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Ultimate Carcinogenic Metabolites from Aromatic and Heterocyclic Aromatic Amines: A Computational Study in Relation to Their Mutagenic Potency

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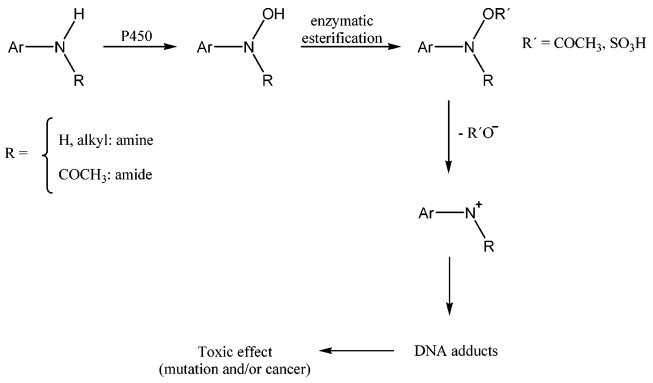
The formation of nitrenium ions from their precursors was examined by density functional theory (DFT) calculations in order to analyze the role of these electrophilic intermediates on the mutagenic activity of the parent amines. The relative reactivities for N−O bond dissociation from the *N*-hydroxy, *N*-acetoxy and *N*-sulfate derivatives of aniline were evaluated. Furthermore, the *N*-acetoxy esters from a set of 17 aromatic and heteroaromatic amines of diverse structure were considered, and correlations were sought between the calculated properties and the reported mutagenic potencies. The mutagenic activity was found to increase when a more negative charge developed at the exocyclic nitrogen of the nitrenium ion (q_N) and with nitrenium ion stability. Different functional correlations were observed for the amine derivatives grouped according to their classification as aromatic (Ar), imidazo-carbocyclic (Imi-C), and imidazo-heterocyclic (Imi-H). The formation of *N*-acetyl nitrenium ions from aromatic amides was also considered and found to be less favorable than nitrenium ion generation from the corresponding amines.

Introduction

Many aromatic amines are either industrial or environmental carcinogens (*1*, *2*). Furthermore, heterocyclic aromatic amines found in cooked meats and protein rich foods have been shown to be mutagenic and carcinogenic (*3*−*8*). The genotoxic potential of these compounds is developed by enzymatic metabolic activation (*9*−*11*). The initial step is the N-oxidation to aryl *N*-hydroxylamines (*9*), which can undergo N−O bond cleavage to arylnitrenium ions under mildly acidic conditions (*11*). Alternatively, further activation of the *N*-hydroxylamine derivative to a *N*-acetoxy or *N*-sulfate ester permits a more facile heterolysis of the N−O bond (*9*). This process generates a highly reactive nitrenium ion, which is the ultimate electrophilic metabolite that covalently binds and damages DNA (*9*−*11*) (Scheme 1). The relative stability of the nitrenium intermediate appears to be a crucial point in determining the biological activity of aromatic amines (*9*).

Aromatic amides follow the same metabolic pathway as aromatic amines (*9*−*11*). For the *N*-hydroxyarylamides intermediates (hydroxamic acids), esterification is required for subsequent reactivity with DNA (*9*). The potent carcinogen 2-acetylaminofluorene (AAF[1]) is the best-studied example and has become a model compound for the study of mutagenic and carcinogenic effects of aromatic amine derivatives (*12*). Although amides generally appear to be no less carcinogenic than amines (*1*), the acetamido derivatives were consistently less mutagenic than their parent amines (*13*, *14*).

Scheme 1. Metabolic Activation for Aromatic Amines and Amides



Many efforts have been made to develop quantitative structure−activity relationships (QSARs) that correlate quantitative bacterial mutagenicity data and quantitative carcinogenicity data for aromatic and heteroaromatic amines with calculated or observed properties of the amines or their derived nitrenium ions (*15*−*24*). Numerous correlations have been found between calculated and experimentally measured chemical properties for the nitreniums and the mutagenic potencies of their parent amines (*17*−*19*). These studies suggest that mutagenicity increases with the rate of nitrenium ion formation from their precursors, that is, with a higher nitrenium intermediate stability,

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[1] Abbreviations: AAF, 2-acetylaminofluorene; QSARs, quantitative structure-activity relationships; PAH, polycyclic aromatic hydrocarbon; DFT, density functional theory; NPA, natural population analysis; NBO, natural bonding orbital; PCM, polarized continuum model; MP, mutagenic potency; Ar, aromatic; Imi-C, imidazo-carbocyclic; Imi-H, imidazo-heterocyclic.

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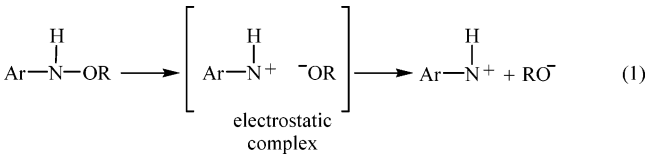
as computed with the semiempirical method AM1. Moreover, recent PM3 and PM5 semiempirical studies have indicated that the production rate and stability of nitrenium ions are more important in influencing mutagenicity than their binding reactions with DNA bases (*25, 26*). However, multiple variable models that include higher level ab initio calculated variables related to nitrenium ion stability have suggested that these variables are of only limited use in regression models (*15, 16, 20*−*22*), and therefore, the importance of nitrenium ion stability in determining the mutagenic potency of amines has been questioned (*22*).

Quantum-mechanical calculations have shown very good agreement with the experimental reactivities of several polycyclic aromatic hydrocarbon (PAH) metabolites (epoxides and diol epoxides, episulfides, imines, and aza-compound derivatives) when applied to the study of the carcinogenic pathways of these compounds (*27*−*32*). Additionally, modeling studies of biological electrophiles from PAHs by density functional theory (DFT) methods have yielded appropriate descriptions of the NMR features and charge delocalization modes of their resulting carbocations (*33*−*37*).

