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Dyes and Pigments 44 (2000) 199–207

DYES and PIGMENTS

Synthesis and evaluation of organic pigments and intermediates. 1. Nonmutagenic benzidine analogs

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Received 20 July 1999; accepted 17 August 1999

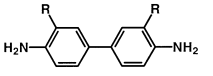
Abstract

The design, synthesis, characterization, and genotoxicity of 4,4′ diaminobiphenyl (benzidine) analogs with substituents in the 3,3′ and/or 2,2′ positions are reported. Analogs containing bulky substituents in the 3,3′ positions significantly reduce or eliminate mutagenic activity, while substituents in the 2,2′-positions increase the dihedral angle across the biphenyl linkage—a property that can be utilized in the design of novel nonmutagenic colorants. 2,2′-Dimethylbenzidine was found to be mutagenic in both the standard Salmonella mammalian mutagenicity assay (Ames test) with metabolic acitivation and the preincubation assay protocol. 2,2′-Dichloro-5,5′-dipropoxybenzidine, 2,2′-dimethoxy-5,5′-dipropoxybenzidine and 2,2′-dimethyl-5,5′-dipropoxybenzidine were nonmutagenic in both assays. The corresponding bis-acetoacetamido derivatives of the latter two compounds were also nonmutagenic. Good yields with minimal purification were obtained for certain diamines, providing potentially useful nongenotoxic intermediates in the synthesis of bisazo and bisazomethine dyes and pigments.

Keywords: Nonmutagenic; Benzidine intermediates; Dihedral angle

1. Introduction

Benzidine **I** is one of the few known human carcinogens [1,2]. Also, certain homologs of benzidine are mutagenic and suspected human carcinogens. In fact, some of the most common benzidine homologs still used in colorant synthesis, such as 3,3′-dimethylbenzidine **II**, 3,3′-dimethoxybenzidine **III**, and 3,3′-dichlorobenzidine **IV** may reasonably be expected to be carcinogenic [3].



	R
I	H
II	CH_3
III	OCH_3
IV	Cl
V	>C_3 alkyl/alkoxy

Colorants prepared from genotoxic intermediates can be either direct acting mutagens or promutagens [4]. By genotoxic is meant interactions between DNA and substances that produce heritable changes in a cell or organism. A promutagen is a compound exhibiting mutagenic activity following metabolic

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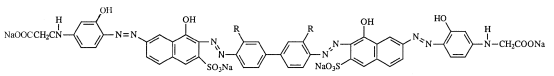
0143-7208/00/$ - see front matter
PII: S0143-7208(99)00078-9

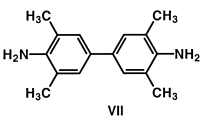
activation. Hence, the manufacture and use of genotoxic intermediates and colorants presents a potential occupational and environmental risk [5,6]. The discovery of the genotoxicity of benzidine and certain congeners has led to legislation in many countries either banning or severely restricting their industrial production [7,8]. These measures have circumvented the design and development of new colorants derived from benzidine-type intermediates. Furthermore, the often exorbitant cost of genotoxicity testing and the now ingrained negative connotation that the 'benzidine' name carries make development of new colorants prepared from benzidines seem imprudent. However, considering the importance of benzidine and its homologs in the synthesis of dyes and pigments during the first half of this century, there is little doubt that the development of nongenotoxic analogs of benzidine is a worthwhile endeavor.

To date, a number of approaches have been attempted, and these have been reviewed recently [4]. One notable development in the design of nongenotoxic aromatic amines stems from work by Shahin et al. [9] who demonstrated that the mutagenicity of aromatic amines can be lowered or eliminated by incorporating bulky alkyl or alkoxy substituents *ortho* to the amino group on a molecule. Similarly, Hunger et al. [10,11] developed nonmutagenic analogs of benzidine of type **V**. These intermediates were employed by Bauer et al. [12] for the synthesis of nonmutagenic water soluble disazo dyes. One application example is the nonmutagenic black ink jet dye **VI** [13].



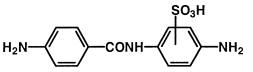
VI; R = OPr

A further example of a nonmutagenic analog of benzidine is 3,3′,5,5′-tetramethylbenzidine **VII**, reported by Holland et al. [14] and also by Genshaw et al. [15] for the detection of blood and glucose, respectively. Compound **VII** is being studied in our laboratories as a potential dyestuff intermediate.



VII

A different approach to reduce the occupational risk of colorants derived from benzidines involves the preparation of alternative dyestuff intermediates that provide technical properties similar to the common benzidines, while exhibiting low genotoxicity. An example of this approach is reported by Ogino et al. [16] in which replacement compounds for benzidine such as **VIII** are disclosed.

