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Carcinogenic carbocyclic and heterocyclic aromatic amines: A DFT study concerning their mutagenic potency

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ABSTRACT

The relative stability of the nitrenium ions derived from a set of 43 aromatic and heteroaromatic amines of diverse structure was evaluated by density functional theory (DFT) calculations in order to examine the role of these electrophilic intermediates on mutagenic activity. Correlations were sought between calculated properties and mutagenic potencies from the literature. Mutagenicity was found to increase with nitrenium ion stability, which was favored by negative charge development at the exocyclic nitrogen of the ion (q_N). Distinct correlation functions were observed for the amines grouped according to their classification as aromatic (Ar), heteroaromatic (HAr), imidazocarbocyclic (ImiAr), imidazoheterocyclic (ImiH), dipyridoimidazoles (PI), and quinoxalines (Qx). The influence of the logarithm of the octanol/water partition coefficient (Log *P*) on the mutagenic potency of the amines selected for this study was also analyzed.

1. Introduction

Aromatic amines (AAs) are industrial and environmental carcinogens [1,2], and exposure occurs in different industrial activities (dyes used in textile, paper, plastic, pharmaceutical, cosmetic, and food industries), as well as in tobacco smoking. Heteroaromatic amines (HAs) found in well-done or over cooked meats and protein rich foods have also been determined to be mutagenic and carcinogenic [3–8]. The biological activity of these compounds is developed by enzymatic metabolic activation [9–11]. The initial step is the N-oxidation to aryl *N*-hydroxylamines [9], which undergo N–O bond cleavage to arylnitrenium ions under mildly acidic conditions [11], whereas further activation to the sulfuric or acetic acid ester of the *N*-hydroxylamine allows a more facile heterolysis of the N–O bond [9–11]. Thus, a highly reactive nitrenium ion is generated, which is the ultimate electrophilic metabolite that covalently binds and alters DNA (Fig. 1) [9–11].

The relative stability of the nitrenium intermediate has been pointed out to be essential in determining the activity of AAs [9]. According to this, several studies have developed quantitative structure–activity relationships (QSARs) intending to correlate quantitative bacterial mutagenicity and carcinogenicity data for AAs and HAs with calculated or observed properties of the amines or their derived nitrenium ions [12–24]. Some of them have proposed that mutagenicity increases with the rate of nitrenium ion formation from their precursors, i.e., with a higher nitrenium intermediate stability, as computed with the semi-empirical methods AM1, PM3 or PM5 [14–19]. On the other hand, multiple variable models including higher level ab initio calculated variables related to nitrenium ion stability have suggested that these variables are of only limited use in regression models [12,13,20–22], questioning the importance of nitrenium ion stability in determining the mutagenic potency of amines [22].

In view of this divergence between lower level semiempirical and Hartree-Fock ab initio results in relation to the importance of nitrenium ions on the mutagenicity of this type of compounds, this topic was recently examined by higher level density functional theory (DFT) calculations, which consider electron correlation [25]. Thus, formation of these electrophilic intermediates from their precursors was analyzed, and correlations were sought between experimental mutagenic potencies reported in the literature and calculated reaction energies and electronic properties for a series of AAs and HAs of different structure. Hence, clear correlations were observed when the results were grouped for compounds of related structure, and each group of amines followed a different functional relationship, showing that mutagenic activity increased with nitrenium ion stability. Furthermore, the charge density at the exocyclic nitrogen of the nitrenium ion (q_N) was strongly correlated with stability, indicating that development of a more negative q_N improved nitrenium ion stability [25]. Afterward, DFT

