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Diversity and abundance of high-molecular-weight azaarenes in PAH-contaminated environmental samples

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Abstract

Azaarenes are N-heterocyclic polyaromatic pollutants that co-occur with polycyclic aromatic hydrocarbons (PAHs) in contaminated soils. Despite the known toxicity of some high-molecular-weight azaarenes, their diversity, abundance, and fate in contaminated soils remain to be elucidated. We applied high-resolution mass spectrometry and mass-defect filtering to four PAH-contaminated samples from geographically distant sites and detected 232 azaarene congeners distributed in eight homologous series, including alkylated derivatives and two hitherto unknown series. Four- and five-ring azaarenes were detected among these series, and the most abundant non-alkylated congeners groups ($C_{13}H_9N$, $C_{15}H_9N$, $C_{17}H_{11}N$, $C_{19}H_{11}N$, and $C_{21}H_{13}N$) were quantified. The profiles of congener groups varied among different sites. Three-ring azaarenes presented higher concentrations in unweathered sites, while four- and five-ring azaarenes predominated in weathered sites. Known toxic and carcinogenic azaarenes, such as benzo[*c*]acridine and dibenzo[*a,h*]acridine, were detected along with their multiple isomers. Our results highlight a previously unrecognized diversity and abundance of azaarenes in PAH-contaminated sites, with corresponding implications for environmental monitoring and risk assessment.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting information

Further information on samples; details on instrumental analysis; mass spectra of azaarene isomers analyzed by qTOF in product ion scan mode (MS/MS); initial PAH concentrations; HPLC-MS/MS analytical parameters for quantification; concentrations of non-alkylated azaarene congeners.

Notes

The authors declare no competing financial interest.

INTRODUCTION

Azaarenes, also known as polycyclic aromatic nitrogen heterocycles (PANH), are the heterocyclic analogues of polycyclic aromatic hydrocarbons (PAHs) in which one or more nitrogen atoms are incorporated into the aromatic structure. While sharing some common properties with PAHs, the presence of nitrogen brings special characteristics to azaarenes, such as greater polarity and higher structural diversity. Some azaarenes are produced for the manufacture of dyes, paints, pharmaceuticals, and pesticides, but they also originate from petrogenic and pyrogenic processes, and therefore they exist in derivatives of coal (tar, creosote) and crude oil.^{1, 2} As a consequence they can potentially co-occur at relatively high concentrations in PAH-contaminated sites such as those associated with coke works, manufactured-gas plants, and wood-treatment facilities.³ However, current practices in risk assessment and remediation overlook the presence and potential risks of contaminants that co-occur with the regulated PAHs, such as oxygenated PAHs and heterocyclic aromatics.^{4, 5} Many azaarenes are toxic; for example, some of the high-molecular-weight (HMW) compounds (containing 4 or more rings) are mutagenic and carcinogenic to animals,^{6, 7} being major contributors of AhR-mediated activities.⁸ In addition, azaarenes are more water-soluble and mobile than PAHs, and thus have been found in groundwater at sites contaminated with creosote⁹ and coal tar.¹⁰ Their toxicity and environmental mobility suggest that azaarenes have implications for human and environmental health.

Azaarenes in contaminated sites have rarely been analyzed, and there is a general lack of knowledge on their environmental behavior and fate.¹ While recent studies have focused on targeted analysis of low-molecular-weight (LMW) azaarenes such as quinoline and acridine, 11–14 limited attention has been paid to the occurrence of isomers of these compounds, methylated derivatives and HMW homologs in contaminated soils.^{15, 16} As a result the overall contribution of azaarenes to toxicity may be significantly underestimated, especially for the HMW compounds. Considering their potential persistence and the risk to public health, it is necessary to fill this gap of knowledge by elucidating the existence and diversity of these pollutants.

