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Mutation Research 491 (2001) 195–209

Genetic Toxicology and Environmental Mutagenesis

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The effects of 4′-alkyl substituents on the mutagenic activity of 4-amino- and 4-nitrostilbenes in *Salmonella typhimurium*

Björn Ludolph [a], Markus Klein [a], Lothar Erdinger [b], Gernot Boche [a,*]

[a] *Philipps-Universität, Marburg Hans-Meerwein Straße, 35032 Marburg, Germany*
[b] *Hygieneinstitut Heidelberg, Im Neuenheimer Feld, 69120 Heidelberg, Germany*

Received 24 October 2000; received in revised form 16 January 2001; accepted 17 January 2001

Abstract

Six derivatives of *trans*-4-aminostilbene bearing different alkyl groups in the 4′-position and six of the corresponding nitro compounds were synthesized and tested for their mutagenic potency in *Salmonella typhimurium* strains TA98 and TA100. Regarding the test series in presence of S9-mix, maximum activity was observed for those *trans*-4-aminostilbenes and *trans*-4-nitrostilbenes bearing small alkyl substituents like methyl and ethyl. More bulky substituents reduced the mutagenic potential in the order *iso*-propyl < *sec*-butyl < *tert*-butyl for the aminostilbenes. The corresponding nitrostilbenes showed a similar trend under these conditions although the mutagenic activity of the *tert*-butyl-substituted compound was unexpectedly high in TA100. In the series without metabolic activation the nitrostilbenes showed a continuous decrease of mutagenic activity with the size of the substituents (methyl > ethyl > *iso*-propyl > *sec*-butyl > *tert*-butyl). These trends have been compared with quantitative structure activity relationship (QSAR) model predictions, leading to the conclusion that steric demand is an important factor for mutagenicity of substituted aminostilbenes and nitrostilbenes. The unexpected result for the *tert*-butyl nitrostilbene tested with metabolic activation may be attributed to a different metabolic pathway.

Keywords: *Salmonella*; Nitrostilbenes; Aminostilbenes; Alkyl substituents; *Para*; Mutagenicity

1. Introduction

Aromatic amines and nitroarenes are used in industrial processes and in the manufacturing of drugs, pesticides and plastics [1]. Several azo compounds that are widely used as dyes can be metabolically cleaved into the corresponding amines [2–4]. Since many aromatic amines are known for their mutagenic and carcinogenic potential attempts have been made to find general strategies to reduce the mutagenic potential of these *N*-substituted aromatics [5]. It is well established that metabolic transformation into electrophilic intermediates (hydroxylamines, nitrenium ions), that form DNA-adducts, is necessary for this class of compounds to develop their genotoxic properties. Thus, useful ways to reduce mutagenic responses should be either to inhibit this activation process or to hinder the binding of the reactive species to the DNA.

Recently we found that bulky alkyl substituents in the *para*-position of 4-aminobiphenyl **1a**–**e** [6,7] (paper in preparation), 4-nitrobiphenyl **2a**–**e** [8] and 2-nitrofluorene **3a**–**e** [8] (Fig. 1) decrease the

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

1383-5718/01/$ – see front matter
PII: S1383-5718(01)00142-5

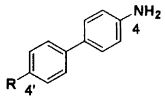
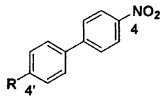
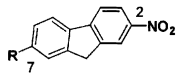
Code	Structure	Code	Structure	Code	Structure	R
1a		2a		3a		-H
1b		2b		3b		-Me
1c		2c		-		-Et
1d		2d		-		-iPr
1e		2e		3e		-tBu

Fig. 1. Structures of recently examined compounds.

mutagenicity in the Ames-test assayed with strains TA98 and TA100 of *Salmonella typhimurium*.

Most remarkably the mutagenicity of those compounds with large substituents like *tert*-butyl (**1e**–**3e**) was almost completely eliminated. In contrast small alkyl substituents like methyl (**1b**–**3b**) generally increased the mutagenic response significantly compared to the parent compounds **1a**–**3a**.

In the early nineties Debnath and Hansch developed quantitative structure activity relationship (QSAR) models to predict to mutagenicity of amino aromatics [9] and nitro aromatics, [10,11] which are based on hydrophobicity ($\log P$) and electronic energies (E_{HOMO}, E_{LUMO}). A comparison of our experimentally determined mutagenicities with those predicted by Debnath and Hansch led to the conclusion that the decrease of mutagenicity with the size of the alkyl substituents is mainly a steric effect [6–8]. For alkyl-substituted compounds the observed mutagenic response was correlated with the modification of the molecular shape. We suggested the steric demand of bulky alkyl groups to interfere with the molecule's ability to intercalate between the planar DNA bases. Regarding the small alkyl groups (methyl, ethyl), steric demand is less important. In this case mutagenicity is determined by the electronic and hydrophobic effects of these substituents. The introduction of bulky *para*-alkyl groups "far away" from the functional group (NH_2 or NO_2) seems to be a promising new method to detoxify mutagenic substances of this class. The chemical properties of the nitro or amino group, or the degree of aromaticity, remain essentially unaffected.

Another amino–aromatic model compound of great interest is *trans*-4-aminostilbene, first tested for its carcinogenic activity in 1948 [12]. Recent QSAR studies of the genotoxic potential of various *trans*-4-aminostilbenes were carried out by Sinsheimer [13,14]. In these investigations mutagenicity could be correlated with the electronic properties of the substituents (in the 4′-position) whereas no correlation was found between hydrophobicity and mutagenicity. The mutagenic potential of 4-nitrostilbene and some of its derivatives was demonstrated by Mullin [15] and Hoobermann [16]. Much effort has also been undertaken to characterize DNA-adducts of 4-aminostilbene derivatives (see Neumann [17], a review of earlier publications is given by Beland and Kadlubar [18]). 4-Aminostilbenes form structurally different adducts if compared to those of the 4-aminobiphenyls or 2-aminofluorenes mentioned above: 4-aminostilbene reacts via the double bond with the nitrogen and oxygen atoms of the DNA bases to form cyclic adducts. Some typical adducts (**4a**–**c**) are shown in Fig. 2.

Is the change of the molecular shape by introduction of bulky alkyl substituents far away from the functional group also suitable to reduce the mutagenicity of 4-amino- and 4-nitrostilbene derivatives as it is for the corresponding amino- and nitrobiphenyles (**1a**–**e** and **2a**–**e**) and aminofluorenes (**3a**–**e**)? To examine this question we investigated the mutagenicity of 4′-alkyl-substituted *trans*-4-amino- and *trans*-4-nitrostilbenes such as **5a**–**f** and **6a**–**f**, respectively (Fig. 3).

