain,[11] Pearson,[12] and Edwards.[13] A theoretical model based on perturbed molecular electrostatic potential, including polarization effects, has been recently presented.[14]

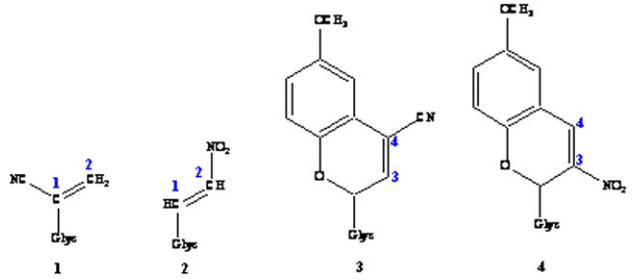
In this work, we will use the global electrophilicity index formerly proposed by Maynard et al.[15] and then formalized by Parr et al.[16] that incorporates the electrostatic contribution through the electronic chemical potential and the polarizability through the chemical softness.

2. Model equations and computational details

The global electrophilicity index, ω, which measures the stabilization in energy when the system acquires an additional electronic charge ΔN from the environment, has been given by the following simple expression.[16]

$$\omega = \frac{\mu^2}{2\eta} = \frac{\mu^2}{2} S \qquad (1)$$

in terms of the electronic chemical potential, μ, and the chemical hardness $\eta = 1/S$, the inverse of the chemical softness S.[8,16,17] Note that the global electrophilicity index encompasses the main factors proposed by LoPachin and DeCaprio[18] to be the determinants of electrophilicity: the electron affinity appears averaged with the ionization potential in a parent concept, namely the electronic chemical potential which is related to electronegativity[8] and the chemical softness which is directly related to polarizability.[19] Inductive substituent effects on the other hand are well described by the electrophilicity index, in the form of local responses at the active site induced by chemical substitution.[20] The global electrophilicity index has been already used as descriptor of biological activity in a wide series of systems.[21,22]



Scheme 2.

The global electrophilicity was evaluated for the series of electrophilic sugars (38 compounds) compiled in Table 1 in their ground states. Ab initio HF/6-31G* calculations were performed using the Gaussian 98 suite of programs[23] to evaluate the electronic quantities[8] required to obtain the electrophilicity index defined in Eq. 1. These quantities may be approached in terms of the one electron energies of the frontier molecular orbital HOMO and LUMO, ε_H and ε_L, as $\mu \approx \frac{\varepsilon_H + \varepsilon_L}{2}$ and $\eta \approx \varepsilon_L - \varepsilon_H$, respectively.[8]

3. Results and discussion

Table 2 summarizes the experimental cytotoxic and antiviral activities, together with the electronic parameters μ and η required to build up the electrophilicity index ω according to Eq. 1.

According to Coles's classification, the softest electrophiles encountered in vivo are polarized double bonds which undergo Michael addition to nucleophiles.[5] Neutral nucleophiles and electrophiles are in general softer than charged electron donors and electron acceptors. The softening of these chemicals is usually traced to the presence of polarizable double bonds. These effects are therefore expected to increase in those electrophiles bearing rich π systems like phenyl groups. On the basis of this classification, the interaction of electrophiles with nucleophilic biological targets is expected to be governed by the HSAB rule,[12,18] which states that hard/hard and soft/soft interaction between a Lewis acid–base pair is preferred over the crossed interactions.[12] However, a close look at the hardness (η) values reveals that the correlation between hardness (or its reciprocal the chemical softness $S = 1/\eta$) is not obvious. Compare for instance compounds **1a** and **3g** in Table 2. Even though these chemicals present a significant difference in hardness (softness) they are experimentally predicted as nonactive antiviral agents. Other comparisons in this direction are also possible from Table 2. This result indicates that hardness or its inverse, the chemical softness, yet important to qualitatively relate the electrophile/nucleophile interaction in biological systems, they do not bear the sufficient information for their potential use in more quantitative predictions of biological activity. However, the ratio electronegativity to hardness that defines the electrophilicity index in Eq. 1 may be a better descriptor in the sense that it contains, apart from the electrostatic component, an explicit polarization term represented by the chemical

Table 1. General structure of electrophilic sugars considered in the present study