In this study, we applied high-resolution mass spectrometry (HRMS) and Kendrick mass defect (KMD) analysis to samples from four different PAH-contaminated sites to reveal the diversity and distribution of azaarene congeners containing one nitrogen atom. Mass-defect

filtering is a nontarget analytical method based on HRMS that has been used to group and illustrate the occurrence of compound classes featuring hetero atoms in complex samples.^{17, 18} Compound classes are distinguished by transforming the exact mass according to a specific mass scale and then calculating the mass defects;^{19, 20} KMD is the most commonly used scale and is based on the monoisotopic and nominal mass of a CH₂ group (described in Supporting Information). The concept has been widely used to identify different compound classes in complex mixtures and environmental samples, such as sulfur-containing^{21, 22} and perfluorinated compounds.^{23, 24}

MATERIALS AND METHODS

Chemicals.

Anhydrous sodium sulfate and high-performance liquid chromatography (HPLC)-grade solvents, including dichloromethane (DCM), acetone, and methanol, were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.).

Acridine, phenanthridine, benzo[*h*]quinoline, benzo[*c*]acridine, and the PAH standard solution (EPA 610 PAH mixture) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Benzo[*a*]acridine was purchased from LGC standards (Teddington, Middlesex, UK). Acridine-d₉ and dibenzo[*a,j*]acridine were from Cambridge Isotope Laboratories (Tewksbury, MA, U.S.A.).

Contaminated sites, sample collection, and solvent extraction.

Four PAH-contaminated samples (three soils and one sediment) were collected from three sites located in North Carolina, U.S.A.,^{25, 26} and one site in Andalucía, Spain.²⁷ Further details on the origin, associated industrial activity, and total PAH concentration and degree of weathering are provided in Table 1. Concentrations of individual PAHs in each sample are listed in Table S1.

Before chemical analysis, all samples were air-dried to constant weight for 48 hours, sieved through 2 mm, and homogenized. All glassware used in the process was clean and autoclaved at 121 °C to avoid contamination. Triplicate 1-g aliquots for each sample were extracted by two successive overnight extractions using 20 mL of a DCM: acetone mixture (1:1, v/v) with agitation on a wrist-action shaker. Prior to extraction, deuterated compounds (100 µg acridine-d₉ and 200 µg anthracene-d₁₀) were spiked into the samples in acetone solution as recovery standards. Sodium sulfate (10 g) was added to dry the sample, and glass beads were added for better extraction efficiency. The collected extracts were filtered through a 0.2 µm pore-size nylon membrane, brought up to 50 mL with DCM and acetone (1:1, v/v), and stored at 4 °C until further analysis.

Instrumental analysis.

Nontarget analysis was performed using an Agilent 1200 series HPLC interfaced with a 6520 accurate mass quadrupole time-of-flight mass spectrometer (qTOF MS, Agilent Technologies, Santa Clara, CA,). Quantitative target analysis on azaarenes was performed using a TSQ Quantum Ultra triple quadrupole mass spectrometer (QQ MS, Thermo Fisher

Scientific, Waltham, MA). Electrospray ionization in positive (ESI+) mode was adopted for both nontarget and target analysis, and the detailed parameters for MS and HPLC are provided in supporting information (SI text and Table S2).

The concentrations of the USEPA-regulated PAHs in the samples were quantified using HPLC with fluorescence detection as described elsewhere.²⁵

Nontarget analysis workflow and mass defect filtering.

High resolution exact mass data were converted to .mzData format, and peak detection and alignment were achieved using the publicly available Scripps XCMS Online platform (<https://xcmsonline.scripps.edu/>). The resulting feature tables were imported and analyzed with an in-house script written in R language (R 3.3.3).

