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Quantum-chemical studies on mutagenicity of aromatic and heteroaromatic amines

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1. ABSTRACT

Arylamines are well-known as widespread industrial and environmental mutagens and carcinogens. Their bioactivity stems from enzymatic metabolic activation to reactive and highly electrophilic intermediates. In this work, computational investigations related to the biological activity of these compounds have been reviewed, especially focusing on studies reporting results from quantum-mechanical calculations. Correlations between relative mutagenicities and structural and electronic features of the parent amines and of their derived nitrenium ion intermediates were examined, with the aim of achieving a clearer comprehension of the main factors determining the genotoxic potential of this type of compounds.

2. INTRODUCTION

Numerous aromatic amines (AAs) present in different industrial activities (dyes used in textile, paper, plastic, pharmaceutical, cosmetic, and food industries), as well as in tobacco smoke, are widely distributed environmental carcinogens (1-3). Similarly, heteroaromatic amines (HAs), found in well-done or over cooked meats and protein rich foods, have also been confirmed as mutagens and carcinogens (4-9). The genotoxic potential of these compounds is developed by enzymatic metabolic activation (Figure 1) (10-12). The initial step involves oxidation of the exocyclic amine nitrogen by cytochrome P450 enzymes to form aryl *N*-hydroxylamines (10), which can undergo N-O bond cleavage to arylnitrenium ions under mildly acidic conditions (12). Additionally, further

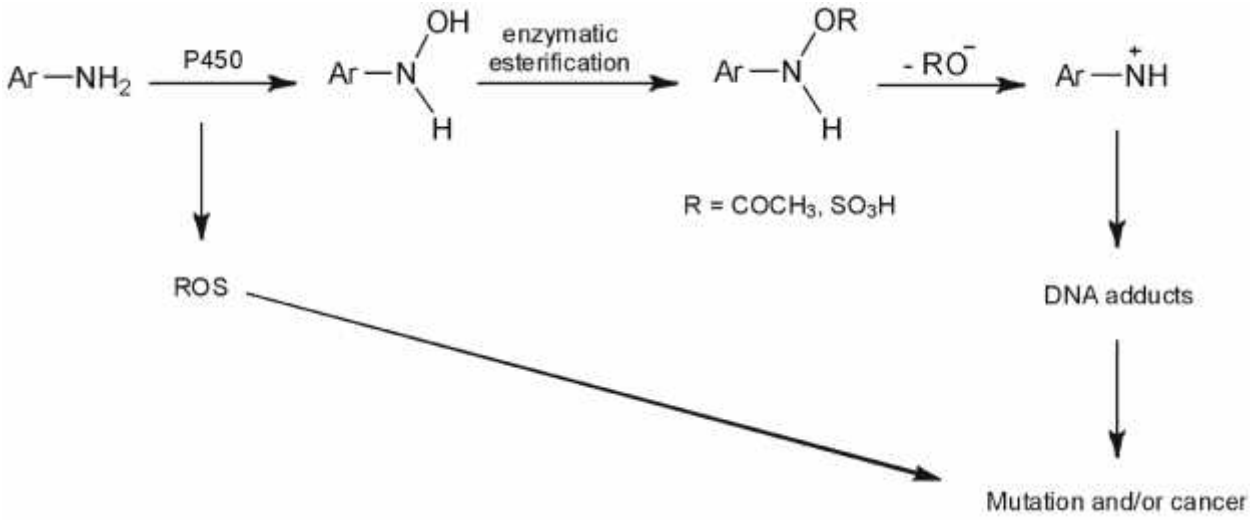


Figure 1. Metabolic activation of aromatic and HAs.

bioactivation of *N*-hydroxylamines to sulfuric or acetic acid esters facilitates the heterolysis of the N-O bond (10-12). The very electrophilic and highly reactive nitrenium ions generated are the ultimate metabolites that covalently bind and modify DNA (10-12). Replication of covalent DNA adducts may lead to mutations and cancer induction (13-15). Besides this major mutagenic pathway, it has been suggested that interaction of a number of AAs and HAs with P450 enzymes and peroxidases causes formation of reactive oxygen species (ROS), which may also provoke DNA mutations and cancer (Figure 1) (16-20).

3. STRUCTURE-ACTIVITY RELATIONSHIPS

During the last decades, many efforts have been made to correlate mutagenicity and carcinogenicity data for AAs and HAs with observed and/or calculated properties of the amines or of their derived nitrenium ions. As an indication of the marked interest in this topic, several structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) studies have been reported (21-43). Some of them, based on the same set of 95 aromatic and HAs, have lately been compared (44). It was found that the QSAR model developed by Maran *et al.* (33) (equivalent to the one of Karelson *et. al.* (38)) was the most adequate to describe the mutagenicity of the 95 amines studied (44). This linear model combined one constitutional descriptor, related to the hydrophobically active surface area of the compounds, and five descriptors derived from quantum-chemical calculations. QSAR studies relative to *in vitro* mutagenicity and animal carcinogenicity of aromatic and HAs have been recently reviewed (45).

According to QSAR analyses having a mechanistic orientation, the toxic activity of amines was shown to correlate with the ease of formation of the *N*-hydroxylamine, with the stability of the nitrenium ion, and with the ease of formation of epoxides on the aromatic ring (46). On the other hand, various studies pointed to the central role of hydrophobicity in the mutagenic and carcinogenic potency of aromatic and HAs (45). Electronic descriptors related to reactivity have also been implied, as the energies of the highest occupied molecular orbital (HOMO) and of the lowest unoccupied molecular orbital (LUMO) (45). The HOMO energy describes the tendency of a molecule to be oxidized, and in this case can account for the propensity to form the hydroxylamine metabolite, whereas the mechanistic reason for the correlation of the mutagenic potential with the LUMO energy is not clear. Regarding steric effects, bulky substituents at the nitrogen amino group, and *ortho* to the amino function, were observed to decrease the activity of arylamines (45). Several authors have found correlations between potency and topological parameters, as the number of fused aromatic rings and the type of ring system (45). In a very recent publication, mutagenicity of arylamines has been ascribed to three factors: (i) high binding affinity in the productive binding mode within the catalytic cavity of P450 enzymes, primarily CYP1A2, (ii) resonance stabilization of the anionic form generated by proton abstraction from the amino group, and (iii) exothermicity of the heterolytic cleavage of N-O bonds of hydroxylamines and their bioconjugates (47).

4. THE ROLE OF NITRENIUM IONS

The relative stability of the nitrenium intermediates has been highlighted to be essential in determining the bioactivity of AAs (10). In line with this, earlier theoretical works have proposed that mutagenicity increases with the rate of nitrenium ion formation, that is, with a greater stability of the nitrenium intermediate, as computed with the semiempirical method AM1 (23-25). Multiple variable models, including variables related to nitrenium ion stability calculated at higher level *ab initio* methods, have later suggested that these variables were of only limited use in regression models (21,22,27-29). The significance of nitrenium ion stability in ruling the mutagenic potency of amines was thus questioned (29).

