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Transformation of mutagenic aromatic amines into non-mutagenic species by alkyl substituents Part I. Alkylation *ortho* to the amino function

Carsten Glende [a], Heimo Schmitt [a], Lothar Erdinger [b], Günter Engelhardt [c], Gernot Boche [a,*]

[a] *Fachbereich Chemie, Philipps-Universität Marburg, Hans-Meerwein Strasse, 35032 Marburg, Germany*
[b] *Hygieneinstitut Heidelberg, Im Neuenheimer Feld, 69120 Heidelberg, Germany*
[c] *BASF AG, Experimental Toxicology and Ecology, 67056 Ludwigshafen/Rhein, Germany*

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Abstract

Alkyl-substituted derivatives of 2-aminonaphthalene (2-AN) **1**, 2-aminofluorene (2-AF) **6** and 4-aminobiphenyl (4-ABP) **11** were synthesized and the mutagenic activity of these compounds determined in *Salmonella typhimurium* strains TA98 and TA100 with and without S9 mix. In the case of the *ortho*-substituted 4-aminobiphenyls **12–15** (3-alkyl = ethyl, *iso*-propyl, *n*-butyl, *tert*-butyl) the substituent with the strongest steric demand (3-*tert*-butyl) shows the strongest influence on the decrease of mutagenicity if compared with the parent compound. In the series of the bis-*ortho*-disubstituted compounds **16–18** (3,5-dimethyl-, 3,5-diethyl- and 3,5-di*iso*propyl-4-aminobiphenyl) generation of non-mutagenic species occurs already with the introduction of two ethyl groups. For the 4-aminobiphenyl derivatives **12–15** and **16–18**, as well as for the 1-alkylated 2-aminofluorenes **7–10** and the 1-alkylated 2-aminonaphthalenes **2–5** a smaller mutagenicity was observed if compared with predicted mutagenicities as calculated by the QSAR equations of Debnath et al. (Environ. Mol. Mutagen. 19 (1992) 37). The largest differences resulted in the cases of the *tert*-butyl substituted compounds. Only with smaller alkyl groups like ethyl the QSAR predictions and the experimentally determined mutagenicities come close to each other. Thus, these results show that appropriate alkyl substitution reduces (eliminates) mutagenicity, secondly, it is necessary to introduce steric parameters to predict the mutagenicity of such compounds correctly.

Keywords: Aromatic amines; 2-Aminonaphthalene; 2-Aminofluorene; 4-Aminobiphenyl; *Salmonella*; Mutagenicity; Alkyl substituents; QSAR

1. Introduction

Aromatic amines are a class of ubiquitous environmental pollutants. They are found, e.g. in tobacco smoke, diesel exhaust and tar, and they are used for preparation of industrial products like azo dyes, pesticides, synthetic materials or pharmaceutical products [1]. To exert their mutagenic and carcinogenic effects aromatic amines require metabolic activation. They are first oxidized by cytochrome P450 to hydroxylamines, and then further activated, e.g. by *O*-acylation [2,3]. From these compounds a highly electrophilic species, the corresponding nitrenium ion, may be generated and react with bionucleophiles. For mutagenicity and carcinogenicity the main target molecule is the deoxyribonucleic acid (DNA) [4]. To investigate the

* Corresponding author. Tel.: +49-6421-282-2030; fax: +49-6421-282-8917.
E-mail address: boche@chemie.uni-marburg.de (G. Boche).

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mutagenicity of such compounds the most widely used bacterial short-time mutagenicity assay is the Ames test with *Salmonella typhimurium* TA98 and TA100, with and without S9 mix. The metabolic activation is simulated by rat liver S9 mix [5].

In many mutagenicity studies of aromatic amines the parent compounds 2-AN **1**, 2-AF **6** and 4-ABP **11** were tested in the above mentioned *Salmonella* strains [6]. In our investigations, the main focus was on the influence of alkyl groups attached *ortho* to the amino function in **1**, **6** and **11**. Therefore, we synthesized homologous series of aromatic alkyl amines with growing steric demand of the alkyl groups. Here, we concentrate on the *ortho*-alkylated 1-alkyl-2-aminonaphthalenes **2–5**, 1-alkyl-2-aminofluorenes **7–10**, 3-alkyl-4-aminobiphenyls **12–15** and 3,5-dialkyl-4-aminobiphenyls **16–18** (Table 1). All samples were tested in the Ames test with *S. typhimurium* tester strains TA98 and TA100, both with and without metabolic activation using rat liver S9 mix.

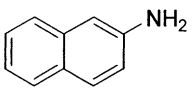
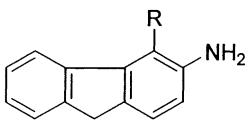
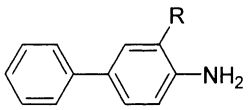
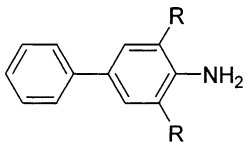
To understand the parameters which influence mutagenicity many investigations have been undertaken [7–10]. One of them are quantitative structure activity relationship (QSAR) studies that try to predict the mutagenicity of chemical compounds. In the case of aromatic amines, Debnath et al. developed QSAR equations for the tester strains TA98 and TA100 with S9 mix activation [7]. The most important factor in these equations is the log *P*-value (octanol–water distribution factor), followed by the electronic parameters E_{HOMO} and E_{LUMO}. We calculated the values of our test compounds and compared them with the experimental data. The aim was to investigate the effect of alkylation, and whether these equations are able to predict the mutagenicity of aromatic amines with sterically demanding alkyl substituents.

2. Experimental section

2.1. General comments

All solvents were reagent grade and used without further purification with the following exceptions. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were dried by distillation from Na/K alloy under argon. Chemicals used were reagent grade and used without

Table 1
Structures of the tested compounds **1–18**

Structure	R	No.	Compound	Abbreviation
	H	**1**	2-Aminonaphthalene	2-AN
	Et	**2**	1-Ethyl-2-aminonaphthalene	1-Et-2AN
	*i*Pr	**3**	1-*i*Propyl-2-aminonaphthalene	1-*i*Pr-2AN
	*n*Bu	**4**	1-*n*Butyl-2-aminonaphthalene	1-*n*Bu-2AN
	*t*Bu	**5**	1-*t*Butyl-2-aminonaphthalene	1-*t*Bu-2AN
	H	**6**	2-Aminofluorene	2-AF
	Et	**7**	1-Ethyl-2-aminofluorene	1-Et-2AF
	*i*Pr	**8**	1-*i*Propyl-2-aminofluorene	1-*i*Pr-2AF
	*n*Bu	**9**	1-*n*Butyl-2-aminofluorene	1-*n*Bu-2AF
	*t*Bu	**10**	1-*t*Butyl-2-aminofluorene	1-*t*Bu-2AF
	H	**11**	4-Aminobiphenyl	4-ABP
	Et	**12**	3-Ethyl-4-aminobiphenyl	3-Et-4ABP
	*i*Pr	**13**	3-*i*Propyl-4-aminobiphenyl	3-*i*Pr-4ABP
	*n*Bu	**14**	3-*n*Butyl-4-aminobiphenyl	3-*n*Bu-4ABP
	*t*Bu	**15**	3-*t*Butyl-4-aminobiphenyl	3-*t*Bu-4ABP
	Me	**16**	3,5-Dimethyl-4-aminobiphenyl	3,5-diMe-4ABP
	Et	**17**	3,5-Diethyl-4-aminobiphenyl	3,5-diEt-4ABP
	*i*Pr	**18**	3,5-Di*i*propyl-4-aminobiphenyl	3,5-di*i*Pr-4ABP

further purification unless noted. Flash chromatography was carried out using Merck silica gel 60 (230–400 mesh) purchased from Merck & Co. Analytical thin layer chromatography (TLC) was performed using Merck precoated silica gel 60 F-254 sheets. All reactions were carried out under argon atmosphere unless otherwise specified.

2.2. Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker ARX-200 and AC-300 spectrometers in $CDCl_3$ solution using tetramethylsilane as internal standard. Mass spectra were recorded with a Variant MAT CH-7-A (EI, 70 eV). Elementary analyses were performed with a Heraeus Rapid Elementalanalyser.

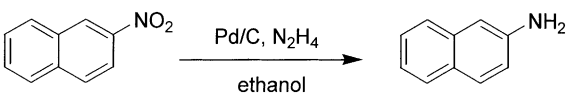
2.3. Chemicals

4-Aminobiphenyl **11** [92-67-1], 4-nitrobiphenyl [92-93-3], 2-aminofluorene **6** [153-78-6], 2-nitrofluorene [607-57-8], 2-nitronaphthalene [581-89-5], 2,6-dimethylaniline [87-62-7], 2,6-diethylaniline [579-66-8], 2,6-diisopropylaniline [24544-04-5] and phenylboronic acid [98-80-6] were purchased from Aldrich Chemical Co.

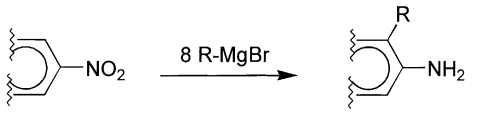
2.4. Synthesis of the test compounds

2-Aminonaphthalene **1** was prepared according to a procedure described by Dewar and Mole [11], see Scheme 1.