Furthermore we compared our experimental results with the predictions of the QSAR models of Debnath and Hansch (applicable for aminoarenes in the presence of S9-mix and nitroarenes in the absence of S9-mix) [11] and Sinsheimer, respectively (applicable

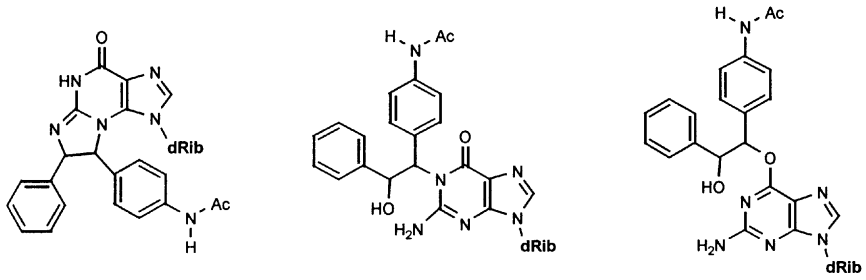


Fig. 2. Structures of typical DNA-adducts of *N*-acetylaminostilbene.

for *trans*-4-aminostilbenes in the presence of S9-mix) [13].

2. Materials and methods

2.1. Instrumentation

NMR spectra were obtained with Bruker spectrometers ARX-200 and AC-300 and are referenced to tetramethylsilane. Mass spectra were recorded with Varian MAT CH-7-A (EI, 70 eV). Elementary analyses were performed by a Heraeus Rapid Elementaranalysator.

2.2. Chemicals

The alkyl-substituted *trans*-4-nitrostilbenes **6a–f** (Fig. 3) were prepared either by Wittig reactions as described by Sinsheimer [12] from (4-nitrobenzyl)-

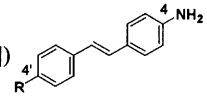
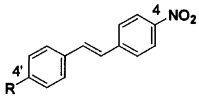
Code	Name (CAS number)	Structure	R	Purity
5a	*trans*-4-Aminostilbene (4309-66-4)		-H	> 99 % *trans*
5b	*trans*-4'-Methyl-4-aminostilbene (7314-08-1])		-Me	"
5c	*trans*-4'-Ethyl-4-aminostilbene (-)		-Et	"
5d	*trans*-4'-*iso*-Propyl-4-aminostilbene (-)		-iPr	"
5e	*trans*-4'-*tert*-Butyl-4-aminostilbene (74518-99-3)		-tBu	"
5f	*trans*-4'-*sec*-Butyl-4-aminostilbene (-)		-sBu	"
6a	*trans*-4-Nitrostilbene (1694-20-8)		-H	> 99 % *trans*
6b	*trans*-4'-Methyl-4-nitrostilbene (24325-70-0)		-Me	> 99 % *trans*
6c	*trans*-4'-Ethyl-4-nitrostilbene (-)		-Et	~ 5 % *cis*
6d	*trans*-4'-*iso*-Propyl-4-nitrostilbene (-)		-iPr	~ 5 % *cis*
6e	*trans*-4'-*tert*-Butyl-4-nitrostilbene (74518-95-9)		-tBu	~ 5 % *cis*
6e'	*trans*-4'-*tert*-Butyl-4-nitrostilbene (74518-95-9)		-tBu	> 99 % *trans*
6f	*trans*-4'-*sec*-Butyl-4-nitrostilbene (-)		-sBu	> 99 % *trans*

Fig. 3. Structures of synthesized compounds.

triphenylphosphoniumbromide and an appropriate substituted benzaldehyde (route A, 72–41% yield), or by Horner-Wadsworth-Emmons reactions as described by Hanna [19] from 4-nitrobenzylphosphonic acid diethylester (prepared according to Deussen [20], 80% yield) and an appropriate benzaldehyde (route B, 71–90% yield). The corresponding benzaldehydes were purchased from Aldrich; 4′-*sec*-butylbenzaldehyde was prepared in two steps from 4′-*sec*-butylaniline by a Sandmeyer transformation (85% yield) and reaction of the obtained 4′-*sec*-butyliodobenzene with BuLi and *N*-formylpiperidine as described by Schlüter [21] (84% yield). The 4-nitrostilbenes **6c**–**6e** contained up to 5% of the corresponding *cis*-isomers, which could not be separated by recrystallization and column chromatography. As *cis*-isomers of 4-nitrostilbene derivatives turn out to be less active than the corresponding *trans*-isomers [16], the *cis*-content should not affect the Ames-test results seriously. However, to investigate the effect of the *cis*-isomer on the mutagenicity a second charge of pure *trans*-**6e**, prepared by iodine-catalyzed isomerization, was also included in the test series (**6e**′).

Reduction of the nitro compounds **6a**–**f** to the *trans*-4-aminostilbenes **5a**–**f** (Fig. 3) was achieved using zinc and ammonium chloride [22] (route C, 71–31% yield) or $SnCl_2$ (route D, 65% yield) [23]. All compounds were structurally characterized by NMR and by MS. Purities were checked by TLC and gas chromatography.

2.2.1. (4-Nitrobenzyl)-triphenylphosphoniumbromide

White precipitate, ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 7.85 − 7.31 (m, 19H), 6.01 (d, $^3J_{H-P}$ = 15.8, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) = 147.2, 135.7, 135.0, 134.4, 132.9, 130.1, 123.1, 117.2 (d, ^1J = 86 Hz), 29.6 (d, ^1J = 42 Hz). ^{31}P NMR (81 MHz, $CDCl_3$): δ (ppm) = 25.5.

2.2.2. trans-4-Nitrostilbene (route A)

Yellow needles, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.20 (d, ^3J = 8.9 Hz, 2H), 7.63 − 7.10 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 146.8, 143.9, 136.2, 133.3, 128.9, 127.0, 126.9, 126.3, 124.1. MS (EI): 225.1 (M^+, 72), 178.1 (100).

2.2.3. trans-4′-Methyl-4-nitrostilbene (route A)

Yellow needles, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.20 (d, ^3J = 8.9 Hz, 2H), 7.60 (d, ^3J = 8.6 Hz, 2H), 7.44 (d, ^3J = 8.14 Hz, 2H), 7.16 (m, 3H), 2.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 146.6, 144.1, 139.0, 133.4, 129.6, 126.9, 126.6, 125.2, 124.1, 21.2. MS (70 eV): *m/z* (%) = 239 (M^+, 100), 178 (52).

2.2.4. 4-Nitrobenzylphosphonic acid diethylester

Red oil, ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) = 8.13 (d, ^3J = 8.8 Hz, 2H), 7.43 (dd, ^3J = 8.8 Hz, $^4J_{P-H}$ = 2.5 Hz, 2H), 4.06 − 3.92 (m, 4H), 3.25 (d, ^3J = 17.3 Hz, 2H), 1.27 (t, ^3J = 6.8 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 146.8 (d, J_{C-P} = 4 Hz), 139.6 (d, J_{C-P} = 9 Hz), 130.5 (d, J_{C-P} = 6.5 Hz), 123.5 (d, J_{C-P} = 3 Hz), 62.3 (d, J_{C-P} = 7 Hz), 33.9 (d, J_{C-P} = 137 Hz), 16.2 (d, J_{C-P} = 6 Hz). MS (70 eV): *m/z* (%) = 273 (M^+, 25), 227 ($M^+ - NO_2$, 11), 137 (M^+ − 4-nitrobenzyl, 36) 136 ($M^+ - PO(OEt)_2$, 25), 109 (M^+, 100).