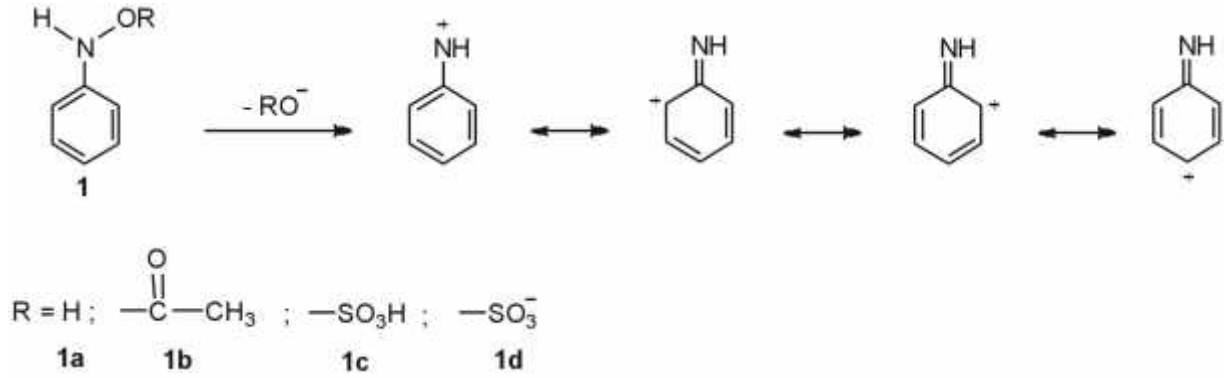


Figure 2. Nitrenium ion formation from aniline derivatives.

Considering the observed discrepancies between semiempirical and Hartree-Fock *ab initio* results, this issue was then examined by using density functional theory (DFT) methods (41,42). Thus, the importance of nitrenium ions on the mutagenicity of this type of compounds was evaluated at a higher level of theory including electron correlation. Formation of these electrophilic intermediates from their precursors was analyzed, and correspondences were explored between experimental mutagenic potencies reported in the literature and calculated reaction energies and electronic properties for a series of AAs and HAs of diverse structure. In this way, clear correlations were observed for compounds of related structure, indicating that mutagenic activity increased with nitrenium ion stability (41-42). Subsequently, DFT calculations were applied in several studies relating the stability of nitrenium ions with mutagenicity (47-55).

4.1. Formation of nitrenium ions

Aniline was selected as the model amine to analyze the reactivity of different precursors towards nitrenium ion generation at the B3LYP/6-31+G* level (41). Calculations on the N-O bond dissociation process were performed for the *N*-hydroxyl derivative (**1a**), as well as for its acetic (**1b**) and sulfuric esters (**1c**) (the deprotonated (negatively charged) analogue **1d** was also considered) (Figure 2) (41). 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 (Figure 2). This fact, evidenced by natural population analysis (NPA) derived charges, the C-N bond distance, and the C-C bond length alternation in the ring, was in agreement with previous reports (56,57). Spectroscopic ^{13}C NMR studies on protonated aromatic imines had shown charge delocalization into the phenyl ring, establishing the ambident carbocationic nature of the iminium ion via the aminocarbenium ion form (58).

The most feasible N-O bond cleavage reaction corresponded to the sulfuric acid ester **1c**, whereas the process from the deprotonated derivative **1d** was much more endothermic. Acetic acid ester **1b** was the second one in reactivity, and the heterolytic dissociation of **1a** resulted the most endothermic reaction (41). These observations were in agreement with the lower reactivity of hydroxyarylamines as compared with their esters (10). In aqueous solution, the charged products were remarkably stabilized, as it would be expected, diminishing the endothermicity of the reaction. Nevertheless, the relative reactivity order for the bond breaking process was identical to the gas-phase trend (41).

Several reactions involved in the mutagenicity of aromatic and HAs were evaluated at the B3LYP/6-31G* level for a set of 312 amines (48). The reactions considered were those corresponding to the generation of a reactive species (hydroxylamines and their conjugates, nitrenium ions, nitrosoarenes), as well as those involving the reaction of these species with DNA. Results showed that formation of nitrenium ions (from hydroxylamines activated either by protonation or conjugation) presented the higher levels of discrimination between active and inactive compounds (48). In this way, chemical reactivity, computed by quantum mechanical methods as energy changes to form reactive intermediates, closely correlated with activity in the Ames test. Solvation corrections by IEFPCM single point energy calculations did not enhance the agreement between computations and experiments (48).

For a large collection of 846 arylamines, the reaction energies of metabolically relevant pathways were calculated at the B3LYP/6-31G* level (50). The nitrenium-forming reaction was determined as the most relevant energetic step for discriminating among mutagenic and nonmutagenic compounds. As shown in previous studies (41,48), solvation energies had an observable effect but did not improve the splitting between both groups. A threshold of 283 kcal/mol for the nitrenium formation energy was suggested for differentiating active (Ames+, lower reaction energies) from inactive (Ames-, higher reaction energies) amines (50). Nitrenium formation energy computations were thus performed for over 14,000 arylamines, with the aim of building a database of potential mutagenicity hazards of use in the pharmaceutical industry (50).

In a very recent study, B3LYP/6-31G* calculations were applied to the rational design of nonmutagenic 4-aminobiphenyls for a drug discovery project (55). For this compound set, 4-aminobiphenyls with

