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Theoretical study of aza-polycyclic aromatic hydrocarbons (aza-PAHs), modelling carbocations from oxidized metabolites and their covalent adducts with representative nucleophiles†

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Protonation of the epoxides, diol epoxides, and dihydrodiols of benzo[*h*]quinoline (BhQ), benzo[*f*]quinoline (BfQ), phenanthrene (Phe), benzo[*c*]phenanthridine (BcPhen), and chrysene (Chry) were studied by DFT at the B3LYP/6-31G* level, and selected cases were calculated with the 6-31+G* diffuse-function augmented basis set for comparison purposes. Bay-region carbocations were formed from *O*-protonated epoxides *via* a barrierless processes. Relative carbocation stabilities were determined in the gas phase and with water as solvent (PCM method). The presence of a heteroatom changes the regioselectivity of epoxide ring opening, in some cases favoring non-bay-region carbocations. The epoxide ring opening mode is also greatly influenced by *N*-protonation. The dications resulting from initial *N*-protonation followed by epoxide protonation were also studied by DFT. Charge delocalization modes in the resulting mono- and dications were derived by GIAO-NMR (based on $\Delta\delta^{13}$C values) and *via* the NPA-derived changes in charges. Relative aromaticity in different rings in the arenium ions was gauged by NICS. In representative cases, the covalent adducts (*syn* and *anti*) formed by reaction of the benzylic carbocations derived from diol epoxides and dihydrodiols with methoxide and methanethiolate anions were studied. Relative energies (in the gas phase and with water as solvent) and geometries of the adducts formed by quenching of the carbocations derived from BhQ and Phe-epoxides with guanine *via* the exocyclic amino group and *via* the N-7 were also investigated computationally. Although aqueous phase calculations change the energy for the addition reactions because of greater stabilization of the reactants, relative reactivity trends remain the same. The data are discussed, taking into account the available experimental results concerning the biological activity of these compounds.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) derived from incomplete combustion of organic material are widespread environmental contaminants and potent mutagens and/or carcinogens.[1] It is well established that PAHs exert their mutagenic activities through their oxidative metabolites, identified as *syn* or *anti*-*trans*-dihydrodiol epoxides.[2] Under appropriate combustion conditions, nitrogen may be incorporated into the aromatic ring systems to form aza-PAHs.[3] A number of such aza-aromatic hydrocarbons were found in significant amounts in urban air particulates, gasoline engine exhaust, tobacco smoke, and effluents from coal combustion processes.[1b,4] Substantial evidence has been obtained suggesting that, like the PAHs, the aza-analogues are also metabolically activated to diol epoxides according to the bay-region theory.[5] The bay-region diol epoxides are the major metabolites responsible for mutagenic and carcinogenic properties. The pathway for metabolic formation of the bay-region diol epoxides of PAHs and aza-PAHs involves oxidation of a terminal, angular benzo ring of the hydrocarbon to form an arene oxide, hydration of the arene oxide to form a *trans*-dihydrodiol, and subsequent epoxidation of the bay-region double bond of the dihydrodiol. The benzylic carbocations generated from these electrophilic diol epoxides by opening of the *O*-protonated epoxide ring are capable of forming covalent adducts with the nucleophilic sites in DNA and RNA, leading to alteration of genetic material.[2a,6]

Experimental data have revealed that the position of the nitrogen heteroatom in the aza-PAHs could have a significant effect on the carcinogenic potencies of their dihydrodiol and bay-region diol-epoxide metabolites. Thus, assessment of the mutagenic activities of several derivatives of benzo[*h*]quinoline (BhQ), benzo[*f*]quinoline (BfQ) and their carbon analogue phenanthrene (Phe) (Fig. 1) showed that, although the BhQ bay-region diol epoxide (with the *anti* isomer more active than the *syn*) was considerably less mutagenic than phenanthrene diol-epoxide, BhQ-7,8-dihydrodiol and Phe-1,2-dihydrodiol were metabolized to a similar extent to mutagenic products (diol epoxides). The data suggested that BhQ-dihydrodiol is in fact more efficiently metabolized than Phe-dihydrodiol, implying

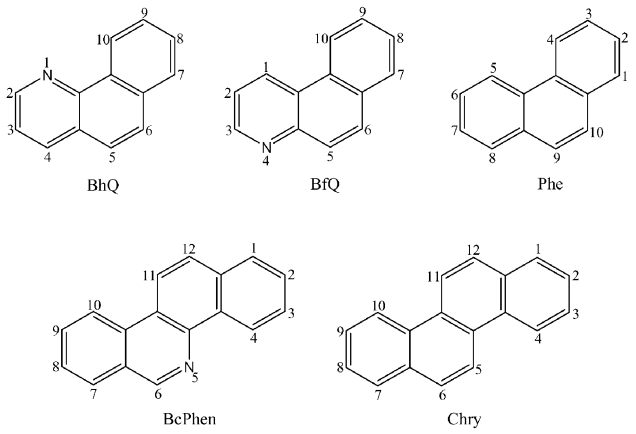

Fig. 1 Structures and numbering of benzo[*h*]quinoline (BhQ), benzo[*f*]quinoline (BfQ), benzo[*c*]phenanthridine and their carbon analogues phenanthrene (Phe) and chysene (Chry).

† Electronic supplementary information (ESI) available: NPA-derived charges, ^{13}C NMR and NICS, superimposed structures of $\mathbf{1}H^+$ and $\mathbf{2}H^+$ adducts with guanine, Cartesian coordinates of the optimized structures for $\mathbf{1}H^+$–$\mathbf{18}H^+$. See http://www.rsc.org/suppdata/ob/b4/b416429f/

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that the presence of nitrogen at the 1-position increases the metabolic activity at the bay-region.[7] The BhQ-bay-region diol epoxide and BhQ-tetrahydroepoxide are considerably more mutagenic than those of BfQ at similar doses.[7] A weak mutagenic potency was inferred for the BfQ *anti* isomer (not available for direct evaluation) according to the activities of related derivatives. The differences in bioactivity between the BhQ and BfQ diol epoxides were explained by Hückel and PMO considerations, whereby diminished reactivity of BfQ-7,8-diol-9,10-epoxide was attributed to the destabilizing effect of placement of a positive charge on the nitrogen heteroatom by resonance of the carbenium ion formed.[7,8]

Recent quantum-mechanical calculations have been successfully applied to the study of the carcinogenic pathways of PAH derivatives, as they have shown good agreement with the experimental reactivities of these compounds.[9] Furthermore, modelling studies of biological electrophiles from PAHs by DFT methods have given a full description of the NMR characteristics and charge delocalization modes on their resulting carbocations.[10]

Considering the relevance of aza-PAHs in the elucidation of the mechanism of chemical carcinogenesis, we have performed a model DFT study on the structural and electronic properties of their reactive intermediates, with the aim of achieving a better understanding of the reactivity of this type of compound. In order to evaluate the influence of the nitrogen atom on reactivity, the bay-region structures selected each presented one nitrogen atom located at a different position on the fused-ring systems. Computations on BhQ and BfQ derivatives were compared with the data obtained for the corresponding carbon analogue (Phe), while benzo[*c*]phenanthridine (BcPhen) calculations were compared with the data for chrysene (Chry).