In this work, computational studies on the carcinogenic derivatives of PAHs and nitrogen-containing PAHs are being continued, focusing on aromatic and heterocyclic amines and amides. Considering the discrepancy mentioned above between semiempirical and Hartree−Fock ab initio results in relation to the importance of nitrenium ions on the mutagenicity of this class of compounds, the problem was now examined by higher level DFT calculations. Hence, aiming to achieve a better understanding of the role of nitrenium ions on the reactivity of aromatic and heteroaromatic amines, the formation of these electrophilic intermediates from their precursors was analyzed. A comparison of the reactivities for N−O bond dissociation of the *N*-hydroxy, *N*-acetoxy, and *N*-sulfate derivatives of aniline was made.

Subsequently, aromatic and heteroaromatic amines and amides of different structure were examined. Particularly, the N−O bond cleavage from the *N*-acetoxy ester to give an electrostatic hydrogen-bonded complex was analyzed (reaction 1). In addition, reaction energies considering the resulting ions at infinite separation allowed taking into account the relative differences in nitrenium ion stability versus complex stability.



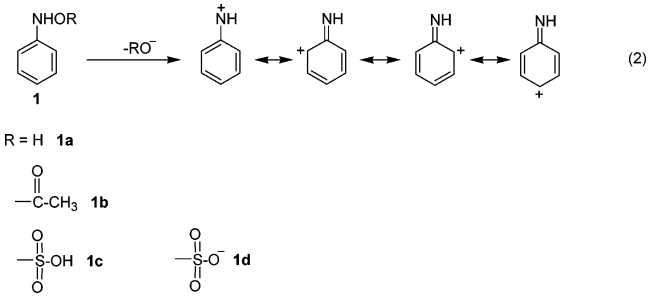
(1)

Correlations were sought between experimental mutagenic potencies reported in the literature and calculated reaction energies and electronic properties.

Computational Methods

DFT calculations were performed with the Gaussian 03 suite of programs (*38*), employing the B3LYP hybrid functional (*39*−*41*) with the 6-31+G(d) basis set. Geometries were fully optimized, and stationary points on the respective potential energy surfaces were characterized as minima (no imaginary frequencies) or transition states (only one imaginary frequency) by harmonic vibrational frequency calculations. Natural bond orbital population analysis (NPA) was evaluated by means of the NBO program (*42*). The solvent effect was estimated by full optimizations with the polarized continuum model (PCM) (*43*−*46*) on the gas-phase optimized geometries.

Scheme 2. Nitrenium Ion Formation from the Hydroxylamine of Aniline and its Esters



(2)

Table 1. Calculations for Scheme 2 (kcal/mol)[a]

derivative	leaving anion	$\Delta E^{\neq}$	ΔE_{rec}[b]	$\Delta E_{r\infty}$[c]
1a	HO^-			190.01 (56.38)
1b	$CH_3CO_2^-$	36.73 (25.15)	35.92 (23.23)	147.40 (26.19)
1c	HSO_4^-	20.27 (5.96)	18.58 (3.87)	111.19 (3.28)
1d	SO_4^{-2}		45.96 (22.45)	252.52 (25.43)

[a] The single point PCM energy calculations are in parentheses. [b] Electrostatic complex as product. [c] Resulting ions at infinite separation.

Results and Discussion

Aniline was selected as the model system to analyze the reactivity of different precursors of the key nitrenium ion intermediate. In this manner, calculations on the N−O bond breaking process were performed for the *N*-hydroxy derivative (**1a**) as well as for its acetic (**1b**) and sulfuric esters (**1c**) (the deprotonated (negatively charged) analogue **1d** was also considered) (Scheme 2). Although the principal activation mechanism appears to be via the sulfuric acid esters (*9*) because of difficulties in their preparation, many studies have focused on the more stable *N*-acetoxyarylamines (*47*), for which there is also evidence of their intermediacy (*9*). Reaction and activation energies for the calculated reactions are shown in Table 1.

An electrostatic complex involving hydrogen-bond interactions between the resulting ions was found on the minimum side corresponding to products on the potential energy surfaces (Figure 1). This complex was more stable than the generated ions at infinite separation. According to the results, the most feasible N−O dissociation reaction corresponded to sulfuric acid ester **1c**, whereas the process from deprotonated derivative **1d** was much more endothermic. Acetic acid ester **1b** was the second one in reactivity. However, no electrostatic complex could be characterized with hydroxyaniline **1a** because the hydroxyl anion reacted with a carbon atom of the aniline nitrenium cation or abstracted a proton. This cleavage reaction was the most endothermic, in agreement with the lower reactivity of hydroxyarylamines as compared with their esters (*9*). Water as solvent remarkably stabilized the charged products diminishing the endothermicity, as expected, but the relative reactivity order remained the same.

The nitrenium ion derived from aniline presented a geometry more consistent with an imino carbenium ion, the dominant canonical form being the one in which the positive charge is localized at the *para*-carbon (Scheme 2), as evidenced by NPA-derived charges, C−N bond distance, and C−C bond length alternation in the ring. These observations are in agreement with previous reports (*48, 49*). Spectroscopic ^{13}C NMR studies on protonated aromatic imines had shown charge delocalization

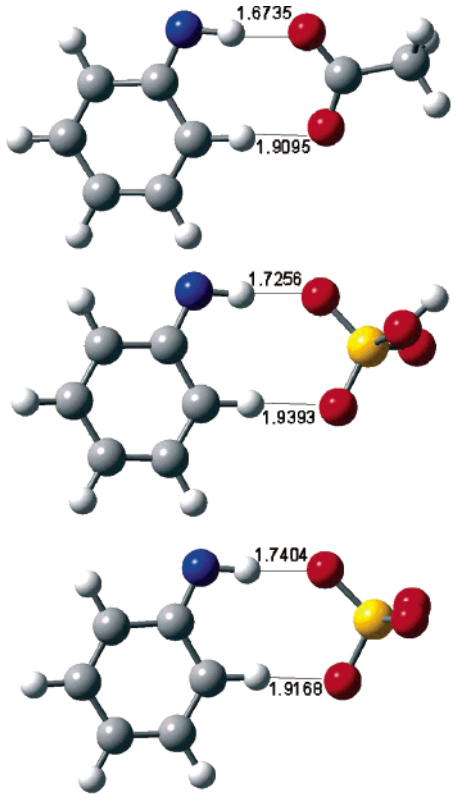


Figure 1. Electrostatic complexes between the nitrenium ion from aniline and the leaving ions studied.

into the phenyl ring, establishing the ambident carbocationic nature of the iminium ion via the aminocarbenium ion form (*50*).

