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Global and local chemical reactivities of mutagen X and simple derivatives

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Abstract Registered by the World Health Organization (WHO), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) is one of the strongest bacterial mutagens ever tested, as highlighted by the Ames *Salmonella typhimurium* TA100 assay. We provide new insights concerning this mutagenic activity on the basis of global and local theoretically defined electrophilicity indices. Our results further support the idea that mutagenicity of MX and its analogues is related more closely to one-electron transfer processes from the electron-rich biological environment than to adduct formation processes. We also stress that, although the Z-open tautomers are intrinsically more electrophilic than furanone ring analogues, the observed mutagenic activity is significantly correlated only to the electrophilicity response of the ring forms. In that context, we also emphasize that it is electrophilicity at the C_α in the α–β unsaturated carbonyl moiety that exhibits a strong correlation with the observed mutagenic activity.

Keywords MX · Electrophilicity · TA100 assay · Global and local indices · Conceptual DFT

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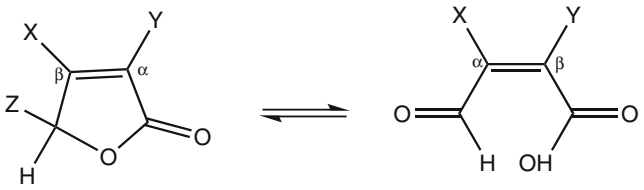
Introduction

3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX, Mutagen-X) **1** and analogue compounds **2**–**24** (see Fig. 1) belong to a group of direct-acting genotoxic disinfection by-products of chlorinated drinking water. MX is formed from the reaction of chlorine with organic compounds (e.g., humic and fulvic acids) present in the raw water [1]. Chlorinated hydroxyfuranones generate mutagenicity in a variety of bacterial strains as evidenced in several short-term assays. These facts have been related (at least partly) to cancer risks associated with chorinated drinking water [2]. In fact, MX has been identified as one of the most potent bacterial mutagens, with mutagenicity values reported in the range of 2,800 to 10,000 revertants/nmol in the Ames *Salmonella typhimurium* TA100 assay [3, 4]. This bacterial reverse mutation assay evaluates the mutagenic properties of compounds. Amino acid-dependent strains of the simplest bacteria are employed. In the absence of an external histidine source, the cells cannot grow in colonies. When reversion of the mutation occurs, colony growth continues due to the production of histidine. Mutagenic compounds thus yield an increased number of revertant colonies in relation to the background level (i.e., spontaneous revertants) [4]. However, analytical difficulties in measuring the low doses of MX encountered in drinking-water lead to uncertainty over whether this species would be genotoxic in vivo. The World Health Organization (WHO) guidelines for drinking water maintain an updated register regarding MX, considering it unnecessary at present to propose a formal guideline value for MX in drinking water [5, 6].

Benigni and co-workers [7, 8], have emphasized the usefulness of quantitative structure-activity relationships (QSAR) for the evaluation/prediction of mutagenicity and carcinogenicity of α–β unsaturated compounds. In the



(only feasible for Z = OH)

Fig. 1 Schematic representation of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) (**1**) and its analogues (**2**–**24**) studied in this work

Compound	X	Y	Z	Compound	X	Y	Z
1	$CHCl_2$	Cl	OH	**13**	CH_2Br	Cl	H
2	$CHCl_2$	Cl	OCH_3	**14**	CH_2Cl	Br	H
3	$CHBr_2$	Cl	OH	**15**	CH_2Cl	H	OH
4	$CHBr_2$	Br	OH	**16**	Cl	Cl	OCH_3
5	CH_2Cl	Cl	OH	**17**	CH_3	Cl	OCH_2CH_3
6	CH_2Br	Br	OH	**18**	CH_3	Cl	OH
7	$CHBr_2$	Cl	H	**19**	CH_3	Br	OH
8	$CHCl_2$	Cl	H	**20**	Cl	Cl	H
9	$CHBr_2$	Br	H	**21**	H	Cl	OCH_2CH_3
10	Cl	Cl	OH	**22**	CH_2Cl	H	H
11	CH_2Br	Br	H	**23**	H	Cl	OH
12	CH_2Cl	Cl	H	**24**	CH_3	H	OH