Charge delocalization modes (positive charge density distributions) in the resulting carbocations were evaluated by GIAO-NMR (*via* computed $\Delta\delta^{13}$C values) and *via* the NPA-derived changes in carbon (and overall charges over CH units) and nitrogen charges. GIAO-NMR chemical shifts for the epoxides and diol epoxides were also computed in water as solvent. Relative aromaticity in various rings in the aza-PAH carbocations was gauged *via* NICS and ΔNICS.

Given the basicity at nitrogen in aza-PAHs and the published data concerning the presence of regions of high H^+ concentration around nucleic acids,[11] it seemed reasonable to assume that *N*-protonation might also become important. It appeared logical to examine how *N*-protonation might influence the regioselectivity of epoxide ring opening and carbocation formation, and the charge delocalization modes in the resulting azonium–carbenium dications.

To model the crucial step of covalent adduct formation, in selected cases, the *syn* and *anti* adducts resulting from quenching of the carbocations derived from the diol epoxides and the dihydrodiols with methoxide and methanethiolate anions, as representative nucleophiles, were studied. For comparison, geometrical features and relative energies of the covalent adducts formed by quenching of the carbocations derived from BhQ and Phe epoxides with guanine *via* the exocyclic amino group and *via* the N-7 were also computed and compared.

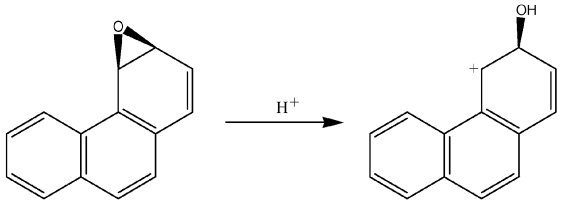
Computational methods

Density functional theory (DFT) calculations were performed with the Gaussian 03 suite of programs,[12] employing the B3LYP[13] functional and the 6-31G*[14] and 6-31+G* split-valence shell basis sets. Geometries were fully optimized and minima were characterized by calculation of the harmonic vibrational frequencies. Natural population analysis (NPA) was evaluated by means of the NBO program.[15] The solvent effect was estimated by the polarized continuum model (PCM)[16] without geometry optimizations, unless specified. NMR chemical shifts and NICS (nuclear independent chemical shift)[17] values were calculated by the GIAO (gauge independent atomic orbitals)[18] method at the B3LYP/6-31G* level. NMR chemical shifts were referenced to TMS (GIAO magnetic shielding tensor = 182.4656 ppm; this value is related to the GIAO isotropic magnetic susceptibility for ^{13}C). NICS values were computed at each ring centroid. Semiempirical calculations were carried out with the AM1 method.[19]

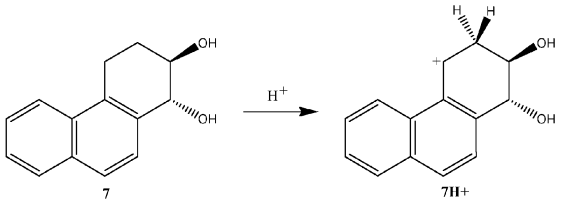
Results and discussion

The epoxides (**1**–**3**), *anti*-diol epoxides (**4**–**6**), and the dihydrodiols (**7**–**9**) derived from Phe, BhQ and BfQ, and those derived from Chry and BcPhen (**10**–**12**, **13**–**15**, **16**–**18**) were calculated at the B3LYP/6-31G* level (Fig. 2). As calculations with the 6-31+G* basis on some of the compounds did not afford significant variations in the computed properties, no diffuse functions were employed for the neutral and positively charged structures.

In every case, epoxide protonation led to the formation of the open carbocations depicted in Fig. 3 *via* a barrierless process, showing that the oxonium ions were not minima on the respective potential energy surfaces. Changes in energy for every ring opening reaction of type (1) are presented in Table 1, as well as some selected NPA-derived charges for the carbocations. Also included in Table 1 are changes in energy for carbocation formation from the dihydrodiols (according to reactions of type (2), which serve as additional models for the generation of benzylic carbocations similar to those formed metabolically *via* diol-epoxides by ring opening), and the NPA-derived charges at the resulting carbocations.



(1)



(2)

According to NPA-derived charge distributions, the positive charge in the resulting carbocations is delocalized throughout the π-system. Nitrogen substitution did not significantly modify the charge distribution pattern. Interestingly, in some cases the presence of the heteroatom changed the regioselectivity of the epoxide ring-opening process, *i.e.*, the relative stability of the two possible carbocations, with preference for the non bay-region carbocation formation. These specific cases will be discussed below.

The NPA-derived heavy atom charges and overall charges over CH and OH units are shown in the supporting information.

Computed GIAO ^{13}C NMR chemical shifts and changes in chemical shifts ($\Delta\delta^{13}$C values) exhibited essentially the same trends as changes in NPA charges, *i.e.*, changes in the chemical shifts to more positive values (carbocations *vs.* neutral compounds) followed the increments in positive charge for the respective positions in every structure. Accordingly, a good agreement was found between the charge delocalization modes depicted by both methods. In this way, charge distribution patterns seem to be well defined and realistic. GIAO NMR data are included in the supporting information.

NICS values (see supporting information) revealed that the ring adjacent to the carbocationic centre is no longer

