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Molecular Modeling of Toxic Indole Derivatives from High Temperature Cooking

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Abstract

More than two decades ago, Japanese scientists discovered a new family of highly mutagenic compounds classified as heterocyclic aromatic amines from roasted meat and grilled Fish. This group of compounds will form the basis of this investigation from a theoretical perspective. In order to simulate high temperature cooking and explore the thermochemical properties of these compounds, high level quantum calculations were employed. Accordingly, the theoretical behaviour of indole derivatives; isoindazole, 1-methyl indole, 4,7-dimethyl isoindazole and carbazole were explored at a pressure of 1 atmosphere over a wide range of pyrolysis temperatures (323-923 K) typically at temperature increments of 50 K. *Ab initio* analytical gradients at MP2 level of theory with 3-21G and 6-31G(d,p) basis sets and Molecular Mechanics (MM) with universal force field (UFF) from *Gaussian 03* computational platform were used for geometry optimization, internal energy calculations, molecular orbitals, and vibrational frequencies. It was observed that the internal energy for isoindazole at 323 K was 75.80 kcal/mol whereas that of carbazole was 123.78 kcal/mol under similar conditions of pressure and temperature. The stability of these molecular compounds decreased with increase in pyrolysis temperature. To make decent conclusions on the potency of these indole derivatives and their effect on human health, toxicity values were estimated using Quantitative Structural Activity Relationship (QSAR) method found in HypeChem computational software. Toxicity indices for isoindazole, 1-methyl indole, 4,7-dimethyl isoindazole and carbazole were -0.16, 0.01, 0.14 and -0.07 respectively, while those for their corresponding radicals were -0.12, -0.21, 0.19 and -0.17. These values point to highly hydrophilic species which indicate that they are very toxic.

Key Words: *Ab initio*, by-product, isoindole, pyrolysis, QSAR, toxicity

Introduction

Health problems caused by mutagenic compounds of the heterocyclic amine (HCA) derivatives resulting from high temperature cooking and other pyrolysis sources is a global concern. More than two decades ago, Japanese scientists discovered a new group of highly mutagenic compounds classified as heterocyclic aromatic amines from broiled and grilled meat, and fish dishes (Jagerstad, Skog, Arvidsson, & Solyakov, 1998). These are chemicals formed when meat, including beef, pork, fish, chicken is cooked using high-temperature procedures including pan

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frying or grilling directly over an open flame. It has been shown in previous studies that rodents fed on a diet supplemented with HCAs developed tumors of the breast, colon, liver, skin, lung, and prostate cancers (Jagerstad et al., 1998). In the search for possible correlation between diet and cancer, the highly mutagenic heterocyclic amines present in cooked foods have attracted a great deal of attention (Doll & Peto, 1981; Jagerstad et al., 1998). The present studies are fascinated with their formation, occurrence in food products, bio-transformation, and carcinogenicity.

HCAs are formed when amino acids (the building blocks of proteins), sugars, and creatine (a substance found in muscle) react at high temperatures (Cross & Sinha, 2004). HCAs become capable of damaging DNA when they are metabolized by specific enzymes in the body, following a process called bioactivation which in turn initiates the development of cancer risks and other cellular damaging consequences (Sugimura, 2004).