establishment of such relationships, lowest unoccupied molecular orbital (LUMO) energies are usually employed as simple indicators of the electrophilic reactivity [9] expected for such a molecular fragment. Structural and electronic properties of MX and related compounds have been examined previously using both experimental and theoretical (e.g., semi-empirical, ab-initio and QSAR) approaches in order to determine why MX is such a potent mutagen in bacteria [10–13]. Within this framework, Tuppurainen and co-workers [12–17] reported a molecular orbital model intended to predict the mutagenicity (in *Salmonella typhimurium* strain TA100) of MX and its analogues on the basis of AM1 semiempirical electronic responses. Significant correlations were found between the experimental mutagenicity and the LUMO energy within a series of MX and its analogues [12–17]. It was argued that the high reductive potential (i.e., an electronic property) of MX should be related closely to the operating mutagenic mechanism. Indeed, it was discovered that hydrophobic (modeled using octanol-water partition coefficient log P), as well as steric (modeled using average molecular polarizability) terms, have no significant effects on the correlation equation relating to biological activity, as measured in terms of ln (TA100), and LUMO energy [13–15]. These results support the hypothesis that the mutagenic ability of MX and related compounds (as is the case for other mutagens) is due to their ability to receive electrons, i.e., mutagenesis involves an electron transfer processes from the DNA electron-rich environment to the lowest empty molecular orbital of the MX compound.

Thus, mutagenicity seems to be concerned primarily only with an intrinsic electronic response in these systems (i.e., direct-acting mutagens). It is also known [18] that (as depicted in Fig. 1) MX can exist structurally as a ring (i.e., furanone) or an open [i.e., (E) and (Z)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid tautomer] form depending on the pH. The ring form predominates in solutions with pH<5.5, whereas the Z-open form will be present at pH>5.5 (e.g., in drinking-water at normal pH) [6, 18, 19]. In the context of correlating biological TA100 responses with LUMO energies associated to the ring and open forms, Cho [10] has stressed that it is indeed the open tautomer, specifically (Z)-2-chloro-4-(dichloromethyl)-4-oxo-butenoic acid, that is the real mutagen at physiological conditions—a conclusion that deserves further detailed attention, as we will show below.

Accumulated evidence so far points to the fact that MX and analogue compounds are expected to behave effectively as electrophiles when interacting with DNA [10–13], although the origin of the specific mutagenic activity remains, to the best of our knowledge, still unresolved. Following ongoing interests [20–23], and in absence of more detailed analysis concerning the electrophilic nature of these compounds, we here resort to a theoretical model defined within the conceptual framework of density functional theory (DFT), intended to quantify intrinsic electrophilicity responses [24, 25]. In order to explore the electronic pattern of reactivity in connection to the experimental mutagenic activity, measured as the natural logarithm of the number of revertants/nmol in the *Salmonella typhimurium* tester strain *TA100* of the Ames assay [4, 17, 19, 26], we study the reactive electrophilic responses, both at the global and local levels, of the series of 24 compounds depicted in Fig. 1. In this set of species, only the hydroxyfuranone series (i.e.,

systems **1**,**3-6**,**10**,**15**,**18**,**19**,**23**, and **24** in Fig. 1) can undergo a tautomeric equilibrium connecting ring and open forms. This series will provide an excellent framework to test the validity of previous conjectures regarding the nature of these mutagenic species [10]. The analysis of both global (i.e., associated to the complete molecular system) and local (i.e., associated to specific sites within the molecular framework) reactivity patterns of electrophilicity will provide, as shown below, useful new insights for a chemical species that is thought to interact with a rich electron environment [25, 27].

Besides offering a powerful computational framework for structural/electronic problems, DFT provides a general conceptual framework for the theoretical development of models rationalizing chemical responses against perturbations [27–32]. In that context, reactivity, selectivity, activation, reaction mechanisms, biological activity, solvation effects, etc., have been addressed using simple tools devised within a perturbative approximation to chemical reactivity [27–30]. Particularly, the (intrinsic) electrophilicity index introduced by Parr et al. [25] measures the maximum energy stabilization that a given system experiences when it is immersed in a sea of electrons (i.e., a perfect electron donor). A comprehensive review (periodically updated) concerning both formal developments and wide range of applicability and predictive ability of such a descriptor is available [27]. Chattaraj and co-workers have indeed emphasized that global and local reactivity descriptors defined within the conceptual DFT framework are helpful towards a deeper understanding of relationships between structure, stability, and reactivity [28]. DFT descriptors have in fact been employed in the exploration of electronic factors entering in quantitative structure-activity (QSAR), structure–property (QSPR), and structure-toxicity (QSTR) relationships [33–36]. Extensions of the electrophilicity into the framework of a spin-polarized version of DFT have also been introduced in order to properly describe both charge-transfer and spin-polarization processes [31, 37].