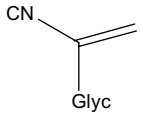
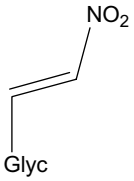
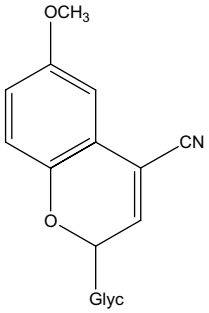
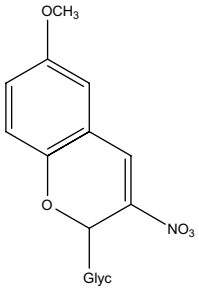
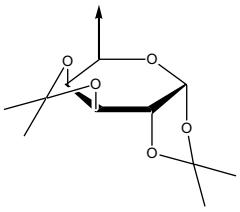
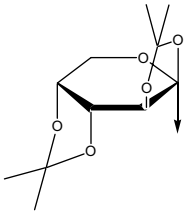
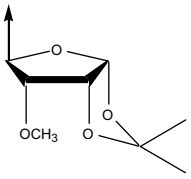
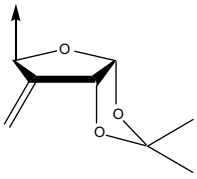
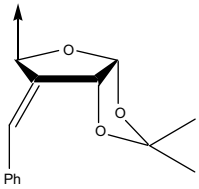
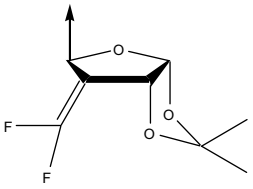
				
	**1a**	**2a**	**3a**	**4a**
	**1b**	**2b**	**3b**	**4b**
	**1c**	**2c**	**3c**	**4c**
	**1d1**	**2d1**	**3d1**	—
	**1d2**	**2d2**	—	—
	**1d4**	**2d4**	**3d4**	—

Table 1 (*continued*)

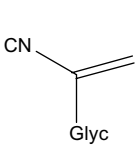
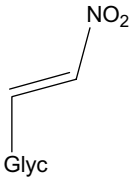
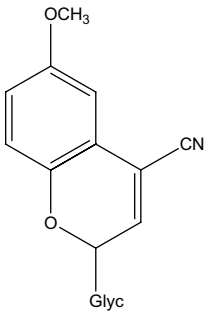
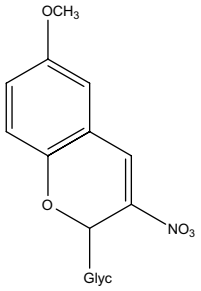
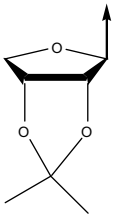
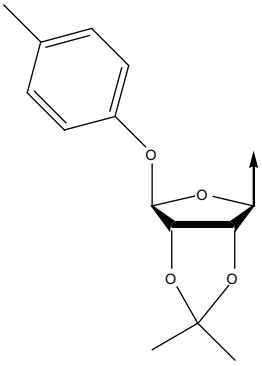
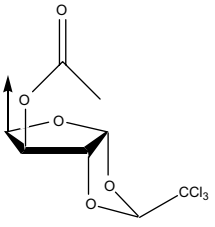
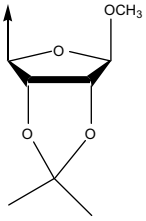
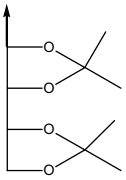
				
	**1e**	**2e**	**3e**	**4e**
	**1f**	**2f**	—	**4f**
	**1g**	**2g**	**3g**	**4g**
	**1h**	**2h**	**3h**	**4h**
	**1i**	**2i**	—	—

Table 2. Cytotoxic activity, antiviral activities, and electronic parameters μ and η and ω of electrophilic sugars[a]