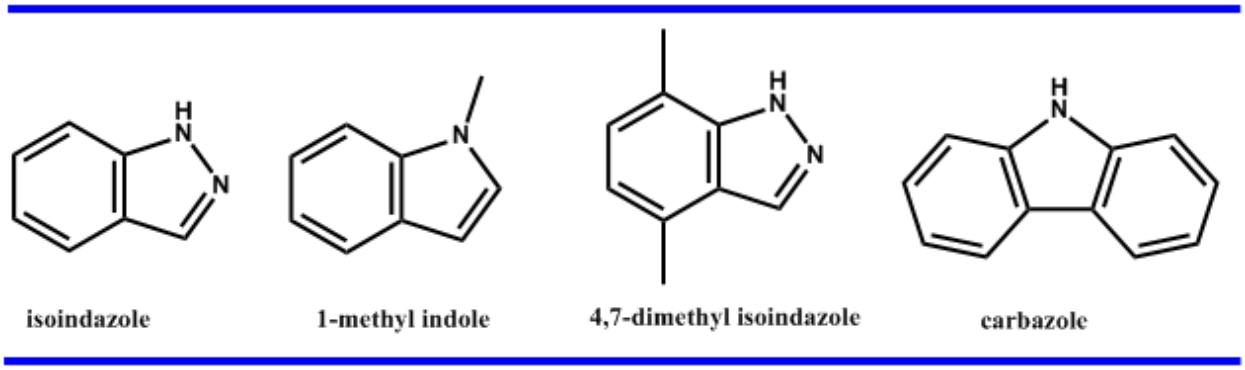

Fig. 1. Structures of indole derivatives investigated in this work

The by-products of high temperature cooking are responsible for serious health problems such as cancer, heart attack, and respiratory problems including emphysema. The indole derivatives (isoindazole, 1-methyl indole, 4,7-dimethyl isoindazole, Carbazole) under study in this work (Fig. 1) are suspected to account for the largest part of the burden of illness and deaths in developing countries from biological complications. Therefore, there is need to categorize these compounds under the new family of highly mutagenic compounds and find ways of controlling their consumption in foods in order to minimize grave biological impacts affecting human health and environmental ecosystems.

Free radical chemistry

Free radical studies have revealed that many neuronal diseases including Parkinson's disease, and aging problems, diabetes, cardiac arrest, prematurity in babies and cancer, are linked to oxidative stress (Majima & Toyokuni, 2012). At high temperatures hydrogen is abstracted from the molecules forming reactive free radicals species which have the potential to react with biological cells leading to cellular assault and subsequently lethal effects. The concept of investigating the thermochemical behavior and toxicity indices of these free radicals is informed by the biological impacts they cause. Stable free radicals have longer life-times resulting to serious biological impacts (Kibet, Khachatryan, & Dellinger, 2012). High temperature cooking therefore is a sink where harmful free radicals are formed. It is therefore important that barbecue, *nyama choma*, and broiled meat lovers should be informed of the dangers of high temperature cooking.

Computational Methodology

This work was carried out using *Gaussian 03* (Frisch, et al., 2004) suite of programs using various levels of computational theories and different basis sets. To compute the energy change for formation of a compound or a radical from its constituents, the following thermodynamic equation (equation 1) is fundamental (Ochtorski, 2000).

$$\Delta rH^0 = \sum \left(\varepsilon_0 - H_{corr}\right)_{products} - \sum \left(\varepsilon_0 - H_{corr}\right)_{reac\,tan\,ts} \qquad (1)$$

where ΔrH^0 is change in enthalpy of the reaction

H_{corr} is correction to the thermal enthalpy

ε_0 is the sum of electronic and thermal enthalpies

Geometry optimization calculations were conducted for isoindole and 1-methyl indole using DFT/B3LYP analytical gradient. The primary objective was to find the arrangement of nuclei for which the potential energy is a minimum. The calculation is said to converge once the minimum energy has been reached on the potential energy surface (PES). Nevertheless, a lot of energy is required to get atoms very close to each other.

Results and Discussion

Figure 2 presents plots of internal energy as a function of pyrolysis temperature at 1 atm for indole derivatives. This graph predicts a linear increase in internal energy with temperature for all the compounds investigated in this study. Clearly, carbazole being a large molecule has the highest internal energies over the whole range of pyrolysis temperature. The smallest molecule, isoindazole has the least internal energies at various temperatures, suggesting that it is the most stable of the four indole derivatives.