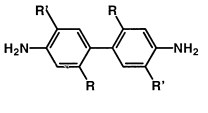


VIII

Despite the aforementioned methods for the generation of nonmutagenic analogs of benzidine, very little work has been directed toward the synthesis and evaluation of organic pigments prepared from nongenotoxic intermediates. It is generally considered that pigments, being generally highly stable and insoluble in most application media, pose low environmental and occupational risk. However, there remains the occupational risk of employing genotoxic diamines such as **II–IV** during pigment manufacture. Also, there is a risk of benzidine congeners remaining unconverted to pigment, presenting not only an occupational risk, but also the environmental risk of genotoxic intermediates being released into receiving waters. This is particularly significant considering the discovery that 3,3′-dichlorobenzidine, used in the manufacture of C.I. Pigment Yellow 12, for example, has been shown to rapidly photodegrade in aqueous solution to a number of products, including benzidine [7]. Furthermore, there is a risk of thermal degradation of pigments employed in melt extrusion processes, thus providing an additional pathway to the release of genotoxic substances.

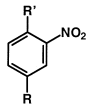
The primary objective of the present study is the development of new pigments prepared from benzidine congeners that have minimal occupational and environmental risks associated with their synthesis and use. We report here the synthesis of benzidine congeners **IX**, via reduction of nitrobenzenes **X**, and provide new mutagenicity data for diamines, 2,2′-dimethylbenzidine **IXa**, 2,2′-dimethyl-5,5′-dipropoxybenzidine **IXf**, 2,2′-dimethoxy-5,5-dipropoxybenzidine **IXg**, and 2,2′-dichloro-5,5′-dipropoxybenzidine **IXh**. Mutagenicity data is also provided for bis-acetoacetamido derivatives

(**XI**). The synthesis of pigments prepared from diamines **IXa–h** will be reported separately.



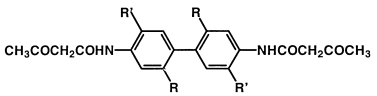
IX

	R	R′
a	CH_3	H
b	H	Pr
c	H	OPr
d	H	OBu
e	H	$OCH_2CH_2OCH_3$
f	CH_3	OPr
g	OCH_3	OPr
h	Cl	OPr



X

	R	R′
a	CH_3	H
b	H	OPr
c	H	OBu
d	H	$OCH_2CH_2OCH_3$
e	H	Pr
f	CH_3	OPr
g	OCH_3	OPr
h	Cl	OPr



XI

	R	R′
a	CH_3	OPr
b	OCH_3	OPr

2. Results and discussion

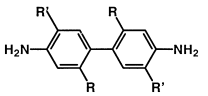
2.1. Synthesis

Table 1 provides some key data on benzidine and congeners currently employed in our studies. The structures of diamines were confirmed by C.I. mass spectrometry and by ^1H NMR. The best reaction yield and simplest purification processes are associated with diamine **IXf**. All diamines were converted to the hydrochloride salt and stored under nitrogen.

The synthesis methods employed were variants of the methods described in the literature [10,11,19,20]. In contrast to the synthesis of benzidine itself, in which a temperature of 115–125°C is commonly employed in the reduction step [21], the preparation of benzidine analogs possessing bulky substituents in the 3,3′-positions requires a lower temperature, commonly 70–80°C. Thermal stability of the hydrazo intermediate was found to decrease when bulky substituents were present in the *ortho, ortho′*-substituted positions, presumably due to steric hindrance. If temperatures exceeding 100°C were employed during the reduction step, the substituted anilino by-product formed as the major product rather than the desired hydrazo intermediate. Scheme 1 shows the main steps to the synthesis of benzidine analogs.

In all cases, the hydrazo intermediate was necessarily washed with dilute mineral acid to remove anilino by-product prior to undertaking the benzidine rearrangement. Removal of the anilino reduction product at a later stage in the synthesis was less efficient. Nevertheless, compounds **IXb–e** required recrystallization, and in the case of **IXc** flash column chromatography was additionally required to provide the pure diamine.