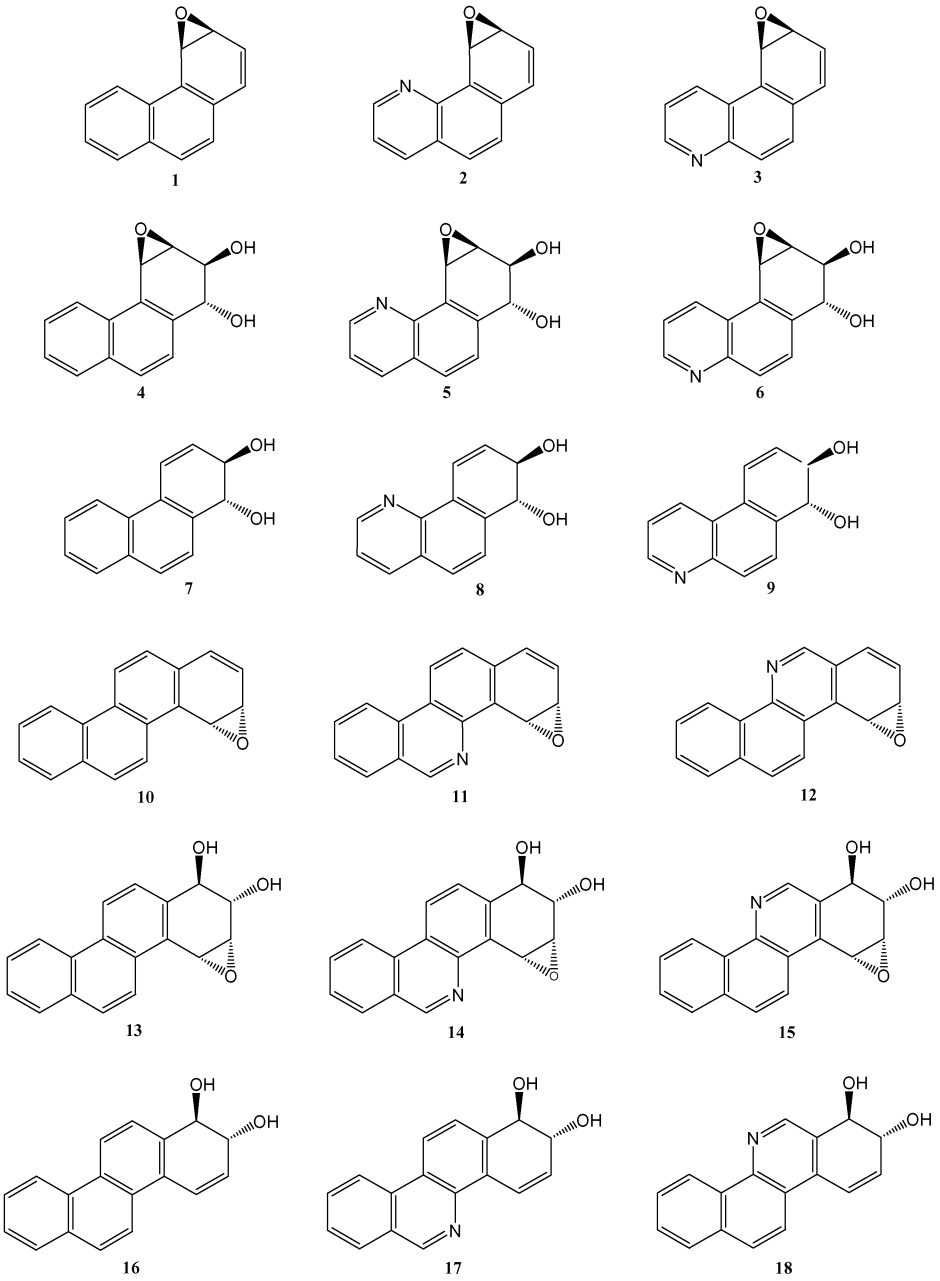


Fig. 2 Studied PAHs and aza-PAHs.

aromatic. This relationship applies to both PAHs and aza-PAHs. Therefore, substitution of a ring carbon atom for nitrogen does not affect the relative aromaticity of the various rings in the carbocations. This finding agrees with the observations made above on the conservation of charge distribution arrangements upon nitrogen substitution.

As was pointed out in the introduction, it seemed relevant and interesting to compute the corresponding azonium–arenium dications ($\mathbf{2}H_2^{2+}$, $\mathbf{3}H_2^{2+}$, $\mathbf{5}H_2^{2+}$, $\mathbf{6}H_2^{2+}$, $\mathbf{11}H_2^{2+}$, $\mathbf{12}H_2^{2+}$, $\mathbf{14}H_2^{2+}$ and $\mathbf{15}H_2^{2+}$). Protonation at the nitrogen had a significant impact on the epoxide opening mode. In this way, for $\mathbf{2}H_2^{2+}$, $\mathbf{3}H_2^{2+}$, $\mathbf{11}H_2^{2+}$ and $\mathbf{12}H_2^{2+}$ the non bay-region carbocation became most stable. For $\mathbf{2}H_2^{2+}$ and $\mathbf{11}H_2^{2+}$ a hydrogen-bond interaction was observed between the proton attached to nitrogen and the hydroxy oxygen, which could explain the higher stability of these species.

In $\mathbf{3}H_2^{2+}$ the NPA charge at nitrogen is more negative than in the less stable bay-region carbocation structure (−0.465 *vs.* −0.452). Moreover, the azonium–oxonium dications $\mathbf{6}H_2^{2+}$ and $\mathbf{15}H_2^{2+}$ were characterized as minima, with $\mathbf{6}H_2^{2+}$ being *ca.* 1 kcal mol^{-1} more stable than the derived azonium–arenium dication. Again, the preferred structure presented a slightly larger negative charge at the nitrogen. The most stable protonated structures for each system are displayed in Fig. 4.

Comparisons between the charge delocalization modes were also made for the computed dihydrodiol bay-region cations ($\mathbf{8}H^+$, $\mathbf{9}H^+$, $\mathbf{17}H^+$ and $\mathbf{18}H^+$) and their *N*-protonated dication counterparts ($\mathbf{8}H_2^{2+}$, $\mathbf{9}H_2^{2+}$, $\mathbf{17}H_2^{2+}$, $\mathbf{18}H_2^{2+}$). No significant changes in charge distribution due to *N*-protonation were found in these cases.

The change in energy for the opening reactions [analogous to reaction (1)] could be considered a measure of the resulting carbocation stability. It is likely that the electrophilic attack of DNA nucleotides by hydrocarbon epoxides is S_N1-like and proceeds through proton-stabilized transition states in which the hydrocarbon exhibits significant carbonium ion character.[6d,20] In relation to this, good correlations have been obtained between calculated relative energies for carbocation formation from PAH derivatives and their reactivity towards nucleic acid.[9] Thus, a clear relationship was observed between the delocalization of