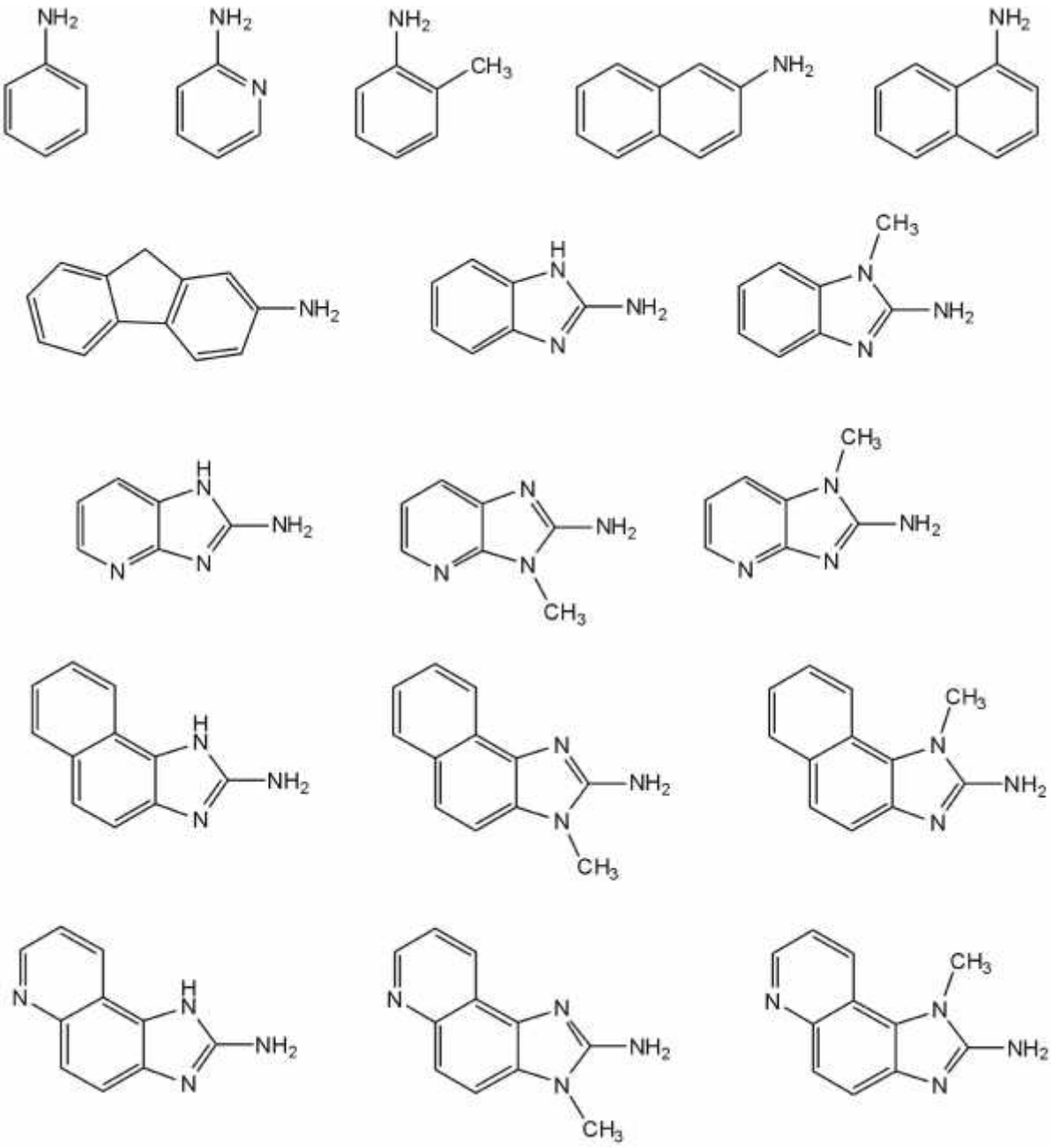
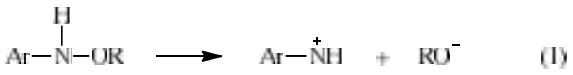


Figure 3. Parent aromatic and HAs studied in reference 41.

a computed energy change for nitrenium ion formation above 145 kcal/mol were inactive in the Ames test (55). These resulted to be the first nonmutagenic derivatives, as no aminobiphenyls that were not positive in an Ames test were previously known.

4.2. Stability of nitrenium ions

Relative nitrenium ion stabilities were calculated for a set of 17 *N*-acetoxy esters of diverse aromatic and HAs by applying DFT methods (41). The parent amines are displayed in Figure 3. Related structures were selected in order to consider the influence of the number of rings, the effect of the nitrogen atom (HAs *vs.* carbocyclic amines), and the presence of a methyl substituent. Relative stabilities were assessed by the computed changes in energy for Reaction 1.



Because of extensive resonance delocalization of the cationic charge through the aromatic system, the resulting structures corresponded to imino carbenium ions (in accordance to references 56 and 57). For every cation,

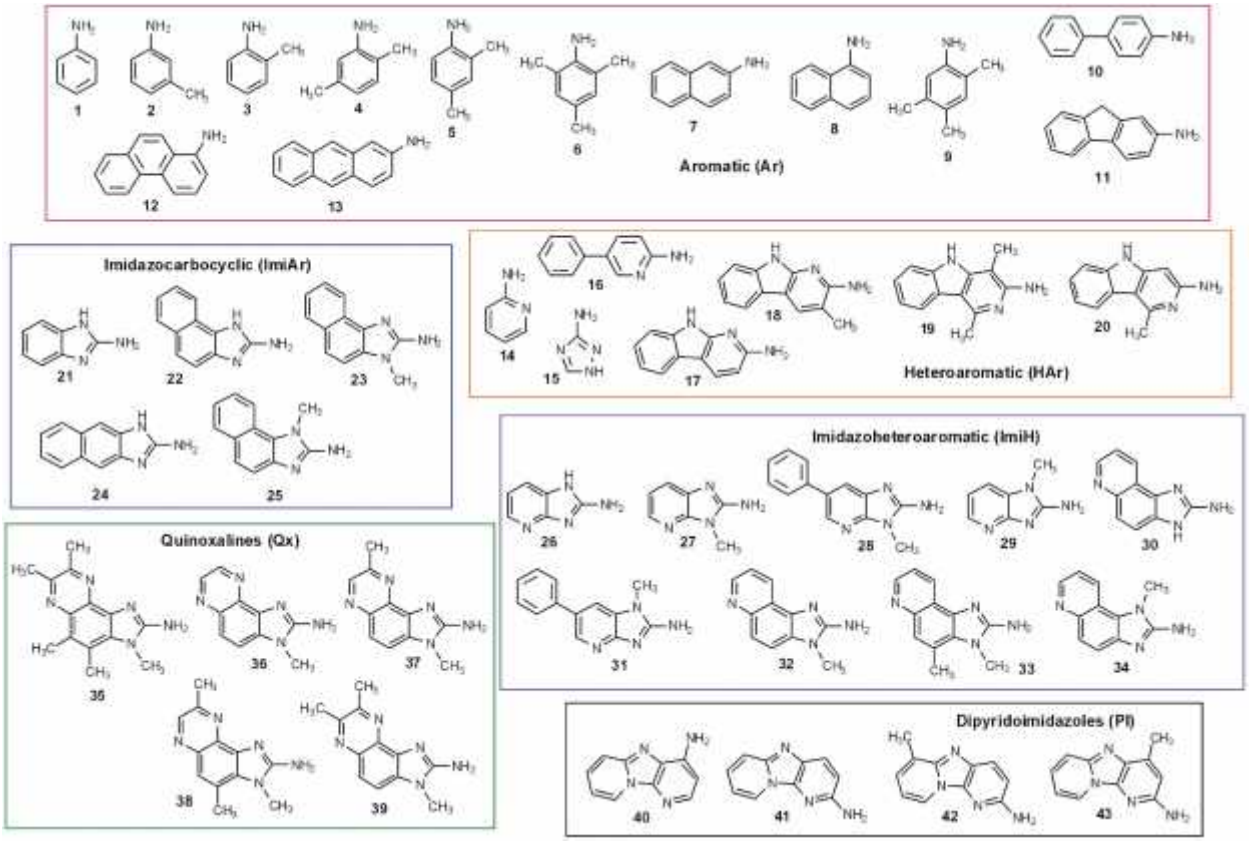


Figure 4. Aromatic and HAs studied in reference 42.