Compound	Cytotoxic activity	Antiviral activity	μ (eV)	η (eV)	ω (eV)
1b	3.449	4.148 (PI)	−3.69	13.49	0.50
1d1	4.316	5.319 (TI)	−3.69	13.39	0.51
1d2	4.452	4.753 (48 h D)	−3.20	11.31	0.45
1d4	4.988	5.290 (24 h D)[b]	−4.08	13.42	0.62
1e	3.892	4.194 (6 h D)	−3.54	13.43	0.47
1i	4.703	5.004 (PI)	−3.91	13.38	0.57
1a	4.148	NA	−3.64	13.55	0.49
1d3	4.753	NA	−3.12	11.73	0.41
1h	4.654	NA	−3.20	12.83	0.40
3g	5.284	NA	−2.93	10.92	0.39
1c	—	NA	−3.49	13.44	0.45
1f	—	NA	−2.86	11.88	0.34
1g	—	A	−4.02	13.86	0.61
2a	—	A	−4.81	12.73	0.91
2b	—	A	−4.96	12.70	0.97
2c	—	A	−4.75	12.64	0.89
2d1	—	A	−4.76	12.43	0.91
2d2	—	A	−5.05	12.34	1.03
2d4	—	A	−5.05	12.34	1.03
2e	—	A	−4.81	12.54	0.92
2f	—	A	−3.76	10.08	0.70
2g	—	A	−5.49	12.75	1.18
2h	—	A	−4.87	12.38	0.96
2i	—	A	−4.15	13.99	0.62
3a	—	NA	−2.07	11.74	0.18
3b	—	NA	−2.28	11.83	0.22
3c	—	NA	−2.15	11.75	0.20
3d1	—	NA	−2.17	11.75	0.20
3d4	—	NA	−2.20	11.80	0.21
3e	—	NA	−1.96	11.69	0.16
3h	—	NA	−2.03	11.73	0.18
4a	—	A	−3.41	9.35	0.62
4b	—	A	−3.58	9.28	0.69
4c	—	NA	−2.61	10.85	0.31
4e	—	NA	−2.48	10.94	0.28
4f	—	NA	−3.09	9.77	0.49
4g	—	NA	−3.01	10.73	0.42
4h	—	A	−3.21	9.52	0.54

[a] For compounds **1b**–**3g** the biological activity was experimentally determined and reported in Ref. 10. TI, total inhibition; PI, partial inhibition; D, delay in the manifestation of viral effect, and NA, no activity.

[b] Measured on retrovirus SV40.

softness. Note for instance the variation of the electronic chemical potential, μ, in Table 2. It seems that a low value of this quantity is related to low or none antiviral activity, whereas enhanced μ values, as for instance in the series (**2**) in Table 2, are qualitatively predicted as active antiviral agents. Note also, that within the series (**3**) and (**4**) the opposite trends are also verified. In the process of forming a covalent bond, the electronic chemical potential should be as important as the chemical hardness which normally acts as a resistance to the charge transfer needed to accumulate electron density at the internuclear region. Therefore, we propose that the electrophilicity index, encompassing both effects, should display a more useful index that may have an interesting potential to build up QSAR equations.

The experimental biological activities have been evaluated for the short subseries of electrophilic sugars **1b**, **1d1**, **1d2**, **1d4**, **1e**, **1i**, **1d3**, **1a**, **1h**, and **3g** (note that compounds **1d2** and **1d3** are geometrical isomers). This database had been already used to perform a theoretical study by Ricca et al.[10] using a model based on the electrostatic potential perturbed by a model nucleophile H^-. The remaining compounds in Table 2 (for which the experimental antiviral and cytotoxic activities have not been evaluated to date) have been introduced to test the predictive value of the present model. All the first 10 compounds shown in Table 2 present cytotoxic activity, yet some of them may or may not present antiviral activity. A first look at Table 2 reveals that compounds presenting global electrophilicity values within the range $0 \leqslant \omega \leqslant 0.5$ eV are inactive as antiviral agents (compounds **1a**, **1d3**, **1h**, and **3g**). On the other hand, compounds **1b**, **1d1**, **1d4**, and **1i** present electrophilicity values greater that the 0.5 eV threshold, while compounds **1d2** and **1e** are predicted as borderline cases.

A better appraisal of the relationship between the electrophilicity values and the experimental cytotoxic and antiviral activities may be obtained from the comparison between these variables shown in Figure 1. We found that the cytotoxic activities expressed as log (minimum cytotoxic concentration (M)) correlated reasonably well with electrophilicity index of the Michael acceptors considered. All 10 compounds experimentally evaluated for cytotoxic activity were split out into two families according to whether or not they further showed antiviral activity. The resulting regression equations for both series are:

$$\log(\text{cytotoxic activity}) = 0.2833 + 0.6780\omega; \quad R = 0.980 \tag{2}$$

and

$$\log(\text{cytotoxic activity}) = 1.2726 - 1.4447\omega; \quad R = 0.960 \tag{3}$$

respectively. Note that the second terms' signs in Eqs. 2 and 3 are opposite to each other. Since the electrophilic-