Grignard reagents were synthesized by standard methods [12]. 1-Alkyl-2-aminonaphthalenes **2–5**, 1-alkyl-2-aminofluorenes **7–10** and 3-alkyl-4-aminobiphenyls **12–15** were synthesized according to a method introduced by Bartoli et al. [13], see Scheme 2 (the Grignard reagent acts simultaneously as an alkylation agent for the aromatic nucleus and a reducing agent for the nitro group). Yields vary in the range of 10–25%.



Scheme 1.



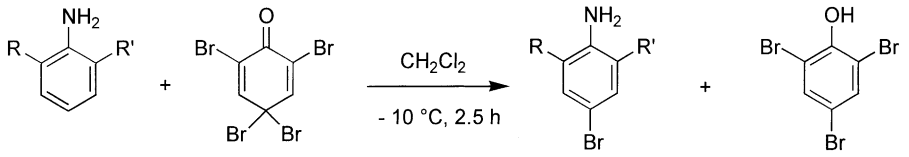
Scheme 2.

3,5-Dialkyl-4-aminobiphenyls **16–18** were synthesized by a modified procedure of Miller and Dugar [14]. 4-Bromo-2,6-dialkylanilines **19–20** were first synthesized by selective bromination methods as described in the literature [15,16]. Yields vary between 80 and 85%. In a second step the *ortho*-disubstituted 4-aminobiphenyls were synthesized by coupling of 4-bromo-2,6-dialkylanilines with phenylboronic acid. Yields were in the range of 35–50%, see Schemes 3 and 4.

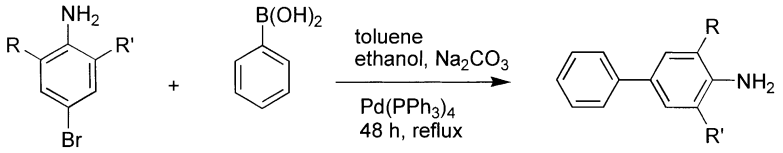
2.4.1. 2-Aminonaphthalene *1*

To a solution of 4.33 g (2.5 mmol) 2-nitronaphthalene in 100 ml ethanol were added 0.72 g Pd/C (10 wt.%) and 18 ml hydrazine hydrate (80 wt.%). After refluxing for 10 min the solution was cooled to room temperature, filtered and the solvent removed. The solid **1** was purified by dissolving in ligroin. Removal of the solvent in vacuo yielded 2.7 g (76%) of the crystalline solid.

Spectroscopic and physical data were in agreement with literature data [17,18].



Scheme 3.



Scheme 4.

*2.4.2. 1-Ethyl-2-aminonaphthalene **2**; typical procedure*

A Grignard solution (ethylmagnesiumchloride) was cooled to −15°C and 0.45 g (2.36 mmol) copper(I)-iodide was added. Then, a solution of 20 mmol of the appropriate nitro compound in 90 ml THF was added slowly. The reaction mixture was stirred at room temperature for 18 h. After careful addition of 10 ml of a 2 N HCl solution at 0°C, the solution was stirred for 20 min and brought to pH 10 by treatment with concentrated aqueous ammonia. The solid residue was separated by filtration and washed with dichloromethane. The combined organic layers were extracted with water (4 × 75 ml), dried ($MgSO_4$), and evaporated to dryness in vacuo. The solid was purified by flash chromatography with $CHCl_3$ as the eluting solvent. 642 mg (19%) dark red crystalline solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.95 (d, 1H, ^3J = 8.6 Hz, H5), 7.80 (d, 1H, ^3J = 8.1 Hz, H4), 7.63 (d, 1H, ^3J = 8.6 Hz, H8), 7.53 (t, 1H, ^3J = 6.7 Hz, H7), 7.34 (t, 1H, ^3J = 6.4 Hz, H6), 6.98 (d, 1H, ^3J = 8.6 Hz, H3), 3.88 (s, 2H, NH_2), 2.98 (q, 2H, ^{3}J = 7.6 Hz, CH_2), 1.32 (t, 3H, ^3J = 7.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 140.6, 132.9, 128.8, 127.3, 126.3, 122.2, 122.0, 119.2, 118.8, 19.3 (CH_2), 13.0 (CH_3). MS (70 eV): *m/z* (%) = 171 (M^+, 57), 156 ($M^+ - CH_3$, 100). Calc. C 84.17%, H 7.65%, N 8.18%; found C 84.33%, H 7.66%, N 7.95%.

Analogously compounds **3–5**, **7–10** and **12–15** were prepared.

*2.4.3. 1-iPropyl-2-aminonaphthalene **3***

468 mg (17%) dark-red oily liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.08 (d, 1H, ^3J = 8.6 Hz, H5), 7.73 (d, 1H, ^3J = 8.0 Hz, H4), 7.55 (d, 1H, ^3J = 8.6 Hz, H8), 7.41 (t, 1H, ^3J = 7.7 Hz, H7), 7.26 (t, 1H, ^3J = 7.4 Hz, H6), 6.90 (d, 1H, ^3J = 8.6 Hz, H3), 3.97 (s, 2H, NH_2), 3.85 (s, 1H, CH), 1.54 (d, 6H, ^3J = 7.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 141.2, 133.0, 129.4, 129.0, 127.7, 125.9, 122.9, 122.5, 121.9, 120.11, 26.5 (CH), 20.7 (CH_3). MS (70 eV): *m/z* (%) = 185 (M^+, 63), 170 ($M^+ - CH_3$, 100). Calc. C 84.28%, H 8.16%, N 7.56%; found C 83.94%, H 8.24%, N 7.80%.

*2.4.4. 1-nButyl-2-aminonaphthalene **4***

1051 mg (27%) reddish-brown viscous liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.84 (d, 1H, ^3J = 8.6 Hz, H5), 7.69 (d, 1H, ^3J = 8.1 Hz, H4), 7.54 (d, 1H, ^3J = 8.7 Hz, H8), 7.41 (t, 1H, ^3J = 7.7 Hz, H7), 7.23 (t, 1H, ^3J = 7.5 Hz, H6), 6.93 (d, 1H, ^3J = 8.7 Hz, H3), 3.82 (s, 2H, NH_2), 2.88 (t, 2H, ^3J = 7.9 Hz, CH_2), 1.66–1.44 (m, 4H, CH_2), 0.98 (t, 3H, ^3J = 7.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 141.0, 133.3, 128.8, 128.7, 127.3, 126.3, 122.4, 122.0, 118.7, 118.1, 31.2 (CH_2), 26.1 (CH_2), 23.3 (CH_2), 14.2 (CH_3). MS (70 eV): *m/z* (%) = 199 (M^+, 26), 157 (21%), 156 ($M^+ - C_3H_7$, 100). Calc. C 84.37%, H 8.60%, N 7.03%; found C 83.95%, H 8.32%, N 7.46%.

*2.4.5. 1-tButyl-2-aminonaphthalene **5***

436 mg (12%) dark-red oily liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.35 (d, 1H, ^3J = 8.9 Hz, H5), 7.69 (d, 1H, ^3J = 8.0 Hz, H4), 7.48 (d, 1H, ^3J = 8.7 Hz, H8), 7.39 (t, 1H, ^3J = 8.0 Hz, H7), 7.25 (t, 1H, ^3J = 7.9 Hz, H6), 6.74 (d, 1H, ^3J = 8.7 Hz, H3), 4.11 (s, 2H, NH_2), 1.81 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 141.9, 134.0, 130.4, 129.1, 128.4, 126.7, 124.5, 122.9, 122.3, 121.4, 37.3, 33.6 (CH_3). MS (70 eV): *m/z* (%) = 199 (M^+, 50), 184 ($M^+ - CH_3$, 100), 144 (24), 143 (33). Calc. C 84.15%, H 8.60%, N 7.03%; found C 84.15%, H 8.67%, N 7.10%.

*2.4.6. 1-Ethyl-2-aminofluorene **7***

495 mg (12%) dark-red oily liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.64 (d, 1H, ^3J = 7.6 Hz, H5), 7.49 (d, 1H, ^3J = 7.1 Hz, H4), 7.46 (d, 1H, ^3J = 8.0 Hz, H8), 7.31 (t, 1H, ^3J = 7.3 Hz, H6), 7.20 (t, 1H, ^3J = 7.4 Hz, H7), 6.74 (d, 1H, ^3J = 8.0 Hz, H3), 3.80 (s, 2H, H9), 3.71 (s, 2H, NH_2), 2.69 (q, 2H, ^3J = 7.6 Hz, CH_2), 1.24 (t, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 143.0, 142.7, 142.1, 132.9, 126.4, 124.8, 124.5, 118.5, 118.2, 114.8, 35.4, 21.4, 12.3 (CH_3). MS (70 eV): *m/z* (%) = 209 (M^+, 100), 194 ($M^+ - CH_3$, 35), 180 ($M^+ - C_2H_5$, 49). Calc. C 86.08%, H 7.22%, N 6.69%; found C 85.61%, H 7.51%, N 6.67%.

2.4.7. 1-iPropyl-2-aminofluorene ***8***

365 mg (9%) dark-red oily liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.63 (d, 1H, ^3J = 7.5 Hz, H5), 7.48 (d, 1H, ^{3}J = 7.3 Hz, H4), 7.45 (d, 1H, ^3J = 8.0 Hz, H8), 7.31 (t, 1H, ^3J = 7.2 Hz, H6), 7.18 (t, 1H, ^3J = 7.4 Hz, H7), 6.73 (d, 1H, ^3J = 8.0 Hz, H3), 3.89 (s, 2H, H9), 3.78 (s, 2H, NH_2), 3.30 (m, 1H, ^3J = 7.2 Hz, CH), 1.43 (d, 6H, ^3J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 143.1, 142.4, 142.2, 133.8, 126.5, 125.0, 124.4, 118.5, 118.3, 116.4, 36.9, 29.1, 20.5 (CH_3). MS (70 eV): *m/z* (%) = 223 (M^+, 100), 208 ($M^+ - CH_3$, 80), 180 ($M^+ - C_3H_7$, 40). Calc. C 86.05%, H 7.67%, N 6.27%; found C 85.73%, H 7.50%, N 6.19%.