the exocyclic nitrogen presented a negative NPA charge density. With nonsymmetrical aryl substituents, alternative orientations of the NH bond generates 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 (59), both isomers being separated by substantial activation barriers (57,60). The most stable isomer for each cation was considered to determine the relative stability order (41). It should be noticed that the traditional nitrenium ion designation will be conserved along this review, in spite of the actual imino carbenium nature of all the species.

No correlation was found between the experimental mutagenic potencies and the calculated changes in energy for nitrenium ion formation (Reaction 1) for the complete set of 17 *N*-acetoxy esters derived from the aromatic and HAs considered (41). In contrast, clear correlations were noticed for groups of compounds of related structure, denoted as aromatic, imidazocarbocyclic, and imidazoheterocyclic. According to this, mutagenic activity was observed to increase with nitrenium ion stability (lower ΔE_r in Reaction 1), and with the development of a more negative charge density at the exocyclic nitrogen of the nitrenium ion (q_N), although each group of structures followed a different functional relationship (41). It was found that ΔE_r and q_N were strongly correlated, and whilst both heteroaromatic sets fitted almost the same function, the aromatic compounds followed another line. Hence, q_N was pointed out as an important factor in determining nitrenium ion stability, which was also favored by the increment in the number of fused rings (resonance effect), and by methyl substitution (hyperconjugation and inductive effects) (41). In aqueous phase, the correlations for each group of amines matched the gas-phase results. Solvation significantly stabilized the charged products, decreasing the endothermicity in Reaction 1.

Subsequently, the previous series of 17 amines in reference 41 was extended to 43 compounds, and the relative stabilities of their corresponding nitrenium ions were evaluated by B3LYP/6-31G* calculations (42). In order to properly represent this group of chemicals, a diverse set of AAs and HAs was selected, covering a wide range of experimental values for mutagenic potencies reported in the literature (5.790 – -3.390) (29). These amines are displayed in Figure 4. Relative nitrenium ion stabilities were gauged by comparing the computed change in energy (ΔE_r) for the formation of the ion from the corresponding parent amine in gas phase (Reaction 2).

$$Ar-NH_2 \xrightarrow{-H^-} Ar-\overset{+}{N}H \qquad (2)$$

Correlations were analyzed between mutagenic potencies expressed as Log*MP* (logarithm of the number of

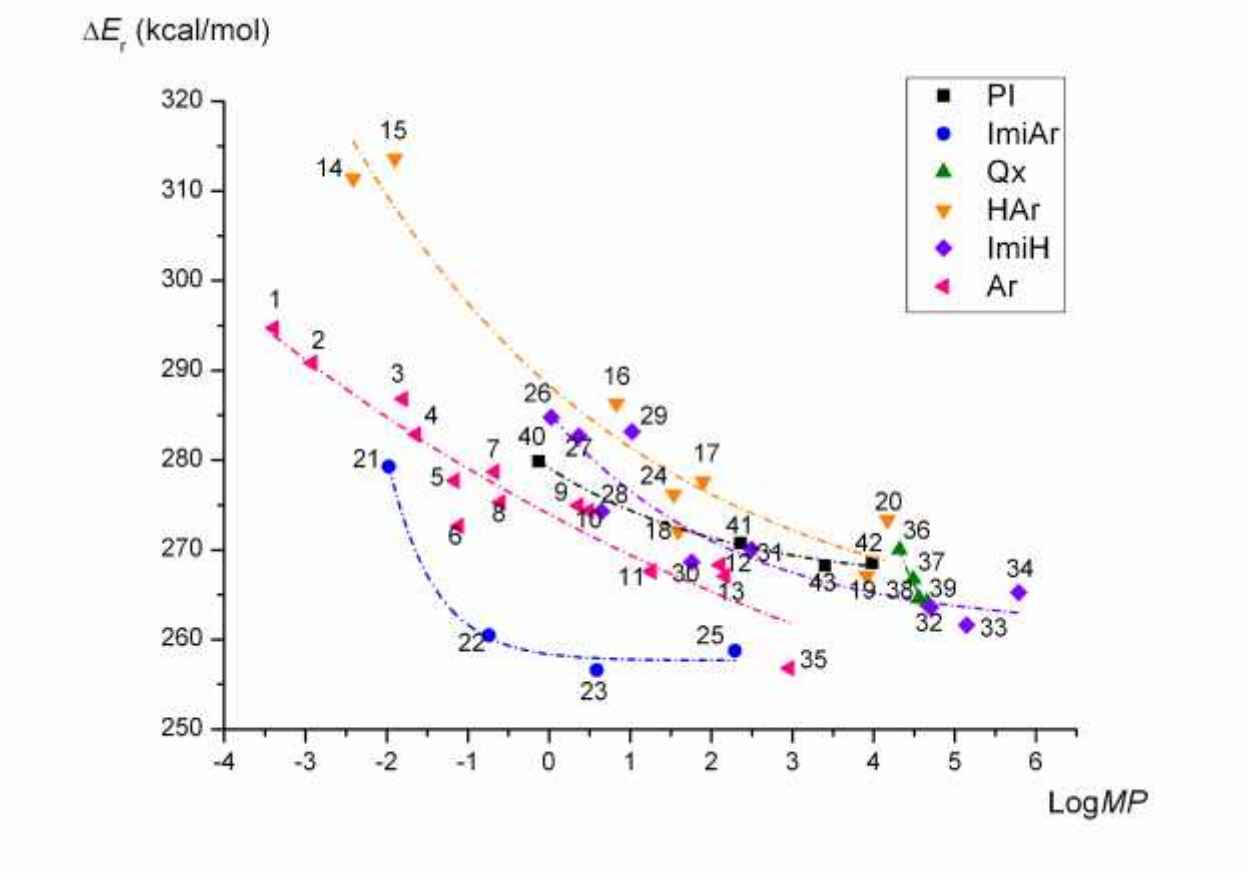


Figure 5. Correlations observed between nitrenium ion stability (ΔE_r) and mutagenic potency (Log*MP*).

histidine revertants *per* nanomole of chemical in the Ames assay for *Salmonella typhimurium* strain TA98 + S9 microsomal preparation) and calculated properties. The theoretical parameters evaluated were ΔE_r (nitrenium ion stability, as defined by Reaction 2), and the NPA charge at the exocyclic nitrogen atom of the nitrenium ion (q_N). As previously noticed (41), better correlations were found for compounds of related structure. Distinct correlation functions were observed for the amines grouped according to their classification as aromatic (Ar), heteroaromatic (HAr), imidazocarbocyclic (ImiAr, amines presenting an imidazole ring fused with a carbocyclic aromatic moiety), imidazoheterocyclic (ImiH, imidazole fused to a heterocyclic system), dipyridoimidazoles (PI), and quinoxalines (Qx) (42).