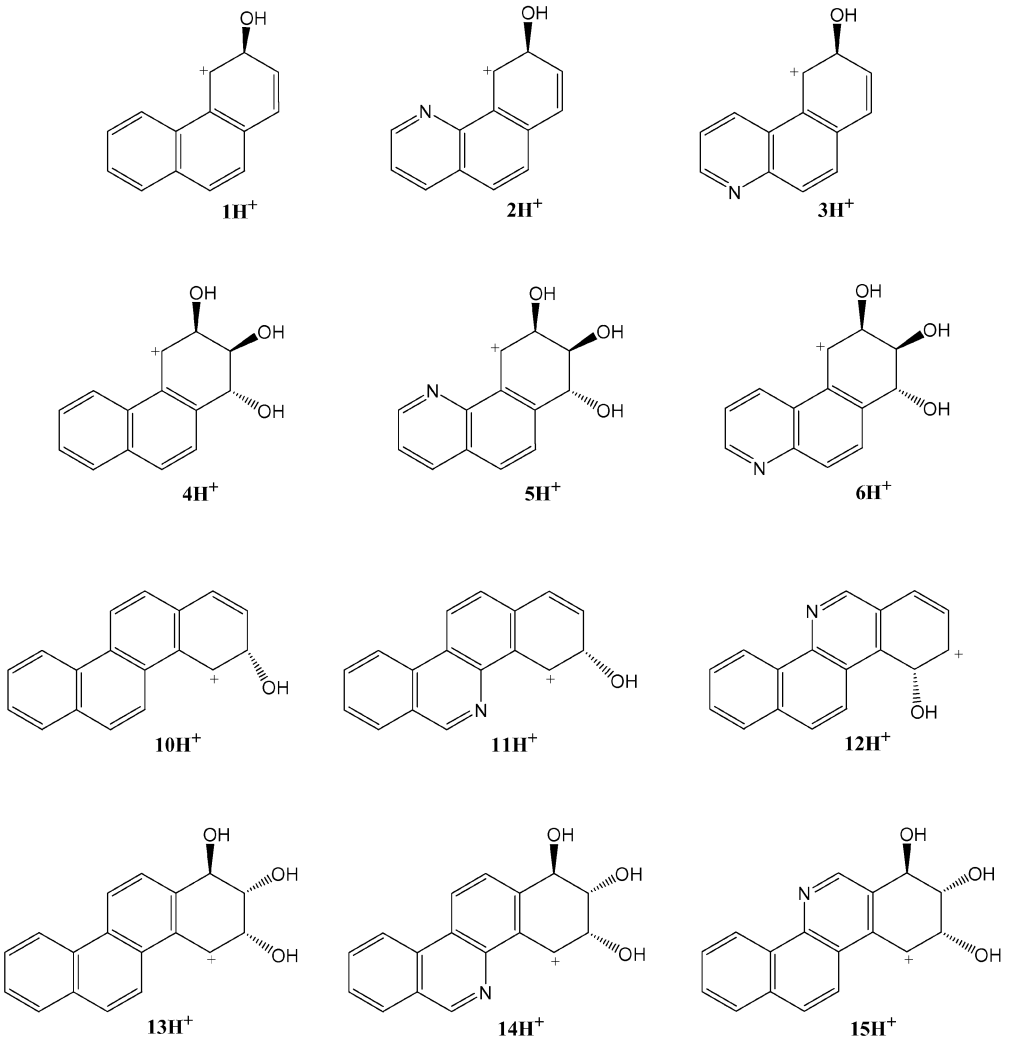


Fig. 3 Studied carbocations from PAHs and aza-PAHs.

the charge in the carbocation (assessed as a decrease in the positive charge at the carbocationic centre) and the ease of its formation.

For the three-ring compounds, ΔE_r values in Table 1 show a decrease in the exothermicity of reaction (1) in the order **1**H$^+$ > **2**H$^+$ > **3**H$^+$, and also in the series **4**H$^+$ > **5**H$^+$ > **6**H$^+$. This tendency was less evident for reaction (2).

These results follow the mutagenic activities observed for the tetrahydro epoxide and the tetrahydro diol-epoxide derivatives of Phe, BhQ and BfQ.[7] The NPA-derived positive charge at the

Table 1 B3LYP/6-31G* calculations for the carbocations in Fig. 3[a]

Compound	ΔE_r/kcal mol^{-1}[b]	ΔE_r/kcal mol^{-1}[c]	NPA charge[d]	NPA charge at N	GIAO-^{13}C NMR[e]
1H$^+$	−234.18 (−165.51)	−227.06	0.243 (0.271)	—	167.6 (172.0)
2H$^+$	−231.97 (−163.22)	−224.98	0.308 (0.313)	−0.449 (−0.475)	178.7 (180.4)
3H$^+$	−228.84 (−161.91)	−221.95	0.254 (0.291)	−0.400 (−0.449)	169.9 (177.1)
4H$^+$	−233.53 (−162.07)	−226.15	0.311 (0.313)	—	182.3 (181.5)
5H$^+$	−230.20 (−157.89)	−223.06	0.361 (0.356)	−0.449 (−0.474)	177.3 (177.6)
6H$^+$	−227.90 (−156.49)	−220.85	0.300 (0.330)	−0.393 (−0.439)	165.2 (171.5)
7H$^+$	−227.43[f]	−219.69	0.342	—	172.6
8H$^+$	−227.87[f]	−220.35	0.375	−0.455	183.2
9H$^+$	−224.36[f]	−216.90	0.316	−0.396	195.0
10H$^+$	−236.06 (−164.43)	−228.93	0.243 (0.277)	—	168.7 (174.8)
11H$^+$	−234.95 (−162.73)	−227.92	0.309 (0.319)	−0.468 (−0.485)	181.0 (183.2)
12H^{+g}	−232.82 (−162.13)	−225.62	0.211 (0.250)	−0.429 (−0.464)	163.1 (171.5)
12H^{+h}	−224.36	−217.77	0.265	−0.348	
13H$^+$	−236.63 (−159.75)	−229.16	0.272 (0.298)	—	161.9 (166.4)
14H$^+$	−227.24 (−156.58)	−220.63	0.361 (0.361)	−0.468 (−0.485)	180.3 (181.0)
15H$^+$	−222.58 (−147.02)	−215.62	0.320 (0.356)	−0.337 (−0.360)	175.4 (180.4)
16H$^+$	−231.81[f]	−224.13	0.292	—	166.4
17H$^+$	−234.24[f]	−226.35	0.370	−0.473	183.1
18H$^+$	−216.30[f]	−209.30	0.336	−0.342	182.1

[a] PCM values in parenthesis (water as solvent). [b] Energy difference between carbocation and the neutral compound [as in reaction(1)]. [c] Including ZPE contribution. [d] At the carbocationic center (carbon plus hydrogen charges). [e] At the carbocationic center. [f] Energy difference between carbocation and the neutral compound (as in reaction 2). [g] Non bay-region carbocation formed. [h] Bay-region carbocation.

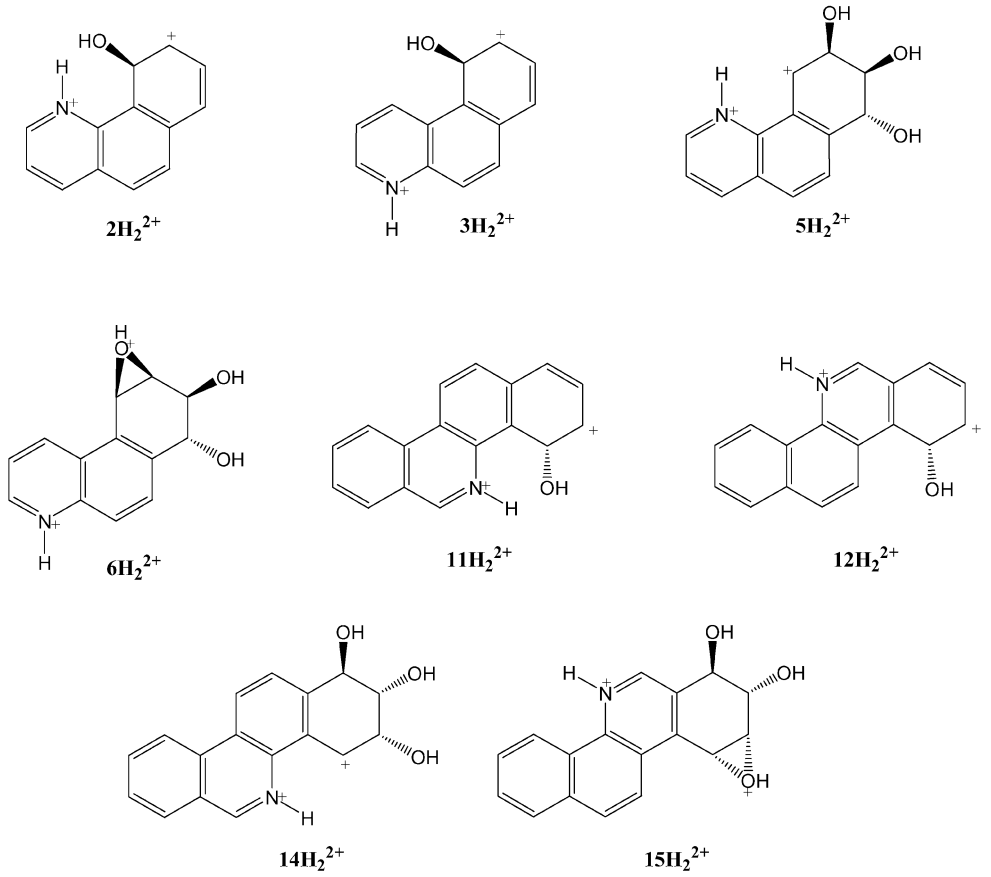


Fig. 4 Lowest energy dications generated from aza-PAHs.