2.2.5. trans-4′-Ethyl-4-nitrostilbene (route B)

Yellow needles, mp 98°C, ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 8.20 (d, ^3J = 8.8 Hz, 2H), 7.60 (d, ^3J = 8.8 Hz, 2H), 7.48 (d, ^3J = 8.1 Hz, 2H), 7.25 (d, ^3J = 16.2 Hz, 1H), 7.25 (d, ^3J = 8.1 Hz, 2H), 7.09 (d, ^3J = 16.3 Hz, 1H), 2.70 (q, ^3J = 7.7 Hz, 2H), 1.29 (t, ^3J = 7.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 146.5, 145.3, 144.0, 133.6, 133.2, 128.3, 127.2, 126.6, 125.3, 124.0, 28.6, 15.3. MS (70 eV): *m/z* (%) = 253 (M^+, 100), 238 ($M^+ - CH_3$, 47), 192 ($M^+ - NO_2$, 16), 191 (21), 178 ($M^+ - CH_3 - NO_2$, 42). HRMS (70 eV): *m/z* calculated for $C_{16}H_{15}NO_2$ 253.1103, found 253.1102. The substance contained 5% *cis*-product (NMR), which could not be separated by recrystallization or column chromatography.

2.2.6. trans-4′-iso-Propyl-4-nitrostilbene (route B)

Yellow needles, mp 134°C, ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 8.20 (d, ^3J = 8.8 Hz, 2H), 7.61 (d, ^3J = 8.8 Hz, 2H), 7.47 (d, ^3J = 8.4 Hz, 2H), 7.25 (d, ^3J = 8.4 Hz, 1H), 7.24 (d, ^3J = 16.3 Hz, 1H), 7.09 (d, ^3J = 16.3 Hz, 1H), 2.93 (p, ^3J = 7.0 Hz, 1H), 1.27 (d, ^3J = 7.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) = 150.0, 146.6, 144.1, 133.8, 133.3, 127.1, 127.0, 126.7, 125.3, 124.1, 34.0,

23.8. MS (70 eV): *m/z* (%) = 267 (M^+, 100), 252 (M^+-CH_3, 75), 235 (14). 178 ($M^+-C_3H_7-NO_2$, 7). CHN: calculated C 76.38%, H 6.41%, N 5.24%, found C 76.51%, H 6.32%, N 5.31%. The substance contained 5% *cis*-product (NMR), which could not be separated by recrystallization or column chromatography.

2.2.7. 4-sec-Butyliodobenzene

Colourless liquid, ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 7.60 (d, ^3J = 12.9 Hz, 2H), 6.93 (d, ^3J = 12.9, 2H), 2.54 (m, 1H), 1.56 (m, 2H), 1.20 (d, ^3J = 6.9 Hz, 3H), 0.80 (t, ^3J = 7.3 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) = 147.3, 137.4, 129.3, 90.7, 41.3, 31.0, 21.7, 12.2. MS (70 eV): *m/z* (%) = 260 (M^+, 55), 231 ($C_8H_8I^+$, 100).

2.2.8. 4-sec-Butylbenzaldehyde

Colourless liquid, ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 9.97 (s, 1H), 7.81 (d, ^3J = 8.0 Hz, 2H), 7.34 (d, ^3J = 8.0 Hz, 2H), 2.67 (m, 1H), 1.63 (m, 2H), 1.26 (d, ^3J = 7.0 Hz, 3H), 0.82 (t, ^3J = 7.4 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) = 191.8, 155.0, 134.5, 129.8, 127.6, 41.9, 35.4, 21.4, 12.0. MS (70 eV): *m/z* (%) = 162 (M^+, 32), 133 ($C_9H_9O^+$, 100). HRMS (70 eV): *m/z* calculated for $C_{11}H_{14}O$ 162.1045, found 162.105.

2.2.9. trans-4′-sec-Butyl-4-nitrostilbene

Yellow needles, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.21 (d, ^3J = 8.9 Hz, 2H), 7.61 (d, ^3J = 8.8 Hz, 2H), 7.48 (d, ^3J = 8.18 Hz, 2H), 7.28 – 7.15 (m, 4H), 2.63 (m, 1H), 1.62 (m, 2H), 1.25 (d, ^3J = 6.9 Hz), 0.84 (t, ^3J = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 148.8, 146.6, 144.1, 133.8, 133.3, 127.6, 127.0, 126.7, 125.3, 124.1, 41.5, 31.0, 21.6, 12.2. MS (70 eV): *m/z* (%) = 281 (M^+, 45), 252 ($C_{16}H_{14}NO_2^+$, 100). HRMS (70 eV): *m/z* calculated for $C_{18}H_{19}NO_2$ 281.1416, found 281.1419.

2.2.10. trans-4′-tert-Butyl-4-nitrostilbene (route A)

Yellow needles, ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) = 8.18 (d, ^3J = 8.7 Hz, 2H), 7.59 (d, ^3J = 8.8 Hz, 2H), 7.48 (d, ^3J = 8.8 Hz, 2H) 7.41 (d, ^3J = 8.7 Hz, 2H), 7.25 (d, ^3J = 16.2 Hz, 1H), 7.08 (d, ^3J = 16.2 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) = 152.2, 146.5, 144.0, 133.3, 133.1, 126.8, 126.6, 125.8, 125.4, 124.0, 34.7, 31.2. MS (70 eV): *m/z* (%) = 281 (M^+, 42), 266 ($C_{17}H_{16}NO_2^+$, 100). HRMS (70 eV): *m/z* calculated for $C_{18}H_{19}NO_2$ 281.1416, found 281.141. The compound prepared via route B contained up to 5% *cis*-product, which could not be separated by recrystallization or column chromatography.

2.2.11. trans-4-Aminostilbene

Off-white precipitate, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.46 (d, ^3J = 7.7 Hz, 2H), 7.34 – 7.30 (m, 4H), 7.20 (tt, ^3J = 7.3 Hz, ^5J = 1.3 Hz, 1H), 7.02 (d, ^3J = 16.3 Hz, 1H), 6.91 (d, ^3J = 16.3 Hz, 1H), 6.66 (d, ^3J = 8.6 Hz, 2H), 3.72 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 146, 136.9, 127.8, 127.5, 126.5, 125.6, 125.3, 124.8, 122.8, 113.7. MS (70 eV): *m/z* (%) = 195.2 (M^+, 100), 167.1 (7). CHN: calculated C 86.12%, H 6.71%, N 7.17%, found C 86.19%, H 6.70%, N 7.31%.

2.2.12. trans-4′-Methyl-4-aminostilbene

Off-white precipitate, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.37 (d, ^3J = 8.1 Hz, 2H), 7.32 (d, ^3J = 8.4 Hz, 2H), 7.13 (d, ^3J = 7.9 Hz, 2H), 6.98 (d, ^3J = 16.3 Hz, 1H), 6.88 (d, ^3J = 16.3 Hz, 1H), 6.66 (d, ^3J = 8.6 Hz, 2H), 3.71 (s, 2H, NH_2), 2.34 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 145, 136.6, 135.2, 129.3, 128.3, 127.7, 127.6, 126.0, 125.1, 115.2, 21.1. MS (70 eV): *m/z* (%) = 209.2 (M^+, 100), 194.2 (16). CHN: calculated C 86.08%, H 7.22%, N 6.69%, found C 85.92%, H 7.09%, N 6.83%.