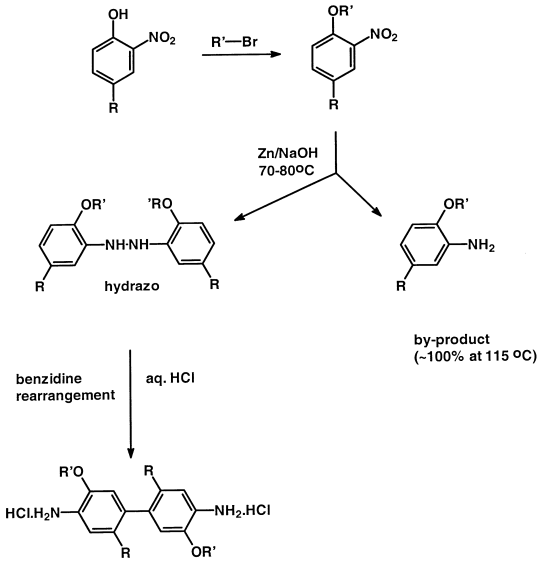
Table 1
Summary of selected data pertaining to benzidine and benzidine analogues



	R	R′	Yield (%)	Mp (°C)	Mutagenicity[a]
I	H	H	–[b]	129	Positive
IXa	CH_3	H	62	129	Positive
IXb	H	Pr	46	Semi-solid	Negative[c]
IXc	H	OPr	51	141–142	Negative[c]
IXd	H	OBu	45	69–71	Negative[c]
IXe	H	$OC_2H_4OCH_3$	44	116–118	Negative[c]
IXf	CH_3	OPr	63	136	Negative[d]
IXg	OCH_3	OPr	53	123	Negative[d]
IXh	Cl	OPr	4.6	106–108	Negative[d]

[a] Standard *salmonella* mammalian assay.
[b] Purchased.
[c] Reported by Hunger et al. [3,4] and confirmed in our laboratories.
[d] Tested in the Prival modification in addition to the standard assay.

The purification steps contributed to yields in the range of 40–51%. However, only minimal purification was required to provide pure tetrasubstituted compounds **IXf** and **IXg**, and these diamines were obtained in yields of 63 and 53%, respectively. Scale up and further optimization of the synthesis procedures may increase the yields further. Tetrasubstituted benzidines have been employed as commercial intermediates in the synthesis of, for example, diarylide pigments. One of the potential benefits of using benzidines with substituents in the 2,2-positions, is that hypsochromic shifts can be achieved by reducing the pi orbital overlap across the biphenyl linkage. Hence, the tetrasubstituted diamines reported here are of particular interest as nongenotoxic benzidine-type intermediates. Surprisingly, compound **IXh** could not be obtained in yields higher than 5%, despite varying temperature, solvent, and reagent addition rates. A procedure identical to that used for the successful synthesis of 2,2α-dichloro-5,5α-dimethoxybenzidine [19] was also attempted, but without success in improving the yield of **IXh**. Tetramethylbenzidine (**VII**) is a nonmutagenic benzidine congener developed as a replacement to benzidine as an analytical reagent in the detection of blood and glucose. Synthesis of **VII** has not been achieved by previous workers [14,15] using the benzidine rearragement procedure, and yields exceeding 20% have not been reported. While a low yield is acceptable in bioanalysis, a significantly higher yield is required for colorant synthesis. Due to the nongenotoxicity of this diamine, synthesis of **VII** was attempted using the reduction conditions reported here. Although the azo and hydrazo intermediate were detected during the zinc/alkali reduction, the benzidine rearrangement procedure produced the anilino product only and not the target diamine. Presumably, steric hindrance in the two adjacent positions to the amino group prevents formation of the biphenyl linkage.



Scheme 1. Synthesis of diamines IXc-h.

In addition to the use of diamines as intermediates to bis-chromogenic colorants, bis-acetoacetanilides may be employed in the synthesis of diarylide-type pigments. For this reason, two such intermediates (**IXa–b**) were synthesized in this study using established procedures [22] and their genotoxicity evaluated.

2.2. Mutagenicity of benzidine and its analogs

The benzidine analogs were evaluated in the standard salmonella mammalian mutagenicity assay [17]. Additionally, diamines **IXf–h** were tested using the preincubation protocol developed by Prival and Mitchell [18]. Figs. 1–9 show the dose response curves for each of the diamines tested. The background count is established by the number of revertant colonies counted for a control test in which no diamine is present. A mutagenic response is recorded if the number of revertant

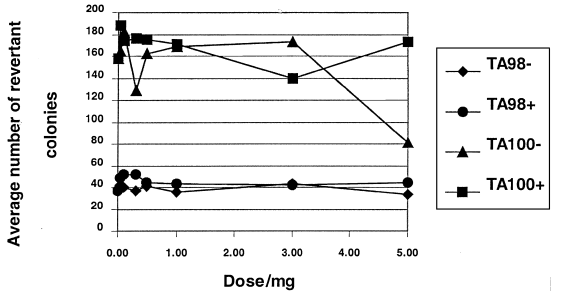


Fig. 1. Dose response of **IXf** using the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