Subsequently, eq 1 type reactions were calculated for a set of 17 esters of diverse aromatic and heteroaromatic amines. The *N*-acetoxy esters were preferred because of their lower computational cost as compared to the sulfuric esters. The parent amines are displayed in Figure 2. The amines were selected with the aim of covering a wide range of experimental values of the mutagenic potencies reported in the literature. Related structures were included in order to account for the effect of the number of rings, the influence of the nitrogen atom (heterocyclic amines vs carbocyclic amines), and the presence of a methyl substituent. In this manner, computed relative reactivities were estimated by the change in energy for the reactions of type 1. The ΔE_r parameters analyzed were those considering the electrostatic complex as product (ΔE_{rec}) as well as the resulting ions at infinite separation ($\Delta E_{r\infty}$). A relative nitrenium ion stability order can be inferred by comparison of the $\Delta E_{r\infty}$ values because the anion is always the same. This treatment allowed differentiation of the influence on the dissociation reaction of the stability of the generated complex in comparison with the ease of the formation of the nitrenium ion. Hence, an alternative analysis considering the availability for hydrogen bonding after N−O cleavage according to the geometrical features of the esters was made. Thus, the stabilization of the generated electrostatic complex was taken into account as another factor that could affect the biological activity, in addition to the consideration of nitrenium ion stability. Optimizations were performed in the gas phase and in water as solvent (PCM optimizations). The results are shown in Table 2.

For calculation of ΔE_r parameters, the most stable hydrogen-bonded complex was searched for each studied system, and the ester conformation preferred was the one leading to that complex via cleavage of the N−O bond. For nitrenium ions, only singlet electronic states were considered because previous calculations at various levels of theory indicate that *N*-arylnitrenium ions are singlet ground states (*48*, *54*). Because of extensive delocalization of the cationic charge by resonance through the aromatic system, the resulting structures more closely resembled imino carbenium ions, as expected according to refs *48* and *49*, and the NPA charge density at the exocyclic nitrogen was negative in all cases. Hence, for nonsymmetrical aryl substituents, alternative orientations of the NH bond give rise to two distinct configurational isomers, designated *syn* if the hydrogen of the NH group is oriented toward the β-ring carbon of higher priority (in the Kahn−Ingold−Prelog sense) and *anti* otherwise (*55*), both isomers being separated by substantial activation barriers (*49*, *56*). In this study, the most stable configuration for each cation was selected. It should be kept in mind that the traditional nitrenium ion designation will be maintained along this work, in spite of the actual imino carbenium nature of all the species.

Considering the values in Table 2, correlations were sought between the mutagenic potencies expressed as log MP (the logarithm of the number of histidine revertants in the Ames assay for *Salmonella typhimurium* strain TA98 + S9 microsomal preparation per nanomole of chemical) and calculated properties. The examined theoretical quantities were both the ΔE_r parameters mentioned above, the NPA charge at the exocyclic nitrogen atom of the nitrenium ion (q_N) and the change in charge density for this nitrogen ($q_{Nnitrenium} - q_{Nester}$, Δq_N). However, because q_N and Δq_N showed similar trends, only q_N was included in further discussions. Because of convenience in their analysis, log MP values were converted to ln MP (natural logarithm).

No apparent relationship was found when the whole set of ln MP values was considered (Figure 3). However, clear correlations were observed when the results were grouped for compounds of related structure. In this manner, a distinct functional dependence of ln MP with the mentioned parameters became noticeable for each group of amines when classified as aromatic (Ar), imidazo-carbocyclic (Imi-C), and imidazo-heterocyclic (Imi-H). According to this, Ar corresponds to aromatic amines (**1**−**5**), whereas heteroaromatic amines are denoted as Imi-C (amines presenting an imidazole ring fused with a carbocyclic aromatic moiety (**6**−**10**)) and Imi-H (imidazole fused to a heterocyclic system (**11**−**17**)). The different relationships of ln MP followed by each set might be ascribed to other aspects affecting the activity that were not taken into account in the present calculations, such as lipophilicity, solubility, and so forth. Furthermore, specific interactions of each type of compound that could take place within the reactive site were disregarded because the biological environment was not included in this model study. Nevertheless, correlations were evident for the three groups of amines considered.

For the Ar group, the charge density q_N for the nitrenium ions presented a linear relationship with ln MP ($r = -0.958$, $r^2 = 0.918$), whereas very good exponential correlations were observed for the heteroaromatic compounds ($r^2 = 0.993$ for Imi-C, and $r^2 = 0.996$ for Imi-H). Therefore, mutagenic activity increased when a more negative charge developed at the exocyclic nitrogen in all cases, although the dependence curve was different for each one of the three groups of amines. These trends were also followed by $\Delta E_{r\infty}$, which can be considered a measure of the relative stability of the nitrenium ions. Thus, the correlation coefficient for Ar was $r = -0.978$ ($r^2 = 0.956$), and for Imi-C and Imi-H were $r^2 = 0.972$ and $r^2 = 0.977$,

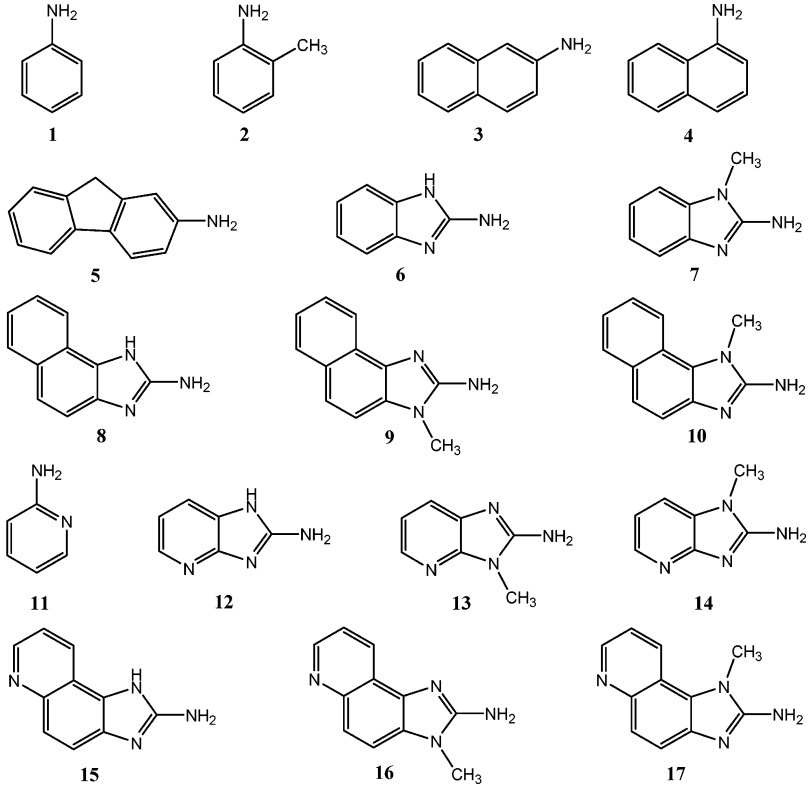


Figure 2. Parent aromatic and heteroaromatic amines considered in this study.