2.2.13. trans-4′-Ethyl-4-aminostilbene

Off-white precipitate, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.40 (d, ^3J = 8.0 Hz, 2H), 7.32 (d, ^3J = 8.3 Hz, 2H), 7.17 (d, ^3J = 7.9 Hz, 2H), 6.98 (d, ^3J = 16.3 Hz, 1H), 6.90 (d, ^3J = 16.3 Hz, 1H), 6.66 (d, ^3J = 8.3 Hz, 2H), 3.71 (s, 2H, NH_2), 2.64 (q, 2H), 1.23 (t, ^3J = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 145.9, 143.1, 135.4, 128.2, 128.0, 127.7, 127.5, 126.0, 125.0, 115.1, 28.5, 15.5. MS (70 eV): *m/z* (%) = 223 (M^+, 100), 208 ($C_{15}H_{15}N^+$, 47). CHN: calculated C 86.05%, H 7.67%, N 6.24%, found C 85.85%, H 7.61%, N 6.27%.

2.2.14. trans-4′-iso-Propyl-4-aminostilbene

Off-white precipitate, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.41 (d, ^3J = 8.2 Hz, 2H), 7.32 (d, ^3J = 8.5 Hz, 2H), 7.20 (d, ^3J = 8.2 Hz, 2H), 6.99 (d, ^3J = 16.3 Hz, 1H), 6.90 (d, ^3J = 16.3 Hz, 1H),

6.65 (d, ^3J = 8.6 Hz, 2H), 3.70 (s, 2H, NH_2), 2.90 (m, ^3J = 6.9 Hz, 1H), 1.26 (t, ^3J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 147.7, 145.9, 135.5, 128.2, 127.8, 127.5, 126.6, 126.0, 125.0, 115.1, 32.8, 23.9. MS (70 eV): *m/z* (%) = 237 (M^+, 100), 222 ($C_{16}H_{16}N^+$, 47). CHN: calculated C 86.03%, H 8.07%, N 5.90%, found C 85.81%, H 8.08%, N 5.61%.

2.2.15. trans-4′-sec-Butyl-4-aminostilbene

Off-white precipitate, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.39 (d, ^3J = 8.2 Hz, 2H), 7.32 (d, ^3J = 8.4 Hz, 2H), 7.14 (d, ^3J = 8.2 Hz, 2H), 6.99 (d, ^3J = 16.3 Hz, 1H), 6.89 (d, ^3J = 16.3 Hz, 1H), 6.67 (d, ^3J = 8.4 Hz, 2H) 3.52 (s, 2H, NH_2), 2.58 (m, 1H), 1.59 (m, 2H), 1.23 (d, ^3J = 6.9 Hz, 3H), 0.83 (t, ^3J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 146.6, 145.6, 135.5, 128.5, 127.7, 127.5, 127.3, 126.0, 125.3, 115.3, 41.4, 31.1, 21.7, 12.2. MS (70 eV): *m/z* (%) = 251 (M^+, 93), 222 ($C_{16}H_{16}N^+$, 100). CHN: calculated C 86.01%, H 8.42%, N 5.57%, found C 85.81%, H 8.31%, N 5.52%.

2.2.16. trans-4′-tert-Butyl-4-aminostilbene

Off-white precipitate, ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.39 − 7.28 (m, 6H), 6.96 (d, ^3J = 16.3 Hz, 1H), 6.87 (d, ^3J = 16.3 Hz, 1H), 6.64 (d, ^3J = 8.5 Hz, 2H), 3.32 (s, 2H, NH_2), 1.29 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 150.0, 145.8, 135.2, 128.4, 127.9, 127.6, 125.8, 125.5, 125.1, 115.3, 34.6, 31.3. MS (70 eV): *m/z* (%) = 251 (M^+, 100), 236 ($C_{17}H_{18}N^+$, 66). HRMS (70 eV): *m/z* calculated for $C_{18}H_{21}N$ 251.1674, found 251.1678.

2.3. Mutagenicity tests

The mutagenicity of all compounds was determined using the Ames-test with the *Salmonella typhimurium* strains TA98(±S9) and TA100(±S9). All compounds were tested in the Hygiene-Institut, Universität Heidelberg, Germany. The Ames-test protocol employed in these investigations followed the standard methodology described by Baron and Ames [24]. Both tester strains (TA98 and TA100) were a gift from Bruce Ames.

In all cases DMSO was used as the diluting solvent. Positive and negative controls (DMSO) were included in each experiment. The test results shown here were obtained using metabolic activation with S9-mix (+S9) and without metabolic activation (−S9). Arochlor-induced S9 from rat liver was purchased from CCR, Germany. The concentration of the S9 fraction in the mix was 10%. For the aminostilbenes a five-point and for the nitrostilbenes a six-point dose-response curve was established using appropriate dilutions of the compound to be tested. Each revertant number presented in Tables 1 and 2 is the average of three values. In order to guarantee optimal comparability all compounds within one series were tested under identical conditions within 1 week. At higher concentrations for most substances cell toxicity resulted in a flattening of the corresponding dose-response curves. Experimental mutation rates (revertant/nmol) are calculated as the slope of a regression analysis over the linear portion of the dose-response curve.

3. Results

3.1. Aminostilbenes **5*a*–*f***

The Ames-test results of the compounds **5a–f** are listed in Table 1. As typically found for amines, without metabolic activation the numbers of revertants do not differ from the solvent controls. A graphical comparison of experimental mutagenicities obtained in the presence of S9-mix is given in Fig. 4. The compounds in Fig. 4 are ordered by growing steric demand of the substituents: methyl < ethyl < *iso*-propyl < *sec*-butyl < *tert*-butyl.

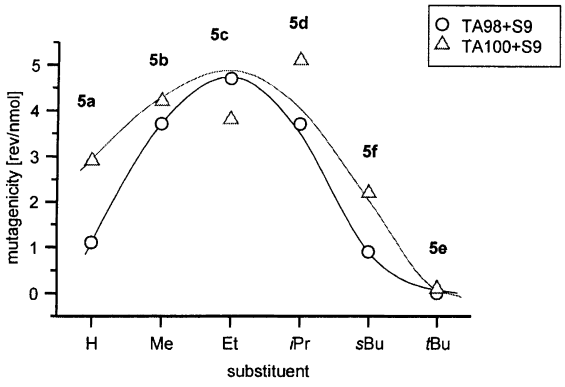


Fig. 4. Experimental mutagenicity of aminostilbenes **5a–f** with S9.