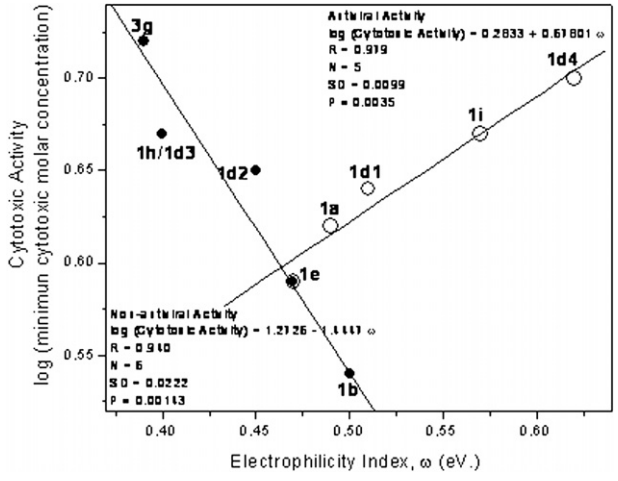


Figure 1.

ity index is positive definite, the change in sign may be traced to an electrophilic activation (+) or electrophilic deactivation (−), respectively. Note that activation/deactivation patterns appear coherently related to the quality of Michael electron acceptor of both series; an effect which be traced to substituent effects.

The antiviral activity was qualitatively denoted as active (A) or nonactive (NA), in Table 2, following the labeling proposed by Ricca et al.[10] It may be seen that low values of electrophilicity of the sugar is consistently associated with a low cytotoxic activity, which is directly associated to the ability of these chemicals to covalently bind the nucleophilic biological target. Note on the other hand that for electrophilicity values around or beyond the threshold 0.5 eV the sugars are consistently predicted as antiviral agents.

Since all the 10 compounds experimentally evaluated correspond to Michael acceptors the results described above may be traced to inductive substituent effects by looking at the general structures shown in Scheme 2. For instance, substitution at the double bond in (**1**) by the moderate electron-withdrawing group –CN results in a moderate electrophilic activation around the threshold 0.5 eV. Incorporation of structures (**2**) shed more insight into the analysis of electrophilic activation by chemical substitution. These structures bear the strong electron-withdrawing group $-NO_2$ at position 2 of the double bond of sugars. Note that this effect results in a significant enhanced electrophilicity (compounds **2a**–**i** in Table 2). These compounds may consistently be predicted as active antiviral agents.

Structures (**3**) and (**4**) are also interesting for discussion. For instance, the subseries (**3**) in Scheme 2 shows a –CN substitution at the Michael region (C_3=C_4) which after comparison with structure (**1**) would lead to a moderate electrophilic activation. However, the presence of a *para*-methoxyphenyl group at position 4 induces an electron-donating effect at the same position, thereby compensating the electrophilic activation of the –CN group (see Scheme 2). Overall, the net result is a global electrophilic deactivation of the sugars (see Table 2).

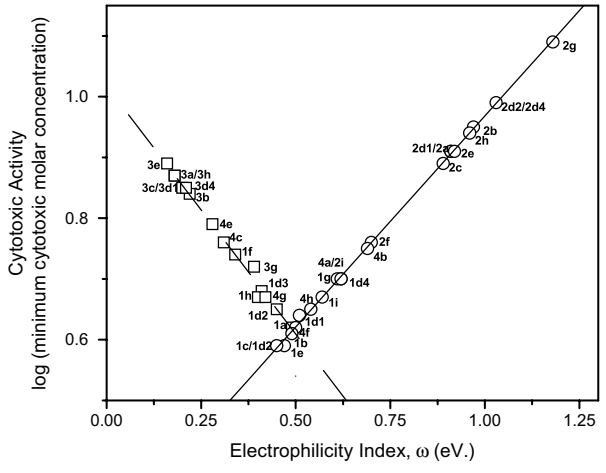


Figure 2.

Table 3. Cross-validation procedure for Eq. 2[a]

Line	Data set	Constant	Slope	R	Cytotoxic activity predicted value
1	{**1a**, **1d4**, **1e**}	0.2727	0.6909	0.995	**1a**: 4.113 (4.148) **1d4**: 5.067 (4.988) **1e**: 3.983 (3.892)
2	{**1d4**, **1e**, **1i**}	0.2423	0.7428	0.998	**1d4**: 5.044 (4.988) **1e**: 3.903 (3.892) **1i**: 4.631 (4.703)
3	{**1d1**, **1d4**, **1e**}	0.2735	0.6934	0.978	**1d1**: 4.238 (4.316) **1d4**: 5.051 (4.988) **1e**: 3.976 (3.892)
4	{**1d1**, **1e**, **1i**}	0.2322	0.7763	0.967	**1d1**: 4.247 (4.316) **1e**: 3.954 (3.892) **1i**: 4.728 (4.703)
5	{**1a**, **1d1**, **1i**}	0.3313	0.5961	0.986	**1a**: 4.201 (4.148) **1d1**: 4.318 (4.316) **1i**: 4.689 (4.703)
6	{**1a**, **1d1**, **1d4**}	0.3337	0.5918	0.995	**1a**: 4.204 (4.148) **1d1**: 4.320 (4.316) **1d4**: 5.019 (4.988)
7	{**1a**, **1d1**, **1e**}	0.0042	1.2500	0.993	**1a**: 4.137 (4.148) **1d1**: 4.382 (4.316) **1e**: 3.906 (3.892)