The following correlation coefficients were obtained: for the Ar group, $r^2 = 0.914$ (13 compounds); for HAr, $r^2 = 0.943$ (8 compounds); for ImiAr, $r^2 = 0.991$ (4 compounds); for Qx, $r^2 = 0.956$ (4 compounds); for PI, $r^2 = 0.994$ (4 compounds); and for the ImiH group, $r^2 = 0.854$, (9 compounds) (42). Mutagenic activity increased with the stability of the nitrenium ions. Correlations for each set are shown in Figure 5. It should be remarked that combination of the different heteroaromatic groups into larger sets afforded very 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), $r^2 = 0.914$. Accordingly, almost all the HAs fitted one curve, indicating that mutagenic activity is significantly influenced by nitrenium ion stability (42).

Mutagenicity was also found to increase with the development of negative charge at the exocyclic nitrogen of the nitrenium ion (q_N) (42). As q_N and ΔE_r were strongly correlated between each other, the negative charge density at the exocyclic nitrogen was signaled as an important factor in determining nitrenium ion stability, and considered as an estimation of the extent of delocalization of the net positive charge within the π system of the cation. Nitrenium ions derived from AAs were more stable than those from the HAs of related structure. Among related heteroaromatic compounds, nitrenium ion stability decreased with the increment in the number of nitrogen atoms. 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 the most stable structures (42).

Nitrenium stability was also favored by the increase in the number of aromatic rings (resonance effect), and by methyl substitution (hyperconjugation and inductive effects) (42). However, for some pairs of amines differing in the number or position of the methyl groups, the stability

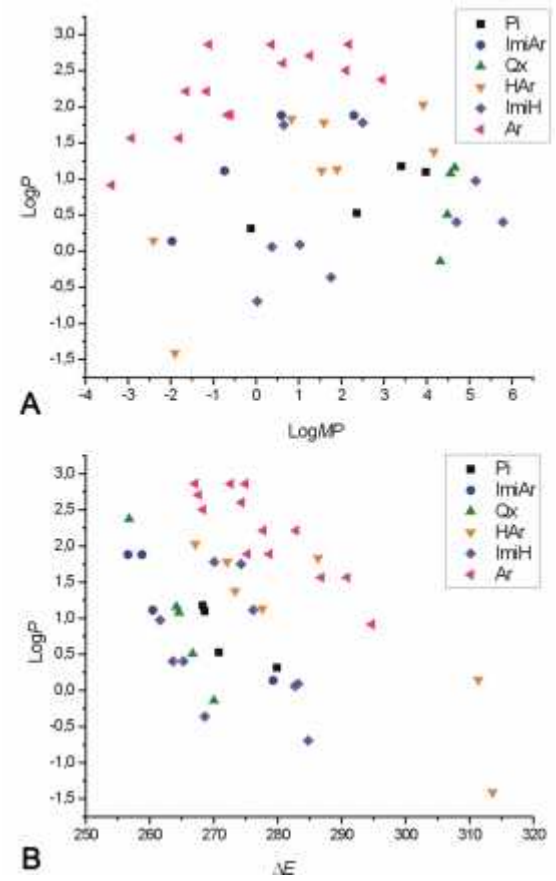


Figure 6. A. Correlations observed between hydrophobicity (Log*P*) and mutagenic potency (Log*MP*). B. Correlations between hydrophobicity (Log*P*) and nitrenium ion stability (ΔE_r).

of the cations did not match the mutagenicity order. According to this, bioactivity should probably be also affected by some other factors, as steric interactions within the active site of the enzymes involved in the activation pathway, or intercalation into the DNA.

More recently, quantum mechanical calculations at different levels of theory (semiempirical, *ab initio*, DFT) were applied to compute the stability of nitrenium ions derived from a set of 257 primary aromatic and HAs (49). These calculations could correctly differentiate between Ames active and inactive compounds, as well as rationalize and predict SAR trends within structurally related chemical series. Therefore, the authors proposed the use of nitrenium ion stability calculations as a valuable tool for medicinal chemists to select nonmutagenic aromatic and heteroaromatic primary amines in preclinical drug discovery programs (49).

5. AROMATIC AMIDES

Aromatic amides follow the same metabolic pathway as aromatic amines (10-12), even though esterification of the *N*-hydroxyarylamides intermediates (hydroxamic acids) is required for subsequent reactivity with DNA (10). Although amides generally appear to be no less carcinogenic than amines (1), the acetamido derivatives were always less mutagenic when compared to their parent amines (61,62). In agreement with this, heterolysis of the N-O bond to give a nitrenium ion was calculated by DFT to be less favorable in the arylamides because of the loss of amide resonance in their precursors (41); but not owing to inductive destabilization of the arylnitrenium ion by the *N*-acetyl group, as proposed earlier according to semiempirical computations (63,64).

Reactions of type 1 were calculated for the *N*-acetoxy esters of acetanilide and 2-acetylaminofluorene at the B3LYP/6-31+G* level in order to compare their results with those from the esters of the respective amines (41). The N-O bond-breaking reaction was more favorable (less endothermic) for the amines (41). The approximately orthogonal conformation adopted by the carbonyl group in the *N*-acetyl nitrenium ions precluded resonance with the aromatic system. According to the NPA charges, nitrenium ions derived from the amides presented less negative charge density at the nitrogen atom than those derived 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. 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. Thus, the acetyl substituent, despite of 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 (41,63,64). On this basis, the formation of *N*-acetyl nitrenium ions seems to be hindered by an unfavorable polarization of the carbonyl group.

6. THE INFLUENCE OF HYDROPHOBICITY

Hydrophobicity plays an important role in the absorption and transport of chemicals to their sites of activation and chemical reaction, as well as in the interaction of the compounds with the bioreceptors. The potential importance of hydrophobic interactions on the genotoxic activity of molecules led to 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 hydrophobicity (30). However, different QSAR studies for AAs and HAs where hydrophatic factors were considered generated contradictory results. Whereas earlier reports indicated that bioactivity was predominantly determined by the hydrophobicity of the amines (30,32), further studies observed that hydropatic factors made only a small contribution to mutagenic potency (22,29).