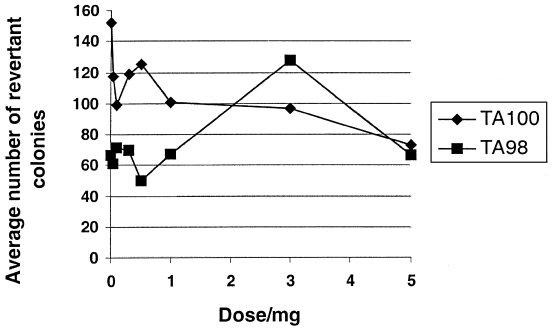


Fig. 2. Dose response of **IXf** using the preincubation modification of the standard mutagenicity assay with bacteria strains TA98 and TA100. DMSO = base count.

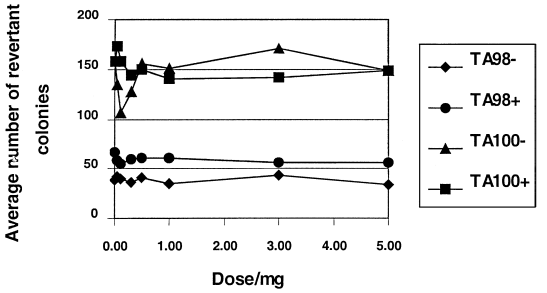


Fig. 3. Dose response of **IXg** using the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

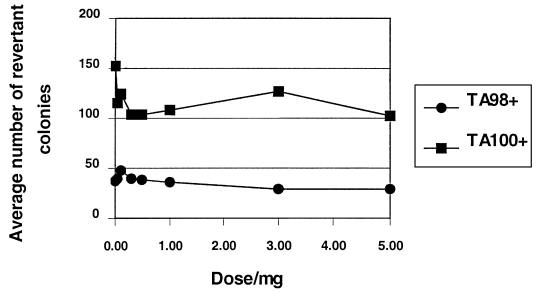


Fig. 4. Dose response of **IXg** using the preincubation modification of the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

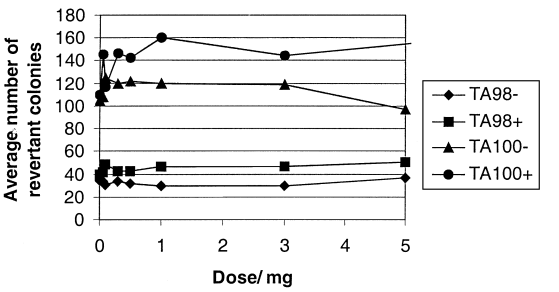


Fig. 5. Dose response of **IXh** using the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

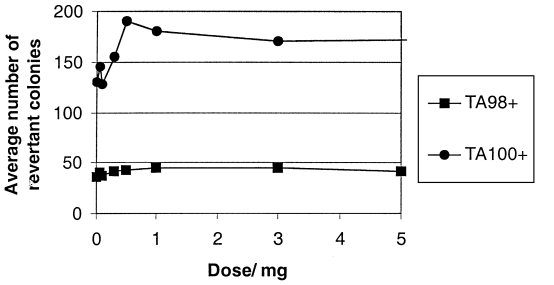


Fig. 6. Dose response of **IXh** using the preincubation modification of the standard mutagenicity assay with bacteria strains TA98 and TA100. DMSO = base count.

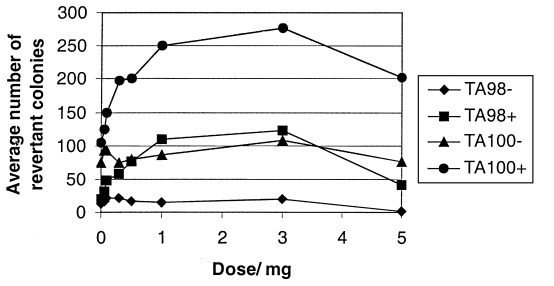


Fig. 7. Dose response of **IXa** using the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

colonies counted is at least twice the background count. As can be seen from Figs. 1–9, all diamines are nonmutagenic in both TA98 and TA100, except **IXa**. The latter diamine was mutagenic in bacteria strain TA98 and TA100 with metabolic activation. This data provides evidence that bulky substituents are required to reduce genotoxicity of benzidine-type compounds irrespective of the presence of substituents in the 2,2′-positions that cause significant increases in the dihedral angle across the biphenyl linkage. Negative mutagenic responses for compounds **XIa–b** were also.