2.4.8. 1-nButyl-2-aminofluorene ***9***

653 mg (14%) orange-brown solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.58 (d, 1H, ^3J = 7.6 Hz, H5), 7.43 (d, 1H, ^{3}J = 6.3 Hz, H4), 7.40 (d, 1H, ^3J = 7.9 Hz, H8), 7.25 (t, 1H, ^3J = 7.3 Hz, H6), 7.12 (t, 1H, ^3J = 7.4 Hz, H7), 6.69 (d, 1H, ^3J = 8.0 Hz, H3), 3.84 (s, 2H, H9), 3.74 (s, 2H, NH_2), 2.60 (t, 2H, ^3J = 7.8 Hz, CH_2), 1.55 (m, 2H, CH_2), 1.42 (m, 2H, CH_2), 0.92 (t, 3H, ^3J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 144.8, 142.5, 141.7, 141.5, 134.7, 131.0, 126.6, 125.0, 124.7, 118.6, 118.4, 115.0, 35.9, 30.4 (CH_2, A), 28.4 (CH_2, B), 23.2 (CH_2, C), 14.1 (CH_3). MS (70 eV): *m/z* (%) = 237 (M^+, 88), 194 ($M^+ - C_3H_7$, 100). Calc. C 86.03%, H 8.07%, N 5.90%; found C 85.93%, H 8.48%, N 5.42%.

2.4.9. 1-tButyl-2-aminofluorene ***10***

271 mg (8%) dark reddish-brown viscous liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.63 (d, 1H, ^3J = 7.6 Hz, H5), 7.50 (d, 1H, ^3J = 8.1 Hz, H4), 7.46 (d, 1H, ^3J = 7.4 Hz, H8), 7.31 (t, 1H, ^3J = 7.4 Hz, H6), 7.20 (t, 1H, ^3J = 7.4 Hz, H7), 6.72 (d, 1H, ^3J = 8.0 Hz, H3), 4.16 (s, 2H, H9), 3.95 (s, 2H, NH_2), 1.69 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 144.8, 142.5, 141.7, 141.6, 134.7, 131.0, 126.6, 125.2, 124.1, 118.8, 118.6, 118.2, 40.8, 37.13, 31.68 (CH_3). MS (70 eV): *m/z* (%) = 237 (M^+, 100), 222 ($M^+ - CH_3$, 70). Calc. C 86.03%, H 8.07%, N 5.90%; found C 86.04%, H 8.27%, N 5.35%.

2.4.10. 3-Ethyl-4-aminobiphenyl ***12***

844 mg (22%) dark red-brownish viscous liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.76–7.41 (m, 7H), 6.87 (d, 1H, ^3J = 8.1 Hz, H5), 3.77 (s, 2H, NH_2), 2.71 (q, 2H, ^3J = 7.5 Hz, CH_2), 1.46 (t, 3H, ^3J = 7.5 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 143.8, 141.7, 131.9, 128.8, 128.4, 127.4, 126.6, 125.6, 115.9, 24.4, 13.3 (CH_3). MS (70 eV): *m/z* (%) = 197 (M^+, 78), 182 ($M^+ - CH_3$, 100). Calc. C 85.24%, H 7.66%, N 7.10%; found C 85.13%, H 7.79%, N 7.01%.

2.4.11. 3-iPropyl-4-aminobiphenyl ***13***

382 mg (9%) dark reddish-brown viscous liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.65–7.33 (m, 7H), 6.79 (d, 1H, ^3J = 8.2 Hz, H5), 3.77 (s, 2H, NH_2), 2.99 (m, 1H, ^3J = 6.2 Hz, CH), 1.38 (d, 6H, ^3J = 6.8 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 142.9, 141.9, 133.0, 132.2, 128.8, 126.7, 126.3, 125.4, 124.5, 116.4, 28.0, 22.5 (CH_3). MS (70 eV): *m/z* (%) = 211 (M^+, 88), 196 ($M^+ - CH_3$, 100). Calc. C 85.26%, H 8.11%, N 6.63%; found C 84.84%, H 8.34%, N 6.93%.

2.4.12. 3-nButyl-4-aminobiphenyl ***14***

254 mg (6%) light brown oily liquid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.68–7.15 (m, 6H), 7.07 (s, 1H, H2), 6.63 (d, 1H, ^{3}J = 8.1 Hz, H5), 3.53 (s, 2H, NH_2), 2.44 (t, 2H, ^3J = 7.9 Hz, CH_2), 1.58–1.31 (m, 4H, CH_2), 0.87 (t, 3H, ^3J = 7.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 142.5, 140.4, 130.6, 127.6, 127.2, 126.1, 125.4, 125.1, 124.5, 114.8, 30.1, 29.9, 21.7, 13.0 (CH_3). MS (70 eV): *m/z* (%) = 225 (M^+, 62), 182 ($M^+ - C_3H_7$, 100). Calc. C 85.29%, H 8.50%, N 6.22%; found C 84.65%, H 8.49%, N 6.10%.

2.4.13. 3-tButyl-4-aminobiphenyl ***15***

408 mg (10%) dark red-brownish solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.62–7.32 (m, 7H), 6.74 (d, 1H, ^3J = 8.1 Hz, H5), 3.88 (s, 2H, NH_2), 1.53 (s, 9H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 144.1, 141.9, 133.9, 131.6, 128.7, 126.6, 126.2, 125.6, 118.2, 34.4, 29.7 (CH_3). MS (70 eV): *m/z* (%) = 225 (M^+, 100), 210 ($M^+ - CH_3$, 70). Calc. C 85.29%, H 8.50%, N 6.22%; found C 84.85%, H 8.37%, N 6.25%.

*2.4.14. 3,5-Dimethyl-4-aminobiphenyl **16**; typical procedure*

A solution of 10 ml toluene, 5 ml Na_2CO_3 (2 M) and 0.17 g (150 μmol) tetra-kis(triphenylphosphine)-palladium was treated with a solution of 1.00 g (5.00 mmol) 4-bromo-2,6-dimethylaniline in ethanol and a solution of 0.67 g (5.50 mmol) phenylboronic acid in ethanol, and refluxed for 48 h. The reaction mixture was separated by filtration and the solid **16** extracted with Et_2O (3 × 20 ml). The organic layer was extracted with brine (3 × 20 ml), dried with $MgSO_4$ and evaporated in vacuo. Repeated column chromatography (petrol ether:ethyl acetate, 8:1) afforded **16**.

0.76 g (39%) violet solid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.62 (d, 2H, ^3J = 7.80, H6), 7.46(m, 3H, H7/8), 7.29 (s, 2H, H3), 3.91 (s, 2H, NH_2), 2.32 (s, 6H, H9). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 142.50, 141.94, 131.63, 129.04, 127.50, 126.97, 122.60, 18.27 (CH_3). MS (70 eV): *m/z* (%) = 197 (M^+, 100%); 182 (18.2%; $M^+ - 15$). Calc. C 85.24%, H 7.66%, N 7.10%; found C 85.06%, H 7.64%, N 7.13%

Analogously compounds **17–18** were prepared.

*2.4.15. 3,5-Diethyl-4-aminobiphenyl **17***

630 mg (32%) reddish-brown oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.47 (d, 2H, ^3J = 7.76 Hz, H6), 7.33–7.25 (m, 3H, H7/8), 7.15 (s, 2H, H3), 3.71 (s, 2H, NH_2), 2.45 (q, 4H, ^3J = 7.41 Hz, H9), 1.21 (t, 6H, ^3J = 7.41 Hz, H10). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 141.20, 139.61, 138.41, 127.57, 127.10, 124.60, 124.48, 24.28 (CH_2), 13.26 (CH_3). MS (70 eV): *m/z* (%) = 225 (M^+, 100%), 210 ($M^+ - 15$, 54.9%), 195 ($M^+ - 30$, 15.5%). Calc. C 85.28%, H 8.50%, N 6.22%; found C 84.30%, H 8.56%, N 6.44%

*2.4.16. 3,5-Diisopropyl-4-aminobiphenyl **18***

980 mg (52%) red-coloured oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.78 (d, 2H, ^3J = 7.78 Hz, H6), 7.68–7.53 (m, 3H, H7/8), 7.50 (s, 2H, H3), 4.02 (s, 2H,NH_2), 3.13 (p, 2H, ^3J = 6.91 Hz, H9), 1.51 (d, 12H, ^3J = 6.91 Hz, H10). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 144.78, 138.64, 134.98, 129.21, 127.65, 126.94, 126.56. MS (70 eV): *m/z* (%) = 253 (M^+, 100%), 237 (76%). Calc. C 85.32%, H 9.15%, N 5.53%; found C 84.98%, H 8.94%, N 5.89%

*2.4.17. 4-Bromo-2,6-dimethylaniline **19**; typical procedure*

2,6-Dimethylaniline (2.42 g, 0.02 mmol) was dissolved in 50 ml dichloromethane and cooled to −10°C. The bromination agents 2,4,4,6-tetrabromocyclohexane (8.20 g, 0.02 mmol), dissolved in 130 ml dichloromethane, was added dropwise and the reaction mixture was stirred for 2.5 h at 20°C. After extraction with 3 × 30 ml 2N NaOH and 3 × 30 ml H_2O, the solvent was evaporated in vacuo. Purification by flash chromatography (petrol ether:ethyl acetate, 8:1) afforded **19**.