Afterwards, the influence of the logarithm of the *n*-octanol/water partition coefficient (Log*P*) on the mutagenic activity of aromatic and HAs was analyzed (Figure 6) (42). Positive correlations were observed for each series of compounds in reference 42, although they were not as good as those achieved between calculated

nitrenium ion stabilities (ΔE_r for Reaction 2) and Log*MP*. In general, within each group of amines, a higher bioactivity was associated with relatively large Log*P* values (Figure 6A). Similarly, the more stable nitrenium ions were those derived from the amines with higher Log*P* values (Figure 6B) (42). On the other hand, when the whole set of compounds was considered, the more active HAs, presenting lower Log*P* values, formed relatively less stable nitrenium ions than the correspondingly related AAs, which are more hydrophobic and less active (42).

Hydrophobicity can differently affect the variety of processes involved in chemical genotoxicity, and this could lead to confusing conclusions if sets of amines of very diverse structure are considered. The observed discrepancies between QSAR studies using hydrophatic factors could arise from this fact (22,29,30,32). Therefore, the influence of hydrophobicity on mutagenic potency seems to be better interpreted when analyzing congeneric amines. Considering related compounds, mutagenicity increases with Log*P*, in correspondence with greater nitrenium ion stability (42). In contrast, when comparing amines that differ in the replacement in an aromatic ring of a carbon atom by nitrogen, an inverse correlation of the mutagenic activity with Log*P* was observed (42).

7. SUMMARY AND PERSPECTIVE

Aromatic and HAs are compounds of particular importance in the pharmaceutical industry and occur frequently as substructures in drug candidates (65). Because of their synthetic feasibility, valuable physicochemical properties, and potential interaction with binding sites of target proteins, they are convenient pharmaceutical building blocks. However, their mutagenic and carcinogenic potential restrict their use in drug discovery programs. Bioactivity of this class of compounds is brought about by complex processes involving a number of metabolic and chemical steps. Nevertheless, the studies presented in this review indicate that current computational methods can provide proper estimations of relative mutagenic potencies, denoting its potential value as predictive tools. A calculated probability of activity in the Ames test can help the rational design of safer drugs. The use of DFT calculations appears particularly encouraging on the basis of their accuracy at moderate computational costs.

Nitrenium ion stability is considered as a key factor in determining mutagenic potency. Since better correlations were obtained for groups of related compounds (41,42), it is fairly probable that the influence on activity of additional aspects, such as solubility, transport, specific interactions with the biological environment, etc., needs to be also considered. In this regard, activation pathways of a series of *para*-substituted anilines and 2-aminopyridines in the CYP1A2 enzyme have been lately investigated via SAR analysis and DFT computations (47). Furthermore, a recent study has shown that the interactions of nitroaromatic compounds and their reductive enzymes play an important role in the formation of nitrenium ions, and correspond to differences in the occurrence of DNA adducts (66).

Additional comparisons of calculated activity trends with data obtained by mutagenicity and tumorigenicity assays, and by DNA binding studies, would be desirable in order to achieve a more comprehensive understanding of SARs between arylamines and their toxicological potential.

8. ACKNOWLEDGEMENTS

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9. REFERENCES

1. Garner, R C, Martin, C N and Clayson, D B. Carcinogenic aromatic amines and related compounds. In: Chemical Carcinogens. American Chemical Society Monogr. 182. Eds: Searle, C E. *American Chemical Society*, Washington, D C, Vol. 1, 175-276 (1982)

2. Later, D W, Pelroy, R A, Stewart, D L, McFall, T, Both, G M, Lee, M L, Tedjamulia, M and Castle, R N: Microbial mutagenicity of isomeric two-, three, and four-rings amino polycyclic aromatic hydrocarbons. *Environ Mutagen* 6, 497-515 (1984)

3. Turesky, R J. Aromatic amines and heterocyclic aromatic amines: from tobacco smoke to food mutagens. In: The Chemical Biology of DNA Damage. Eds: Geacintov, N E, Broyde, S. *Wiley-VCH Verlag*, Weinheim. 157-183 (2010)

4. Adamson, R H, Farb, A, Virmani, R, Snyderwine, E G, Thorgeirsson, S S, Takayama, S, Sugimura, T, Dalgard, D W and Thorgeirsson, U P. Studies on the carcinogenic and myocardial effects of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in nonhuman primates. In: Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens, 23rd Intl Symp Princess Takamatsu Cancer Res Fund. Eds: Adamson, R H, Gustafsson, J -A, Ito, N, Nagao, M, Sugimura, T, Wakabayashi, K, Yamazoe, Y. *Princeton Scientific Publishing Co Inc*, Princeton, N J. 260-627 (1995)

5. Felton, J S, Wu, R, Knize, M G, Thompson, L H and Hatch, F T. Heterocyclic amine mutagenicity/carcinogenicity: influence of repair, metabolism, and structure. In: Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens, 23rd Intl Symp Princess Takamatsu Cancer Res Fund. Eds: Adamson, R H, Gustafsson, J -A., Ito, N, Nagao, M, Sugimura, T, Wakabayashi, K, Yamazoe, Y. *Princeton Scientific Publishing Co Inc*, Princeton, N J. 50-58 (1995)

6. Felton, J S, Knize, M G, Hatch, F T, Tanga, M J and Colvin, M E: Heterocyclic amine formation and the impact of structure on their mutagenicity. *Cancer Lett* 143, 127-134 (1999)

7. Sugimura, T: Overview of carcinogenic heterocyclic amines. *Mutat Res* 376, 211-219 (1997)

8. Schut, H A J and Snyderwine, E G: DNA adducts of heterocyclic amine food mutagens: implications for

mutagenesis and carcinogenesis. *Carcinogenesis* 20, 353-368 (1999)

9. Weisburger, J H: Comments on the history and importance of aromatic and heterocyclic amines in public health. *Mutat Res* 506/507, 9-20 (2002)

10. Kadlubar, F F and Beland, F A. Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamides. In: Polycyclic Hydrocarbons and Carcinogenesis, ACS Symposium Series 283. Eds: Harvey, R G. *American Chemical Society*, Washington, D C. 341-370 (1985)

11. Beland, F A and Kadlubar, F F. Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons. In: Handbook of Experimental Pharmacology. Eds: Cooper, C S, Grover, P L. *Springer-Verlag*, Heidelberg. Vol 94, Part 1, 267-325 (1990)

12. Kadlubar, F F, Miller, J A and Miller, E C: Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis. *Cancer Res* 37, 805-814 (1977)