Theoretical models

Let us recall that by considering the variation of the electronic energy in a given system immersed in a perfect, structureless, electron donor environment (i.e., a sea of electrons, with μ_e=0 and η_e=0), Parr et al. [25] identified the intrinsic electrophilicity power ω of the system as the negative of the net electronic energy stabilization, namely [27, 30, 31, 37]:

$$\omega = \frac{\mu^2}{2\eta} \tag{1}$$

This amount arises by minimizing the electronic energy change $\Delta E(\Delta N)$ with respect to the amount of electrons, ΔN, transferred from the bath. The external potential to the electronic system, $\nu(r)$, is kept "fixed" or is not allowed to vary during the electron transfer. It accounts (in the absence of other fields) for the total coulomb interaction at electronic position r due to nuclei α fixed at position R_α, i.e.,

$$\nu(\mathrm{r}) = \sum_{\alpha} \frac{Z_\alpha}{|\mathrm{r} - \mathrm{R}_\alpha|} \tag{2}$$

Under those conditions, the optimum amount of electrons, ΔN^*, is predicted to be [25, 38]:

$$\Delta N^* = -\frac{\mu}{\eta} \tag{3}$$

and ω in Eq. 1 become identified as $-\Delta E(\Delta N^*)$. In this formulation, the electronic chemical potential, μ, measures the escaping tendency of the electronic cloud from equilibrium (i.e., the negative of electronegativity), whereas the molecular hardness, η, is associated to the resistance of the electron cloud to experience changes in a charge transfer process [29], namely:

$$\mu = \left(\frac{\partial E}{\partial N}\right)_{\nu(\mathrm{r})}; \eta = \left(\frac{\partial^2 E}{\partial N^2}\right)_{\nu(\mathrm{r})} \tag{4}$$

Finite difference approximation to μ and η provides that [29]:

$$\mu = -\frac{I + A}{2}; \eta = I - A, \tag{5}$$

where I and A represent the first ionization potential and electron affinity of the system, respectively. Within the simplest operational approximation to Eq. 5, we used the negative of the frontier molecular orbital energies, i.e., the highest occupied (HO) and the lowest unoccupied (LU) molecular orbitals (MO), respectively, as approximations to I and A. However, we have to stress that, within the Kohn-Sham formalism, orbital energies have no physical meaning except that implied by Janak's theorem (i.e., the derivative of the total energy with respect to the orbital occupation number) [39]. From the long-range behavior of electron density, and expected only for the exact effective potential in DFT theory, the HOMO energy will indicate the exact ionization potential. Within the framework of Hartree-Fock theory, the above gross approximation is justified in terms of the scope of validity of Koopmans' theorem [40]. This level of approximation, despite its well-known outcomes, is suitable enough for the analysis of relative qualitative reactivity trends, as intended in the present work. Hence, Eq. 5 is operationally evaluated as:

$$\mu \approx \frac{\varepsilon_L + \varepsilon_H}{2} \quad ; \eta \approx \varepsilon_L - \varepsilon_H, , \tag{6}$$

where ε_H and ε_L are the HOMO and LUMO energies, respectively. Note from Eq. 4 that μ and η constitute only

the global responses of the electronic system against a global perturbation in the number of electrons. In order to determine the site of attack by nucleophiles and/or by electron donors on these mutagenic compounds, we use the Fukui function as the key local descriptor, defined as [29]:

$$f(\mathbf{r}) = \left[\frac{\delta\mu}{\delta v(\mathbf{r})}\right]_N = \left[\frac{\partial\rho(\mathbf{r})}{\partial N}\right]_{v(\mathbf{r})}, \quad (7)$$

which can be used to project the global response into the local molecular framework. Given the discontinuity of energy with respect to variations in the number of electrons, two functions arise, i.e., $f^+(\mathrm{r})$ and $f^-(\mathrm{r})$, which give the local response of a system against nucleophilic and electrophilic attacks, respectively. The Fukui functions are the most important regioselectivity indicators for frontier controlled reactions in conceptual DFT. Condensed-to-atoms Fukui descriptors f_k^+ and f_k^-, can be obtained immediately within a frozen-core molecular orbital approximation [41–43] by using the basis-set $\{\chi_\mu(\mathbf{r})\}$ linear expansion of the frontier molecular orbital α,

$$f_k^\alpha = \sum_{\mu\in k}\sum_{\nu} c_{\mu,\alpha}c_{\nu,\alpha}S_{\mu\nu}, \quad (8)$$

where $c_{\mu,\ \alpha}$ and $c_{\nu,\alpha}$ are the expansion coefficients, and $S_{\mu\nu}$ refers to the overlap integrals between the basis-set functions, i.e., $S_{\mu\nu} = \int \chi_\mu(\mathbf{r})\chi_\nu(\mathbf{r})\mathbf{dr}$. This procedure allows us to obtain intrinsic electrophilic (i.e., for α=HOMO) and nucleophilic (i.e., for α=LUMO) responses of the system without additional calculations of the anion or cation species [41–43]. The condensation procedure of Eq. 8 is useful only when localized basis functions are used, being attractive because it does not require calculations of the charged species [44]. Other schemes of condensation could certainly be helpful for researchers interested in using conceptual DFT for QSAR models, such as those defined within topologically defined regions of electronic localization [45, 46]. Here, we use the electrophilic Fukui function $f^+(\mathbf{r})$, as a suitable local normalized-to-unity projector quantity, providing in this way the local counterpart for the electrophilicity, ω_k^+, condensed on atom k in the molecular system [27, 31]