3.41 g (85%) violet solid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 6.98 (s, 2H, H3), 3.47 (s, 2H, NH_2), 2.07 (s, 6H, H5). ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 143.39, 131.14, 125.04, 117.58, 18.65 (CH_3). MS (70 eV): *m/z* (%) = 201 (M^+, 88%), 199 (87%), 186 (5%), 184 (6%), 120 (100%). Calc. C 47.84%, H 5.02%, N 6.97%; found C 47.62%, H 5.31%, N 6.83%.

Analogously compounds **20** and **21** were prepared

*2.4.18. 2,6-Diethyl-4-bromoaniline **20***

3.77 g (85%) deep green oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 6.94 (s, 2H, H3), 3.47 (s, 2H, NH_2), 2.32 (q, 4H, ^3J = 7.59 Hz, H5), 1.14 (t, 6H, ^3J = 7.59 Hz, H6). ^{13}C NMR ($CDCl_3$, 75 MHz,) δ = 142.21, 131.09, 130.23, 111.67, 25.78 (CH_2), 14.25 (CH_3). MS (70 eV): *m/z* (%) = 229 (M^+, 62%), 227 (61%), 214 (82%), 212 (72%), 149 (63%), 134 (100%). Calc. C 52.43%, H 6.16%, N 6.11%; found C 52.14%, H 6.32%, N 5.95%.

*2.4.19. 2,6-Diisopropyl-4-bromoaniline **21***

4.17 g (81%) deep red oil.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.02 (s, 2H, H3), 3.59 (s, 2H, NH_2), 2.84 (p, 2H, ^3J = 6.92 Hz, H5), 1.15 (d, 6H, ^3J = 6.92 Hz, H6-H7). ^{13}C NMR

($CDCl_3$, 75 MHz) δ = 139.10, 134.35, 125.51, 110.87, 27.69 (CH), 22.01 (CH_3). MS (70 eV): *m/z* (%) = 257 (M^+, 66%), 255 (61%), 242 (97%), 240 (100%), 177 (20%), 162 (56%), 146 (23%). Calc. C 56.01%, H 7.05%, N 5.44%; found C 56.42%, H 7.19%, N 4.82%

2.5. Computational methods

E_{HOMO} and E_{LUMO} of **1–18** were calculated by the semi-empirical molecular orbital method AM1, after optimizing the starting geometries by MM2 force field calculations [19]. The initial geometry was built from standard bond lengths and angles. All calculations were performed using the program MOPAC Version 6.0. Hydrophobicity is expressed in terms of log *P* as calculated with the program KOWWIN Version 1.54.

2.6. Mutagenicity tests

The mutagenic activity of the aromatic amines **1–18** was evaluated in *S. typhimurium* TA98 and TA100, with and without metabolic activation (S9 mix), using the procedure of Maron and Ames [5]. Tester strains *S. typhimurium* TA98 and TA100 were gifts from Bruce Ames, University of California, Berkeley, CA. Aroclor induced S9 rat liver mix was purchased from CCR, Germany. In all cases, DMSO was used as the diluting solvent, and dosage solutions were prepared immediately prior to testing. Incubations were carried out in triplicate; solvent controls were run with each experiment. Compounds were mostly tested at five concentrations both with TA98 and TA100. Each data point represents the average value of three parallel test experiments. Appropriate vehicle controls (DMSO) were conducted both with and without metabolic activation. The positive control for TA98 + S9 was 2-aminoanthracene (2-AA), for TA98-S9 4-nitro-*o*-phenylenediamine (NOPD) or nitropyrene (NP), for TA100 + S9 2-AA, and for TA100 − S9 *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) or sodium azide (NaN_3). Compounds were considered to be mutagenic if the average number of histidine revertants obtained at any dose level assayed was at least twice that of the solvent control. Revertants per nanomoles (rev/nmol) were calculated from the linear slope of the increasing part of the dose-response curve (linear regression analysis). Cytotoxicity is indicated by thinning or absence of background lawn.

3. Results

3.1. Salmonella assays

The mutagenicities (log rev/nmol) of the arylamines (**1**, **6**, **11**) and the alkylated arylamines (**2–5**, **7–10**, **12–18**) as determined in the Ames tests are summarized in Table 2.

Details of the mutagenicity tests of 2-AN **1** and its alkylated derivatives **2–5**, 2-AF **6** and the alkylated 2-aminofluorenes **7–10**, 4-ABP **11** and the alkylated 4-aminobiphenyls **12–15** as well as the *ortho*- dialkylated 4-aminobiphenyls **16–18**, are shown in Tables 3–6.

3.1.1. 2-Aminonaphthalene *1* and its ortho-alkyl derivatives *2–5*

The number of revertants induced by the test compounds **1–5** in TA98 and TA100 with and without metabolic activation are shown in Table 3.

The parent compound 2-AN **1** shows in the tester strain TA98 with (+S9) and without (−S9) metabolic

Table 2
Experimental mutagenicities (log rev/nmol) of compounds **1–18**

Number	Compound	log rev/nmol	
		TA98 + S9	TA100 + S9
1	2-AN	−1.43	0.29
2	1-Et-2AN	0.36	1.22
3	1-*i*Pr-2AN	−0.62	0.68
4	1-*n*Bu-2AN	−0.29	1.04
5	1-*t*Bu-2AN	<−2	−0.82
6	2-AF	1.54	1.03
7	1-Et-2AF	1.85	1.08
8	1-*i*Pr-2AF	2.23	1.29
9	1-*n*Bu-2AF	2.86	0.82
10	1-*t*Bu-2AF	0.07	−0.26
11	4-ABP	0.65	0.92
12	3-Et-4ABP	0.17	0.89
13	3-*i*Pr-4ABP	−0.01	0.97
14	3-*n*Bu-4ABP	−0.17	0.27
15	3-*t*Bu-4ABP	−0.39	0.40
16	3,5-DiMe-4ABP	−1.34	−0.37
17	3,5-DiEt-4ABP	−1.71	−0.30
18	3,5-Di*i*Pr-4ABP	−1.95	−1.40

Table 3
Number of revertants of 2-aminonaphthalene **1** and the *ortho*-alkylated 2-aminonaphthalenes **2**–**5** in TA98 and TA100[a]

Number	Compound	Dose (μg per plate)	Revertants			
			TA98		TA100	
			+S9	−S9	+S9	−S9
1	DMSO		42	37	106	108
	Positive control		691^{α}	1129^{β}	992^{α}	1002^{β}
	2-AN	20	53	31	381	126
		100	70	39	429	143
		500	37	37	315	214
		2500	15	34	50	32
		5000	tox^{T}	tox^{T}	tox^{T}	tox^{T}
2	DMSO		42	37	106	108
	Positive control		691^{α}	1129^{γ}	992^{α}	1002^{β}
	1-Et-2-AN	20	356	30	1139	112
		100	684	29	1554	114
		500	558	35	1317	130
		2500	tox^{T}	tox^{T}	tox^{T}	tox^{T}
		5000	tox^{A}	tox^{A}	tox^{A}	tox^{A}
3	DMSO		47	32	124	117
	Positive control		516^{α}	1021^{γ}	793^{α}	707^{γ}
	1-*i*Pr-2-AN	0.8	34	32	152	132
		4	44	29	205	140
		20	56	28	635	166
		100	168	29	2700	159
4	DMSO		33	27	113	114
	Positive control		1005^{α}	1011^{γ}	1431^{α}	1025^{β}
	1-*n*Bu-2AN	5	80	27	306	128
		20	289	28	1198	141
		100	584	26	1644	127
		500	tox^{T}	tox^{T}	tox^{T}	tox^{T}
		2500	$tox^{A,P}$	$tox^{A,P}$	$tox^{A,P}$	$tox^{A,P}$
5	DMSO		42	37	106	108
	Positive control		691^{α}	1129^{γ}	992^{α}	1002^{β}
	1-*t*Bu-2AN	20	42	35	196	115
		100	39	31	204	126
		500	23	13	43^{R}	29^{R}
		2500	tox^{T}	tox^{T}	tox^{A}	tox^{A}
		5000	tox^{T}	tox^{T}	tox^{A}	tox^{A}

[a] tox^{T}: thinning of background lawn; tox^{A}: absence of background lawn; P: precipitation of test compound; R: reduced number of revertants by thinning of background lawn; α: 2-AA, (2.5 μg); β: MNNG, (5 μg); γ: NOPD, (10 μg).

activation no mutagenic activity. The regression analysis of the linear portion of the dose-response curve leads to a value of −1.43 log rev/nmol (TA98 + S9). In the case of TA98 − S9 cytotoxic effects are observed at a concentration of 5000 μg per plate. In TA100 + S9, a different situation is seen: the number of revertants shown in Table 3 lead to 0.29 log rev/nmol (Table 2) which clearly indicates mutagenic activity. Cytotoxicity is seen at a concentration of 5000 μg per plate. Without S9 mix no activity could be found in TA98 and TA100. In the case of 1-Et-2AN **2**, in both tester strains with S9 mix, an increase of mutagenicity is observed as compared to **1**, see Tables 2 and 3: in TA98 + S9 the