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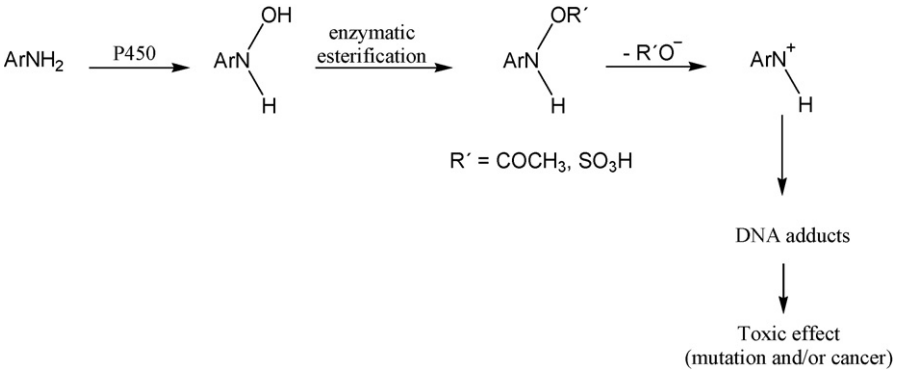

Fig. 1. Metabolic activation for aromatic amines.

calculations on the stability of isomeric nitrenium ions derived from benzanthrone were applied to explain their differences in mutagenicity [26].

Chemical–biological relationships have crucial importance in the elucidation of the reaction mechanisms involved in chemical carcinogenesis, and are also helpful for predicting the potential activity of new compounds. Considering the significance of ascertaining relationships between molecular structure and biological activity, the present work represents a continuation of our computational studies on carcinogenic derivatives. Hence, with the aim of achieving a better understanding of the role of nitrenium ions on the reactivity of AAs and HAs, the previous series of 17 amines [25] is now extended to 43 compounds. The relative stabilities of their corresponding nitrenium ions were evaluated.

The possible importance of hydrophobic interactions on the genotoxic activity of molecules have caused the use of hydrophatic factors in a variety of mutagenicity QSARs. Particularly, earlier QSARs for the mutagenicity of amines had established that activity was primarily determined by their hydrophobicity [27]. Taking this into account, the role of the logarithm of the *n*-octanol/water partition coefficient (Log *P*) on the mutagenic activity of the amines in this study was analyzed.

2. Computational methods

All calculations were performed using standard techniques with the Gaussian 03 package of programs [28]. The B3LYP hybrid functional [29–31] was employed in conjunction with the polarization-function augmented 6–31G(d) split-valence shell basis set. All geometries were fully optimized without imposing symmetry constraints. Optimized structures were subsequently checked with respect to being true minima on the respective potential energy surfaces (equilibrium structures, no imaginary frequencies) by harmonic vibrational frequency calculations at the same level and with the same basis set used for the optimization procedure. Natural population analysis (NPA)-derived charges were computed at the same level of theory by means of the NBO program [32].

3. Results and discussion

In order to properly represent this group of chemicals, a diverse set of AAs and HAs was selected, covering the wide range of experimental values for mutagenic potencies reported in the literature (5.790 to −3.390) [22], which is within the normal scope for organic compounds. Moreover, related structures were included to account for the influence of the nitrogen atom, the effect of the number of rings, and the presence of methyl substituents. HAs are generally classified into two structural groups: IQ type (aminoimidazoazaarenes), and non-IQ type, based on the presence or absence of an imidazoquinoline, imidazoquinoxaline, or imidazopyridine ring system [33,34]. The amines studied are displayed in Fig. 2.

Relative nitrenium ion stabilities were estimated by comparing the computed change in energy (ΔE_r) for the formation of the ion from the corresponding parent amine in gas-phase (reaction 1). In the previous study, water as solvent significantly stabilized the nitrenium ions decreasing the endothermicity of the reactions [25]. However, aqueous-phase calculated stabilities and properties showed the same trends as those indicated by gas-phase results, and pointed to similar conclusions. Therefore, only gas-phase calculations were carried out in the present work as a way to reduce the computational costs. Reaction energies for the calculated reactions are shown in Table 1.

$$\text{Ar-NH}_2 \xrightarrow{-H^-} \text{Ar-}\overset{+}{\text{N}}\text{H} \quad (1)$$