Since accurate, monoisotopic atomic masses are defined in relation to ^{12}C , there are small differences between the nominal mass and the accurate mass of other elements (“mass defects”).^{19, 28} Molecules with different numbers of hydrogens and other heteroatoms each have unique mass defects. Therefore, it is feasible to distinguish between compound classes by transforming the exact mass according to a specific mass scale (in this case the Kendrick scale based on CH_2) and then calculating the mass defects. The following equations were used as the basis for mass-defect filtering (where X represents a halogen atom if present):

$$\text{Kendrick mass(KM)} = \text{exact mass} \times (14/14.01565)$$

$$\text{Kendrick mass defect(KMD)} = \text{nominal mass} - \text{KM}$$

$$\text{Double-bond equivalents(DBE)} = \text{C} - \text{H}/2 - \text{X}/2 + \text{N}/2 + 1$$

According to previous knowledge on mass-defect filtering, the CH_2 -based KMD difference between neighboring series of analytes of interest (here, those containing one N atom) is 0.0134.²⁰ Because the Kendrick mass defect was defined by the difference of CH_2 units, the neighboring series on the Kendrick scale should differ in their DBE by 1. By calculation and accurate mass measurements on standards, we determined that the KMD of acridine and phenanthridine ($\text{C}_{13}\text{H}_9\text{N}$, DBE 10) is 0.1205, and the KMD of benzo[*a*]acridine ($\text{C}_{17}\text{H}_{11}\text{N}$, DBE 13) is 0.1607, which is equal to $0.1205 + 3 \times 0.0134$. Another frequently detected signal, probably an azapyrene ($\text{C}_{15}\text{H}_9\text{N}$, DBE 12), featured a KMD of 0.1473, equal to $0.1205 + 2 \times 0.0134$. Therefore, the KMD differences of these detected compounds were in agreement with the theoretical pattern. Accordingly, within the mass range of interest ($100 < m/z < 500$), we selected data that fit in the series by having KMD values that differed with those of known compounds by increments of 0.0134. The resulting sets of series represented KMDs from 0.1071 to 0.2009, corresponding to DBE from 9 to 16. The diversity and intensity of all the detected azaarenes are illustrated by plotting the KMD versus Kendrick mass, known as a Kendrick mass defect plot. Partial transparent bubble plots (transparency = 1/4) based on peak area data were created using the ggplot2 package in R.

RESULTS AND DISCUSSION

PAH quantification and distribution profiles.

Samples from the four contaminated sites varied greatly in the concentrations of the EPA-regulated PAHs, with total PAH concentrations ranging between 147 and 7,430 $\mu\text{g/g}$ dry weight (Table 1). The individual PAH concentration profiles (Table S1) revealed that KM and HS samples were highly enriched in HMW PAHs relative to the LMW compounds, with HMW/LMW PAH concentration ratios of 42.6 and 22.9, respectively (Table 1). These results indicated a higher degree of weathering compared to FS and SC samples (HMW/LMW PAH ratios of 3.1 and 0.63, respectively),^{29, 30} which contained high concentrations of LMW PAHs.

Diversity of azaarenes.

The solvent extracts of the samples from the four sites were analyzed by HRMS followed by KMD filtering. Eight homologous series of azaarenes, with DBE values ranging from 9 to 16, were tentatively identified and are presented in KMD plots (Figure 1). The identities of eight detected azaarenes were confirmed by authentic standards, or from their spectral characteristics by MS/MS fragmentation when the standards were not available. In general, the non-alkylated compounds featured a loss of m/z 28, consistent with a fragment of H_2CN (Figure 2 and Figure S1), in accordance with a previously reported method for azaarene analysis.³¹ The possible mechanism is that the nitrogen atom was protonated by electrospray ionization, and the loss of H_2CN corresponds to the protonated HCN group. Methylated azaarenes were also confirmed by the consecutive loss of alkyl groups (m/z 15, $-\text{CH}_3$) and H_2CN (Figure 2). Therefore, we concluded that the series we observed from KMD plots were azaarene homologs and their alkylated derivatives. According to the widely accepted levels of confidence in nontarget analysis,³² the compounds confirmed with authentic standards would be among level 1 (highest), while others identified by MS/MS and KMD analysis would fit in level 3 (tentative candidates). The formulas and the respective number of isomers detected for each congener group in the 4 samples are listed in Table 2.