$$\omega_k^+ = \omega f_k^+ \quad (9)$$

Equation 9 indicates that the most electrophilic site will be related to both the global and local distribution of electrophilic power, allowing us to compare the relative proclivity reactivities among the series of related compounds.

Methods and computational details

Molecular structures of MX and its analogues in Fig. 1 were fully optimized at the B3LYP/6-311G(d) level of theory [24] using the GAUSSIAN 09 package of programs [47]. The nature of stationary points on the explored potential surfaces were confirmed through frequency calculations. The global quantities i.e., hardness, chemical potential, amount of charge transfer, and electrophilicity, were evaluated using Eqs. 6, 3 and 1, respectively. Condensed-to-atoms indices, i.e., Fukui and electrophilicity, were evaluated from Eqs. 8 and 9, respectively. Simple in-house-written routines were employed to evaluate both the global and local descriptors directly from Single Point results using the options "SCF = Tight IOP(3/33=1) and POP = Full" [43]. The Supporting Information with this work reports all optimized geometries and the resulting condensed-to-atoms Fukui and electrophilicities indices, as well as the full regression ANOVA results statistics (Tables S1–7) concerning simple and multilinear regression analysis correlating mutagenic activities with both global and local reactivities.

Results and discussion

As mentioned above, compounds in Fig. 1 with substituent Z = OH can also exist in Z-tautomeric open forms (B) [10]. Structures B exhibit two motifs associated to a C_α–C_β unsaturated carbonyl fragment that enhances (in principle) the electrophilic nature as compared to the ring tautomer. However, by examining the geometrical parameters of the optimized structures [i.e., 24 ring forms (A) and 11 Z-tautomers (B)], it can be noted that the substitution pattern does not affect significantly the interatomic distances in the active motif in any structure, in either the A or B forms. The open forms exhibit two α–β unsaturated motifs. In that case, the electronic properties of the active C_α–C_β unsaturated carbonyl fragment correspond to those whose planarity will favor an enhanced electron delocalization as indicated in Fig. 1. Therefore, it seems reasonable to expect that the mutagenic activity observed for these compounds should be associated only to the variation in intrinsic electronic properties induced in those regions through (exclusively) the substitution pattern. These facts further justify the view that attention should be focused only on the electronic responses, as measured through the global and local descriptors, on the mutagenic reported activities.

Mutagenic activity and global electrophilicity

Table 1 reports the global electronic indices, including frontier molecular orbital energies, chemical potential, chemical hardness, maximum amount of charge transfer, and electrophilicity (all in eV), associated to the ring conformations of MX and its analogues. Table 2 reports the corresponding information associated to the 11 Z-open tautomers. In the case of the 24 ring forms, the simple linear correlation

Table 1 Global electronic properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and its analogues evaluated at the B3LYP/6-311G(d) level of theory. Energy values in eV

Compound	Ln (TA100)[a]	E_{HOMO}	E_{LUMO}	μ	η	ω	ΔN_{max}
1	8.748	−8.2916	−2.7149	−5.5032	5.5767	2.72	0.99
2	8.648	−8.2252	−2.6632	−5.4442	5.5620	2.66	0.98
3	8.607	−7.992	−2.7666	−5.3793	5.2254	2.77	1.03
4	7.966	−7.881	−2.7494	−5.3152	5.1315	2.75	1.04
5	6.361	−8.0924	−2.4626	−5.2775	5.6298	2.47	0.94
6	6.04	−7.7789	−2.5723	−5.1756	5.2066	2.57	0.99
7	5.198	−8.0499	−2.7127	−5.3813	5.3372	2.71	1.01
8	5.176	−8.2369	−2.6259	−5.4314	5.611	2.63	0.97
9	4.86	−7.8897	−2.7010	−5.2953	5.1887	2.70	1.02
10	4.094	−7.8714	−2.2390	−5.0552	5.6325	2.27	0.90
11	2.109	−7.7256	−2.4956	−5.1106	5.2300	2.50	0.98
12	1.593	−8.014	−2.3331	−5.1736	5.6809	2.36	0.91
13	1.374	−7.9087	−2.5051	−5.2069	5.4036	2.51	0.96
14	1.371	−7.8113	−2.3138	−5.0625	5.4975	2.33	0.92
15	1.351	−7.9098	−2.2343	−5.0721	5.6755	2.27	0.89
16	0.993	−7.8363	−2.1685	−5.0024	5.6679	2.21	0.88
17	0.742	−7.6214	−1.6977	−4.6595	5.9236	1.83	0.79
18	0.405	−7.681	−1.7954	−4.7382	5.8856	1.91	0.81
19	0.405	−7.4815	−1.7883	−4.6349	5.6932	1.89	0.81
20	0.113	−7.8388	−2.0441	−4.9415	5.7947	2.11	0.85
21	−0.223	−7.9158	−1.9459	−4.9308	5.9699	2.04	0.83
22	−1.187	−7.8203	−2.0790	−4.9496	5.7413	2.13	0.86
23	−1.603	−8.0097	−2.0545	−5.0321	5.9552	2.13	0.84
24	−3.507	−7.5942	−1.5282	−4.5612	6.0660	1.71	0.75