Table 2. Mutagenic Potencies for the Amines in Figure 2 and Calculated Properties for Their *N*-Acetoxy Esters

		change in energy (kcal/mol)[a]		NPA charge	
amine	log MP	ΔE_{rec}[b]	$\Delta E_{r\infty}$[c]	q_N[a]	Δq_N[d]
1	−3.390[e]	35.92 (21.60)	147.40 (25.62)	−0.342 (−0.412)	−0.001
11	−2.410[e]	48.55 (36.92)	162.62 (41.07)	−0.307 (−0.381)	0.035
6	−1.973[e]	34.78 (15.98)	132.79 (15.48)	−0.456 (−0.532)	−0.081
2	−1.800[e]	32.42 (16.82)	139.47 (20.37)	−0.384 (−0.435)	−0.046
8	−0.740[f]	28.84 (6.34)	118.37 (4.84)	−0.502 (−0.576)	−0.125
3	−0.670[g]	28.57 (15.83)	131.57 (17.81)	−0.413 (−0.489)	−0.077
4	−0.600[g]	25.98 (8.98)	128.04 (12.58)	−0.430 (−0.509)	−0.090
7	−0.430[e]	28.52 (13.50)	126.22 (14.72)	−0.477 (−0.535)	−0.089
12	0.030[e]	40.63 (22.64)	140.62 (22.42)	−0.437 (−0.512)	−0.067
13	0.370[e]	32.81 (18.82)	133.64 (20.74)	−0.458 (−0.518)	−0.080
9	0.590[e]	19.68 (2.38)	109.70 (2.60)	−0.524 (−0.585)	−0.130
14	1.022[e]	32.42 (19.96)	134.50 (21.95)	−0.466 (−0.518)	−0.082
5	1.260[h]	25.34 (8.03)	120.39 (9.78)	−0.442 (−0.525)	−0.121
15	1.760[f]	31.31 (11.30)	124.94 (10.26)	−0.490 (−0.553)	−0.114
10	2.290[e]	21.66 (4.72)	111.89 (3.92)	−0.524 (−0.581)	−0.128
16	4.700[e]	22.15 (7.39)	116.39 (8.34)	−0.510 (−0.560)	−0.118
17	5.790[e]	23.71 (9.19)	118.08 (9.35)	−0.513 (−0.560)	−0.118

[a] In water as solvent in parenthesis (PCM optimizations). [b] ΔE_{rec} = Energy$_{electrostatic\ complex}$ − Energy$_{ester}$. [c] $\Delta E_{r\infty}$ = Energy$_{ions\ at\ infinite\ separation}$ − Energy$_{ester}$. [d] Δq_N = Charge$_{N(nitrenium)}$ − Charge$_{N(ester)}$. [e] From data collected in ref *22*. [f] From ref *51*. [g] From data collected in ref *52*. [h] From ref *53*.

respectively. At the same time, q_N and $\Delta E_{r\infty}$ appeared to be strongly correlated to each other. In this case, the results for both heteroaromatic groups fitted almost the same line, and the aromatic compounds followed another linear function (Figure 3). Hence, the charge density at the exocyclic nitrogen is pointed out as an important factor in determining nitrenium ion stability. For the imidazole compounds, $\Sigma\Delta q_N$ (the summation of the changes in charge density for all nitrogen atoms in the molecule, that is, the exocyclic nitrogen and nitrogen atoms of the imidazole ring) also correlated with ln MP and $\Delta E_{r\infty}$. These observations are consistent with previous computational results on aza-polycyclic aromatic hydrocarbons (aza-PAHs), where the relative stability of carbocations derived from these compounds was found to increase with a more negative charge at N, independent of the degree of delocalization of the cation (assessed by the charge density at the carbocationic center) (*30*, *31*).

In cases where q_N (and $\Delta E_{r\infty}$) versus ln MP followed exponential correlations, the corresponding inverse functions, that is, ln MP as a function of q_N (or $\Delta E_{r\infty}$), were, consequently, logarithmic. In this manner, ln MP could be plotted against ln q (or ln $\Delta E_{r\infty}$). For a better comparison, the q_N (and $\Delta E_{r\infty}$) versus ln MP representations were preferred in order to include all groups of amines within the same Figure.

Considering ΔE_{rec}, a behavior similar to $\Delta E_{r\infty}$ was observed, and the same types of relationships (linear, exponential) were shown in every case (Figure 4). However, slightly lower correlations with ln MP and q_N were found, particularly for the heteroaromatic families, although a good correspondence between ΔE_{rec} and q_N was found for the aromatic amines (r^2 =