Table 1
Experimental mutagenicity data of aminostilbenes **5a**–**f**

Code	Dose (μg/plate)	Revertants, induction factors							
		TA98−S9		TA98+S9		TA100−S9		TA100+S9	
Positive control[a]		3017		1311		1032		1740	
Negative control	DMSO	35		60		102		102	
5a	0	35	1.0	60	1.0	102	1.0	102	1.0
	6.25	34	1.0	92	1.5	101	1.0	189	1.8
	12.5	30	0.9	104	1.7	117	1.1	265	2.6
	25	39	1.1	200	3.3	129	1.3	435	4.3
	50	37	1.1	328	5.4[b]	134	1.3	848	8.3[b]
	100	49	1.4	469	7.8	182	1.8	469	4.6
5b	0	35	1.0	60	1.0	102	1.0	102	1.0
	6.25	33	0.9	160	2.7	100	1.0	265	2.6
	12.5	49	1.4	283	4.7[b]	102	1.0	355	3.5[b]
	25	43	1.3	383	6.3	98	1.0	496	4.9
	50	38	1.1	541	9.0	113	1.1	775	7.6
	100	39	1.1	642	10.6	121	1.2	597	5.9
5c	0	35	1.0	60	1.0	102	1.0	102	1.0
	6.25	34	1.0	172	2.8	95	0.9	154	1.5
	12.5	36	1.0	322	5.3[b]	106	1.0	265	2.6
	25	34	1.0	465	7.7	108	1.1	476	4.7[b]
	50	33	0.9	546	9.1	117	1.1	651	6.4
	100	32	0.9	557	9.2	99	1.0	594	5.8
5d	0	35	1.0	60	1.0	102	1.0	102	1.0
	6.25	40	1.1	149	2.5	87	0.9	227	2.2
	12.5	33	0.9	255	4.2	102	1.0	332	3.3
	25	36	1.0	445	7.4[b]	113	1.1	641	6.3[b]
	50	27	0.8	387	6.4	101	1.0	610	6.0
	100	32	0.9	396	6.6	89	0.9	458	4.5
5e	0	35	1.0	60	1.0	102	1.0	102	1.0
	6.25	38	1.1	51	0.9	105	1.0	118	1.2
	12.5	41	1.2	58	1.0	94	0.9	112	1.1
	25	28	0.8	59	1.0	96	0.9	114	1.1
	50	37	1.1	61	1.0	92	0.9	119	1.2
	100	29	0.8	57	1.0	94	0.9	107	1.0
5f	0	35	1.0	60	1.0	102	1.0	102	1.0
	6.25	33	1.0	104	1.7	99	1.0	162	1.6
	12.5	36	1.0	114	1.9	96	0.9	213	2.1[b]
	25	30	0.9	159	2.6[b]	93	0.9	295	2.9
	50	33	1.0	151	2.5	93	0.9	319	3.1
	100	27	0.8	130	2.1	77	0.8	211	2.1

[a] TA98−S9 1-nitropyren 2.5 μg; TA100−S9 sodium azide 5 μg; TA98+S9 and TA100+S9 2-aminoanthracene 2.5 μg.
[b] Indicates the highest dose used in regression analysis.

In TA98+S9 the mutagenic potential increases from the parent compound **5a** to the ethyl-substituted compound **5c**, which is strongly mutagen. In contrast activity drops continuously from **5c** to **5e** with increasing steric demand of the substituents. While the number of revertants exceeds the solvent control up to 1.5-fold for the *sec*-butyl compound **5f**, no revertants above the solvent controls and no cell toxicity are observed for the sterically even more demanding *tert*-butyl compound **5e**. The compound **5e** is non-mutagenic at all concentrations. As shown in Fig. 4 the mutagenicity in TA100+S9 is within the same range as in strain TA98+S9. In TA100+S9 also a similar trend in the mutagenicity is observed. Again the activity of the *sec*-butyl compound **5f** and the *tert*-butyl compound **5e** is lower than those of all other 4-aminostilbenes.

Table 2
Experimental mutagenicity data of nitrostilbenes **6a–f**

Code	Dose (μg/plate)	Revertants, induction factors							
		TA98−S9		TA98+S9		TA100−S9		TA100+S9	
Positive control[a]		2450		1661		1206		1830	
Negative control	DMSO	36		47		176		130	
6a	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	55	1.6	90	1.9	308	1.8	125	1.0
	3.1	76	2.1	92	2.0	460	2.6	124	1.0
	6.25	118	3.3	95	2.0	774	4.4[b]	148	1.1
	12.5	162	4.5[b]	105	2.2	625	3.5	195	1.5
	25	183	4.9	136	2.9	0	0.0	411	3.2
	50	209	5.0	225	4.8	0	0.0	773	6.0[b]
6b	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	54	1.5	235	5.0	301	1.7	493	3.8
	3.1	59	1.7	267	5.7	359	2.0	631	4.9
	6.25	86	2.4[b]	344	7.3	471	2.7[b]	945	7.3[b]
	12.5	101	2.8	585	12.5[b]	491	2.8	1062	8.2
	25	105	2.9	774	16.5	522	3.0	0	0.0
	50	118	3.3	254	5.4	520	3.0	0	0.0
6c	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	–	–	435	9.3	–	–	433	3.3
	3.1	41	1.1	541	11.5	238	1.4	668	5.1
	6.25	59	1.6	681	14.5[b]	298	1.7[b]	1150	8.9[b]
	12.5	58	1.6	981	20.9	299	1.7	619	4.8
	25	76	2.1[b]	1069	22.7	312	1.8	0	0.0
	50	78	2.2	1145	24.4	315	1.8	0	0.0
	100	142	4.0	–	–	412	2.3	–	–
6d	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	–	–	94	2.0	–	–	191	1.5
	3.1	41	1.1	94	2.0	234	1.3	226	1.7
	6.25	40	1.1	111	2.4	245	1.4[b]	358	2.8
	12.5	48	1.3	171	3.6	248	1.4	788	6.1[b]
	25	46	1.3	259	5.5	249	1.4	1156	8.9
	50	41	1.1	536	11.4[b]	249	1.4	375	2.9
	100	61	1.7	–	–	265	1.5	–	–
6e	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	–	–	135	2.9	–	–	365	2.8
	3.1	36	1.0	140	3.0	183	1.0	589	4.5
	6.25	41	1.1	160	3.4	213	1.2	795	6.1[b]
	12.5	36	1.0	212	4.5[b]	273	1.5[b]	909	7.0
	25	40	1.1	246	5.2	270	1.5	923	7.1
	50	44	1.2	298	6.3	260	1.5	973	7.2
	100	52	1.5	–	–	259	1.5	–	–
6e′	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	–	–	124	2.6	–	–	394	3.0
	3.1	37	1.0	145	3.1	237	1.3	511	3.9
	6.25	41	1.1	188	4.0	267	1.5	857	6.6[b]
	12.5	43	1.2	205	4.4[b]	285	1.6[b]	1022	7.9
	25	42	1.2	243	5.2	240	1.4	1125	8.7
	50	43	1.2	291	6.2	236	1.3	873	6.7
	100	48	1.3	–	–	236	1.3	–	–

Table 2 (*Continued*)

Code	Dose (μg/plate)	Revertants, induction factors							
		TA98−S9		TA98+S9		TA100−S9		TA100+S9	
6f	0	36	1.0	47	1.0	176	1.0	130	1.0
	1.6	–	–	74	1.6	–	–	170	1.3
	3.1	37	1.0	74	1.6	226	1.3	208	1.6
	6.25	37	1.0	119	2.5	230	1.3	264	2.0
	12.5	42	1.2	126	2.7	233	1.3[b]	389	3.0
	25	53	1.5	196	4.2[b]	254	1.4	686	5.3[b]
	50	76	2.1	246	5.2	275	1.6	897	6.9
	100	90	2.5	–	–	253	1.4	–	

[a] TA98−S9 1-nitropyren 2.5 μg; TA100−S9 sodium azide 5 μg; TA98+S9 and TA100+S9 2-aminoanthracene 2.5 μg.
[b] Indicates the highest dose used in regression analysis.