Table 4
Number of revertants of 2-aminonaphthalene **6** and the *ortho*-alkylated 2-aminofluorenes **7–10** in TA98 and TA100[a]

Number	Compound	Dose (μg per plate)	Revertants			
			TA98		TA100	
			+S9	−S9	+S9	−S9
6	DMSO		16	17	102	110
	Positive control		527^{α}	352^{γ}	1448^{α}	920^{β}
	2-AF	5	17	9	453	125
		20	49	6	1289	31^{R}
		100	29	8	869	12^{R}
		500	16	3	tox^{A}	tox^{A}
7	1-Et-2AF	5	20	10	386	153
		20	75	10	1305	293
		100	82	24	1483	608
		500	tox^{A}	tox^{A}	816	tox^{A}
8	DMSO		31	37	102	110
	Positive control		1555^{α}	965^{γ}	1448^{α}	920^{b}
	1-*i*Pr-2AF	5	81	33	419	149
		20	166	53	1825	264
		100	737	172	1933	452
		500	tox^{A}	tox^{A}	tox^{A}	tox^{A}
9	DMSO		33	27	113	114
	Positive control		1005^{α}	1011^{γ}	1431^{α}	1025^{β}
	1-*n*Bu-2AF	5	192	31	170	114
		20	601	28	650	124
		100	1198	27	1061	110
		500	1153^{P}	28^{P}	363^{P}	75^{P}
		2500	396^{P}	$22^{P,R}$	38^{P}	44^{P}
10	DMSO		16	17	102	110
	Positive control		527^{α}	352^{γ}	1448^{α}	920^{β}
	1-*t*Bu-2AF	5	9	10	135	115
		20	18	8	154	110
		100	18	6	184	112
		500	tox^{A}	tox^{A}	43^{R}	3^{R}

[a] tox^{A}: absence of background lawn; P: precipitation of test compound; R: reduced number of revertants by thinning of background lawn; α: 2-AA, (2.5 μg); β: MNNG, (5 μg); γ: NOPD, (10 μg).

mutagenicity of **2** (0.36 log rev/nmol) is nearly 60 times higher than that of **1** (−1.43 log rev/nmol). Similarly, in TA100 + S9 a strong mutagenic potential is revealed (1.22 log rev/nmol). In both strains, without metabolic activation, no mutagenicity is detected. Thinning of background lawn was observed for both tester strains, TA98 and TA100, with and without metabolic activation at concentrations of 2500 μg per plate. Absence of background lawn is found at 5000 μg per plate. 1-*Iso*propyl-2-aminonaphthalene **3** shows no mutagenic activity in TA98 − S9 and in TA100 − S9. In TA98 + S9, for **3** a linear slope up to a concentration of 100 μg per plate is observed, leading to −0.62 log rev/nmol, which indicates weak mutagenicity. In TA100 + S9, up to a concentration of 100 μg per plate, the mutagenic properties of 1-*iso*propyl-2-aminonaphthalene **3** led to a value of 0.68 log rev/nmol clearly indicating mutagenic behavior of **3**. Cytotoxicity is neither observed in TA98 nor in TA100. For the *n*-butyl derivative **4** in TA98 with S9 mix, a value of −0.29 log rev/nmol, and in TA100 with S9 mix, 1.04 log rev/nmol are observed. Without S9 mix also compound **4** shows no mutagenicity. Above 500 μg per plate cytotoxicity is detected in

Table 5
Number of revertants of 4-aminobiphenyl **11** and the *ortho*-alkylated 4-aminobiphenyls **12–15** in TA98 and TA100[a]

Number	Compound	Doses (μg per plate)	Revertants			
			TA98		TA100	
			+S9	−S9	+S9	−S9
11	DMSO		39	28	113	114
	Positive control		950^{α}	842^{γ}	1431^{α}	1025^{β}
	4-ABP	5	188	28	336	115
		20	576	28	1093	146
		100	572	27	1116	148
		500	375^{R}	21	1281	141
		2500	tox^{A}	tox^{A}	tox^{A}	tox^{A}
12	DMSO		36	32	134	12
	Positive control		721^{α}	1176^{γ}	479^{α}	1051^{β}
	3-Et-4ABP	2.40	–	–	184	–
		4.75	88	–	290	–
		9.5	$tox^{A,C}$	–	395	–
		19	304	–	876	–
		37.5	449	30	1573	145
		75	603	33	–	142
		150	671	38	–	152
		300	691	41	–	69
13	DMSO		31	37	102	110
	Positive control		1555^{α}	965^{γ}	1448^{α}	920^{β}
	3-*i*Pr-4-ABP	5	37	28	176	103
		20	115	23	945	99
		100	487	25	1539	113
		500	tox^{A}	tox^{A}	tox^{A}	tox^{A}
14	DMSO		33	27	113	114
	Positive control		1005^{α}	1011^{γ}	1431^{α}	1025^{β}
	3-*n*Bu-4-ABP	5	53	34	132	115
		20	96	31	269	112
		100	334	24	921	79
		500	293	29	65^{R}	26^{R}
		2500	82^{P}	$16^{P,R}$	$tox^{A,P}$	$tox^{A,P}$
15	DMSO		41	32	125	123
	Positive control		1379^{α}	1176^{γ}	1837^{α}	1051^{β}
	3-*t*Bu-4-ABP	37.5	116	27	643	103
		75	177	17	964	104
		150	304	17	1053	101
		300	325	21	1493	92

[a] tox^{A}: absence of background lawn; P: precipitation of test compound; R: reduced number of revertants by thinning of background lawn; C: contamination; (–): no measurement at this concentration; α: 2-AA, (2.5 μg); β: MNNG, (5 μg); γ: NOPD, (10 μg).

both tester strains, and at a concentration of 2500 μg per plate precipitation of the test compound together with the absence of background lawn is observed. In contrast to compounds **2–4**, the *tert*-butyl derivative **5** shows in TA98, with metabolic activation, no mutagenic potential (≤2 log rev/nmol for TA98 + S9). Also in TA100 + S9 no mutagenicity is found (−0.82 log rev/nmol). In TA98 with and without S9 mix cytotoxicity sets in at a concentration of 2500 μg per plate indicated by thinning of the background lawn. At the same concentration absence of background lawn is observed for TA100 + S9 and TA100 − S9. Thus, all

Table 6
Number of revertants of the *ortho*-dialkylated 4-aminobiphenyls **16–18** in TA98 and TA100[a]

Number	Compound	Dose (μg per plate)	Revertants			
			TA98		TA100	
			+S9	−S9	+S9	−S9
16	DMSO		48	31	106	118
	Positive control		1167^{α}	1487^{δ}	1612^{α}	898^{ϵ}
	3,5-DiMe-4ABP	10	44	30	118	108
		20	57	34	150	111
		100	70	49	184	123
		500	143	33	219	38
		2500	tox^{T}	tox^{T}	24	tox^{T}
17	3,5-DiEt-4ABP	10	46	30	117	107
		20	57	34	150	122
		100	57	38	152	129
		500	57	39	150	123
		2500	57	42	55	36
18	3,5-Di*i*Pr-4ABP	10	49	30	136	100
		20	48	30	117	103
		100	53	30	136	116
		500	54	30	137	90
		2500	60	40	131	107

[a] tox^{T}: thinning of background lawn; α: 2-AA, 2.5 μg; δ: NP 2.5 μg; ϵ: NaN_3 (5 μg).

alkyl-substituted derivatives of 2-AN **1**, except the *tert*-butyl derivative **5**, are in TA98 + S9 of slightly higher mutagenicity than 2-AN **1**. The same trend is observed for the tester strain TA100 + S9. Interestingly 1-*t*butyl-2-aminonaphthalene **5** shows in TA98 and TA100, with and without metabolic activation, no mutagenicity.

*3.1.2. 2-Aminofluorene **6** and its ortho-alkyl derivatives **7–10***

The number of revertants induced by the test compounds **6–10** in TA98 and TA100 with and without metabolic activation are shown in Table 4. [1]

2-AF **6**, as reported in the literature [20], is a mutagenic compound. This is clearly illustrated by the log rev/nmol values in TA98 + *S*9 (1.54 log rev/nmol) and TA100 + S9 (1.03 log rev/nmol), see Table 2. Without S9 mix, in both tester strains, a positive response was not seen. In TA100 with and without S9 mix, absence of background lawn was observed at a concentration of 500 μg per plate. In TA98 − S9, the number of revertants was at all concentrations (5–500 μg per plate) lower than the solvent control. The ethyl derivative 1-ethyl-2-aminofluorene **7** is in TA98 + S9 more mutagenic (1.85 log rev/nmol) than the non-alkylated **6**, see Table 2. In TA100 + S9, the mutagenicity 1.08 log rev/nmol is in the range of **6**. At concentrations of 500 μg per plate in strain TA98 with and without S9 mix and in TA100 with S9 mix, cytotoxicity was indicated by the absence of background lawn. In TA98 without S9 mix no mutagenicity is found. In TA100 − S9, mutagenicity is clearly seen as indicated by the number of revertants, see Table 4. The *iso*-propyl-substituted 1-*i*Pr-2AF **8** is even more mutagenic than **6** and **7** in both tester strains with S9 mix: 2.23 log rev/nmol (TA98 + S9) and 1.29 log rev/nmol

[1] In the test series of the 2-aminofluorenes **6–10** in TA98, due to the quality of the tester strain, the number of revertants in the case of the parent compound 2-AF **6** was much smaller than reported in the literature [20]. Since, the results of the alkyl-substituted **7–10** should be similarly affected, the revertants of 2-AF **6** were related to the literature value of 34.6 rev/nmol [20], and the same relation as found for **6** was used for the determination of the values of **7–10**. As one can see from Fig. 2, the determined values of **6–10** in TA98 + S9 show exactly the same tendency as those correctly determined in TA100+S9. Lack of compounds **7–10**, unfortunately, did not allow a second mutagenicity study in TA98 + S9.