In total, 232 congeners were detected and tentatively identified (except for the location of the N atom in a given congener for which there were no standards) within these 8 homologous series. Each series containing as many as 4 congener groups, corresponding to the non-alkylated and the mono-, di-, and tri-methylated (C1-C3) derivatives. The deeper color in the first three positions of each line in the KMD plots implied higher isomer diversities for the non-alkylated and the C1 and C2 azaarenes. Of the eight homologous series listed in Table 2, six of them could be related to known azaarene structures previously detected in atmospheric particulate matter³¹ or during the thermal degradation of nylon polymers.³³ The other two homolog series (DBE = 11 and 14), tentatively corresponding to phenylquinoline isomers ($\text{C}_{15}\text{H}_{11}\text{N}$) and phenylacridine isomers ($\text{C}_{19}\text{H}_{13}\text{N}$), were tentatively identified here for the first time, with no data being available regarding their detection in other complex matrices. We note that, except for the standards, the names used here are example chemicals that represent the congener groups with a certain molecular formula and ring number, but do not indicate identified structures. For instance, “azaapyrene isomers” represent the formula $\text{C}_{15}\text{H}_9\text{N}$, which can also include azafluoranthene. Because

these congener groups were only tentatively identified and not confirmed with authentic standards, we have used their common names (*e.g.* azapyrene, azabenz[*a*]pyrene) instead of the more specific IUPAC nomenclature.

Figure 3 depicts the extracted ion chromatograms (EIC) for the five major non-alkylated congener groups in the SC sample labeled A, B and C for the cata-condensed groups C₁₃H₉N (DBE 10, 3-ring), C₁₇H₁₁N (DBE 13, 4-ring), and C₂₁H₁₃N (DBE 16 5-ring), respectively, and E and F for the peri-condensed groups C₁₅H₉N (DBE 12, 4-ring) and C₁₉H₁₁N (DBE 15, 5-ring), respectively. For each EIC, multiple chromatographic peaks were observed, corresponding to the different isomers. This high diversity of azaarene isomers derives from both their distinctive carbon skeleton as well as the position of the substituted nitrogen atom. The substitution of a given isomer with methyl groups amplifies the structural diversity, resulting in a large number of isomers detected for some methylated congener groups. Such diversity of azaarene isomers has never been reported for environmental samples, and suggests that conventional target analysis of azaarenes based on available standards might result in a biased and very limited estimation of their abundance.

In general, these five congener groups presented the largest bubbles in KMD plots (Figure 1), indicating their predominance among azaarene congener groups in the four samples. The intensities (abundances) of methylated azaarenes decreased with increasing level of methylation, indicating that non-alkylated and mono-methylated derivatives accounted for the major signal intensities among these azaarene congeners. However, because the relative response factors (RRF) might differ for various compounds, the estimation of concentrations exclusively based on peak intensities might not be accurate.³⁴ More-accurate quantification was achieved using commercially available standards; each standard was used to quantify all isomers of the standard itself (*i.e.*, response factors were assumed to be equivalent for each isomer).

Abundances of azaarenes.

Quantification of non-alkylated azaarenes was based on the MS/MS transition from [M+H]⁺ to [M+H-28]⁺, corresponding to the loss of a CH₂N group, and further confirming the identities of azaarene isomers. Authentic standards for acridine, phenanthridine, benzo[*h*]quinoline, benzo[*a*]acridine, benzo[*c*]acridine, and dibenzo[*a,j*]acridine were used to quantify their series of isomers, and semi-quantification of azapyrene and azabenz[*a*]pyrene isomers was based on the RRF of benzo[*a*]acridine and dibenzo[*a,j*]acridine, respectively. Concentrations of quantifiable isomers in each series are shown in Table S3.