3.2. Nitrostilbenes **6*a*–*f***

The revertant numbers of the nitrostilbene series **6a**–**f** in the presence and in the absence of S9-mix are summarized in Table 2.

3.2.1. Absence of S9-mix

A graphical comparison of the mutagenicities of compounds **6a**–**f** without S9-mix is shown in Fig. 5. In both *Salmonella* strains the parent compound **6a** and the methyl-substituted **6b** derivative are highly mutagenic. The activity of **6a** and **6b** is in TA100 markedly higher than in TA98, in good agreement with former results of Hoobermann [16]. Concerning the influence of the alkyl substituents in both strains a continuous decrease of mutagenicity is observed from the parent compound **6a** to the *tert*-butyl-substituted nitrostilbene **6e**. Notably there is no significant difference between the *tert*-butyl-nitrostilbene containing 5% *cis*-product **6e** and the pure *tert*-butyl-nitrostilbene **6e′**. In TA98 the number of revertants of **6e** and **6e′** remain below twice the solvent control at all concentrations (see Table 2) but exceed the solvent control up to 2.5-fold in case of the *sec*-butyl-substituted compound **6f** at a dose of 100 μg. In TA100 the revertants of **6d**, **6e**, **6e′** and **6f** do not reach twice the solvent control at any concentration tested. In the absence of S9-mix all these compounds must be classified as weakly or non-mutagenic according to the definition of Ames [24].

3.2.2. Presence of S9-mix

In the presence of S9-mix a strong increase of mutagenicity in both strains is observed for all compounds if compared with the same test series in the absence of S9-mix (see Table 2). In Fig. 6 the mutagenicity of the compounds **6a**–**f** with S9-mix is shown. In strain TA98 the mutagenicity increases from the

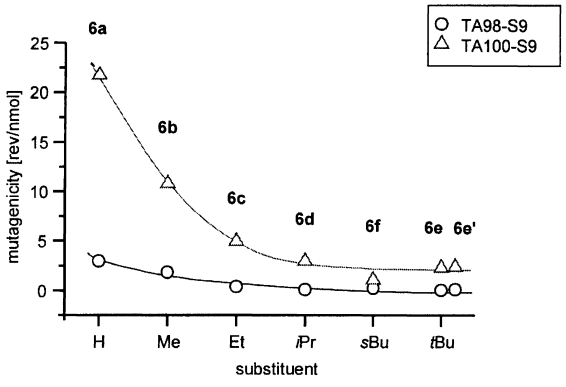


Fig. 5. Experimental mutagenicity of nitrostilbenes **6a**–**f** without S9.

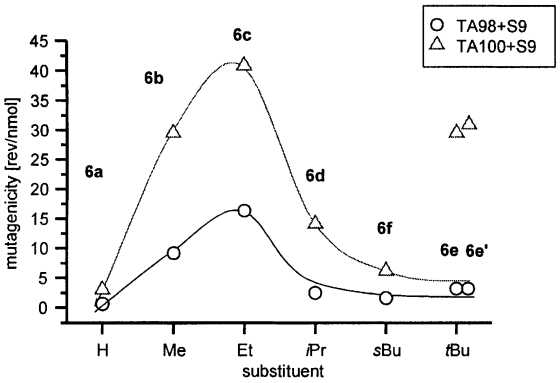


Fig. 6. Experimental mutagenicity of nitrostilbenes **6a**–**f** with S9.

parent compound **6a** to the ethyl-substituted derivative **6c**. Similar to the aminostilbene series larger alkyl groups generally reduce the mutagenicity with the size of the attached substituent. Compounds **6d–f** are markedly less active than the corresponding derivatives bearing smaller alkyl groups. Compared to the *sec*-butyl-nitrostilbene **6f** a slightly enhanced activity for the *tert*-butyl-nitrostilbenes **6e** and **6e′** is found. In strain TA100 the same overall trend is followed but the differences in activity between the compounds are much larger. Maximum activity again is reached for the ethyl nitrostilbene **6c**, followed by a decrease from **6c** to **6f**. Remarkably the *tert*-butyl-substituted compounds **6e** and **6e′** exhibit an exceptional behavior under these conditions. Instead of being non-mutagenic, **6e** and **6e′** are highly active. Apart from this experiment we have never observed a similar effect for any other *tert*-butyl compound. As in the test series without S9-mix one finds only a minor difference between the *cis*-containing 4′-*tert*-butyl-4-nitrostilbene **6e** and the pure *trans*-compound **6e′** in both strains TA98 and TA100. This finding suggests that *cis*-4′-*tert*-butyl-4-nitrostilbene is just as, or less, mutagenic than *trans*-4′-*tert*-butyl-4-nitrostilbene. Further investigations using pure *cis*-4-nitrostilbene derivatives have to clarify this point.

4. Discussion

In the last two decades QSAR studies have revealed a number of chemical and biological factors that affect mutagenicity, including structural and chemical properties like hydrophobicity ($\log P$) and energies of the frontier orbitals (E_{HOMO}, E_{LUMO}) [25,26]. Based on these parameters Debnath and Hansch developed empirical equations that allow to predict the mutagenicity of aminoarenes [9] (in the presence of S9-mix) and nitroarenes (in the absence of S9-mix) in TA98 [10] and TA100 [11]. The influence of $\log P$ and orbital energies on the processes underlying mutagenesis has been discussed by several authors [9–11,25,26]. In general it is assumed that electronic factors determine whether the mutagen is active or not whereas the $\log P$ of a certain mutagen correlates with the magnitude of its mutagenic potency [25].

Applying the Debnath/Hansch model to predict the mutagenicity of *N*-substituted stilbenes in quantitative terms one has to be careful due to the special reactivity of stilbenes with the DNA via the double bond (see Fig. 2). It has to be noted further that some of the alkyl-substituted 4-aminostilbenes investigated in this work have a higher hydrophobicity than those that were used in their QSAR study.

4.1. Aminostilbenes 5a–f

The mutagenicities of the aminostilbenes **5a–f** according to the Debnath/Hansch model are listed in Table 3. They are compared graphically with our experimental results in Fig. 7.