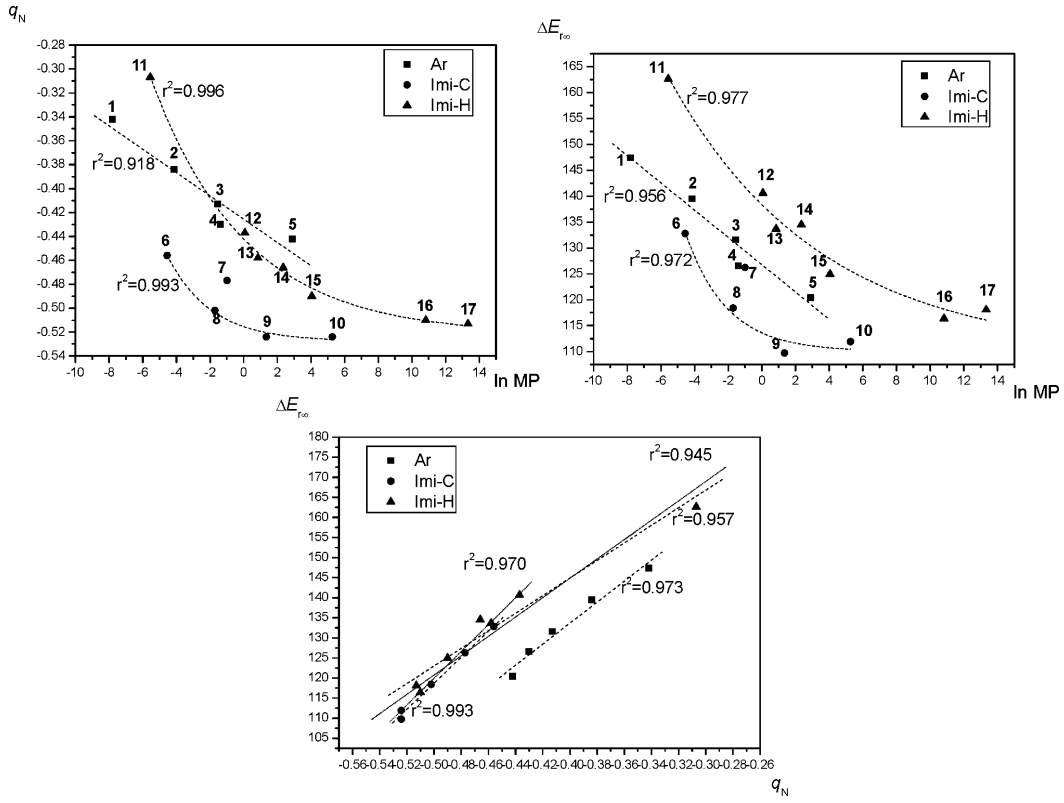


Figure 3. Correlations between ln MP, q_N, and $\Delta E_{r\infty}$.

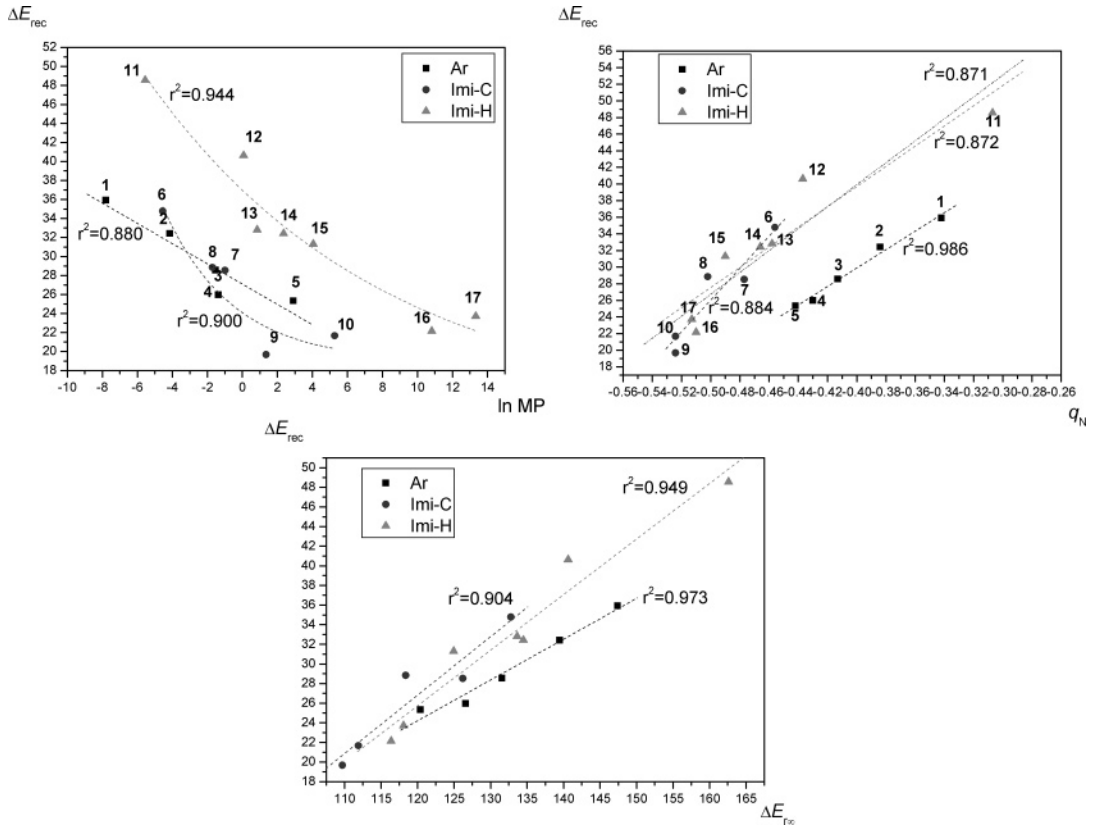


Figure 4. Correlations of ΔE_{rec} with ln MP, q_N, and $\Delta E_{r\infty}$.

0.986). According to this, the mutagenic potency seemed to be better explained by nitrenium ion stability than by the electrostatic complex stabilization. Compared to the nitrenium ion case, the charge density at the exocyclic nitrogen was less important in influencing the stability of the complex, which is mainly stabilized by the attainment of hydrogen-bond interactions.