[a]Experimental data for lnTA100 are taken from [12]

analysis of experimental mutagenic activities [12] and global electrophilicity (see Fig. 2 and Table S1) reveals that,

$$lnTA100 = (9.493 \pm 1.188)\omega_R - (19.315 \pm 2.805), \quad (10)$$

where the F test for overall significance of such a linear model allows us to conclude that a significant portion (i.e., 74.4 %) of the variation in LnTA100 can be explained satisfactorily by the variation in global electrophilicity ω_R. The individual *t*-test for the slope and intercept reveals indeed that the linear relationship between LnTA100 and ω_R is acceptable at the 0.05 level of significance. The confidence intervals at the 95 % level for both the slope and intercept parameters do not include zero—also in agreement with a satisfactory F-test of these parameters. The global electrophilicity corresponds to the total lowering in electronic energy that a given system experiences when it

Table 2 Global electronic properties of the open forms associated to the hydroxyfuranone systems evaluated at the B3LYP/6-311G(d) level of theory. Energy values in eV

Compound	Ln (TA100)[a]	E_{HOMO}	E_{LUMO}	μ	η	ω	ΔN_{max}
1	8.748	−7.7261	−3.138	−5.4321	4.5881	3.22	1.18
3	8.607	−7.5438	−3.1323	−5.3381	4.4115	3.23	1.21
4	7.966	−7.5482	−2.8632	−5.2057	4.6850	2.89	1.11
5	6.361	−7.8562	−3.2254	−5.5408	4.6308	3.31	1.20
6	6.04	−7.7112	−3.1271	−5.4191	4.5840	3.20	1.18
10	4.094	−7.9367	−3.2003	−5.5685	4.7364	3.27	1.18
15	1.351	−7.6883	−3.2221	−5.4552	4.4662	3.33	1.22
18	0.405	−7.5909	−2.8417	−5.2163	4.7492	2.86	1.10
19	0.405	−7.5618	−2.7516	−5.1567	4.8102	2.76	1.07
23	−1.603	−7.6271	−3.2101	−5.4186	4.417	3.32	1.23
24	−3.507	−7.3708	−2.7913	−5.081	4.5794	2.82	1.11

[a]Experimental data for lnTA100 are taken from [12]

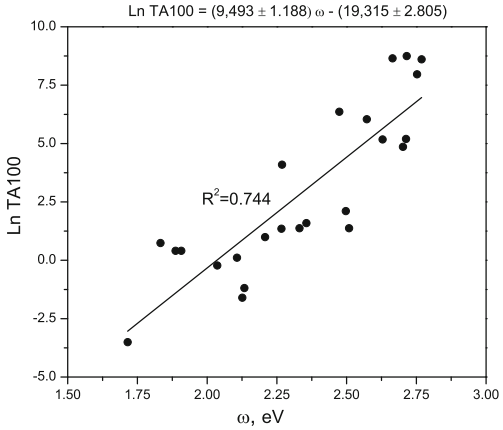


Fig. 2 Plot of ln(TA100) against global electrophilicity (eV) for the set of ring-forms of MX and its analogues, n=24. Complete ANOVA results are reported in Table S1 in the Supplementary material