The $\log P$ values and the electronic energies were calculated as previously described [27]. In both strains the mutagenicity is predicted to increase continuously with the size of the alkyl group. Orbital energy calculations for the aminostilbenes **5a–f** revealed that

Table 3
Experimental and predicted mutagenicity of aminostilbenes **5a–f**

Code	$\log P$[a]	E_{HOMO}	E_{LUMO}	σ^+	Log revertants/nmol				
					TA98+S9			TA100+S9	
					Experimental	Sinsheimer's prediction	Debnath/Hansch's prediction	Experimental	Debnath/Hansch's prediction
5a	3.61	−8.017	−0.351	0.00	0.02	0.75	1.09	0.47	1.71
5b	4.15	−7.963	−0.344	−0.31	0.57	0.47	1.74	0.63	2.26
5c	4.65	−7.967	−0.341	−0.30	0.67	0.48	2.27	0.58	2.71
5d	5.06	−7.977	−0.323	−0.28	0.57	0.50	2.69	0.71	3.05
5e	5.52	7.958	−0.325	−0.27[b]	−1.47	0.50	3.31	−1.09	3.50
5f	5.55	7.967	−0.338	−0.26	−0.03	0.51	3.24	0.35	3.53

[a] $\log P$ values calculated with KOWWIN.
[b] Value extrapolated.

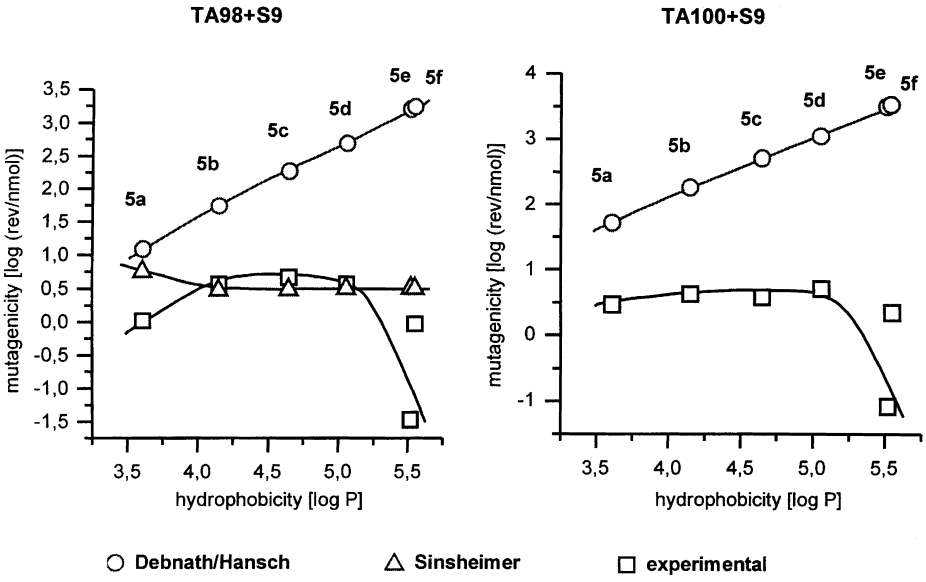


Fig. 7. Experimental and predicted mutagenicity of aminostilbenes **5a**–**f**.

alkyl substituents exert only a minor influence on the HOMO and LUMO energies (see Table 3), ruling out that electronic factors cause the predicted increase of mutagenic activity. An analysis of the parameters used to calculate the mutagenicity indicated that hydrophobicity almost exclusively accounts for the predicted increase of mutagenicity in the aminostilbene series investigated in this work. For the first compounds of the test series (**5a**–**c**) the experimental data agree decently well with the prediction (see Fig. 7). However, regarding the derivatives with larger alkyl groups (**5d**–**f**), the deviations between experimental and predicted mutagenicities become larger, growing with the size of the substituent. Hydrophobicity alone, clearly does not correlate with the experimental results as shown in Table 3. Although the *tert*-butyl-substituted **5e** is approximately as hydrophobic as the *sec*-butyl-substituted **5f**, **5e** is less active than **5f**. Likewise **5f** is more hydrophobic than the higher mutagenic *iso*-propyl-substituted **5d**.

In 1994 Sinsheimer developed a QSAR equation describing the mutagenic activity of 4′-substituted 4-aminostilbenes in TA98+S9 based on the Hammet coefficients σ^+ of the attached substituents [14]. We also applied this rather simple QSAR model to the aminostilbenes **5a**–**f** as shown in Table 3 and Fig. 7. The σ^+ values were taken from [28]. Since the σ^+ values are very similar for different alkyl groups, Sinsheimer's model essentially predicts no significant change in mutagenicity for the differently alkylated compounds. For **5b**–**d** with small alkyl groups these predictions agree very well with the experimental results in absolute numbers, whereas neither the activity increase from **5a** to **5c** nor the activity drop for **5e**–**f** is predicted correctly. Summarizing these comparisons, both models are unsuitable to predict the low mutagenicity of compounds bearing large alkyl substituents. Thus, in addition to orbital energies, hydrophobicity and Hammet coefficients σ^+, other parameters should be considered in order to explain the experimental activities of **5e** and **5f**.

Apart from the parameters discussed above compounds **5a**–**f** strongly differ in the steric demand of their substituents. Since the mutagenicity decreases with the size of the attached alkyl groups we conclude that the deviations between prediction and experiment must be attributed mainly to steric factors. This conclusion is in accordance with our previous findings for the alkyl-substituted amino- and nitrobiphenyls (**1a**–**f**, **2a**–**f**) and nitrofluorenes (**3a**, **b**, **e**). As discussed for these compounds [8] most probably the reduced ability of the sterically demanding aminostilbenes to intercalate well into DNA accounts for the reduced mutagenicity.

Table 4
Experimental and predicted mutagenicity of nitrostilbenes **6a–f**

Code	log P[a]	E_{LUMO}	Log revertants/nmol			
			TA98−S9		TA100−S9	
			Experimental	Debnath/Hansch's prediction	Experimental	Debnath/Hansch's prediction
6a	4.2	−1.356	0.47	0.39	1.34	1.38
6b	4.75	−1.339	0.26	0.57	1.03	1.90
6c	5.24	−1.333	−0.42	0.52	0.38	2.19
6d	5.66	−1.332	−1.00	0.21	0.00	2.18
6e	6.11	−1.329	−1.52	−0.44	0.36	1.79
6e′	6.11	−1.329	−1.40	−0.44	0.36	1.79
6f	6.15	−1.333	−0.64	−0.50	0.00	1.74

[a] Log P values calculated with KOWWIN.

4.2. *Nitrostilbenes* **6*a–f***

The nitrostilbenes **6a–f** tested without S9-mix showed a continuous decrease of the activity with the size of the alkyl substituents in both *Salmonella* strains. Only in TA100−S9 the *tert*-butyl compounds **6e** and **6e′** are found to be slightly more active than the preceding compounds. In Table 4 the experimental and predicted values according to Debnath/Hansch's QSAR model are compared. A graphical comparison is given in Fig. 8 for both strains.