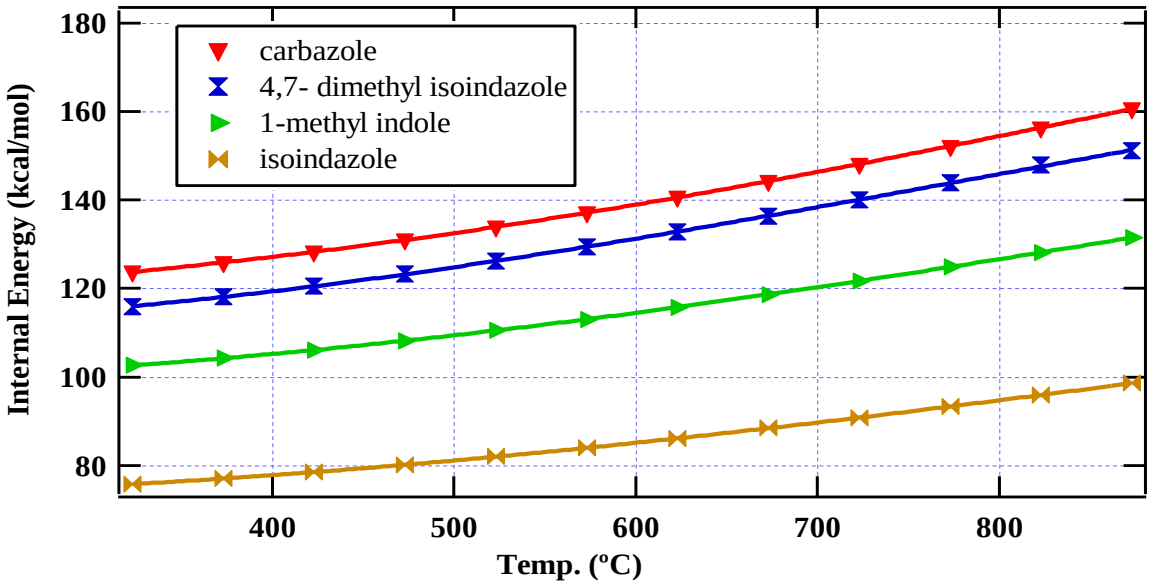

Fig. 2. Internal energy as a function of pyrolysis temperature for indole derivatives

Evidently, Fig. 3, *vide infra*, shows that DFT and MP2 give approximately similar internal energies for a given type of molecule. These analytical gradients from a literature standpoint usually give accurate results. HF and Molecular Mechanics (MM) give somewhat exaggerated results because they do not take into account electron correlation functions. Semi-empirical data (SEMI) yield comparable results to those of DFT and MP2. Remarkably, all these methods were employed to fully investigate the thermochemical characteristics of the indole derivatives in this work. The internal energies predict the vibrational behavior of molecules at a given temperature and subsequently estimate the stability of molecules. Notably, the smaller basis set 3-21G and the larger basis set 6-31G gave approximately similar internal energies for all the molecules investigated in this study (Fig. 3). The universal force field (UFF) of molecular mechanics gave results that are slightly higher than expected. The restricted and the unrestricted force fields of semi-empirical calculations yield similar results for individual molecules (Fig. 3).