carbocationic center is smaller for $\mathbf{1}H^+$ than for $\mathbf{2}H^+$, *i.e.*, the degree of stabilization by delocalization is larger for $\mathbf{1}H^+$, in accordance to its greater ease of formation. However, in the case of $\mathbf{3}H^+$ the ΔE_r does not correlate with the charge at the carbocationic center, as the last one is smaller than for $\mathbf{2}H^+$. On the other hand, it can be noted that $\mathbf{3}H^+$ presents a less negative charge at the nitrogen atom than $\mathbf{2}H^+$. The same observations apply to the diol epoxides, that is, $\mathbf{5}H^+$ is more stable than $\mathbf{6}H^+$ not because of a greater delocalization of the positive charge but as a consequence of its more negatively charged nitrogen atom. Regarding Chry and BcPhen derivatives, carbocation formation is more favored in the order $\mathbf{10}H^+ > \mathbf{11}H^+ > \mathbf{12}H^+$, and $\mathbf{13}H^+ > \mathbf{14}H^+ > \mathbf{15}H^+$. This trend was not observed for reaction (2) (compounds $\mathbf{16}H^+$, $\mathbf{17}H^+$, $\mathbf{18}H^+$).

Selected BcPhen derivatives present the nitrogen heteroatom in the bay region where the benzylic cation is formed (BcPhen-3,4-epoxide **11** and BcPhen-1,2-diol-3,4-epoxide **14**), or in the other bay region, more distant to the carbocationic center (BcPhen-9,10-epoxide **12** and BcPhen-7,8-diol-9,10-epoxide **15**). It should be noted, however, that **12** unexpectedly opened to give the non bay-region carbocation $\mathbf{12}H^+$ upon epoxide protonation. The bay-region carbenium ion ($\mathbf{12}H^{+\prime}$) was computed to be *ca.* 8 kcal mol^{-1} less stable. Comparison of NPA charges for both isomeric carbocations shows that in the most stable ($\mathbf{12}H^+$) the nitrogen has a somewhat greater negative charge, and the positive charge is more delocalized (Fig. 5). The same observation (that a larger negative charge on N accounts for a greater stability of the carbocation) was found for the corresponding diol epoxide compounds.

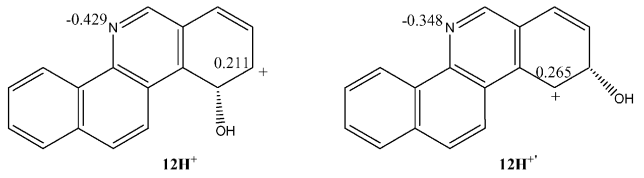
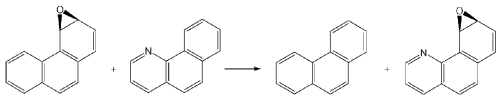


Fig. 5 NPA charges for both isomeric carbocations derived from **12**H.

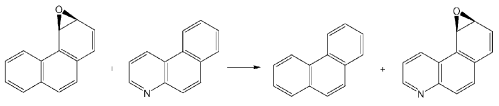
Aqueous phase calculations (PCM method with water as solvent) afforded the same reactivity pattern, although the ring opening reactions were less exothermic owing to the large solvation energy of the proton (despite the greater stabilization of the carbocations as compared to neutral epoxides). The same observations concerning conservation of delocalization mode trends were made regarding charge densities and NMR chemical shifts.

According to these results, the relative ease of formation of the carbocation by opening of the protonated epoxide and, consequently, the reactivity of the parent aza-PAH, increases with placement of a larger negative charge on the nitrogen heteroatom. On the other hand, the presence of one N atom within the aromatic ring structure produces a greater polarization of the charge distribution and, as a result, a minor degree of delocalization of the positive charge in the carbenium ion. Therefore, the lower activity of the benzoquinoline derivatives compared to the phenanthrenes could be explained by this reasoning. It is remarkable that inclusion of the solvent effect did not alter these observations.

In order to obtain some insight as to the effect of the nitrogen atom on the metabolic activation of aza-PAHs, isodesmic reactions (3) and (4) were calculated as a measure of the relative ease of formation of the aza-epoxides. As almost no energy differences were observed for the epoxides derived from Phe, BhQ and BfQ, it can be inferred that formation of these intermediates is not as relevant as carbocation formation in determining the relative reactivity of the ultimate metabolites.



(3)



(4)

DFT Study of covalent adducts of carbocations with representative nucleophiles

In order to simulate the crucial step of aza-PAH adduct formation, calculations of the nucleophilic reactions with MeO^- and MeS^- were performed for the carbocations **4**H$^+$, **5**H$^+$ and **6**H$^+$. These reactions were considered as models for evaluation of the reactivity trends for these carbocations toward nucleophiles. In this way, the thermodynamical tendency of each carbocation to react with the nucleophilic sites of DNA could be estimated.

Both *syn* and *anti* methoxy adducts were considered (Fig. 6). The 6-31+G* basis set was employed to give a proper description of the anions by inclusion of diffuse functions. The corresponding ΔE_r and ΔG_r for these reactions showed similar trends (see Table 2). Different rotamers in the corresponding adducts resulting from rotation of the C–OMe bond were computed by AM1, allowing for diverse dispositions of the methyl group. However, as differences in the heat of formation among these conformational isomers were lower than 2 kcal mol^{-1}, DFT calculations on all of these structures were not considered necessary in order to compare relative carbocation reactivities.

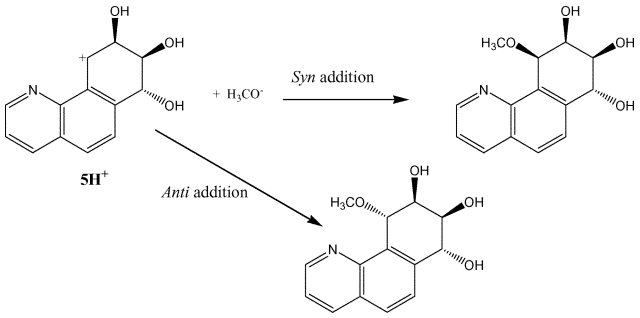


Fig. 6 Calculated nucleophilic reactions with MeO^-.

For **4**H$^+$ and **6**H$^+$ formation of the *anti* product was favored. On the other hand, attainment of a hydrogen-bond interaction between the nitrogen and one hydrogen atom of the –OMe group determined a noticeable preference for the *syn* product in the case of **5**H$^+$.

In aqueous phase calculations, the decrease in the exothermicity of the reactions due to the more efficient solvation of the charged reactants is especially noteworthy. Nevertheless, these reaction energies do not correlate with the known relative experimental activities of these compounds.[7] In this way, these results agree with the evidence proposing that epoxide ring opening mechanism for PAH activation could become substantially S_N1-like or proceed through proton stabilized transition states that present significant carbocationic character.[6d,20] Accordingly, the reactivity of this type of compounds would be predominantly governed by the feasibility of carbocation formation.