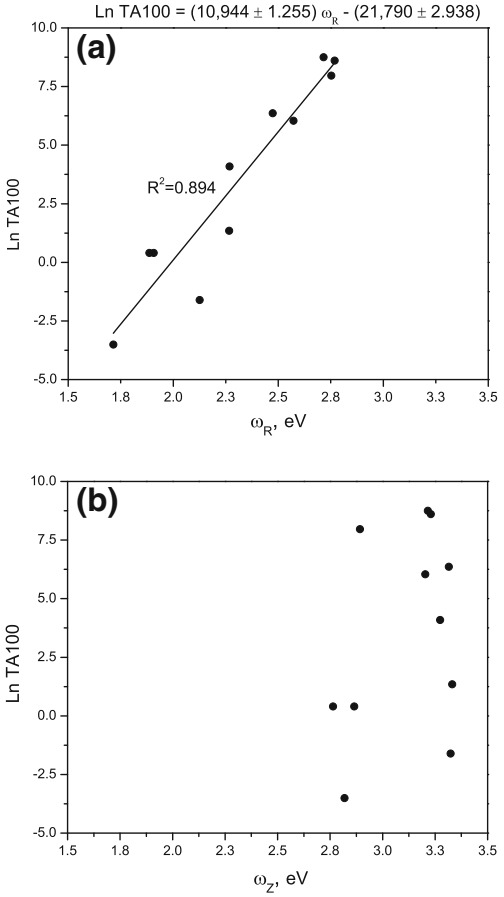


Fig. 3 Plot of ln(TA100) against the global electrophilicity (eV) associated to the ring (ω_R) and the open forms (ω_Z), of the hydroxyfuranone systems, n=11. Complete ANOVA results are reported in Tables S2 and S3, respectively

sucks the optimum (maximum) amount of electrons given in Eq. 3. Note from Table 1 that this maximum amount of charge transfer is predicted to be just one electron along the entire series of MX analogues.

These results further stress the interpretation that the mechanism of MX mutagenicity could in fact be being controlled by one-electron reduction processes [12]. Note simply from Eqs. 1 and 3 that $\omega=0.5\mu\Delta N^{*}$. In the case of the 11 hydroxyfuranones, the z-open forms are predicted to have a noticeable increment (i.e., from 5.0 % in compound **4** to 64 % in compound **24**) in intrinsic electrophilic character as compared to the ring tautomers (see Table 2). However, we found that the reported mutagenic activity can be correlated linearly only to the global electrophilicities of the ring forms, as revealed in Table S2 and Fig. 3a. Table S3 and Fig. 3b evidences the absence of linear correlation of mutagenic activity and the electrophilicity of open forms for the examined hydroxyfuranone series, including MX. The fact that mutagenic activity, expressed as lnTA100, is not linearly related to the electrophilicity of the Z-tautomers (Fig. 3b) opposes previous conjectures [10]. We stress here that the overall mutagenic activity should be effectively related to the ring forms of the MX compounds, which is indeed not the predominant form in drinking water!. The proportions of the two tautomeric forms depend on the pH and it is thus difficult to theoretically predict the ratio of their concentrations. However, the activation barrier between these tautomeric forms has been determined at the current level of theory to be about 27.0 kcal mol^{-1} for the chloro-hydroxyfuranone systems. Note further that, in the presence of a tautomeric equilibrium, we also could resort to a multilinear model that combines the global electrophilicities of both the ring and open tautomers. In that case, the best model we can explore indicates that,

$$lnTA100 = (12.078 \pm 1.674)\omega_R - (7.823 \pm 1.256)\omega_Z. \quad (11)$$

As reported in Table S4, the F test for overall significance of such a multilinear model (i.e., F=50.5 with a P-value= 2.9E−5), reveals that 91.8 % of the variation in LnTA100 can be explained satisfactorily by the variations in both ω_R and ω_Z. Also, the individual t-test for the slopes reveals indeed that the linear relationship between LnTA100 and ω_R and ω_Z are acceptable at the 0.05 level of significance. Also, the confidence intervals at the 95 % level for both slope parameters do not include zero, in agreement with the satisfactory F-test of these parameters. Note, however, that only 79.8 % of the variation in Ln TA100 is explained by the variation in electrophilicity of both tautomers, taking into account sample size (n=24) and the number of independent variables (2). These results confidently suggest that mutagenicity in hydroxyfuranones (as measured by LnTA100) will increase on average by 12.1 units for each unit increase in ω_R, net of the effects of changes due to ω_Z. Correspondingly, LnTA100 will decrease, on average, by 7.8 units for each unit increase in ω_Z, net of the effects of changes due to ω_R. These results and the standard deviation

value confirm that, along this series subject to tautomeric equilibrium, the greater effect on increasing mutagenicity should be associated necessarily to the ring forms, which is low at the pH of drinking water conditions [5, 6].