(TA100 + S9), see Table 2. Cytotoxicity is found at 500 μg per plate. Both, for TA98 and TA100 without S9 mix mutagenicity is observed (Table 4). 1-*n*Bu-2AF **9**, the *n*-butyl-substituted derivative, shows the highest mutagenicity in this series: in TA98 + S9 it is 20 times higher (2.86 log rev/nmol) than that of the parent compound **6** (Table 2). In TA100 + S9, **9** has a lower mutagenic activity (0.82 log rev/nmol) compared to **8** (1.29 log rev/nmol). Precipitation of the test compound was observed at concentrations of 500 and 2500 μg per plate. The only alkyl-substituted derivative having lower mutagenicity than the unsubstituted **6** (1.54 log rev/nmol in TA98 + S9, 1.03 log rev/nmol in TA100 + S9) in both tester strains, is 1-*t*Bu-2AF **10**: 0.07 log rev/nmol in TA98 + S9 and −0.26 log rev/nmol in TA100 + S9 (Table 2). The latter value together with the number of revertants in Table 4 indicates weak mutagenicity. Cytotoxicity is observed at concentrations of 500 μg per plate for TA98 with and without S9 mix. In TA100, a reduced number of revertants caused by a reduction of the background lawn is observed at the same concentration. As expected, mutagenicity is not observed without metabolic activation. In conclusion, with increasing log *P*-values of compounds **6–9**, mutagenicity in general increases. In the case of the *tert*-butyl-substituted **10**, however, the sterically crowded substituent leads to a reduction almost an elimination of mutagenicity.

*3.1.3. 4-Aminobiphenyl **11** and its ortho-alkylated derivatives **12–15***

The number of revertants induced by the test compounds **11–15** in TA98 and TA100 with and without metabolic activation are shown in Table 5.

As expected, the parent compound 4-aminobiphenyl **11** requires metabolic activation to become a mutagen. In both tester strains with S9 mix, **11** shows a positive mutagenic response, being stronger in TA100 (0.92 log rev/nmol) than in TA98 (0.65 log rev/nmol), see Tables 5 and 2. Up to a concentration of 20 μg per plate, in TA98 + S9, the mutagenicity of 4-ABP **11** rises linearly, while at concentrations of 2500 μg per plate absence of background lawn is observed in both tester strains with and without S9 mix. In both strains, without S9 mix, no mutagenicity was recorded (Table 5). 3-Et-4ABP **12** shows clearly mutagenic properties in TA98 (0.17 log rev/nmol) and in TA100 (0.89 log rev/nmol), in the presence of S9 mix. In the absence of S9 mix no mutagenicity was observed in TA98, but in TA100 mutagenicity is seen as indicated by the number of revertants (Table 5). 3-*i*Pr-4ABP **13** is non-mutagenic in both tester strains without activation. The number of revertants per plate are in the range of the negative control. The compound shows mutagenicity in TA98 + S9 (−0.01 log rev/nmol) and TA100 + S9 (0.97 log rev/nmol), see Tables 5 and 2. At concentrations of 500 μg per plate **13** is cytotoxic in both tester strains with and without metabolic activation. 3-*n*Bu-4ABP **14** is non-mutagenic without S9 mix (Table 5). With S9 mix, **14** showed in TA98 (−0.17 log rev/nmol) and in TA100 (0.27 log rev/nmol) a lower mutagenicity than **11**. In TA100 with and without S9 mix cytotoxicity was observed at concentrations of 2500 μg per plate. The compound with the bulkiest alkyl group within this series, 3-*t*Bu-4-ABP **15**, reveals in both tester strains with S9 mix (−0.39 log rev/nmol for TA98, and 0.40 log rev/nmol for TA100) reduced mutagenicity as compared to **11**, especially in TA98. When 3-*t*Bu-4ABP **15** was tested without S9 mix, the compound was non-mutagenic in both strains. In contrast to 1-*t*Bu-4AN **5**, the mutagenic activity of 1-*t*Bu-4ABP **15** in TA98 + S9 was not completely extinguishable. A similar observation is made in TA100 + S9.

In conclusion, also in the alkylated 4-aminobiphenyl test series **12–15**, the mutagenicity decreases with increasing steric demand of the *ortho*-alkyl substituents.

*3.1.4. 3,5-Dialkyl-4-aminobiphenyl **16–18***

The number of revertants induced by the test compounds **16–18** in TA98 and TA100 with and without metabolic activation is given in Table 6.

It was shown above that due to alkylation *ortho* to the amino function mutagenicity of arylamines may decrease, especially in the case of bulky substituents. Double-alkylation *ortho* to the amino function should enhance this effect. Indeed, 3,5-DiMe-4ABP **16** confirms this suggestion: **16** shows very weak mutagenic potential in TA98 + S9 (−1.34 log rev/nmol), see Table 2. In comparison to the parent compound 4-ABP **11** (0.65 log rev/nmol), mutagenicity is nearly nine times lower. In TA100 + S9 weak mutagenicity is also observed (−0.37 log rev/nmol). In both tester strains, without metabolic activation, a significant

number of mutated revertants was not observed. **16** exhibits cytotoxicity in TA98 + S9, TA98 − S9 and TA100 − S9 at concentrations of 2500 μg per plate. 3,5-DiEt-4ABP **17**, in TA98 with S9 mix, shows no mutagenicity (−1.71 log rev/nmol), see Table 2. In the strain TA100 + S9 the mutagenicity value (−0.30 log rev/nmol) is in the range of 3,5-DiMe-4ABP **16**. 3,5-Di*i*Pr-4ABP **18**, in TA98 and TA100 with and without metabolic activation, is non-mutagenic (−1.95 log rev/nmol for TA98 + S9, and −1.40 log rev/nmol for TA100 + S9).

In conclusion, double-alkylation *ortho* to the amino function with (bulky) alkyl groups causes an enhanced decrease of mutagenicity in the 4-aminobiphenyl derivatives **16–18**.

3.2. Comparison of predicted and experimental mutagenicities

The aim of a QSAR is to predict the mutagenic potential of chemical compounds as a function of their structure. Debnath et al. developed empirical correlations for aromatic amines tested in the *Salmonella* strains TA98 and TA100 with metabolic activation [7]. According to their results mutagenic activity of an aromatic amine is mainly determined by its hydrophobicity (log *P*) and HOMO and LUMO energies. The correlations for aromatic and heteroaromatic amines, assayed in TA98 and TA100 with rat liver S9 mix, are shown in Eqs. (1) and (2).

$$\log \mathrm{TA98} = 1.08 \log P + 1.28\, E_{\mathrm{HOMO}} - 0.73\, E_{\mathrm{LUMO}} + 1.46\, I_{\mathrm{L}} + 7.20 \quad (1)$$

$$\log \mathrm{TA100} = 0.92 \log P + 1.17\, E_{\mathrm{HOMO}} - 1.18\, E_{\mathrm{LUMO}} + 7.35 \quad (2)$$

The strongest influence results from hydrophobicity (log *P*). Increasing hydrophobicity increases mutagenicity linearly. In the case of TA98 mutagenic activity is furthermore influenced by the indicator variable I_{L}. It is 1 for compounds containing three or more fused rings, and 0 for all other species. The electronic properties (E_{HOMO} and E_{LUMO}) have only smaller influence on the mutagenicity. They account for 17% in TA100 and 4% in TA98. Nevertheless, there exists a positive correlation between mutagenic activity and E_{HOMO}. Compounds with higher E_{HOMO}

Table 7
The log *P*, E_{LUMO}, E_{HOMO} values, and calculated mutagenicities of **1–18** in comparison with the experimental mutagenicities

Number	Compound	log *P*	E_{LUMO} (eV)	E_{HOMO} (eV)	log rev/nmol			
					TA98 + S9		TA100 + S9	
					Calculated	Expected	Calculated	Expected
1	2-AN	2.28[a]	−0.175	−8.227	−0.33	−1.43	0.00	0.29
2	1-Et-2AN	3.29	−0.169	−8.127	0.82	0.36	1.07	1.22
3	1-*i*Pr-2AN	3.71	−0.172	−8.102	1.28	−0.62	1.49	0.68
4	1-*n*Bu-2AN	4.27	−0.171	−8.124	1.88	−0.29	1.98	1.04
5	1-*t*Bu-2AN	4.16	−0.179	−8.052	1.79	<−2	1.97	−0.82
6	2-AF	3.14[a]	−0.098	−8.107	1.75	1.54	0.87	1.03
7	1-Et-2AF	4.14	−0.073	−8.060	2.87	1.85	1.82	1.08
8	1-*i*Pr-2AF	4.55	−0.051	−8.023	3.34	2.23	2.21	1.29
9	1-*n*Bu-2AF	5.12	−0.065	−8.045	3.94	2.86	2.72	0.82
10	1-*t*Bu-2AF	5.01	−0.041	−7.997	3.86	0.07	2.65	−0.26
11	4-ABP	2.86[a]	0.045	−8.274	−0.33	0.65	0.25	0.92
12	3-Et-4ABP	3.88	0.073	−8.214	0.82	0.17	1.22	0.89
13	3-*i*Pr-4ABP	4.30	0.076	−8.210	1.28	−0.01	1.61	0.97
14	3-*n*Bu-4ABP	4.86	0.072	−8.212	1.88	−0.17	2.13	0.27
15	3-*t*Bu-4ABP	4.75	0.108	−8.170	1.79	−0.39	2.03	0.40
16	3,5-DiMe-4ABP	3.93	0.088	−8.166	0.92	−1.34	1.31	−0.37
17	3,5-DiEt-4ABP	4.92	0.096	−8.150	2.01	−1.71	2.23	−0.30
18	3,5-Di*i*Pr-4ABP	5.75	0.114	−8.144	2.90	−1.95	2.98	−1.40