There were obvious differences in the abundances of congener groups among samples (Figure 4). Consistent with the degree of weathering observed for PAHs, the KM sample was particularly enriched in the HMW azaarenes, with congener groups B, C and F at the highest concentrations (20.5, 21.4 and 16.9 µg/g, respectively). In contrast, the relatively unweathered SC sample was highly enriched in 3-ring azaarenes, with a concentration (320 µg/g) at least four times higher than that of any of the 4- and 5-ring congener families (ranging between 7.5 and 72 µg/g). The less-contaminated FS and HS samples presented a more balanced distribution among the different azaarene congener groups, ranging between

1.0 and 2.8 µg/g for FS, and between 2.0 and 9.7 µg/g for HS. In general, except for the SC sample, the most abundant azaarene congener groups were those with four or five rings. The varied profiles in congener groups demonstrated that the occurrence and environmental prevalence of azaarenes might be site-specific and affected by weathering processes. Consequently, the exclusive analysis of compounds from one congener group, such as the most frequently targeted 3-ring compounds, will likely lead to underestimation of the environmental impact of azaarenes.

Within each of the congener groups, we analyzed the isomer distribution among the four samples. In congener group A (C₁₃H₉N), acridine (A1) was the most abundant among the five quantifiable isomers at all four sites; this contrasts with results from a study¹² on three PAH-contaminated soil samples in which benzo[*h*]quinoline was at higher concentration than acridine. The average concentration of acridine was as high as 281 µg/g (SC sample), dramatically higher than those previously reported for other PAH-contaminated soils^{12, 15} (ranging from 0.18 to 10 µg/g). For the FS, HS, and KM samples, the concentrations of acridine were 0.9, 1.0, and 3.2 µg/g, respectively, similar to concentrations reported in other studies.

In congener group B (C₁₇H₁₁N), seven isomers were quantified; in general, benzo[*a*]acridine (B1) and benzo[*c*]acridine (B6) were the predominant isomers. In accordance with their lower degree of contamination, the FS and HS samples presented the lowest concentrations among the four sites (respectively 1.0 and 2.8 µg/g for benzo[*a*]acridine, and 0.90 and 1.0 µg/g for benzo[*c*]acridine). The heavily contaminated KM and SC samples had higher concentrations (12.2 and 13.1 µg/g for benzo[*a*]acridine, and 4.2 and 33.9 µg/g for benzo[*c*]acridine, respectively). Compared to the data from the only published study on PAH-contaminated soil that considered these compounds,¹⁵ the concentrations in the KM sample were comparable, while the concentrations in the SC sample were substantially higher. Consistent with results in the earlier study, the concentration of benzo[*c*]acridine was higher than that of benzo[*a*]acridine in the FS, HS and SC samples.

Congener group C (C₂₁H₁₃N) comprised five quantified isomers. Isomers C3 and C4, which co-eluted in a single peak, were the most abundant in all of the samples. According to the chromatographic profiles previously described by Lintelmann et al.³¹ during the analysis of azaarenes in air particulate matter, these compounds were tentatively identified as dibenzo[*a,h*]acridine and dibenzo[*a,c*]acridine. The latter is the only 5-ring azaarene for which there is previously published data for contaminated soil,¹⁵ with concentrations in two soils (0.4 and 0.7 µg/g) comparable to those detected here in the FS and HS samples.

Regarding the peri-condensed groups of congeners, 5 congeners in group E (C₁₅H₉N) and 13 congeners in group F (C₁₉H₁₁N) were semi-quantified. Within group E, isomers E1 (0.55–31 µg/g), E4 (0.4–12 µg/g), and E5 (0.13–14 µg/g) had relatively higher concentrations, the last two tentatively corresponding to 4-azapyrene (E4) and 1-azafluoranthene (E5) based on their LC elution profiles.³¹ Within the diverse congener group F (C₁₉H₁₁N), F1 (0.17–2.2 µg/g), F6 (0.17–3.8 µg/g), F8 (0.39–4.6 µg/g) and F10 (0.26–4.6 µg/g) were the major isomers. However, due to a lack of authentic standards, we could not establish their identities. To our knowledge, this is the first report of peri-

condensed 5-ring azaarenes in contaminated soil. Several peri-condensed, unsubstituted 5-ring PAHs such as benzo[*a*]pyrene, benzo[*b*]fluoranthene, and benzo[*k*]fluoranthene are known human carcinogens; therefore, the presence of their azaarene analogues in contaminated soils should not be ignored.