For nitrenium ions only singlet electronic states were considered since previous calculations at various levels of theory indicated that *N*-arylnitrenium ions are singlet ground states [35,36]. Due to extensive delocalization of the cationic charge by resonance through the aromatic system, the nitrenium ion structures more closely resembled imino carbenium ions. This was evidenced by NPA-derived charges (the NPA charge density at the exocyclic nitrogen was negative in all cases), the C–N bond distance, and C–C bond length alternation in the rings. These observations are in agreement with those of previous reports [25,36,37]. 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 [38], both isomers being separated by substantial activation barriers [37,39]. In this study the most stable configuration for each cation was considered. It should be noticed 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 data in Table 1, correlations were looked for between mutagenic potencies expressed as Log MP (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 theoretical quantities examined were ΔE_r (nitrenium ion stability, as defined by reaction 1), and the NPA charge at the exocyclic nitrogen atom of the nitrenium ion (q_N). As previously noticed [25], better correlations were found when the results were grouped for compounds of related structure. Thus, distinct functional dependences of Log MP with the parameters mentioned were observed

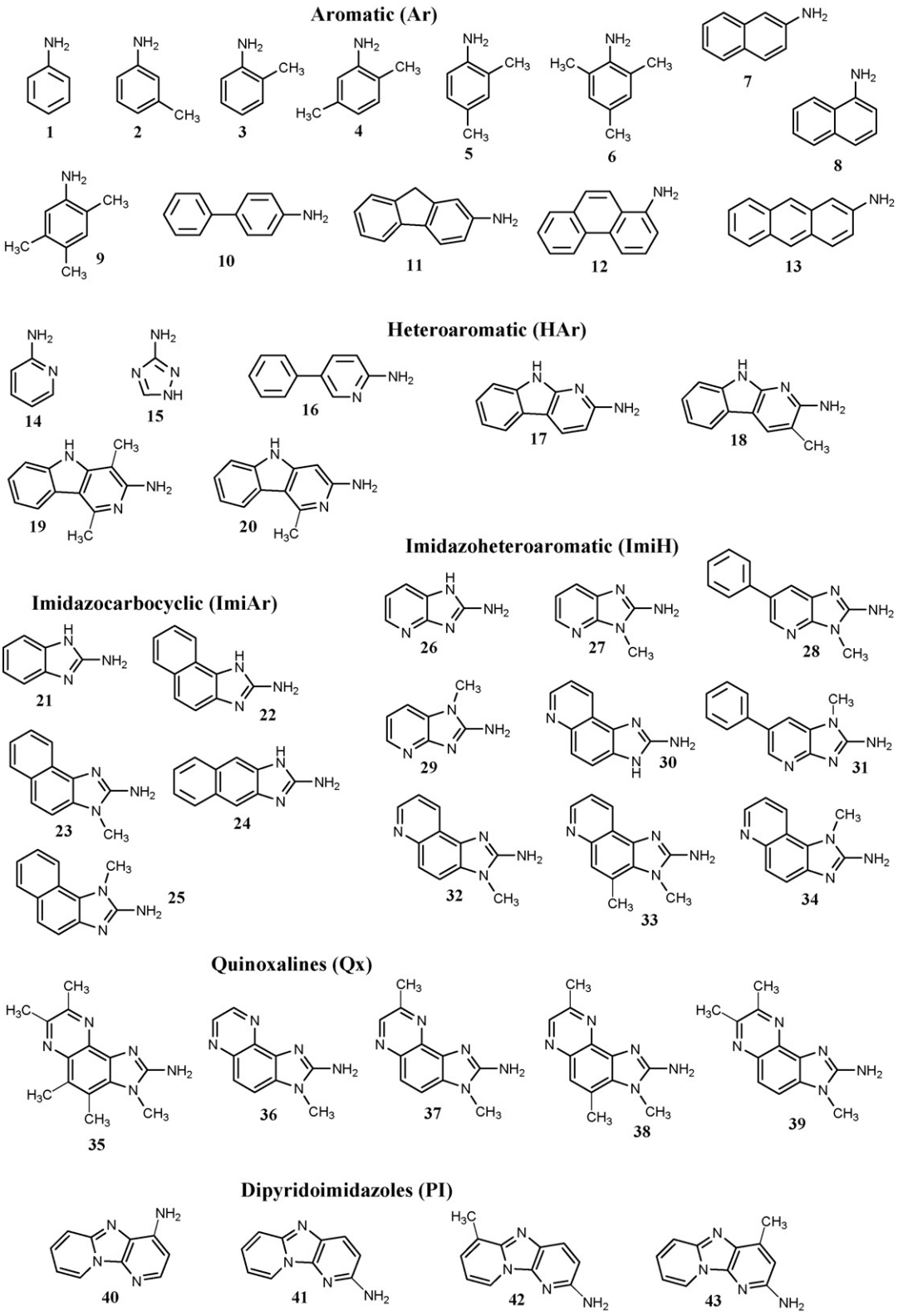

Fig. 2. AAs and HAs studied.

for each group of amines when classified as: aromatic (Ar, **1**–**13**), heteroaromatic (HAr, **14**–**20**), imidazocarbocyclic (ImiAr, amines presenting an imidazole ring fused with a carbocyclic aromatic moiety, **21**–**25**), imidazoheterocyclic (ImiH, imidazole fused to a heterocyclic system, **26**–**34**), quinoxalines (Qx, **35**–**39**), and dipyridoimidazoles (PI, **40**–**43**).