Local electrophilicity, ω_k^+

The above results emphasize that the global reactivity of the ring forms of MX and analogues becomes the relevant factor, at least within the context of the current limited set of analyzed systems and within the validity of the naive theoretical approximations already mentioned. Hence, by considering the local counterparts, we will focus only on the 24 ring form species. However, we explored the existence of correlations of biological activity with respect to the condensed electrophilicities calculated on the 11 open forms (despite the fact that there is no correlation with global responses), given these are the ones that are predominant at physiological pH. In contrast to the case of ring forms, the biological activity is linearly correlated only to the local electrophilicities at the beta carbon in the open forms. We would argue strongly that it is the global electrophilicity that should be the primary response to be considered because local electrophilicity simply corresponds to the local mapping of such global responses.

It is well known that the β and α-carbon sites of $\alpha-\beta$-unsaturated carbonyl compounds are prone to be attacked by nucleophiles and electron donors. For the entire series, our results for the condensed-to-atom Fukui indices, f_k^+, reveal that values associated to the $\alpha-\beta$-unsaturated carbonyl moieties in fact always become the higher ones, suggesting that a direct nucleophilic attack on the carbonyl is certainly disfavored. Table 3 reports f_k^+ and ω_k values only on the $\alpha-\beta$ region (full data is available as indicated in the Supplementary material). Note that, for each individual species, the local electrophilicity on the β-carbons is predicted always to be greater (ranging from 1 % in system **7** to 76 % in system **17**) than that associated to the α-carbons, along the entire series of studied compounds. The mutagenic activity was therefore correlated independently to the local electrophilicity in each center. Results for the correlation of mutagenic activity and the reactivity at the α carbon are reported in Table S5 and Fig. 4a. Table S6 and Fig. 4b report the corresponding results for the β-center. We found that the reported mutagenic activity is related linearly only to the electrophilic reactivity at the α center,

$$lnTA100 = (32.534 \pm 4.217)\omega_{C_\alpha,R} - (14.173 \pm 2.246), \quad (12)$$

with an F test for overall significance of such a linear model (i.e., F=59.52 with a P-value=1.0E−7) allowing us to conclude that a significant portion (i.e., 73.0 %) of the variation in LnTA100 can be explained satisfactorily by the variation of

Table 3 Local electronic properties associated to the open and ring forms of hydroxyfuranone systems evaluated at the B3lyp/6-311G(d) level of theory. Energy values in eV

		Ring form		Open form	
Compound	Atom	f^+	ω^+	f^+	ω^+
1	C_α	0.2465	0.67	0.1978	0.64
	C_β	0.2818	0.77	0.3065	0.99
3	C_α	0.2359	0.65	0.1903	0.61
	C_β	0.2453	0.68	0.3008	0.97
4	C_α	0.2361	0.65	0.1746	0.50
	C_β	0.2484	0.68	0.3537	1.02
5	C_α	0.2272	0.56	0.1994	0.66
	C_β	0.2880	0.71	0.2652	0.88
6	C_α	0.2138	0.55	0.1853	0.59
	C_β	0.2416	0.62	0.2722	0.87
10	C_α	0.2228	0.51	0.1983	0.65
	C_β	0.3421	0.78	0.2470	0.81
15	C_α	0.2446	0.55	0.2111	0.70
	C_β	0.2920	0.66	0.2562	0.85
18	C_α	0.1986	0.38	0.1983	0.57
	C_β	0.3444	0.66	0.2366	0.68
19	C_α	0.2000	0.38	0.1891	0.52
	C_β	0.3483	0.66	0.2466	0.68
23	C_α	0.2251	0.48	0.2255	0.75
	C_β	0.3773	0.80	0.2268	0.75
24	C_α	0.2149	0.37	0.2061	0.58
	C_β	0.3514	0.60	0.2270	0.64

C_α, R. The individual t-test for the slope and intercept also reveals that the linear relationship between LnTA100 and $\omega_{C\alpha,R}$ is confident at the 0.05 level of significance. The confidence intervals at the 95 % level for both the slope and intercept parameters do not include zero, also in agreement with the satisfactory F-test of these parameters. The fact that mutagenic activity, expressed as lnTA100, is not related linearly to the electrophilicity at the β carbon center allow us to conjecture that adduct formation via Michael-type addition processes in the mutagenic mechanism might be not relevant. In order to further characterize the local reactive responses in that context, we have explored a multilinear model that combines the local electrophilicities of both the α and β carbon centers, obtaining the model,

$$lnTA100 = (25.718 \pm 4.867)\omega_{C_\alpha,R} - (15.375 \pm 3.789)\omega_{C_\beta,R}. \quad (13)$$

The corresponding statistics are reported in Table S7. The F test for overall significance of such a multilineal model (i.e., F=32.11 with a P-value=4.09E−7), reveals that 74.5 % of the variation in LnTA100 can be explained