Overall, the detection of azaarene isomers at relatively high concentrations in four independent and geographically distant samples suggests that they might be prevalent and abundant in sites contaminated with coal tar and creosote. Congener groups B, C, and F encompass some known or suspected human carcinogens, such as benzo[*c*]acridine and 10-azaB[*a*]P.^{6, 35, 36} The high concentrations and isomer diversity of these congener groups therefore suggests that they could significantly contribute to the overall toxicity and/or genotoxicity of PAH-contaminated soils. We suggest that future analysis and monitoring of azaarenes should also include HMW compounds, such as benzo[*c*]acridine, azapyrenes, and/or dibenzo[*a,h*]acridine.

Environmental significance.

This work highlights the application of nontarget analytical methods such as mass defect filtering that can help select and prioritize important compound classes from complex environmental samples. By applying these nontarget analytical techniques we have demonstrated the occurrence of a wide range of N-heterocyclic aromatics in soil and sediment samples contaminated with coal tar and creosote, many at relatively high concentrations. Azaarenes have received far less attention than unsubstituted PAHs in contaminated environmental systems, and they currently are not among the regulated contaminants in these systems. Their biotransformation, biodegradation, and fate in the environment were not well understood, and further research would be warranted. In a companion manuscript, we illustrate that many of these compounds are recalcitrant to biodegradation (Tian, Vial et al, submitted). Given the known toxicological properties of several azaarenes, the diversity, abundance and, in some cases, persistence in environmental samples of a wide range of previously unknown compounds suggests that further attention be paid to this overlooked class of contaminants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Kendrick mass defect (KMD) plots of azaarene congeners obtained from the four contaminated sites. (a) Soil sample from a manufactured-gas plant site in Salisbury, NC (FS sample, coal tar contamination); (b) Soil sample from the Holcomb Creosote Superfund site, NC (HS sample); (c) Sediment sample from the Kerr-McGee Superfund site, NC (KM sample, creosote contamination). (d) Soil sample from a wood-treatment facility in Andalucía, Spain (SC sample, creosote contamination). The five groups of non-alkylated azaarenes of greatest interest (most abundant congeners) are labeled as follows: A ($C_{13}H_9N$), E ($C_{15}H_9N$), B ($C_{17}H_{11}N$), F ($C_{19}H_{11}N$), and C ($C_{21}H_{13}N$). Each horizontal series of bubbles corresponds to a homologous series of congeners that share the same basic structure, while the overlapping bubbles are isomers with identical molecular formula and position on the KMD plot. The color darkened by overlapping suggests the emergence of multiple isomers, and the areas of the bubbles are proportional to the intensities.

Figure 2. MS/MS fragmentation patterns of two tentatively identified compounds C₁₉H₁₁N (F8, above) and C₂₀H₁₃N (below). The structures were inferred from MS/MS and literature data. MW: monoisotopic molecular weight.

Figure 3.

Extracted ion chromatograms (EICs) of the SC sample, corresponding to the five major congener groups. Structures are shown for peaks that corresponded to available standards:

A1, acridine; A4, phenanthridine; A5, benzo[*h*]quinoline; B1, benzo[*a*]acridine; B6, benzo[*c*]acridine; C1, dibenzo[*a,j*]acridine. Structures E4 (azapyrene) and E5 (azafluoranthene) were inferred from literature data.

Figure 4. Concentrations (µg/g dry weight) of the five major azaarene congener groups in each of the samples. Note the differences in the y-axis scales.

Table 1.