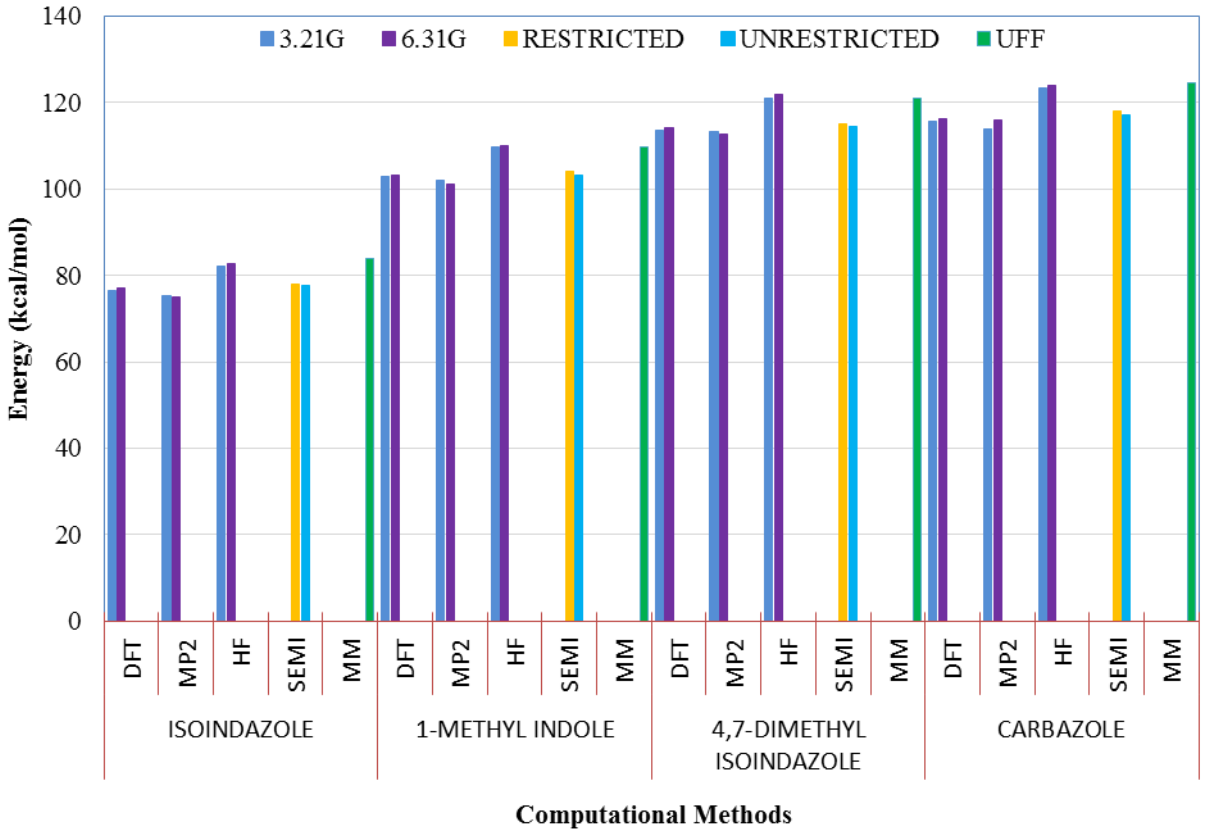

Fig. 3. Internal energy variations using different computational analytical gradients

The optimization process presented in Fig. 4 shows steps towards achieving an optimized structure for 1-methyl indole. This molecule required 17 steps to attain a structure of minimum energy, usually referred to as the global energy. Clearly, this is a fascinating quantum mechanics result that cannot easily be accomplished by experimental. The smallest molecule (isoindole) took 12 optimization steps to attain a structure of minimum energy. Therefore, the larger the molecule, the more the optimization steps and the longer the computational time scale.

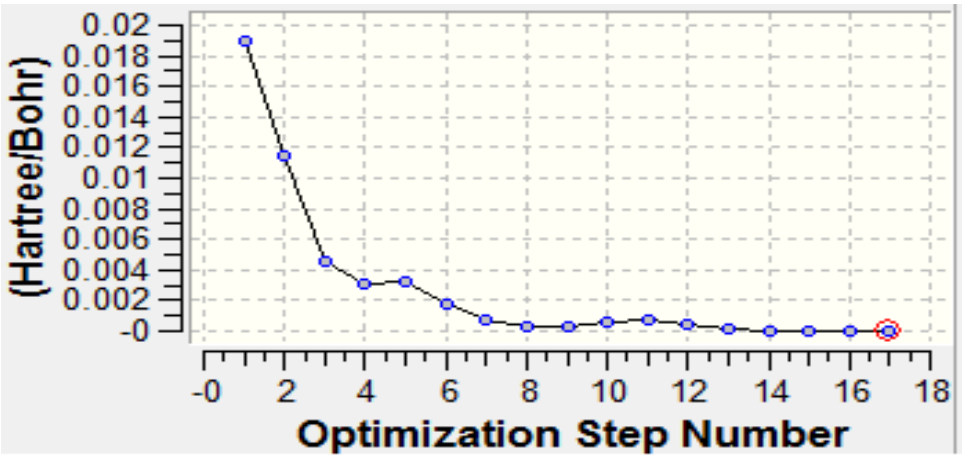

Fig. 4. The optimization plot for 1-methyl indole (the red circle is the optimization level)

All the other three indole derivatives (isoindazole, 4,7-dimethyl isoindazole, Carbazole) were also optimized in the same manner when computing the internal energies presented in Fig. 3. As an example only the optimization curve for 1-methyl indole has been presented in this work (Fig. 4). Moreover, the optimized structures for isoindole and 1-methyl indole are presented in Fig. 5 also as examples.