Although methyl group substitution enhances the number of hydrogen atoms available for hydrogen bonding, no additional stabilization of the electrostatic complexes in comparison with nitrenium ion stabilization was observed. Thus, both ΔE_r values were affected in similar amounts for compounds having the same structure but differing by the presence of a methyl (**6** and **7**; **8**,

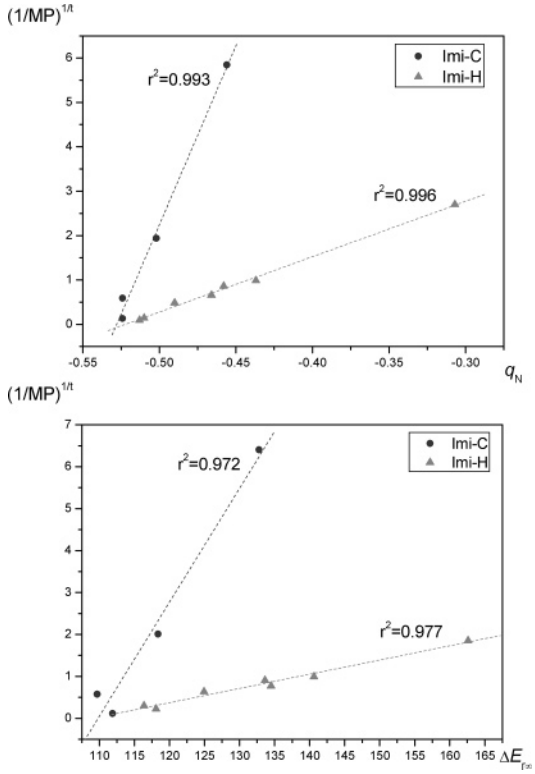


Figure 5. Linear correlations of the mutagenic potency with q_N and $\Delta E_{r\infty}$ for the heteroaromatic amines.

9, and **10**; **12**, **13**, and **14**; **15**, **16**, and **17**).

It should be mentioned that amine **7** was excluded from the correlations of ln MP. A bibliographic search provided another measurement for its mutagenic potency (log MP = 0.868) (*19*), although this value gave an even worse correspondence. Nevertheless, for the correlations where ln MP was not involved, that is, those concerning both ΔE_r values and q_N, **7** perfectly fitted into the corresponding curves. It is interesting to note that the mutagenicity order predicted by the present calculations is **6** < **7** < **8**, which is in agreement with the mutagenic potencies for the Imi-H related compounds (**12** < **14** < **15**). Considering this, the activity order yielded by these computations for the corresponding Imi-C amines looks reasonable and supports the reliability of the employed methods of calculation as well as the potential predictive capabilities of the structure−activity relationships established in this study.

In cases where exponential correlations were found for a calculated parameter y (both ΔE_r parameters and q_N for the heteroaromatic compounds) as a function of ln MP, the equations were of the type $y = Ae^{-\ln MP/t} + y_0$. Therefore, a linear relationship of $(1/MP)^{1/t}$ with y can be derived, that is, $(1/MP)^{1/t} = (y/A) - (y_0/A)$. In this manner, the increase of the mutagenic activity with the development of the negative charge at the exocyclic nitrogen (and with the stability of the nitrenium ion) can be visualized in a linear form, where very good correlation coefficients were obtained (Figure 5).

In the aqueous phase, the correlations for each group of amines were fairly similar to the gas-phase results (Figure 6). Moreover, because the correlation coefficients did not show a clear preference for any of the two sets of results, it could be assumed that solvation did not significantly affect the analysis. On this basis the gas-phase calculations seem to be adequate to understand the behavior of the system. The high correlation observed in water between both ΔE_r parameters is noteworthy because the results for the three groups of amines fitted a single line ($r = 0.989$, $r^2 = 0.978$). The solvent significantly stabilized the charged products, decreasing the endothermicity of both reactions, especially when the two separated ions were generated ($\Delta E_{r\infty}$). In water, the electrostatic complexes presented longer (less stable) hydrogen bonds and consequently were more similar to the separate ions. In this manner, consideration of the solvent effect also points to nitrenium ion stability ($\Delta E_{r\infty}$) as the major factor determining the reactivity of the amines.

Stability of the Nitrenium Ions. As stated above, the charge at the exocyclic nitrogen (q_N) was found to be very significant for nitrenium ion stability. When comparing aromatic amine **1** with the heteroaromatic one of related structure (**11**), the heteroaromatic nitrenium ion presented the lowest stability. Moreover, for related Imi-C and Imi-H compounds, the Imi-H nitrenium ions were the least stable. In all cases, q_N and Δq_N were more negative for the aromatic and Imi-C compounds than for the Imi-H ones. For the latter, the change in charge density at the nitrogen atom of the heteroaromatic ring was always positive. These observations are in concert with previous results, where the relative stability of the carbocations was found to increase with a more negative charge at N (*30*, *31*). This effect diminished as the fused system increased in size, improving delocalization (Figure 7). Thus, the difference in stability between **1** and **11** was the highest, followed by **6** and **12**, whereas the relative stability of **8** and **15** was the lowest among the three pairs.

In addition, the following observations were made by analyzing the effect of the molecular structure on $\Delta E_{r\infty}$ values in Table 2. First, the increase in the number of fused rings favored the ion stability by resonance. This fact can be seen by comparing the relative stability series **1** < **3**, **4** < **5**, **1** < **6** < **8**, and **11** < **12** < **15**.

Substitution by a methyl group favored nitrenium ion stability by hyperconjugation and inductive effects. However, in some cases where the amines differed in the methyl position, the stability of the ions did not follow the mutagenicity order. Hence, for the **9**−**10**, **13**−**14**, and **16**−**17** pairs, some other factors not contemplated by the present calculations must also be affecting biological activity.

Amide Derivatives. Even though 2-acetylaminofluorene (AAF), originally intended for use as a pesticide but never marketed because of its great carcinogenicity, is one of the best known and most potent carcinogens derived from aromatic amines (*12*), mutagenicity has generally been observed to decrease by acetylation of the amine group (*13*, *14*). Heterolysis of the N−O bond to give a nitrenium ion has been calculated to be less favorable in the arylamides because of the loss of amide resonance in their precursors and not because of inductive destabilization of the arylnitrenium ion by the *N*-acetyl group (*57*, *58*). These computations were performed at the AM1 and HF/3-21G levels, together with single point MP2/6-31G*//HF/3-21G calculations.