Description of the PAH-contaminated samples used in this study

Site ID	Origin	Industrial activity	PAH conc. ^a	Weathering ratio ^b
FS	Salisbury, NC	Manufactured gas plant	421 ± 27.8	3.1
HS	Holcomb Superfund Site, NC	Wood treatment facility	147 ± 19.9	22.9
KM	Kerr McGee Superfund Site, NC	Wood treatment facility	2,490 ± 64	42.6
SC	Andalucía, Spain	Wood treatment facility	7,430 ± 315	0.6

^aSum of the concentrations (µg/g) of 14 of the 16 PAHs in the US EPA list of priority pollutants, excluding acenaphthylene and indeno[1,2,3-*cd*]pyrene. Data are means and standard deviations of triplicates. The KM sample was surface sediment from an area adjacent to contaminated soil, whereas the other samples were soils.

^bWeathering ratio = HMW PAHs (4–6 rings)/ LMW PAHs (2–3 rings)

Table 2.

Detailed information on azaarene congeners detected in the samples

example chemical	formula	DBE	exact mass (Da)		# of isomers
			M	[M+H] ⁺	
Azafluorene	C ₁₂ H ₉ N	9	167.0735	168.0806	4
C1-azafluorene	C ₁₃ H ₁₁ N	9	181.8910	182.0962	4
C2-azafluorene	C ₁₄ H ₁₃ N	9	195.1048	196.1118	6
Acridine	C ₁₃ H ₉ N	10	179.0735	180.0806	6
C1-acridine	C ₁₄ H ₁₁ N	10	193.0891	194.0965	11
C2-acridine	C ₁₅ H ₁₃ N	10	207.1048	208.1120	19
C3-acridine	C ₁₆ H ₁₅ N	10	221.1204	222.1275	3
phenylquinoline	C ₁₅ H ₁₁ N	11	205.0891	206.0965	7
C1-phenylquinoline	C ₁₆ H ₁₃ N	11	219.1048	220.1117	7
C2-phenylquinoline	C ₁₇ H ₁₅ N	11	233.1204	234.1277	8
Azapyrene	C ₁₅ H ₉ N	12	203.0735	204.0807	7
C1-azapyrene	C ₁₆ H ₁₁ N	12	217.0891	218.0965	18
C2-azapyrene	C ₁₇ H ₁₃ N	12	231.1048	232.1120	15
C3-azapyrene	C ₁₈ H ₁₅ N	12	245.1204	246.1275	4
benzo[<i>a</i>]acridine	C ₁₇ H ₁₁ N	13	229.0891	230.0965	13
C1-benzo[<i>a</i>]acridine	C ₁₈ H ₁₃ N	13	243.1048	244.1120	16
C2-benzo[<i>a</i>]acridine	C ₁₉ H ₁₅ N	13	257.1204	258.1278	13
C3-benzo[<i>a</i>]acridine	C ₂₀ H ₁₇ N	13	271.1361	272.1433	1
phenylacridine	C ₁₉ H ₁₃ N	14	255.1048	256.1118	5
C1-phenylacridine	C ₂₀ H ₁₅ N	14	269.1204	270.1278	1
azabenz[<i>a</i>]pyrene	C ₁₉ H ₁₁ N	15	253.0891	254.0965	17
C1-azabenz[<i>a</i>]pyrene	C ₂₀ H ₁₃ N	15	267.1048	268.1122	22
C2-azabenz[<i>a</i>]pyrene	C ₂₁ H ₁₅ N	15	281.1204	282.1276	3
dibenzo[<i>a</i> , <i>j</i>]acridine	C ₂₁ H ₁₃ N	16	279.1048	280.1120	10
C1-dibenzo[<i>a</i> , <i>j</i>]acridine	C ₂₂ H ₁₅ N	16	293.1204	294.1277	7
C2-dibenzo[<i>a</i> , <i>j</i>]acridine	C ₂₃ H ₁₇ N	16	307.1361	308.1433	5
Total					232