For comparison, the addition of methanethiolate anion to the bay-region carbocations derived from dihydrodiols **7**H$^+$ and **8**H$^+$ was also investigated (Fig. 7, Table 2). Again, the AM1 heats of formation for different rotamers of the adducts were computed. Since they differed by less than 1 kcal mol^{-1}, not all the rotational isomers were subsequently calculated at the DFT level.

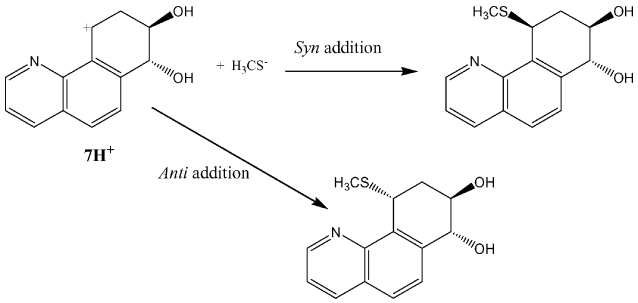


Fig. 7 Calculated nucleophilic reactions with MeS^-.

With MeS^- as nucleophile, formation of the *anti* product was favored for both **7**H$^+$ and **8**H$^+$. In this case, the longer MeS–C and H_3C–S bond distances provoked similar interactions between the nitrogen in **8**H$^+$ and one hydrogen atom of the –SMe group for both products, determining the usual preference for the *anti* product.

Furthermore, the adducts of **1**H$^+$ and **2**H$^+$ with guanine were computed at the B3LYP/6-31G* level. It has been established that bay-region diol epoxides derived from PAHs predominantly form stable adducts with DNA by *cis* or *trans* addition to the exocyclic amino groups of deoxyguanosine and deoxyadenosine.[21] Moreover, depurinating products that are lost from DNA by cleavage of the glycosidic bond are formed by reaction with the N-7 of guanine, and at N-3 and N-7 of adenine.[22] According to this, the adducts arising from reactions with the exocyclic nitrogen and with the N-7 of guanine were calculated for both carbocations. The *trans* products were selected, as this attack mode is preferred for *anti*-diol epoxides.[23] Conformational searches by rotation of the generated C–N bond were performed by AM1 calculations in order to find the most stable rotamer for each compound. In this way one global minimum was found for each N-7 adduct, while two isomers close in energy were identified for reactions with the exocyclic nitrogen, with the new C_{PAH}–$N_{guanine}$ bond and the carbonyl group on the same or opposite side of the guanine moiety. These lower energy structures were subsequently optimized by DFT calculations (Table 3, Figs. 8 and 9).

In all cases, guanine adducts with both **1**H$^+$ and **2**H$^+$ presented very similar conformations. A perpendicular arrangement between the two aromatic moieties was the more

Table 2 B3LYP/6-31+G* calculations for nucleophilic additions[a]

Compound	Nucleophile	Addition	ΔE_r/kcal mol^{-1}	ΔG_r/kcal mol^{-1}[b]
4H$^+$	H_3C–O^-	*syn*	−175.12 (−59.74)	−157.38
		anti	−177.73 (−62.88)	−159.62
5H$^+$	H_3C–O^-	*syn*	−183.04 (−64.40)	−164.62
		anti	−176.48 (−63.48)	−158.59
6H$^+$	H_3C–O^-	*syn*	−180.78 (−65.93)	−162.84
		anti	−182.98 (−69.34)	−164.80
7H$^+$	H_3C–S^-	*syn*	−146.52 (−38.13)	−131.87
		anti	−148.88 (−40.25)	−133.69
8H$^+$	H_3C–S^-	*syn*	−149.46 (−40.69)	−134.67
		anti	−151.18 (−43.16)	−137.12

[a] PCM values in parenthesis (water as solvent). [b] $T = 298.15$ K, 1 atm.

Table 3 B3LYP/6-31G* calculations for guanine adducts

Carbocation	Reacting nitrogen of guanine	Total energy/hartree		Relative energy/kcal mol^{-1}		$\Delta E_{reaction}$/kcal mol^{-1} [a]	
		Gas phase	Aqueous phase	Gas phase	Aqueous phase	Gas phase [b]	Aqueous phase
1H$^+$	Exocyclic[c]	−1157.27626	−1157.32382	3.74	4.27	215.64 (224.19)	264.63
	Exocyclic[d]	−1157.27842	−1157.33063 (−1157.33262)	2.38	0.00	214.28 (221.11)	260.36
	N-7	−1157.28222	−1157.32680 (−1157.32914)[e]	0.00	2.40	211.90 (220.80)	262.76
2H$^+$	Exocyclic[c]	−1173.31666	−1173.36596	1.68	3.99	213.95 (222.47)	262.61
	Exocyclic[d]	−1173.31717	−1173.37232	1.36	0.00	213.63 (220.84)	258.62
	N-7	−1173.31934	−1173.36863	0.00	2.32	212.27 (220.33)	260.94

[a] $\Delta E_{reaction}$ = Energy$_{Adduct}$ − Energy$_{Guanine}$ − Energy$_{Carbocation}$. [b] $\Delta G_{reaction}$ in parenthesis (T = 298.15 K, 1 atm). [c] C_{PAH}–$N_{guanine}$ bond and the carbonyl group on the same side of the guanine moiety. [d] C_{PAH}–$N_{guanine}$ bond and the carbonyl group on opposite sides of the guanine moiety. [e] PCM optimization.

Fig. 8 Adducts between guanine and **1**H$^+$; (a) exocyclic N, C–N bond and carbonyl group on opposite sides of the guanine moiety; (b) exocyclic N, C–N bond and carbonyl group on the same side of the guanine moiety; (c) N-7 isomer.

favored disposition for every adduct. According to gas-phase calculations, the most stable isomer was the N-7 product for both carbocations, being *ca.* 2 kcal mol^{-1} lower in energy than the preferred conformation for the exocyclic adduct. This fact can be ascribed to the stabilization brought about by two intramolecular hydrogen bonds in the N-7 product (between the guanine carbonyl oxygen and the two bay-region hydrogens in **1**H$^+$), *vs.* one hydrogen bond in the exocyclic one. Especially noticeable is the deviation in the aromatic systems array that takes place in the **2**H$^+$ adduct in order to attain the second hydrogen-bond interaction, as this species has only one bay-region hydrogen atom. Superimposed structures are shown in the supporting information. Among both pairs of exocyclic isomers, the most stable is the one that permits the achievement of the only intramolecular hydrogen bond with the more perpendicular arrangement of the aromatic moieties. On the other hand, aqueous-phase calculations (both single points and optimizations) reverted the relative stability of the adducts, with the exocyclic adduct becoming the most stable by about 2 kcal mol^{-1}. Optimizations in aqueous phase yielded a decrease in energy of less than 2 kcal mol^{-1} as compared with the single-point PCM computations of the gas-phase geometries.

Reaction energies for the formation of each type of adduct with both carbocations (measured as the energy difference between the adduct minus guanine and carbocation total energies) were comparable. The same observation was made for the ΔG_r values. Inclusion of the solvent caused an increase in the endothermicity of the reactions, presumably due to a better solvation of the carbocations.