Thus, for the Ar group, ΔE_r presented an exponential relationship with Log MP, the correlation coefficient being $r^2 = 0.914$ (13 compounds). For the heteroaromatic compounds, very good exponential correlations were observed ($r^2 = 0.943$ for HAr (8 compounds), $r^2 = 0.991$ for ImiAr (4 compounds), $r^2 = 0.956$ for Qx (4 compounds), and $r^2 = 0.994$ for PI (4 compounds)). For the ImiH

Table 1
Experimental and calculated properties for the amines in Fig. 2

Amine	Log MP	ΔE_r (kcal/mol)	q_N	Group	Log P[a]
1	−3.390[a]	294.71	−0.343	Ar	0.915
2	−2.920[a]	290.84	−0.350	Ar	1.564
14	−2.410[a]	311.40	−0.311	HAr	0.145
21	−1.973[a]	279.29	−0.460	ImiAr	0.140
15	−1.900[a]	313.58	−0.327	HAr	−1.413
3	−1.800[a]	286.81	−0.385	Ar	1.564
4	−1.640[a]	282.86	−0.395	Ar	2.213
5	−1.160[a]	277.72	−0.408	Ar	2.213
6	−1.110[a]	272.64	−0.425	Ar	2.862
22	−0.740[b]	260.46	−0.509	ImiAr	1.114
7	−0.670[c]	278.70	−0.411	Ar	1.889
8	−0.600[c]	275.28	−0.428	Ar	1.889
40	−0.126[a]	279.89	−0.425	PI	0.315
26	0.030[a]	284.78	−0.443	ImiH	−0.695
9	0.361[a]	274.93	−0.413	Ar	2.862
27	0.370[a]	282.70	−0.463	ImiH	0.061
10	0.490[d]	274.34	−0.425	Ar	2.603
23	0.590[a]	256.60	−0.527	ImiAr	1.881
28	0.650[a]	274.27	−0.499	ImiH	1.749
16	0.830[a]	286.31	−0.411	HAr	1.833
29	1.022[a]	283.17	−0.472	ImiH	0.093
11	1.260[d]	267.60	−0.438	Ar	2.708
24	1.540[a]	276.16	−0.490	ImiAr, HAr	1.114
18	1.591[e]	272.09	−0.455	HAr	1.781
30	1.760[b]	268.62	−0.494	ImiH	−0.363
17	1.900[a]	277.61	−0.426	HAr	1.132
12	2.110[a]	268.31	−0.469	Ar	2.503
13	2.170[a]	267.10	−0.459	Ar	2.863
25	2.290[a]	258.77	−0.528	ImiAr	1.881
41	2.360[a]	270.77	−0.449	PI	0.529
31	2.497[e]	270.07	−0.514	ImiH	1.781
35	2.960[a]	256.79	−0.526	Qx, Ar	2.377
43	3.400[a]	268.25	−0.453	PI	1.178
19	3.915[e]	267.12	−0.470	HAr	2.027
42	3.980[a]	268.56	−0.454	PI	1.098
20	4.170[a]	273.33	−0.437	HAr	1.378
36	4.320[a]	270.03	−0.497	Qx	−0.139
37	4.489[f]	266.72	−0.502	Qx	0.510
38	4.560[a]	264.57	−0.510	Qx	1.079
39	4.660[a]	264.20	−0.506	Qx	1.159
32	4.700[a]	263.62	−0.514	ImiH	0.404
33	5.150[a]	261.61	−0.521	ImiH	0.973
34	5.790[a]	265.26	−0.517	ImiH	0.404