13. Cho, B. Structure-function characteristics of aromatic amine-DNA adducts. In: The Chemical Biology of DNA Damage. Eds: Geacintov, N E, Broyde, S. *Wiley-VCH Verlag*, Weinheim. 217-238 (2010)

14. Hoffmann, G R and Fuchs, R P P: Mechanisms of frameshift mutations: insight from aromatic amines. *Chem Res Toxicol* 10, 347-359 (1997)

15. Kulkarni, S A, Moir, D and Zhu, J: Influence of structural and functional modifications of selected genotoxic carcinogens on metabolism and mutagenicity. A review. *SAR QSAR Environ Res* 18, 459-514 (2007)

16. Siraki, A G, Chan, T S, Galati, G, Teng, S and O´Brien, P J: *N*-oxidation of aromatic amines by intracellular oxidases. *Drug Metab Rev* 34, 549-564 (2002)

17. Makena, P S and Chung, K –T: Evidence that 4-aminobiphenyl, benzidine, and benzidine congeners produce genotoxicity through reactive oxygen species. *Environ Mol Mutagen* 48, 404-413 (2007)

18. Blair, I A: DNA adducts with lipid peroxidation products. *J Biol Chem* 283, 15545-15549 (2008)

19. Shafirovich, V and Geacintov, N E. Role of free radical reactions in the formation of DNA damage. In: The Chemical Biology of DNA Damage. Eds: Geacintov, N E, Broyde, S. *Wiley-VCH Verlag*, Weinheim. 81-104 (2010)

20. Knutson, C G and Marnett, L J. DNA damage caused by endogenously generated products of oxidative stress. In: The Chemical Biology of DNA Damage. Eds: Geacintov, N E, Broyde, S. *Wiley-VCH Verlag*, Weinheim. 105-129 (2010).

21. Novak, M and Rajagopal, S: Correlations of nitrenium ion selectivities with quantitative mutagenicity and carcinogenicity of the corresponding amines. *Chem Res Toxicol* 15, 1495-1503 (2002)

22. Knize, M G, Hatch, F T, Tanga, M J, Lau, E Y and Colvin, M E: A QSAR for the mutagenic potencies of twelve 2-amino-trimethylimidazopyridine isomers: structural, quantum chemical, and hydropathic factors. *Environ Mol Mutagen* 47, 132-146 (2006)

23. Ford, G F and Herman, P S: Relative stabilities of nitrenium ions derived from polycyclic aromatic amines. Relationship to mutagenicity. *Chem -Biol Interact* 81, 1-18 (1992)

24. Ford, G F and Griffin, G R: Relative stabilities of nitrenium ions derived from heterocyclic amine food carcinogens: relationship to mutagenicity. *Chem -Biol Interact* 81, 19-33 (1992)

25. Sabbioni, G and Wild, D: Quantitative structure-activity relationships of mutagenic aromatic and heteroaromatic azides and amines. *Carcinogenesis* 13, 709-713 (1992)

26. Bhat, K L, Hayik, S, Sztandera, L and Bock, C W: Mutagenicity of aromatic and heteroaromatic amines and related compounds: a QSAR investigation. *QSAR Comb Sci* 24, 831-843 (2005)

27. Hatch, F T, Colvin, M E and Seidl, E T: Structural and quantum chemical factors affecting mutagenic potency of aminoimidazo-azaarenes. *Environ Mol Mutagen* 27, 314-330 (1996)

28. Hatch, F T and Colvin, M E: Quantitative structure-activity (QSAR) relationships of mutagenic aromatic and heterocyclic amines. *Mutat Res* 376, 87-96 (1997)

29. Hatch, F T, Knize, M G and Colvin, M E: Extended quantitative structure-activity relationships for 80 aromatic and heterocyclic amines: structural, electronic, and hydropathic factors affecting mutagenic potency. *Environ Mol Mutagen* 38, 268-291 (2001)

30. Debnath, A K, Debnath, G, Shusterman, A J and Hansch, C: A QSAR of the role of hydrophobicity in regulating mutagenicity in the Ames test: 1. Mutagenicity of aromatic and heteroaromatic amines in Salmonella typhimurium TA98 and TA100. *Environ Mol Mutagen* 19, 37-52 (1992)

31. Cash, G G, Anderson, B, Mayo, K, Bogaczyk, S and Tunkel, J: Predicting genotoxicity of aromatic and heteroaromatic amines using electrotopological state indices. *Mutat Res* 585, 170-183 (2005)

32. Benigni, R, Giuliani, A, Franke, R and Gruska, A: Quantitative structure-activity relationships of mutagenic and carcinogenic aromatic amines. *Chem Rev* 100, 3697-3714 (2000)

33. Maran, U, Karelson, M and Katritzky, A R: A comprehensive QSAR treatment of the genotoxicity of heteroaromatic and aromatic amines. *Quant Struct -Act Relat* 18, 3-10 (1999)

34. Basak, S C, Mills, D R, Balaban, A T and Gute, B D: Prediction of mutagenicity of aromatic and heteroaromatic amines from structure: a hierarchical QSAR approach. *J Chem Inf Comput Sci* 41, 671-678 (2001)

35. Basak, S C and Mills, D: Prediction of mutagenicity utilizing a hierarchical QSAR approach. *SAR QSAR Environ Res* 12, 481-496 (2001)

36. Gramatica, P, Consonni, V and Pavan, M: Prediction of aromatic amines mutagenicity from theoretical molecular descriptors. *SAR QSAR Environ Res* 14, 237-250 (2003)

37. Fang, Y, Feng, Y and Li, M: Optimal QSAR analysis of the carcinogenic activity of aromatic and heteroaromatic amines. *QSAR Comb Sci* 27, 543-554 (2008)

38. Karelson, M, Sild, S and Maran, U: Nonlinear QSAR treatment of genotoxicity. *Mol Simul* 24, 229-242 (2000)

39. Toporov, A A and Toporova, A P: Prediction of heteroaromatic amine mutagenicity by means of correlation weighting of atomic orbital graphs of local invariants. *J Mol Struct Theochem* 538, 287-293 (2001)

40. Vračko, M, Mills, D and Basak, S C: Structure-mutagenicity modeling using counter propagation neural networks. *Environ Toxicol Pharmacol* 16, 25-36 (2004)

41. Borosky, G L: Ultimate carcinogenic metabolites from aromatic and heterocyclic aromatic amines: a computational study in relation to their mutagenic potency. *Chem Res Toxicol* 20, 171-180 (2007)

42. Borosky, G L: Carcinogenic carbocyclic and heterocyclic aromatic amines: a DFT study concerning their mutagenic potency. *J. Mol. Graphics Modell* 27, 459-465 (2008)