[a] Experimental log *P*.

energies are more easily oxidized, which leads to faster transformation into the corresponding hydroxylamines and, thus, e.g. *O*-acylhydroxylamines, the ultimate mutagens. The QSAR correlations as developed by Debnath et al. are based on a large number of aromatic amines with different aromatic ring systems. Different (alkyl) substituents, however, have not been considered. It is noticed that "steric effects and other structural factors, which have not been built into the QSARs, must be responsible for some of the observed variation in activity" [7]. In our investigations, we exactly studied these effects of alkyl substituents *ortho* to the amino function, and it is obvious from the results shown above that steric effects dramatically influence the mutagenicity of the compounds tested.

In Table 7, log *P*-values and electronic properties (E_{HOMO} and E_{LUMO}) of compounds **1–18** are listed along with their calculated and experimental mutagenicities (log rev/nmol).

4. Discussion

As shown in Table 7 hydrophobicity of a certain compound increases with the number and the size of the alkyl groups attached *ortho* to the amino function. The highest log *P*-values are calculated for compounds containing the *n*-butyl group (**4**: 4.27, **9**: 5.12 and **14**: 4.86), and for the disubstituted species **16–18** (**16**: 3.93, **17**: 4.92 and **18**: 5.75). The log *P*-values of compounds with linear alkyl side chains are higher than those of the isomeric branched ones (1-*n*Bu-2AN **4**: 4.27 versus 1-*t*Bu-2AN **5**: 4.16). The LUMO and the HOMO energies are slightly raised by alkyl groups because of their +I-effects (4-ABP **11**: $E_{LUMO} = 0.045$ eV, $E_{HOMO} = -8.274$ eV versus 3-Et-4ABP **12**: $E_{LUMO} = 0.073$ eV, $E_{HOMO} = -8.214$ eV). The only exception is 1-*t*Bu-2AN **5** showing a slightly lower LUMO ($E_{LUMO} = -0.179$ eV) than the parent compound 2-AN **1** ($E_{LUMO} = -0.175$ eV). *Ortho*-disubstituted aromatic amines show a stronger

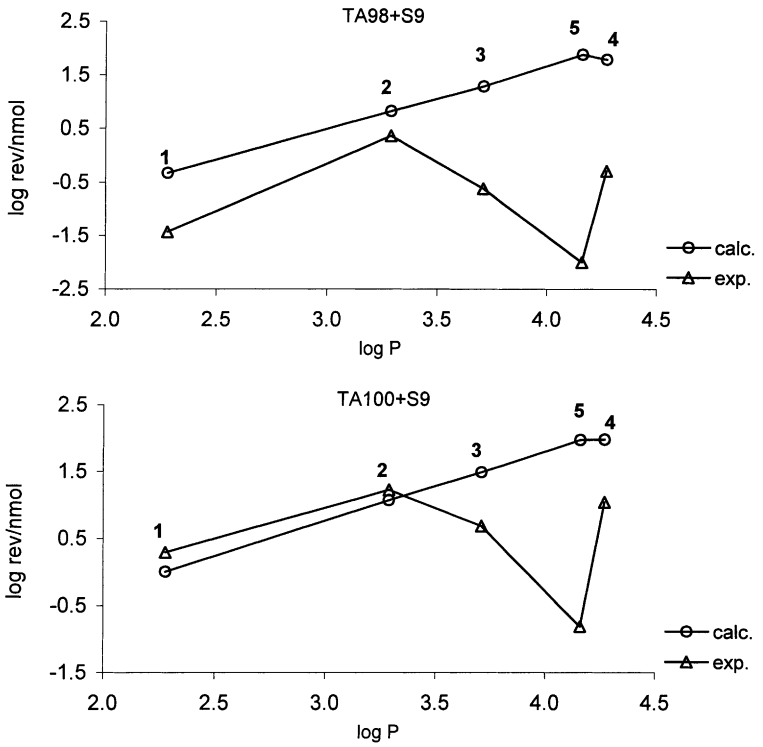


Fig. 1. Experimental and calculated mutagenicities of compounds **1–5**.

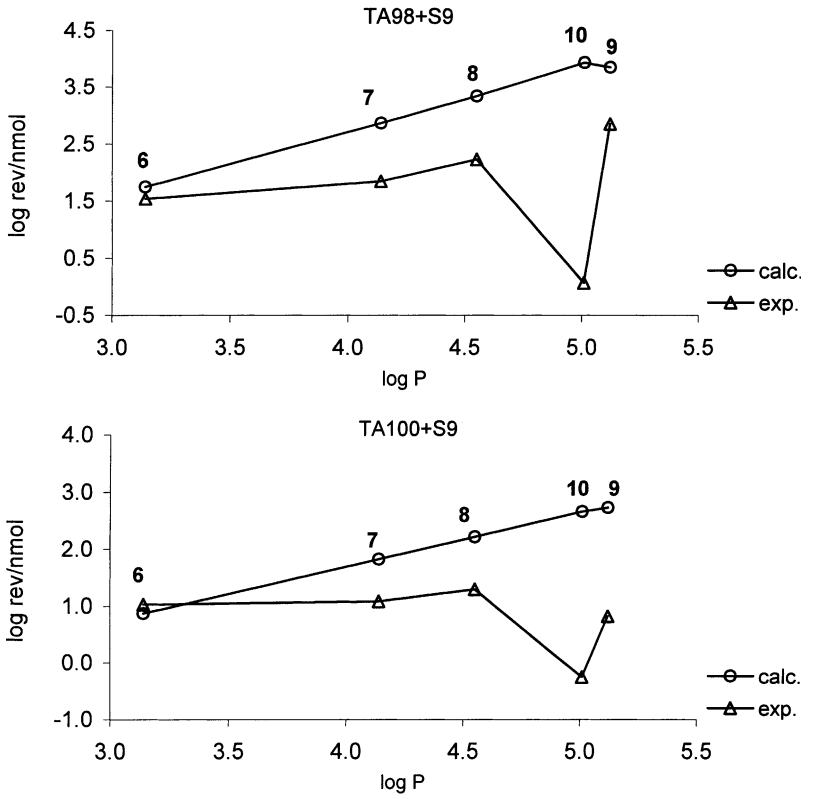


Fig. 2. Experimental and calculated mutagenicities of compounds **6–10**.

influence on the orbital energies than monosubstituted ones, see, e.g. 3,5-DiMe-4ABP **16** (E_{LUMO} = 0.088 eV, E_{HOMO} = −8.166 eV). In Figs. 1–4 the calculated and the measured mutagenicity values (log rev/nmol) are graphically compared with each other.

In Fig. 1, the parent compound 2-aminonaphthalene **1** and the alkylated derivatives **2–5** are shown. For both *Salmonella* strains TA98 and TA100, with metabolic activation, the Debnath–Hansch correlations predict a linear increase of mutagenicity with increasing hydrophobicity resulting from the alkyl groups (Et < *i*Pr < *t*Bu < *n*Bu). In the case of the parent compound 2-AN **1** and of 1-Et-2AN **2** mutagenicity is decently predicted. However, with growing steric demand of the alkyl groups, the predicted and the experimental values differ increasingly. The graphs show strong deviations in the case of the *iso*-propyl-substituted **3**, the *n*-butyl-substituted **4**, and especially the *tert*-butyl-substituted **5**. In the case of 1-*t*Bu-2-AN **5**, in TA98 + S9, the experimental value deviates from the straight line by nearly 3.8 units (log rev/nmol; this corresponds to a factor of ∼6000). These results nicely illustrate that the decrease of mutagenicity in compounds **2–5** is strongly related to the steric influence of the alkyl groups. The steric demand of the *n*-butyl group is in the range between that of the ethyl and the *iso*-propyl groups [21]. Therefore, and because of its high hydrophobicity, 1-*n*Bu-2AN **4** shows higher mutagenic activity than 1-*i*Pr-2AN **3** and 1-*t*Bu-2AN **5**. The only species showing smaller mutagenicity than the parent compound 2-AN **1**, is 1-*t*Bu-2AN **5** with its sterically strongly demanding *tert*-butyl substituent [21]. Related results were also observed in the case of the tester strain TA100 + S9, see also Fig. 1.