[a] From data collected in Ref. [22].
[b] From Ref. [43].
[c] From data collected in Ref. [27].
[d] From Ref. [44].
[e] From data collected in Ref. [15].
[f] From data collected in Ref. [16].

group, the correlation coefficient was somewhat lower (r^2 = 0.854, 9 compounds). Therefore, mutagenic activity increased with the stability of the nitrenium ions, although the dependence curve was different for each one of the groups of amines. Correlations are shown in Fig. 3.

Interestingly, combination of the distinct heteroaromatic groups into larger sets afforded remarkably good correlations, some of them even better than those for the separate groups. Thus, for HAr + Qx (12 compounds), r^2 = 0.953; for HAr + Qx + PI (16 compounds), r^2 = 0.933; for HAr + Qx + PI + ImiH (25 compounds), r^2 = 0.914. Hence, almost all the HAs fitted one curve. These observations reinforce the hypothesis that mutagenic activity is strongly influenced by nitrenium ion stability.

On the other hand, addition of the ImiAr family to the last group of 25 compounds resulted in a weaker correlation. However, inclusion of compound **24** to the HAr group gave better results than with ImiAr. These facts could stem from other aspects (not considered in these calculations) that possibly affect the activity, such as solubility, transport, and specific interactions with the biological environment. Another interesting example was compound **35**, which did not fit into the Qx correlation, but successfully added to the Ar group (r^2 = 0.904), probably due to its high lipophilicity.

Mutagenicity was also found to increase with negative charge development at the exocyclic nitrogen of the nitrenium ion (q_N). At the same time, q_N and ΔE_r were strongly correlated between each other (Fig. 3). In this case, the linear correlation coefficient for Ar was r = 0.968 (r^2 = 0.938), for HAr, r = 0.990 (r^2 = 0.980), for ImiAr, r = 0.943 (r^2 = 0.890), for ImiH, r = 0.935 (r^2 = 0.874), for Qx, r = 0.984 (r^2 = 0.968), and for the PI family, r = 0.997 (r^2 = 0.995). The negative charge density at the exocyclic nitrogen is therefore seen as a significant factor in determining nitrenium ion stability, and can be considered a measure of the extent of delocalization of the net positive charge within the π system of the cation. These observations are consistent with those in the earlier study [25], as well as 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, independently of the degree of delocalization of the cation (assessed by the charge density at the carbocationic center) [40,41].

It is worthy of note that those amines having a terminal imidazole ring (ImiAr, ImiH and Qx) appeared to follow the same correlation function for ΔE_r *vs.* q_N, while a second correlation was observed for the remaining groups (Ar, HAr, and PI) (Fig. 3).