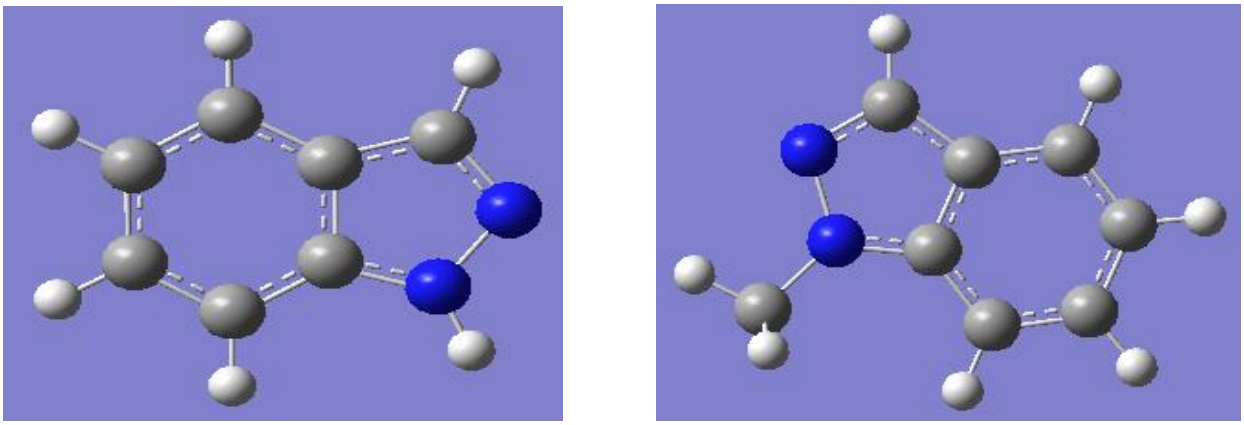

Fig. 5. Optimized Structures for isoindole and 1-methyl indole conducted at 298.15 K.

Toxicity Indices

The toxicity indices for the combustion by-products presented in this work were estimated using HyperChem computational platform (HyperChem®, 2002). HyperChem computational software contains a suite of computational programs such as quantitative structural activity relationship (QSAR). QSAR method is used to calculate the relative toxicity indices of a compound and determine its toxicity in humans and the environmental ecosystems. Hydration energies for indole derivatives were also determined using QSAR. Hydration energies are fundamental in predicting solvation properties of a compound in biological systems. Higher hydration energies lead to a higher probability that a compound may cause oxidative reactions with body cells and consequently cell damage. The hydration energy plays a critical role in determining the ionic

mobilities of compounds. The higher the hydration energy of a component, the greater is its solubility in water. Furthermore, the extent of hydration energies depends upon the size of the species. Smaller ionizable species are easily hydrated giving rise to greater hydrated ionic radius and less ionic mobility. The logarithm of the octanol-water partition coefficient $(\log P)$ is an important parameter which affects metabolic reactions, biological transport properties, and intrinsic toxicity (Smith & Hansch, 2000). The quantitative structural activity relations for indole derivatives investigated in this study are reported in Table 1.

Table 1. Quantitative structural activity relationship (QSAR) parameters

Compounds		Toxicity index (logP)	P	Hydration energy (kcal/mol)	Molar Mass (g/mol)
1.	1 methyl indole	0.01	1.023	-1.91	131.18
	1-methyl indolyl radical	-0.21	0.617	-3.53	130.17
2.	4,7-dimethyl isoindazoyl	0.14	1.38	-4.81	146.19
	4,7-dimethyl isoindazoyl radical	0.19	1.549	-3.05	145.18
3.	Isoindazole	-0.16	0.692	-7.83	118.14
	Isoindazoyl radical	-0.12	0.759	-5.86	117.13
4.	Carbazole	-0.07	0.851	-4.85	167.21
	Carbazoyl radical	-0.17	0.676	-3.37	166.20

Log P value is generally a predictor of toxicity, the range of log P values associated with optimum toxicity is influenced both by the chemical structure of the compound and by the particular type of toxicity. Therefore from the table above we can deduce that logP values are very high pointing to highly hydrophilic compounds. The level of toxicity decreases in the order; 4,7-dimethyl isoindazole > 1-methyl indole > Carbazole > isoindazole. This shows the rate of radical formation is very high in 4,7-dimethyl isoindazole and low in Carbazole.

Molecular Orbitals

HOMO and LUMO are acronyms for highest occupied molecular orbital and lowest unoccupied molecular orbital, respectively. HOMO and LUMO are sometimes referred to as frontier orbitals. These orbitals are the pair that lies nearest in energy of any pair of orbitals in any two molecules, which permits them to interact more strongly. Computational calculations therefore predict areas where interactions can occur to cause a reaction.