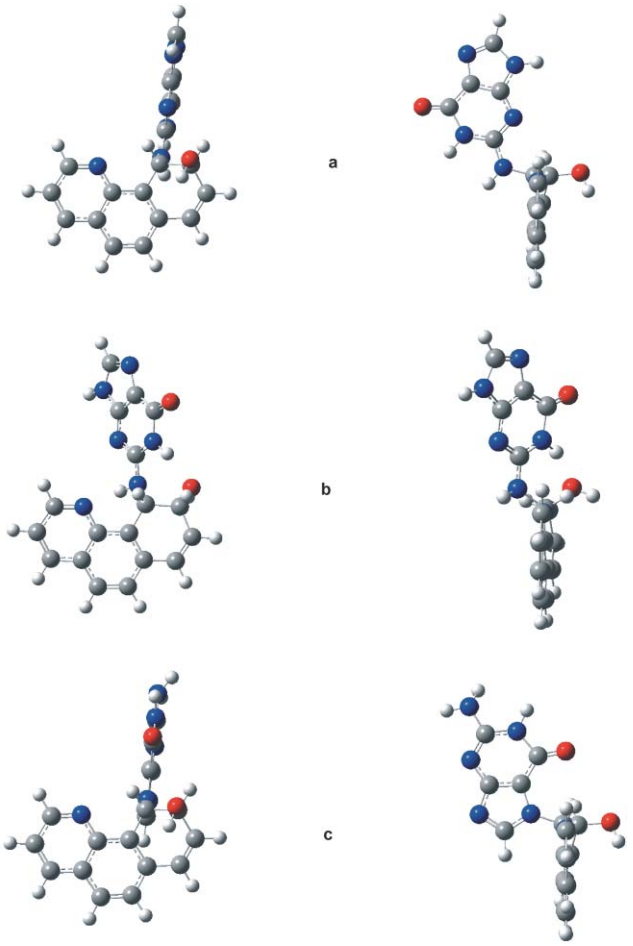


Fig. 9 Adducts between guanine and $2H^+$; (a) exocyclic N, C–N bond and carbonyl group on opposite sides of the guanine moiety; (b) exocyclic N, C–N bond and carbonyl group on the same side of the guanine moiety; (c) N-7 isomer.

Comparative discussion and summary

According to the present calculations, nitrogen substitution did not significantly modify the charge density distribution, as determined by NPA-derived charges and the GIAO-NMR chemical shifts. Therefore, similar reactivity patterns are expected to be followed by both PAH and aza-PAH compounds. The calculated relative ease of formation, *i.e.*, the stability of the carbocations correlated with the mutagenic activities observed for tetrahydro epoxide and tetrahydro diol epoxide derivatives of Phe, BhQ and BfQ. In all cases, a larger negative charge on N accounted for a greater stability of the carbocation.

The ΔE_r and ΔG_r values for reactions between the carbocations of Phe derivatives and those derived from aza-PAHs with nucleophiles did not correlate with the known relative experimental activities of these compounds. This fact reinforces the notion that the stability of the generated carbocation is the determining factor for its reactivity, by an S_N1-like process (epoxide ring opening). Thus, as stated above, the experimental activity order Phe > BhQ > BfQ could be explained by the relative stabilities of their derived carbocations.

Aqueous-phase computations modified the change in energy for the addition reactions due to greater stabilization of the reactants, although inclusion of the solvent did not alter the relative reactivity trends. The change in the preferred product of the addition reactions with guanine due to solvent effect is noteworthy. Whereas the N-7 adducts were most stable in the gas phase, in water as solvent the most stable adducts were those formed by reaction with the exocyclic nitrogen of guanine, in accordance with the experimental observations. Therefore, the present model DFT study has provided a very reasonable description of the reactivity of this type of compounds.

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References

1 (*a*) International Agency for Research on Cancer, *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Polycyclic Aromatic Compounds, Part 1, Chemical Environmental and Experimental Data*, IARC, Lyon, 1983; (*b*) D. Hoffmann and E. L. Wynder, in *Air Pollution*, ed. A. C. Stern, Academic Press, New York, 1977, vol. 2, pp. 361–455.

2 (*a*) R. G. Harvey, in *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*, Cambridge University Press, Cambridge, UK, 1991; (*b*) M. Nordquist, D. R. Thakker, H. Yagi, R. E. Lehr, A. W. Wood, W. Levin, A. H. Conney and D. M. Jerina, in *Molecular Basis of Environmental Toxicity*, ed. R. S. Bhatnagar, Ann Arbour Science Publishers, Ann Arbour, MI, 1980, pp. 329–357.

3 (*a*) Committee on Biologic Effects of Atmospheric Pollutants, *Particulate Polycyclic Organic Matter, Biological Effects of Atmospheric Pollutants*, National Academy of Sciences, Washington, DC, 1972; (*b*) B. L. Van Duuren, J. A. Bilboa and C. A. Joseph, *J. Natl. Cancer Inst.*, 1960, **25**, 53–61.

4 (*a*) M. Dong, D. D. Locke and D. Hoffmann, *Environ. Sci. Technol.*, 1997, **11**, 612–618; (*b*) I. Schmeltz and D. Hoffmann, *Chem. Rev.*, 1977, **77**, 295–311; (*c*) International Agency for Research on Cancer, *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds*, IARC, Lyon, 1972, vol. 3; (*d*) M. Dong, I. Schmeltz, E. La Voie and D. Hoffman, in *Carcinogenesis: Polynuclear Aromatic Hydrocarbons*, ed. P. W. Jones and R. I. Freudenthal, Raven Press, New York, 1978, vol. 2, pp. 97–108.

5 (*a*) A. W. Wood, R. L. Chang, W. Levin, D. E. Ryan, P. E. Thomas, R. E. Lehr, S. Kumar, M. Schaefer-Ridder, U. Engelhardt, H. Yagi, D. M. Jerina and A. H. Conney, *Cancer Res.*, 1983, **43**, 1656–1662; (*b*) W. Levin, A. W. Wood, R. L. Chang, S. Kumar, H. Yagi, D. M. Jerina, R. E. Lehr and A. H. Conney, *Cancer Res.*, 1983, **43**, 4625–4628; (*c*) R. L. Chang, W. Levin, A. W. Wood, S. Kumar, H. Yagi, D. M. Jerina, R. E. Lehr and A. H. Conney, *Cancer Res.*, 1984, **44**, 5161–5164; (*d*) A. W. Wood, R. L. Chang, W. Levin, S. Kumar, N. Shirai, D. M. Jerina, R. E. Lehr and A. H. Conney, *Cancer Res.*, 1986, **46**, 2760–2766; (*e*) R. L. Chang, W. Levin, A. W. Wood, N. Shirai, A. J. Ryan, C. C. Duke, D. M. Jerina, G. M. Holder and A. H. Conney, *Cancer Res.*, 1986, **46**, 4552; (*f*) A. W. Wood, R. L. Chang, M. Katz, A. H. Conney, D. M. Jerina, H. C. Sikka, W. Levin and S. Kumar, *Cancer Res.*, 1989, **49**, 6981; (*g*) R. E. Lehr, A. W. Wood, W. Levin, A. H. Conney and D. M. Jerina, in *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure–Activity Relationships*, ed. S. K. Yang and B. D. Silverman, CRC Press, Boca Raton, FL, 1988, pp. 31–58; (*h*) R. L. Chang, A. W. Wood, S. Kumar, R. E. Lehr, S. Naohiro, D. M. Jerina and A. H. Conney, *Carcinogenesis*, 2000, **21**, 1997–2003; (*i*) D. Warshawsky, *J. Environ. Sci. Health, Part C*, 1992, **10**, 1–71; (*j*) R. L. Chang, S. Battista, C. Q. Wong, S. Kumar, P. L. Kole, H. C. Sikka, S. K. Balani, D. M. Jerina, A. H. Conney and A. W. Wood, *Carcinogenesis*, 1993, **14**, 2233–2237; (*k*) R. L. Chang, A. W. Wood, J. G. Xie, M. T. Huang, X. Cui, P. L. Kole, H. C. Sikka, S. K. Balani, D. M. Jerina, S. Kumar and A. H. Conney, *Proc. Am. Assoc. Cancer Res.*, 1996, **37**, 128.