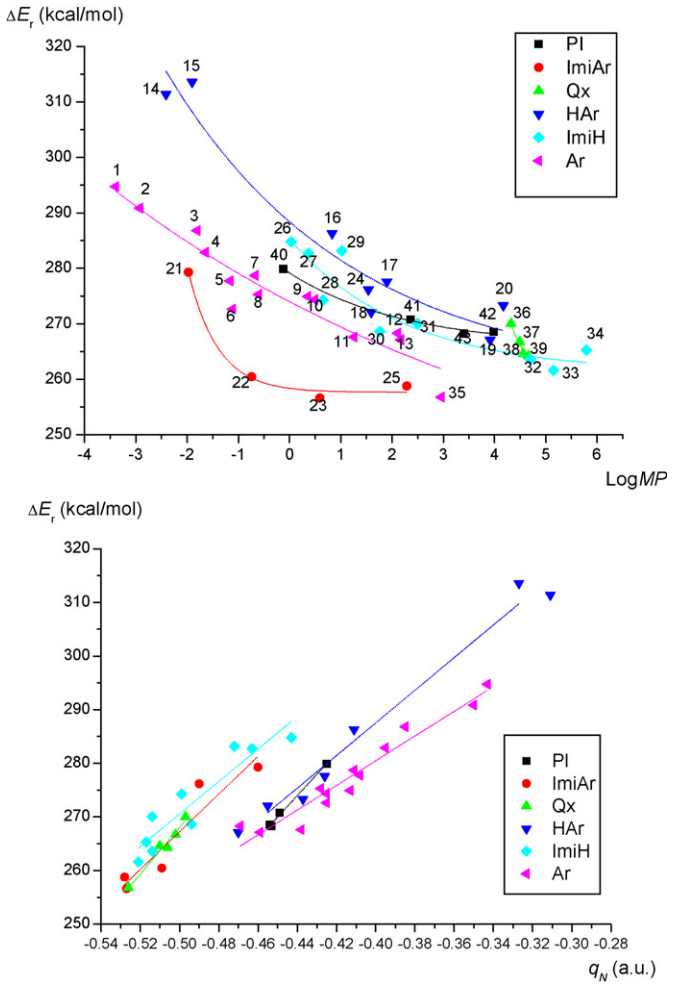

Fig. 3. Correlations between Log MP, ΔE_r, and q_N.