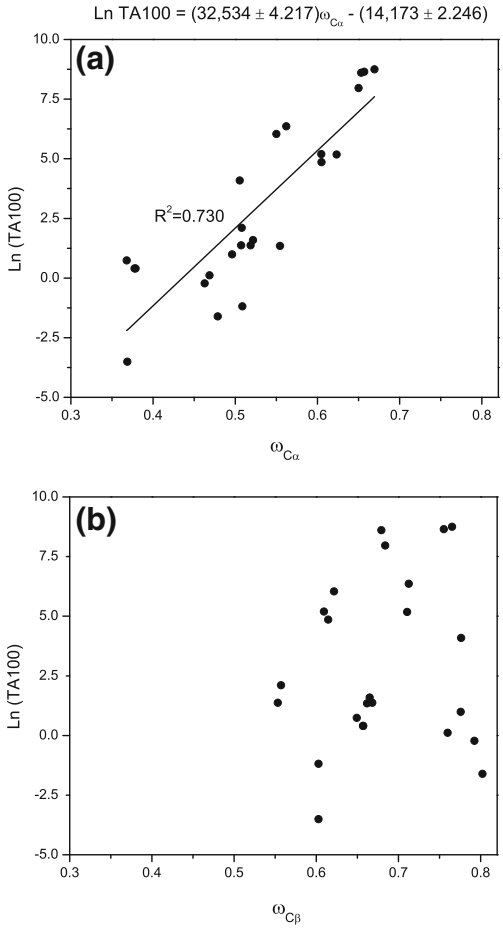


Fig. 4 Plot of ln(TA100) against the local electrophilicity (eV) at the α and β carbon associated to the ring-forms of MX and its analogues, n= 24. Complete ANOVA results are reported in Tables S5 and S6, respectively

satisfactorily by the variations of both $\omega_{C\alpha,\,R}$ and $\omega_{C_\beta,R}$. The individual t-test for the slopes reveals indeed that the linear relationship between LnTA100 and $\omega_{C_\alpha,R}$ and $\omega_{C_\beta,R}$ are confident at the 0.05 level of significance. The confidence intervals at the 95 % level for both slope parameters do not include zero, also in agreement with satisfactory F-test of these parameters. Note, however, that only 68.8 % of the variation in Ln TA100 is explained by the variation in electrophilicity of both centers, taking into account the sample size (n=24) and number of independent variables (2). These results confidently suggest that mutagenicity (as measured by LnTA100) will increase on average by 25.7 units for each unit increase in $\omega_{C_\alpha,R}$, net of the effects of changes due to $\omega_{C_\beta,R}$. Correspondingly, LnTA100 will decrease, on average by 15.4 units for each unit increase in $\omega_{C_\beta,R}$, net of the effects of changes due to $\omega_{C_\alpha,R}$. This result reveals (in contrast to the intrinsic reactivity) that, along this series of MX and analogue species, the greater effect on increasing mutagenicity is necessarily associated to the enhancement of the local electrophilicity pattern at the C_α center. The above results further contribute to supporting the idea that, regarding intrinsic reactivity, it is the electrophilicity response mapped at the $C_{\alpha,\,R}$ center that will play the most important role in the context of rationalizing the mutagenic activity for MX and related analogues. In this context, it should be highlighted that the global and local electrophilicity trends are similar within the context of rationalizing the observed biological activity along the series of MX direct-acting mutagens.

Conclusions

This work used global and local electrophilicity descriptors to further explore new insights associated to the mutagenic activity of MX and simple related analogues. The main results emphasize that, in agreement with previous findings, the open forms are intrinsically more electrophilic than the ring tautomeric-related ones. However, the experimental mutagenic activity of MX compounds seems better correlated to the intrinsic electrophilicity pattern of the ring forms, at both the global and local levels. Validation of the resulting QSAR models was carried out by examining part of the training set, which also provides satisfactory linear correlations. Open forms do not show satisfactory correlations, at least at the current level of theory and current approximations with TA100. Concerning the local aspects of reactivity, it is the C_α site of ring forms that exhibits the greatest proclivity to interact with electrons (or nucleophiles). It can therefore be expected that, if MX species interacts with fragments of DNA, Michael-type reactions or interactions might be regretted at the, until now, unrevealed active site of DNA. It should be noted that many factors can govern the regioselectivity, including for instance steric hindrance, changes in the external potential of reagents when approaching one another, and the existence of secondary interactions orientating the reaction differently. The above results are related exclusively to the intrinsic electronic responses, as measured through the Fukui and electrophilic responses. Based on the wide accumulated evidence related to applications of the theoretical electrophilicity index [27, 28, 30, 31, 37], it is expected that the present results become similar by including solution phase [42, 48, 49], or along a varying reaction coordinate [50], under the scope of validity of the above approximations.

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