For TA98 and TA100 the QSAR model predicts first an increase of the mutagenic activity with increasing size of the substituents, a maximum around those substances with a log P value of 4.9 (TA98) or 5.4 (TA100), followed by a decrease for the more hydrophobic *sec*-butyl and *tert*-butyl-substituted nitrostilbenes **6d–f**. Since electronic energies are not influenced significantly by different alkyl substituents, the predicted trend is, as for the aminostilbenes, mainly determined by the increasing hydrophobicity of the molecules. In Debnath/Hansch's QSAR model

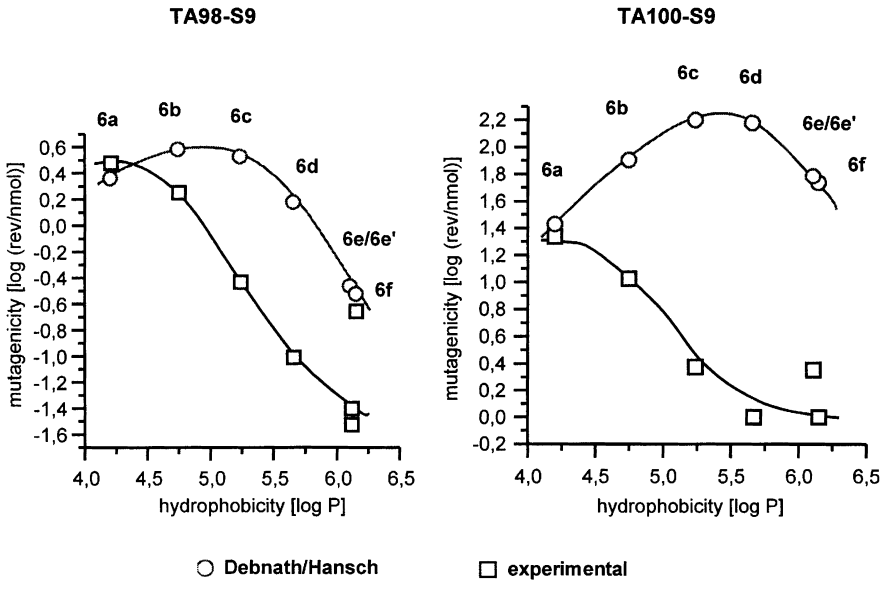


Fig. 8. Experimental and predicted mutagenicity of nitrostilbenes **6a–f**.

mutagenic activity of nitroarenes is bilinearly dependent on the hydrophobicity and causes a reduction of mutagenicity for compounds with log *P* values exceeding the optimum. In fact this decrease of mutagenic activity for very hydrophobic compounds as **6d–f** is corroborated by our experimental results. Nevertheless there are still large gaps between the curves in Fig. 8 (more so in TA100 than in TA98) confirming additional effects to be important here as well. Our investigations suggest that mainly steric effects cause these deviations. As can be seen from the striking differences between predicted and experimental results especially in TA100, even for sterically less demanding compounds like **6b** and **6c** the Debnath/Hansch model seems to be generally less suitable to predict mutagenicity of nitrostilbene derivatives in TA100 correctly. As mentioned before, this may be due to the special reactivity of nitrostilbenes via the double bond.

Apart from a recent CoMFA study, which considers mutagenicities of nitroarenes in TA98(±S9) and TA100(±S9) [29], there exists no simple 2D-QSAR model for 4-nitrostilbenes tested with metabolic activation. The overall experimental trends (see Fig. 6) in the presence of S9-mix are comparable to the results obtained for the aminostilbenes in both *Salmonella* strains (see Fig. 4). Again the trend for the nitrostilbenes turns out to be determined by hydrophobicity and steric demand of the substituents as this is the case for the aminostilbenes. However, striking exceptions are the 4′-*tert*-butyl-4-nitrostilbenes **6e** and **6e**′ in TA100+S9, which are much more mutagenic than the *sec*-butyl- and *iso*-propyl-substituted compounds **6f** and **6d**. It is therefore obvious that the mutagenic activity of the *tert*-butyl compound **6e** in TA100+S9 cannot be determined exclusively by the factors already discussed (hydrophobicity, electronic energies and steric demand). This exceptional behavior points to another factor. The strong increase of mutagenicity of all nitrostilbenes tested in the presence of S9 may be caused by an additional oxidative activation process. In principal this activation can occur at the benzylic position of the alkyl groups or at the double bond instead of the nitrogen atom. Indeed, Hoobermann showed that mutagenicity of 4′-methyl-4-nitrostilbene **6b** in the nitroreductase deficient strains TA98NR, with S9-mix added, is much stronger than for 4-nitrostilbene **6a** [16]. This activation is not believed to proceed via the corresponding hydroxylamine. A benzylic activation is also supported by the findings of Hanna who described that P450 mediated metabolization of *N*-acetyl-4-aminostilbenes bearing a 4′-methyl or 4′-*tert*-butyl substituent takes place at the alkyl groups predominately. In the case of 4′-*tert*-butyl-*N*-acetyl-4-aminostilbene oxidation occurred almost exclusively at the aliphatic alkyl group producing a primary alcohol, and not at the *N*-acetylamino group. Thus, we suggest a different metabolic pathway to be operative in the presence of S9-mix, which does not involve the hydroxylamine as the important intermediate. Since no metabolic studies on 4′-alkyl-substituted 4-nitrostilbenes have been performed so far we cannot explain doubtlessly the unexpected high mutagenicity of 4′-*tert*-butyl-4-nitrostilbene **6e** at the moment. This area has to be the subject of further research.

A definite reason for the reduced mutagenic potential of the stilbenes with sterically demanding alkyl groups (like *iso*-propyl or *tert*-butyl) can neither be given. Concerning the molecular shape it has been demonstrated that a flat aromatic system, capable to intercalate in DNA, is an important structural feature for a molecule to react as frameshift mutagen [30]. We discussed this topic recently to explain the steric effect of the alkyl-substituted nitrobiphenyls **2a–f** and nitrofluorenes **3a–f** [8]. Since adducts of *N*-substituted stilbenes are quite different (see Fig. 2) from adducts of, e.g. amino- or nitrobiphenyls, it is not clear whether a similar explanation holds here, too. Apart from intercalation into DNA the stilbenes' bulky alkyl groups may also interfere with its binding to the *N*-esterification enzymes, which is essential to exhibit the mutagenic potential. Experimentally, however, it is obvious that sterically demanding alkyl groups reduce the mutagenicity of amino- and nitrostilbenes.

5. Conclusion

Summarizing the results, it turns out that alkyl groups that are located far away from the functional groups $-NH_2$ and $-NO_2$, respectively, exhibit a size-dependent effect on the mutagenicity of amino- and nitrostilbenes (**5a–f**, **6a–f**). Therefore, these results confirm the effects we found recently for the amino- **1a–f** and nitrobiphenyls **2a–f** [8] and nitrofluorenes **3a–f** [8]. Based on the results of this and our

recent work, the strategy to reduce the mutagenicity by changing the molecular shape seems to be of promising general applicability for *N*-substituted aromatic compounds. However, there are limitations. Apart from steric considerations, the mutagenic potential is also influenced by several other parameters like hydrophobicity, and experimentally observed mutagenicity is the result of various factors. A striking example for an "exception from the rule" is the unexpected high mutagenicity of the *tert*-butyl nitrostilbenes **6e** and **6e′** in TA100 in the presence of S9-mix that may be explained by a different metabolic pathway.

Acknowledgements

We thank the BASF AG, Ludwigshafen, Germany for continuous financial support.

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