[a] In the last column the experimental cytotoxic activity value is given in parentheses for comparisons for each substrate.

Table 4. Cross-validation procedure for Eq. 3[a]

Line	Data set	Constant	Slope	R	Cytotoxic activity predicted values
1	{**1d2**, **1e**, **3g**}	1.3167	−1.5192	0.972	**1d2**: 4.296 (4.452) **1e**: 4.005 (3.892) **3g**: 5.299 (5.284)
2	{**1b**, **1d3**, **1h**}	1.2418	−1.4011	0.988	**1b**: 3.477 (3.449) **1d3**: 4.649 (4.753) **1h**: 4.801 (4.654)
3	{**1e**, **1h**, **3g**}	1.2679	−1.4474	0.962	**1e**: 3.869 (3.892) **1h**: 4.886 (4.654) **3g**: 5.051 (5.284)
4	{**1d2**, **1d3**, **1h**}	0.8767	−0.5000	0.866	**1d2**: 4.484 (4.452) **1d3**: 4.695 (4.753) **1h**: 4.750 (4.654)
5	{**1d2**, **1h**, **3g**}	1.3167	−1.5192	0.972	**1d2**: 4.296 (4.452) **1h**: 5.117 (4.654) **3g**: 5.299 (5.284)
6	{**1d2**, **1e**, **1h**}	1.0767	−1.0000	0.866	**1d2**: 4.233 (4.452) **1e**: 4.042 (3.892) **1h**: 4.749 (4.654)
7	{**1d2**, **1d3**, **1e**}	1.2575	−1.3928	0.929	**1d2**: 4.273 (4.452) **1d3**: 4.857 (4.753) **1e**: 4.007 (3.892)

[a] In the last column the experimental cytotoxic activity value is given in parentheses for comparisons for each substrate.

Note also that within the subseries (**4**) in Scheme 2, the effect of chemical substitution by the strong electron-withdrawing group $-NO_2$ causes, in three out of seven compounds, electrophilic activation even in the presence of the deactivating CH_3O-ϕ fragment (see

Scheme 2). Eqs. 2 and 3 were then used to predict the antiviral activity for compounds not experimentally evaluated to date which are shown in Figure 2.

In order to further test the stability of the statistical analysis we have performed a simple cross-validation procedure to guarantee the stability of the regression Eqs. 2 and 3. The validation is performed by randomly choosing a subset of compounds that have known experimental activity. A reduced regression analysis is performed and the remaining members of each series are predicted with different regression equations. This validation analysis is reported in Tables 3 and 4. Unfortunately, the number of molecules for which the experimental biological activity is known is reduced, but we feel that even in this case the present procedure is reliable. The reliability of the reported regression equations is reinforced by the cross-validation procedure: the statistical parameters of Eqs. 2 and 3 are consistently upper and lower bounded by the corresponding statistical parameters of the randomly generated regression equations.

4. Concluding remarks

The electrophilicity of sugars may be classified as Michael acceptors on the basis of the electrophilicity index encompassing both, the electrostatic contribution driven by the electronic chemical potential and the polarization term represented by the chemical softness. We have shown that quantitative relationship between the electrophilicity index of sugar at their ground sates and their biological activity may be established, thereby showing that this molecular reactivity index has a potential usefulness to build up QSAR equations. The model is promising in the sense that (i) it may be easily implemented from a very simple model of electronic structure that may be obtained from standard quantum chemical methods, and (ii) it contains the main ingredients proposed by Coles as the determinants of the optimum interaction between electrophiles and biological nucleophilic targets, namely their chemical softness and the electrostatic contribution represented by the electronic chemical potential. Furthermore, electrophilic activation/deactivation patterns induced by electron-withdrawing and electron-donating groups at the series of Michael acceptors are consistently ordered, in good agreement with the experimental cytotoxic and antiviral activities and with a high predictive value.

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