43. Chung, K -T, Kirkovsky, L, Kirkovsky, A and Purcell, W P: Review of mutagenicity of monocyclic aromatic amines: quantitative structure–activity relationships. *Mutat Res* 387, 1-16 (1997)

44. Restrepo, G, Basak, S C and Mills, D: Comparison of QSARs and characterization of structural basis of bioactivity using partial order theory and formal concept analysis: a case study with mutagenicity. *Curr Comput -Aided Drug Des* 7, 109-121 (2011)

45. Benigni, R and Bossa, C: Mechanism of chemical carcinogenicity and mutagenicity: a review with implications for predictive toxicology. *Chem Rev* 111, 2507-2536 (2011)

46. Benigni, R and Bossa, C: Flexible use of QSAR models in predictive toxicology: a case study on aromatic amines. *Environ Mol Mutagen* 53, 62-69 (2012)

47. Shamovsky, I, Ripa, L, Börjesson, L, Mee, C, Nordén, B, Hansen, P, Hasselgren, C, O´Donovan, M and Sjö, P: Explanation for main features of structure–genotoxicity relationships of aromatic amines by theoretical studies of their activation pathways in CYP1A2. *J Am Chem Soc* 133, 16168-16185 (2011)

48. Leach, A G, Cann, R and Tomasi, S: Reaction energies computed with density functional theory correspond with a whole organism effect; modelling the Ames test for mutagenicity. *Chem Commun* 1094-1096 (2009)

49. Bentzien, J, Hickey, E R, Kemper, R A, Brewer, M L, Dyekjær, J D, East, S P and Whittaker, M: An *in silico* method for predicting Ames activities of primary aromatic amines by calculating the stabilities of nitrenium ions. *J Chem Inf Model* 50, 274-297 (2010)

50. McCarren, P, Bebernitz, G R, Gedeck, P, Glowienke, S, Grondine, M S, Kirman, L C, Klickstein, J, Schuster, H F and Whitehead, L: Avoidance of the Ames test liability for aryl-amines via computation. *Bioorg Med Chem* 19, 3173-3182 (2011)

51. Arlt, V M, Glatt, H, Gamboa da Costa, G, Reynisson, J, Takamura-Enya, T and Phillips, D H: Mutagenicity and DNA adduct formation by the urban air pollutant 2-nitrobenzanthrone. *Toxicol Sci* 98, 445-457 (2007)

52. Reynisson, J, Stiborová, M, Martínek, V, Gamboa da Costa, G, Phillips, D H and Arlt, V M: Mutagenic potential of nitrenium ions of nitrobenzanthrones: correlation between theory and experiment. *Environ Mol Mutagen* 49, 659-667 (2008)

53. Arlt, V M, Phillips, D H and Reynisson, J: Theoretical investigations on the formation of nitrobenzanthrone-DNA adducts. *Org Biomol Chem* 9, 6100-6110 (2011)

54. Kim, E J, Matuszek, A M, Yu, B and Reynisson, J: Theoretical investigations into the role of aryl nitrenium ions´ stability on their mutagenic potential. *Aust J Chem* 64, 910-915 (2011)

55. Birch, A M, Groombridge, S, Law, R, Leach, A G, Mee, C D, and Schramm, C: Rationally designing safer anilines: the challenging case of 4-aminobiphenyls. *J Med Chem* 55, 3923-3933 (2012)

56. Novak, M and Lin, J: Correlation of azide/solvent selectivities for nitrenium ions with ab initio hydration energies: understanding the kinetic lability of nitrenium ions in aqueous solution. *J Org Chem* 64, 6032-6040 (1999)

57. Ford, G P, Herman, P S and Thompson, J W: *Syn* and *anti* aryl nitrenium ions. *J Comput Chem* 20, 231-243 (1999)

58. Olah, G A and Donovan, D J: Carcinogen chemistry. 2. Carbon-13 nuclear resonance spectroscopic study of the ambident carbocationic nature of iminium ions and its relevance to the aminoalkylating ability of related chemical carcinogens. *J Org Chem* 43, 860-865 (1978)

59. Ford, G P and Herman, P S: Comparison of the relative stabilities of polycyclic aryl nitrenium ions and arylmethyl cations: ab initio and semiempirical molecular orbital calculations. *J Mol Struct (Theochem)* 236, 269-282 (1991)

60. Cramer, C J, Dulles, F J and Falvey, D E: Ab initio characterization of phenylnitrenium and phenylcarbene: remarkably different properties for isoelectronic species. *J Am Chem Soc* 116, 9787-9788 (1994)

61. Connor, T H, Ramanujam, V M S, Rinkus, S J, Legator, M S and Trieff, N M: The evaluation of mutagenicities of 19 structurally related aromatic amines and acetamides in *Salmonella typhimurium* TA98 and TA100. *Mutat Res* 118, 49-59 (1983)

62. Trieff, N M, Biagi, G L, Ramanujam, V M S, Connor, T H, Cantelli-Forti, G, Guerra, M C, Bunce, 3rd, H and Legator, M S: Aromatic amines and acetamides in Salmonella typhimurium TA98 and TA100: a quantitative structure-activity relation study. *Mol Toxicol* 2, 53-65 (1989)

63. Ford, G P and Herman, P S: Alkyl and acyl substituent effects on nitrenium ion stabilities: ab initio molecular orbital calculations. *J Mol Struct (Theochem)* 204, 121-130 (1990)

64. Ford, G P and Herman, P S: Conformational preferences and energetics of N-O heterolyses in aryl nitrenium ion precursors: ab initio and semiempirical molecular orbital calculations. *J Chem Soc Perkin Trans* 2, 607-616 (1991)

65. Mirza, A, Desai, R and Reynisson, J: Known drug space as a metric in exploring the boundaries of drug-like chemical space. *Eur J Med Chem* 44, 5006-5011 (2009)

66. Stiborová, M, Martínek, V, Svobodová, M, Sístková, J, Dvorák, Z, Ulrichová, J, Simánek, V, Frei, E, Schmeiser, H H, Phillips, D H and Arlt, V M, Mechanisms of the different DNA adduct forming potentials of the urban air pollutants 2-nitrobenzanthrone and carcinogenic 3-nitrobenzanthrone. *Chem Res Toxicol* 23, 1192-1201 (2010)

Abbreviations: AA: aromatic amine; HA: heteroaromatic amine; ROS: reactive oxygen species; SAR: structure-activity relationship; QSAR: quantitative structure-activity relationship; HOMO: highest occupied molecular orbital; LUMO: lowest unoccupied molecular orbital; DFT: density functional theory; NPA: natural population analysis; Ar: aromatic; HAr: heteroaromatic; ImiAr: imidazocarbocyclic; ImiH: imidazoheterocyclic; PI: dipyridoimidazoles; Qx: quinoxalines

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