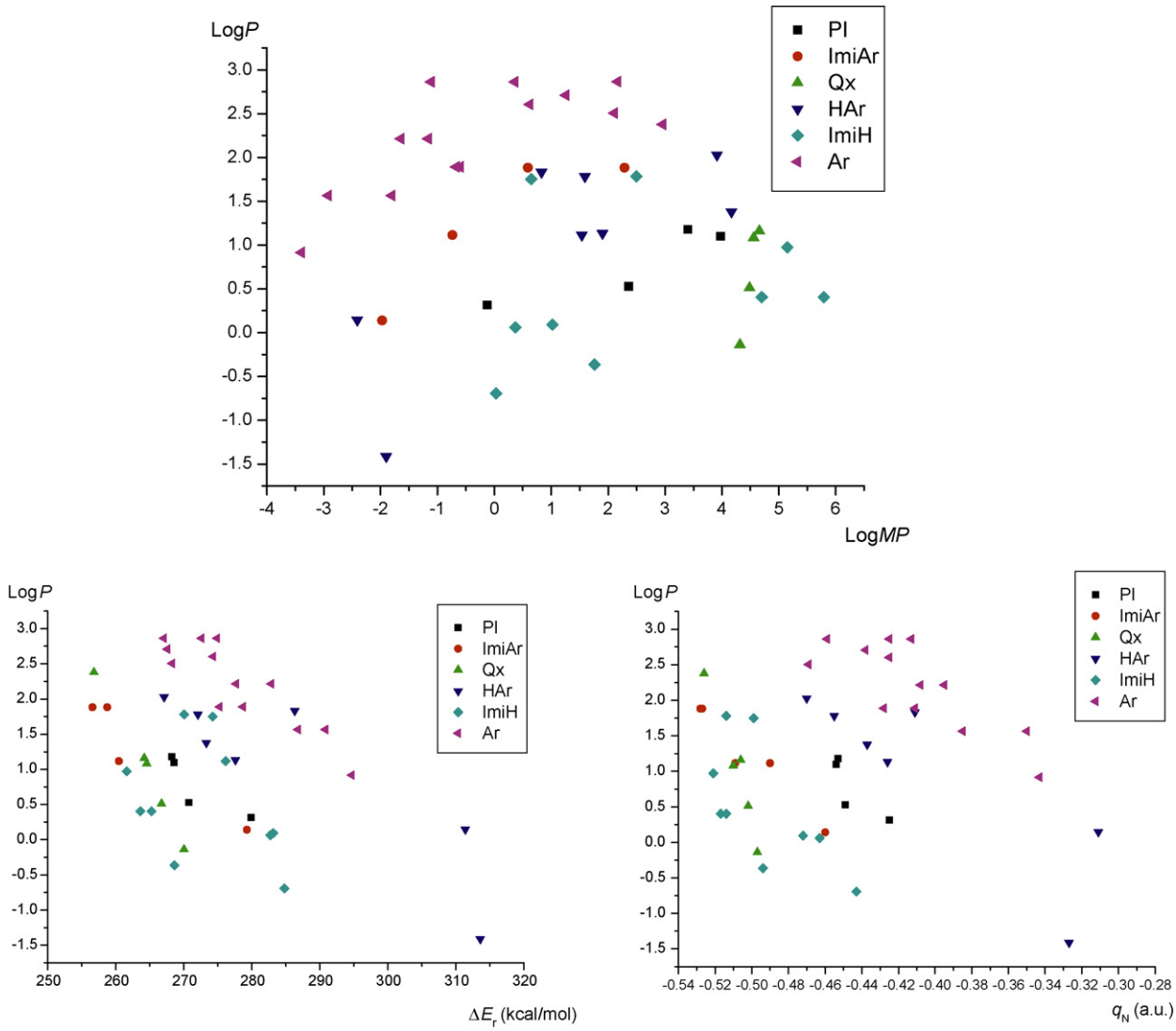

Fig. 4. Correlations between Log *P*, Log MP, ΔE_r, and q_N.

3.1. Nitrenium ion stability

When comparing the relative stability of nitrenium ions derived from AAs with those from the HAs of related structure (**1**–**14**, **10**–**16**), the aromatic cations were the most stable. Moreover, among related heteroaromatic compounds (**21**–**26**, **22**–**30**, **23**–**32**–**36**, **25**–**34**), nitrenium ion stability decreased when the number of nitrogen atoms increased. In all cases, q_N was more negative for most stable structures.

The increase in the number of rings favored the cation stability by resonance. This fact can be seen by comparing the relative stability series **1** < **7**, **8**, **10** < **11**, **12**, **13**; **1** < **21** < **22**, **24**; and **14** < **16**, **26** < **17**, **30**, **40**, **41**.

Substitution by methyl groups favored nitrenium ion stability by hyperconjugation and inductive effects. In this way, stabilization by methyl addition can be observed in the series **1** < **2**, **3** < **4**, **5** < **6**, **9**; **17** < **18**, **20** < **19**; **22** < **23**, **25**; **26** < **27**, **29**; **30** < **32**, **34** < **33**; **36** < **37** < **38**, **39** < **35**; and **41** < **42**, **43**. However, for some pairs of amines differing in the number or position of methyls, the stability of the cations did not follow the mutagenicity order. This could indicate that other factors not contemplated by these calculations should be affecting the activity, such as steric interactions within the active site of the enzymes involved in the activation pathway, or intercalation into the DNA. Similar observations have recently been made concerning the important difference in mutagenic potency between **28** and **31**, which only differ in the position of the methyl group [42].

3.2. The role of hydrophobicity

Hydrophobicity plays an important role in the absorption and transport of the chemicals to their sites of activation and chemical reaction, as well as in the interaction of the compounds with the bioreceptors responsible for activation. Different QSAR studies for AAs and HAs where hydrophatic factors were considered have led to contradictory conclusions. Whereas earlier reports indicated that bioactivity was predominantly determined by the hydrophobicity of the amine [27,23], more recent results revealed that hydropatic factors made only a small contribution to mutagenic potency [13,22].

In this work, the influence of hydrophobicity on mutagenic activity was examined by plotting Log *P* as a function of Log MP (Fig. 4). Log *P* data were taken from Ref. [22]. Although positive correlations could be observed for each series of compounds, they were not as good as those between ΔE_r and Log MP. As a general trend, a higher activity was associated with relatively large Log *P*

values within each group. Similarly, the lower ΔE_rs and more negative q_Ns data (indicative of more stable nitrenium ions) corresponded to higher Log *P* values (Fig. 4). The higher hydrophobicity of the AAs in relation to the HAs was also evidenced.