In this work, reactions of type 1 were also calculated for the *N*-acetoxy esters of acetanilide (**18**) and AAF (**19**) at the higher B3LYP/6-31+G* level in order to compare their results with those from **1** and **5**, respectively (Figure 8). The results are displayed in Table 3. The N−O bond-breaking reaction was more favorable (less endothermic) for the amines. Accordingly, the activation energy for **1** was lower than the value calculated for **18**. The approximately orthogonal conformation adopted by the carbonyl group in the *N*-acetyl nitrenium ions precludes resonance with the aromatic system. According to the NPA charges, nitrenium ions derived from the amides presented a minor negative charge at the nitrogen atom than those derived

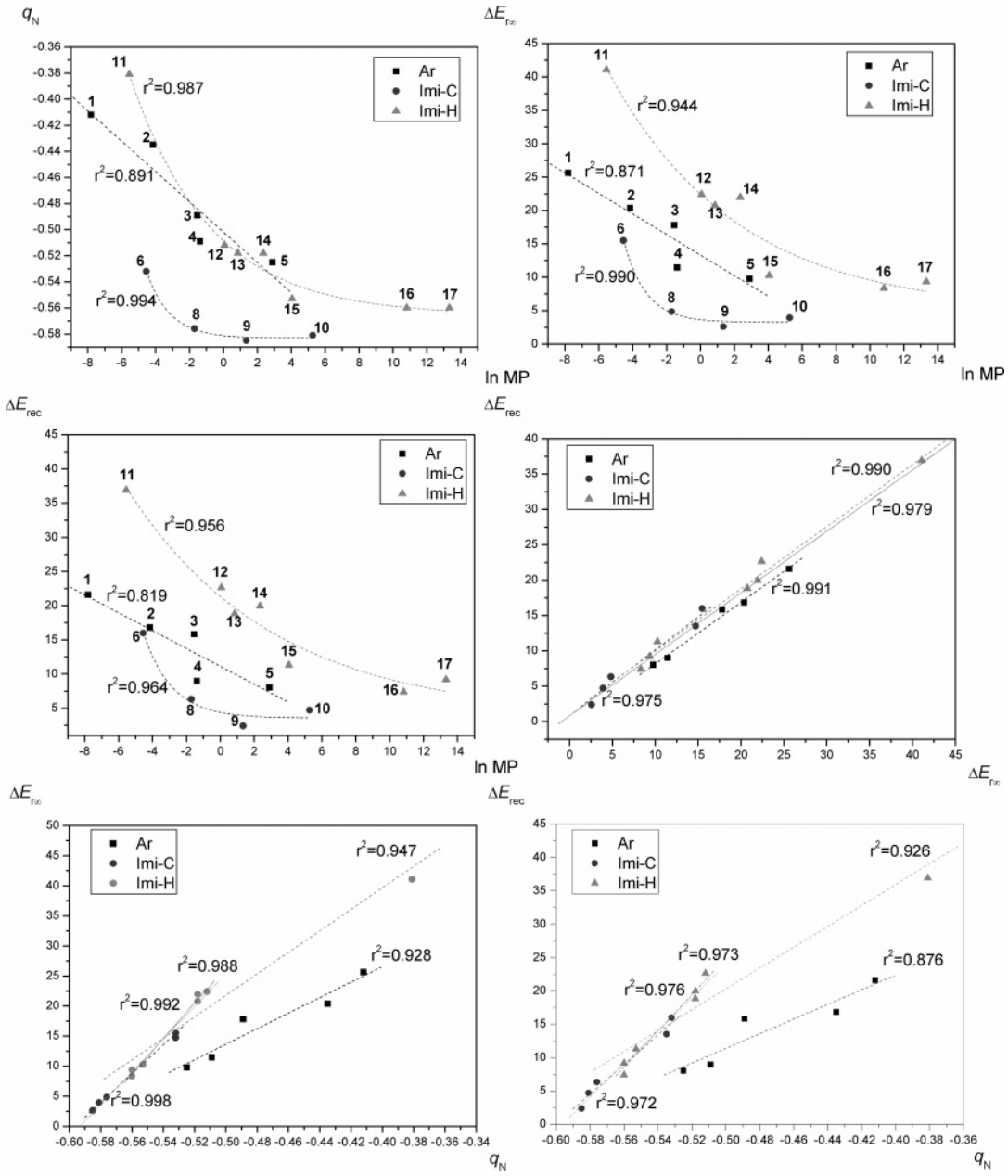


Figure 6. Correlations in water as solvent.

from the amines. However, the change in charge density for the exocyclic nitrogen (Δq_N) was more favorable (more negative) for the nitrenium ion derived from acetanilide than that from aniline, and Δq_N was almost similar when comparing **5** and **19**. In contrast, electron donation brought about by the acetyl group resulted in an important decrease of the negative charge at the oxygen atom of the carbonyl (Table 3). These observations are in accord with refs *57* and *58*, where the acetyl substituent, despite being generally regarded as a powerful electron-withdrawing group, in the nitrenium ion derived from acetanilide was found to act as a σ-electron donor of almost identical strength as a methyl group. On this basis, the formation of *N*-acetyl nitrenium ions seems to be hindered by an unfavorable polarization of the carbonyl group. These results agree with the reduction in mutagenicity observed for the acetamido derivatives in comparison with the corresponding amines (*13*, *14*).

Conclusions

The formation of a nitrenium ion, the ultimate electrophilic metabolite from aromatic and heteroaromatic amines that covalently binds to DNA, was more plausible through the N−O dissociation reaction from an ester of the parent amine than from the related hydroxylamine, in accordance with known experimental reactivity. Particularly, sulfuric acid esters were more suitable precursors than acetic esters. A hydrogen-bonded electrostatic complex between the resulting ions was found as a product on the potential energy surface. These complexes were more stable than the ions at infinite separation.

For the series of 17 *N*-acetoxy esters derived from the aromatic and heteroaromatic amines considered, no correlation for the complete set was found between the experimental mutagenic potencies and the calculated properties such as the reaction energy for the electrostatic complex (ΔE_{rec}) or nitrenium ion formation ($\Delta E_{r\infty}$) and the charge density at the exocyclic nitrogen of the nitrenium ion (q_N). In contrast, clear correlations were observed when the results were grouped for compounds of related structure, classified as aromatic (Ar), imidazo-carbocyclic (Imi-C), and imidazo-heterocyclic (Imi-H). Thus, the mutagenic activity increased with the development of a more