The same tendency as for **1–5** was similarly observed in the case of the alkylated 2-aminofluorenes **7–10** if compared with the parent compound **6**, see

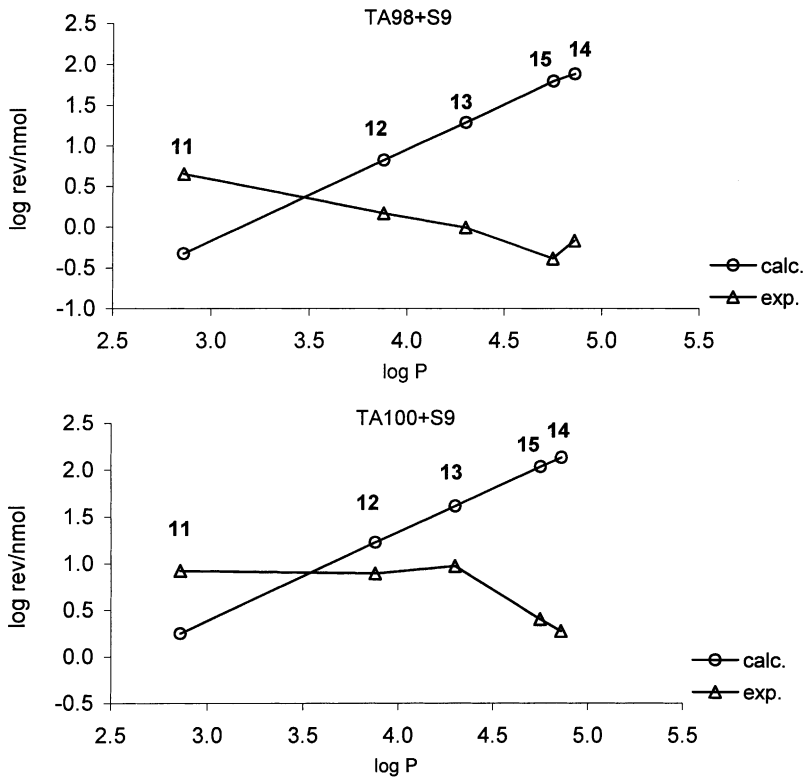


Fig. 3. Experimental and calculated mutagenicities of compounds **11–15**.

Fig. 2. The diagrams for both tester strains (Fig. 2) reveal that the compounds bearing bulkier alkyl groups have less mutagenic activity than predicted by the Debnath–Hansch equations (Eqs. (1) and (2)). In contrast to the series of **1–5** only weak increase of mutagenicity was observed due to the ethyl group in **7**. As in the case of 1-*n*Bu-2AN **4**, see Fig. 1, also 1-*n*Bu-2AF **9** in TA98+S9 shows stronger mutagenic activity than the other alkylated species **7**, **8** and **10**, see Fig. 2. Apparently, the strong hydrophobicity of **9** dominates here over the steric demand of the *n*-butyl group. The *tert*-butyl substituted **10** has again the lowest mutagenicity value within the 2-AF series. It was, however, not possible to suppress completely the mutagenic activity in the case of **10**.

The results of the *ortho*-alkylated 4-aminobiphenyls **12–15** (Fig. 3) and the dialkylated **16–18** (Fig. 4) show similarly the importance of steric factors on mutagenicity. Again growing steric demand of the alkyl groups leads to decreased mutagenicity. The experimental and the predicted values deviate strongly in these cases: for 3-*t*Bu-4ABP **15** the Debnath–Hansch correlation predicts a mutagenicity in TA98 + S9 of 1.79 log rev/nmol, while the experimental data lead to −0.39 log rev/nmol. The largest difference is observed for the dialkyl compounds **16–18**, and here especially for 3,5-Di*i*Pr-4ABP **18** (calculated: 2.90 log rev/nmol; experimental: −1.95 log rev/nmol). These results strongly support the necessity to include steric parameters into the QSAR correlations if reasonable mutagenicity values are to be excepted for compounds bearing sterically demanding substituents.

Similar observations were made by Klein et al. in the case of *ortho*-alkylated nitrobiphenyls [22]. In their work the synthesis of non-mutagenic derivatives of 4-nitrobiphenyls is reported by introducing bulky alkyl groups, as similarly shown here. The predicted mutagenicities of alkyl-substituted nitro compounds are also too high, because the existing equations for the calculation of mutagenicities likewise do also

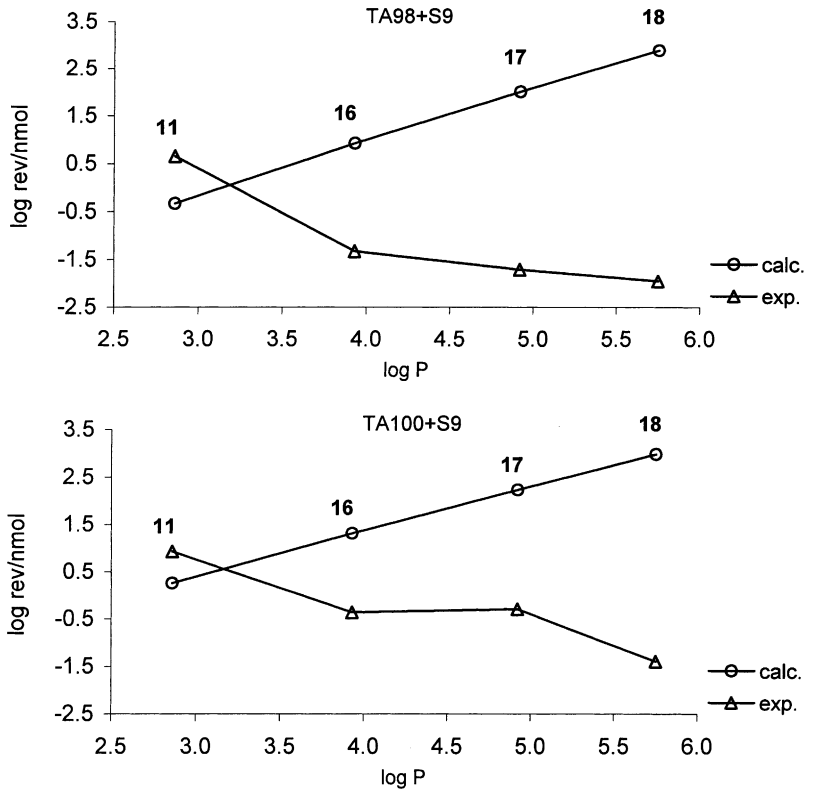


Fig. 4. Experimental and calculated mutagenicities of compounds **11**, **16**–**18**.

not take into account steric effects of aliphatic substituents.

4.1. Reduced and increased mutagenicity of ortho-alkyl substituted aromatic amines

One reason for the decrease of the mutagenicity of arylamines with sterically demanding alkyl groups as the *iso*-propyl and *tert*-butyl substituents could be the steric hindrance of the metabolic oxidation of the amino group by enzymes, which is a pre-requisite for the transformation of the non-mutagenic aromatic amines into mutagens. If the oxidation step to the respective hydroxylamine is blocked, or aggravated, the amine is not transformed into its ultimate mutagen. In the literature similar cases are mentioned, e.g. the completely different mutagenicity of 1-aminonaphthalene and 2-aminonaphthalene: 1-aminonaphthalene is non-mutagenic, while 2-aminonaphthalene is mutagenic. In contrast, in the case of the respective hydroxylamines, it is 1-hydroxylaminonaphthalene that shows a 10-fold higher induction rate of tumors than 2-hydroxylaminonaphthalene [23]. Related results are observed for the 2-amino and 3-amino positional isomers of 4-aminobiphenyl **11** and their hydroxylamino derivatives [24], and for 3-*t*Bu-4ABP **15** [25].

What is the reason for the comparatively high mutagenicity of the ethyl-substituted arylamines 1-Et-2AN **2**, 1-Et-2AF **7** and 3-Et-4ABP **12**, as observed in this work, and for 3-methyl-4-aminobiphenyl as reported by Bayoumy et al. [26], and for *ortho*-alkylated anilines as described by Beland et al. [27]? First, the steric hindrance caused by the methyl and the ethyl group is small if compared to the increase of the log *P*-value resulting from these substituents. One should also consider that *ortho*-alkyl groups, due to their $+I$-effect, stabilize the intermediate nitrenium ions. Indeed, one has observed a linear relationship between

mutagenicity and the stability of the respective nitrenium ion [28]. Secondly, substituents like methyl, and probably ethyl, are oxidized, leading to the corresponding benzylic alcohol which are transformed by sulfation or phosphation into ultimate mutagens which react with DNA [29]. A prominent example for such a metabolic pathway was observed in the case of 1-methylpyrene [30,31]. Thus, methyl- and ethyl-substituted arylamines possibly are not only activated via oxidation of the amino group, but also via oxidation of the small alkyl groups bonded to the aromatic rings.

In summary, it is shown in this work that the mutagenicity of aromatic amines decreases with growing steric demand of the alkyl groups in the *ortho*-position of the amine functionality. Generally, steric demand leads to lower mutagenicities than predicted by the Debnath–Hansch equations (Eqs. (1) and (2)). Thus, these QSAR correlations are not appropriate to evaluate the mutagenicity of aromatic amines substituted with such alkyl groups.

Due to these findings, it was possible in the cases of 1-*t*Bu-2AN **5**, 3,5-DiEt-4ABP **17** and 3,5-Di*i*Pr-4-ABP **18** to synthesize non-mutagenic derivatives of rather mutagenic parent aromatic amines.

The investigations of aromatic amines bearing alkyl groups "far away" from the amino functionality will be discussed in a following paper.

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