6 (*a*) P. B. Hulbert, *Nature*, 1975, **256**, 146–148; (*b*) S. K. Yang, D. W. McCourt and H. V. Gelboin, *J. Am. Chem. Soc.*, 1977, **99**, 5130–5135; (*c*) T. C. Bruice and P. Y. Bruice, *Acc. Chem. Res.*, 1976, **9**, 378–384; (*d*) S. M. Fetzer, C.-R. Huang, R. G. Harvey and P. R. LeBreton, *J. Phys. Chem.*, 1993, **97**, 2385–2394 and references therein.

7 S. Kumar, H. C. Sikka, S. K. Dubey, A. Czech, N. Geddie, C.-X. Wang and E. J. LaVoie, *Cancer Res.*, 1989, **49**, 20–24.

8 R. E. Lehr and D. M. Jerina, *Tetrahedron Lett.*, 1983, **24**, 27–30.

9 (*a*) G. L. Borosky, *J. Org. Chem.*, 1999, **64**, 7738–7744; (*b*) G. L. Borosky, *Helv. Chim. Acta*, 2001, **84**, 3588–3599; (*c*) G. L. Borosky, *J. Comput. Chem.*, 2003, **24**, 601–608.

10 (*a*) T. Okazaki, K. K. Laali, B. Zajc, M. K. Lakshman, S. Kumar, W. M. Baird and W.-M. Dashwood, *Org. Biomol. Chem.*, 2003, **1**, 1509–1516; (*b*) K. K. Laali and P. E. Hansen, *J. Org. Chem.*, 1997,

62, 5804–5810; (*c*) K. K. Laali and M. Tanaka, *J. Org. Chem.*, 1998, **63**, 7280–7285; (*d*) K. K. Laali, T. Okazaki, S. Kumar and S. E. Galembeck, *J. Org. Chem.*, 2001, **66**, 780–788; (*e*) K. K. Laali, T. Okazaki and R. G. Harvey, *J. Org. Chem.*, 2001, **66**, 3977–3983; (*f*) K. K. Laali, T. Okazaki and P. E. Hansen, *J. Org. Chem.*, 2000, **65**, 3816–3828; (*g*) K. K. Laali, T. Okazaki and M. M. Coombs, *J. Org. Chem.*, 2000, **65**, 7399–7405; (*h*) Review: K. K. Laali and T. Okazaki, *Annu. Rep. NMR Spectrosc.*, 2002, **47**, 149–214; (*i*) K. K. Laali in *Carbocation Chemistry*, ed. G. A. Olah and G. K. S. Prakash, Wiley, 2004, ch. 9; (*j*) T. Okazaki and K. K. Laali, *Org. Biomol. Chem.*, 2003, **1**, 3078–3093; (*k*) T. Okazaki and K. K. Laali, *J. Org. Chem.*, 2004, **69**, 510–516.

11 (*a*) G. Lamm and G. R. Pack, *Proc. Nat. Acad. Sci. U. S. A.*, 1990, **87**, 9033–9036; (*b*) S. Hanlon, L. Wong and G. R. Pack, *Biophys. J.*, 1997, **72**, 291–300.

12 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, Y. Nakajima, O. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yasyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannemberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. A. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, *Gaussian 03, Revision B.05*, Gaussian, Inc., Wallingford, CT, 2003.

13 (*a*) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652; (*b*) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785–789; (*c*) B. Miehlich, A. Savin, H. Stoll and H. Preuss, *Chem. Phys. Lett.*, 1989, **157**, 200.

14 P. C. Hariharan and J. A. Pople, *Theor. Chim. Acta*, 1973, **28**, 213–222.

15 D. A. Liotard, G. D. Hawkins, G. C. Lynch, C. J. Cramer and D. G. Truhlar, *J. Comput. Chem.*, 1995, **16**, 422.

16 (*a*) S. Miertus, E. Scrocco and J. Tomasi, *Chem. Phys.*, 1981, **55**, 117; (*b*) M. T. Cances, V. Mennucci and J. Tomasi, *J. Chem. Phys.*, 1997, **107**, 3032; (*c*) V. Barone, M. Cossi and J. Tomasi, *J. Comput. Chem.*, 1998, **19**, 404.

17 P. v. R. Schleyer, C. Maerker, A. Dransfeld, H. Jiao and N. J. v. E. Hommes, *J. Am. Chem. Soc.*, 1996, **118**, 6317–6318.

18 (*a*) K. Wolinski, J. F. Hinton and P. Pulay, *J. Am. Chem. Soc.*, 1990, **112**, 8251; (*b*) R. Dichfield, *Mol. Phys.*, 1974, **27**, 789.

19 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902–3909.

20 (*a*) N. T. Nashed, A. Bax, R. J. Loncharich, J. M. Sayer and D. M. Jerina, *J. Am. Chem. Soc.*, 1993, **115**, 1711; (*b*) N. T. Nashed, S. K. Balani, R. J. Loncharich, J. M. Sayer, D. Y. Shipley, D. L. Whalen and D. M. Jerina, *J. Am. Chem. Soc.*, 1991, **113**, 3910; (*c*) N. T. Nashed, T. V. S. Rao and D. M. Jerina, *J. Org. Chem.*, 1993, **58**, 6344; (*d*) J. W. Keller and C. Heildelberger, *J. Am. Chem. Soc.*, 1976, **98**, 2328.

21 (*a*) D. M. Jerina, A. Chadha, A. M. Cheh, M. E. Schurdak, A. W. Wood and J. M. Sayer, in *Biological Reactive Intermediates IV. Molecular and Cellular Effects and Their Impact on Human Health*, ed. C. M. Witmer, R. Snyder, D. J. Jollow, G. F. Kalf, J. J. Kocsis and I. G. Sipes, Plenum Press, New York, 1991; (*b*) A. Dipple, in *DNA Adducts: Identification and Biological Significance*, ed. K. Hemminki, A. Dipple, D. E. G. Shuker, F. F. Kadlubar, D. Segerbaeck and H. Bartsch, IARC Publications, Lyon, 1994.

22 E. L. Cavalieri and E. G. Rogan, in *The Handbook of Environmental Chemistry: PAHs and Related Compounds*, ed. A. H. Neilson, Springer, Heidelberg, 1998, vol. 3J, pp 81–117.

23 S. K. Agarwal, J. M. Sayer, H. J. C. Yeh, L. K. Pannell, B. D. Hilton, M. A. Pigott, A. Dipple, H. Yagi and D. M. Jerina, *J. Am. Chem. Soc.*, 1987, **109**, 2497–2504 and references therein.