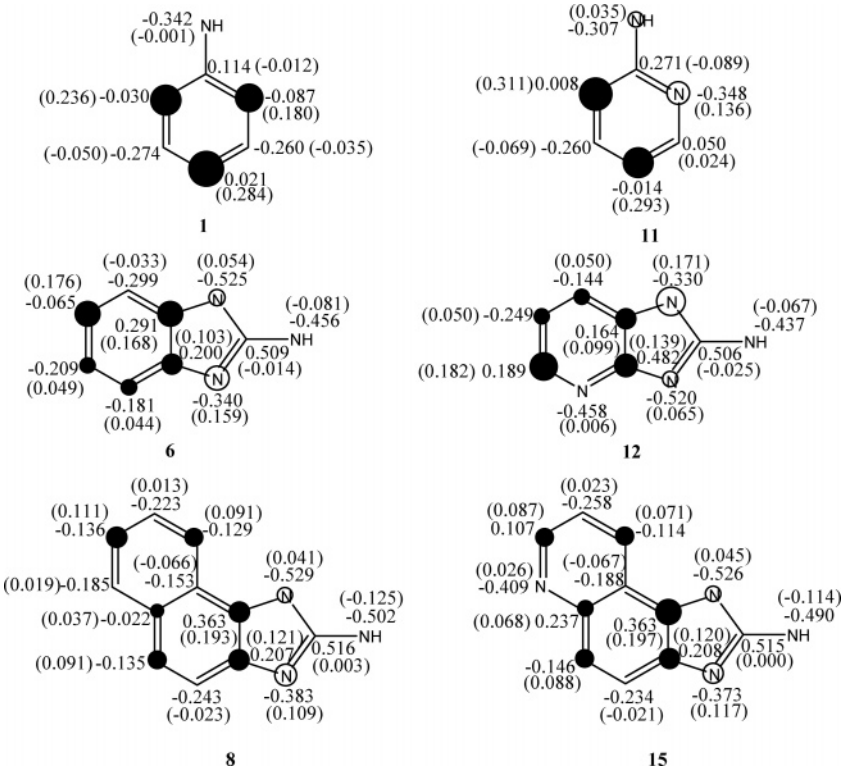


Figure 7. Computed gas-phase NPA heavy atom charge densities (Δcharges relative to the neutral ester in parentheses) for nitrenium ions. (The dark circles are roughly proportional to the magnitude of C Δcharges and white circles to N Δcharges; the threshold was set to 0.030.)

Table 3. Mutagenic Potencies for the Amides in Figure 8 and the Corresponding Amines and Calculated Results for Their *N*-Acetoxy Esters

			change in energy (kcal/mol)[a]		NPA charges			
amine	log MP	$\Delta E^{\neq a}$	ΔE_{rec}[b]	$\Delta E_{r\infty}$[c]	q_N	Δq_N[d]	q_O	Δq_O[e]
1	−3.390[f]	36.73 (25.15)	35.92 (23.23)	147.40 (26.19)	−0.342	−0.001	-	-
18	[g]	38.37 (31.75)	38.34 (31.58)	147.59 (33.96)	−0.223	−0.047	−0.469	0.133
5	1.260[h]	-	25.34 (3.06)	120.39 (3.17)	−0.442	−0.121	-	-
19	1.186[f]	-	29.67 (15.94)	119.07 (12.71)	−0.319	−0.116	−0.523	0.068

[a] The single point PCM energy calculations are in parenthesis. [b] ΔE_{rec} = Energy$_{electrostatic\ complex}$ − Energy$_{ester}$. [c] $\Delta E_{r\infty}$ = Energy$_{ions\ at\ infinite\ separation}$ − Energy$_{ester}$. [d] Δq_N = Charge$_{N(nitrenium)}$ − Charge$_{N(ester)}$. [e] Δq_O = Charge$_{O(nitrenium)}$ − Charge$_{O(ester)}$. [f] From data collected in ref *22*. [g] No indication for mutagenic effects (*59*). [h] From ref *53*.

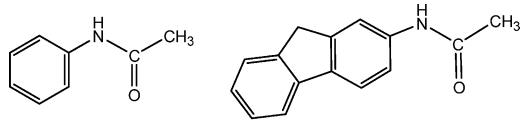


Figure 8. Parent aromatic amides considered in this study.

negative q_N and with the decrease of $\Delta E_{r\infty}$, although each group of amines followed a different functional relationship of the mutagenic potency with the parameters mentioned. Furthermore, q_N and $\Delta E_{r\infty}$ were strongly correlated to each other, both heteroaromatic groups fitting almost the same line, whereas the aromatic compounds followed another line. Hence, q_N is pointed out as an important issue in determining nitrenium ion stability. However, lower correlations were observed with ΔE_{rec}.

The solvent significantly stabilized the charged products, decreasing the endothermicity of both reactions, especially $\Delta E_{r\infty}$. Nevertheless, aqueous-phase calculations pointed to similar conclusions than gas-phase results. Moreover, ΔE_{rec} followed a single line correlation with $\Delta E_{r\infty}$ for all of the compounds studied, a further indication of the importance of nitrenium ion stability in influencing the mutagenic potency of amines. According to this, more costly computations taking into account the solvent effect would not be crucial for the study of this system.

Heteroaromatic nitrenium ions were less stable than the respective aromatic ones when comparing aromatic and heteroaromatic related structures. Imi-C derived nitrenium ions were also more stable than the related Imi-H intermediates because of their more negative q_N values. In addition to a more negative value of q_N, nitrenium ion stability was also favored by the increase in the number of fused rings (resonance effect) and by methyl substitution (hyperconjugation and inductive effects).

The formation of *N*-acetyl nitrenium ions from amides was found to be a process less favorable than nitrenium ion generation from the corresponding amines. This fact can be ascribed to the loss of negative charge density at the oxygen atom by polarization of the carbonyl group of the amides.

According to the present computations, nitrenium ion stability is a key factor in determining mutagenic potency. Better correlations were obtained for related compounds (aromatic and heteroaromatic). This might be attributed to the fact that other aspects that could affect activity, such as lipophilicity and solubility as well as specific interactions with the biological environment, were not taken into account in these calculations. Nevertheless, the computational results for each group of compounds in this study agree fairly well with the measured activity of the amines under consideration. Thus, the low correlations with ab initio nitrenium ion stabilities observed in

previous regression models (*22*) could have been based on a consideration of the set of amines of very different structure.

The present results suggest that DFT calculations can provide reasonable estimations of relative mutagenic potencies for structurally related compounds. Accordingly, theoretical investigations of this type could also be of predictive value. Further studies involving more aromatic and heteroaromatic amines are currently in progress, aiming to extend the observations from this work to a broader spectrum of compounds.

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Supporting Information Available: Cartesian coordinates for optimized geometries presented in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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