On the other hand, considering the whole set of compounds, the most active structures were those presenting a comparatively low hydrophobicity, i.e., HAs. This remark is in line with the higher mutagenicity of the HAs when compared to their structurally related AAs, which was not explained by the relative nitrenium ion stabilities. That is, the more active HAs, which present lower Log *P* values, formed less stable nitrenium ions, while the corresponding more hydrophobic and less active AAs formed comparatively more stable cations. These observations applied to the following pairs of compared Ar *vs.* HAr structures: **1**–**14**; **10**–**16**; **11**–**17**; ImiAr *vs.* ImiH: **21**–**26**; **22**–**30**; **23**–**32**; **25**–**34**. However, an additional replacement of carbon by nitrogen did not follow this criteria, as shown by the pairs **17**–**41** (HAr *vs.* PI), **32**–**36** (ImiH *vs.* Qx).

According to the present results, discrepancies between the QSAR studies mentioned above could arise from relative differences in the influence of hydrophobicity on the various processes it rules. Therefore, its overall effect seems not to be so definite when a very diverse set of amines is considered. However, when analyzing related compounds, mutagenicity appears to increase with Log *P*, which correlates with greater nitrenium ion stability. On the other hand, when comparing related amines that differ by the replacement of a carbon atom by nitrogen in an aromatic ring, an inverse correlation of the mutagenic activity with Log *P* is observed.

4. Concluding remarks

For the series of 43 AAs and HAs considered, evident correlations were noticed between the mutagenic potency and nitrenium ion stability (ΔE_r) when the results were grouped for compounds of related structure, classified as aromatic (Ar), heteroaromatic (HAr), imidazocarbocyclic (ImiAr), imidazoheterocyclic (ImiH), quinoxalines (Qx), and dipyridoimidazoles (PI). Furthermore, almost all the HAs fitted one curve. Nitrenium stability was strongly correlated with the charge density at the exocyclic nitrogen (q_N). Hence, q_N, which allows an estimation of the degree of delocalization of the positive charge within the π system, is indicated as an important factor in determining nitrenium ion stability. The present calculations strongly reinforce previous conclusions drawn for a more limited set of compounds [25].

When comparing Ar and HAr related structures, the heteroaromatic nitrenium ions were less stable than their respective aromatic cations. Among related heteroaromatic compounds, nitrenium ion stability decreased when the number of nitrogen atoms increased. In this way, ImiAr derived ions were more stable than the related ImiH and Qx intermediates. In all cases, q_N was more negative for most stable structures. Nitrenium stability was also favored by the increment in the number of aromatic rings (resonance effect), and by methyl substitution (hyperconjugation and inductive effects).

In general, within each group of amines a higher activity was observed for relatively large Log *P* values. Thus, the more stable nitrenium ions were those derived from the amines with higher Log *P* values. On the other hand, the more active HAs, which present lower Log *P* values, formed relatively less stable nitrenium ions than those of the corresponding aromatic compounds, which are more hydrophobic and less active. Therefore, the influence of hydrophobicity on mutagenic potency was better interpreted when related compounds were taken into account. Between amines of the same group, mutagenicity increased with Log *P*, while when comparing amines that differ by the replacement of a carbon atom by nitrogen, an inverse correlation of the mutagenic activity with Log *P* was observed.

The computational results in this study are in good agreement with the measured activity of the amines under consideration. Thus, nitrenium ion stability is considered as a key factor in determining mutagenic potency. Better correlations were obtained for groups of related compounds (aromatic, heteroaromatic), suggesting the influence on activity of other aspects not considered by these calculations (solubility, steric interactions with the biological environment, etc.). Bioactivity of amines is brought about by complex processes involving a number of metabolic and chemical steps. Nevertheless, the present study indicate that DFT calculations can provide reasonable estimations of relative mutagenic potencies for structurally related compounds, encouraging their use as a predictive tool.

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