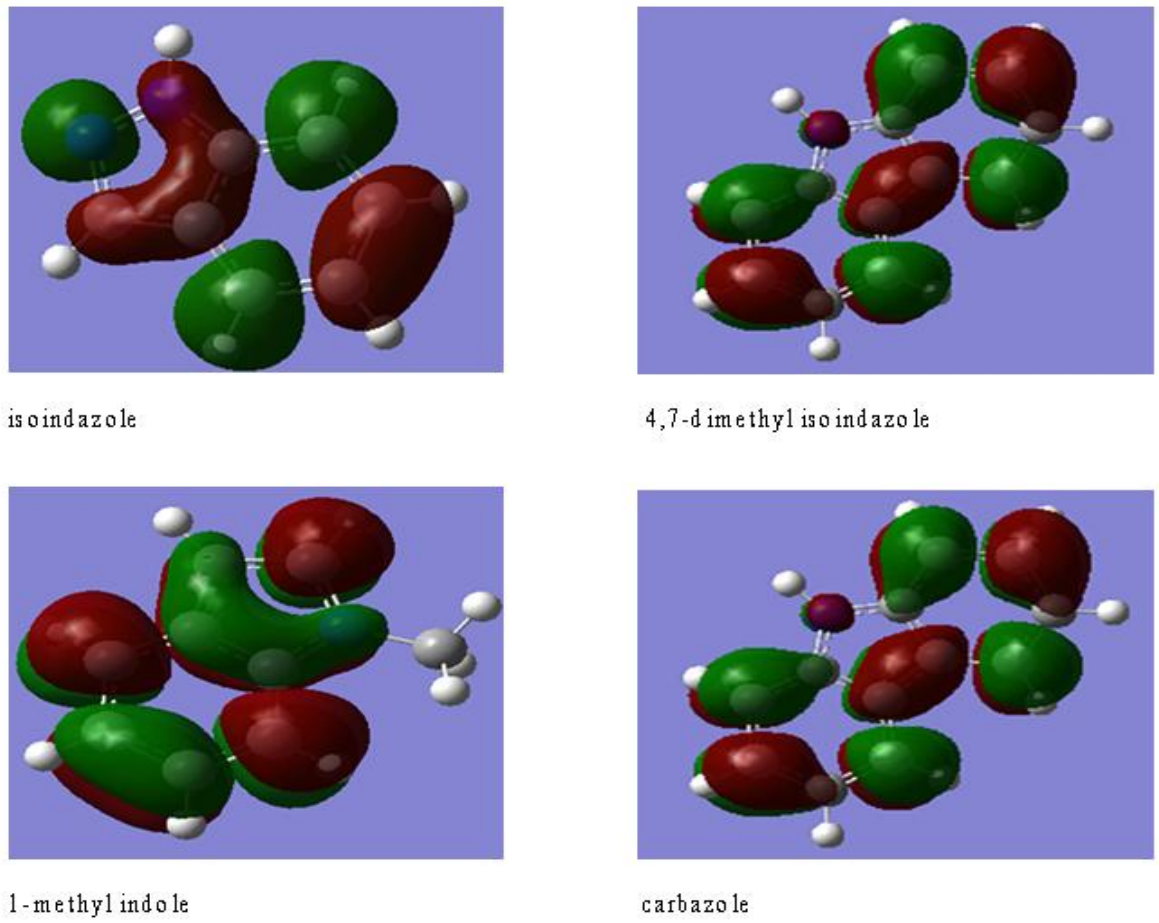

Fig. 6. Molecular orbital showing electron density around the constituent atoms of indole derivatives examined in this study

The molecular orbitals and electron density potentials for isoindazole, 1-methyl indole, 4,7-dimethyl isoindazole and carbazole are presented explicitly in Fig. 6. The red shades indicate regions of high electron density while the green shades show regions of low electron density. Interestingly, smaller molecules such as isoindazole and 1-methyl indole have a high tendency to react because of the high electron density around them. On the other hand, large molecules such as carbazole have high steric effects and thus low electron density in the region of possible interaction with other molecules. Nevertheless, their bulky nature implies that they are highly hydrophobic as predicted by QSAR data presented in Table 1.

Conclusion

Computational modeling of indole derivatives has provided an understanding into their bio-hazardous behavior, their thermochemistry, electronic properties, and subsequently their toxicities. It is evident from the quantitative activity relationship (QSAR) data, that most of the molecular compounds and their corresponding free radicals are highly hydrophilic. This implies that they can readily react with biological compounds causing cellular injury resulting to fatal consequences. From the thermochemical data, it was found that carbazole exhibited a high

internal energy over the whole range of pyrolysis temperature and predictably the most unstable of the four indole derivatives examined in this work. This suggests that carbazole, supported by low QSAR data is the most toxic compound. The formation of these mutagenic compounds in high temperature cooking is a serious health concern that must be tackled through scientific research and informing the general public on better cooking procedures.

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