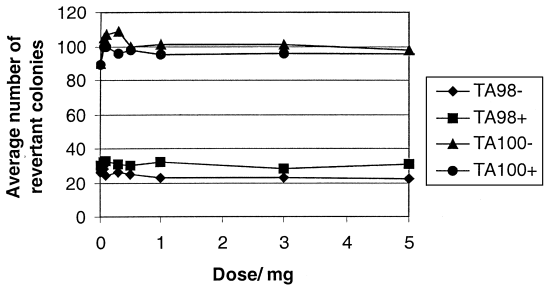


Fig. 8. Dose response of **XIa** using the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

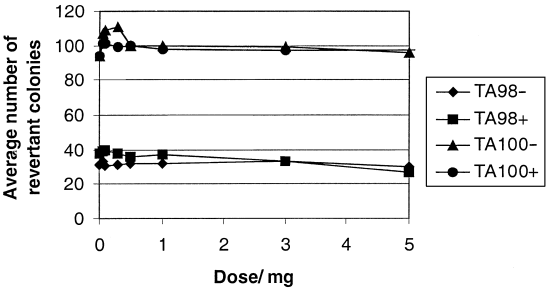


Fig. 9. Dose response of **XIb** using the standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

Following the discovery of the nonmutagenicity and ease of synthesis of dimaines **IXf** and **IXg**, these diamines were selected as candidates for a detailed study into the synthesis of environmentally responsible pigments from nongenotoxic intermediates.

3. Experimental

3.1. General

Unless specified, all chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI), and were used without further purification. The structures of the alkoxy nitrobenzenes and the benzidine analogs prepared were confirmed by ^{1}H NMR and CI or EI mass spectrometry. The purity of the benzidine analogs was also determined by combustion analysis. ^{1}H NMR spectra were recorded on a General Electrical GN 300 MHz spectrophotometer. CI and EI mass spectra were recorded on a Hewlett Packard 5985B GC mass spectrometer. Mutagenicity testing was conducted using the standard Salmonella mammalian assay [17], and the Prival modification [18]. Melting points were uncorrected.

3.2. Synthesis

3.2.1. Preparation of 2-nitro-n-propoxybenzene (Xb)

1-Bromopropane (21.6 g, 0.176 mol) and finely ground anhydrous potassium carbonate (16.2 g, 0.117 mol) was added to a stirred solution of 2-nitrophenol (16.6 g, 0.117 mol) in 2-methoxyethanol (80 ml) at 25°C. The red–orange mixture was stirred under reflux for 7 h and the resultant pale yellow mixture was cooled, filtered, and concentrated by rotary evaporation. The residue was dissolved in ethyl acetate (80 ml) and the solution was washed twice with NaOH (20 ml of 5% w/w), then dried (sodium sulfate) and evaporated to dryness. The pale yellow-orange liquid was purified by vacuum distillation, bp 112°C/3 mm Hg to give a clear, pale yellow liquid (20.1 g, 95% yield).

3.2.2. Preparation of 2-n-butoxynitrobenzene (Xc)

The procedure described for **Xb** was employed except that *n*-bromobutane was used as the alkylating agent. The product was obtained as a pale yellow liquid, bp 117°C/4 mm Hg (18.7 g, 82% yield).

3.2.3. Preparation of 2-(β-methoxyethoxy)nitrobenzene (Xd)

The procedure described for **Xb** except 2-bromoethylmethyl ether was as the alkylating agent. Compound **Xd** was obtained as a pale yellow liquid, bp 139°C/4 mm Hg (11.8 g, 61% yield).

3.2.4. Preparation of 4-methyl-2-nitro-n-propoxybenzene (Xf)

The procedure described for **Xb** was employed except that 4-methyl-2-nitrophenol was employed. The product was obtained as a pale yellow liquid, bp 104°C/3 mm Hg (85% yield).

3.2.5. Preparation of 4-methoxy-2-nitro-n-propoxybenzene (Xg)

The procedure described for **Xb** was employed except that 4-methoxy-2-nitrophenol was employed.

The product was obtained as a pale yellow liquid, bp 115–117°C/ 4 mm Hg (91% yield).

3.2.6. *Preparation of 4-chloro-2-nitro*-n-*propoxybenzene* (**Xh**)

The procedure described for **Xb** was employed except that 4-chloro-2-nitrophenol was employed. The product was obtained as a pale yellow liquid, bp 108–110°C/0.35 mm Hg (86% yield).

3.2.7. *Preparation of 3,3′-di*-n-*propoxybenzidine* (**IXc**)

To a stirred solution of 2-nitropropoxybenzene (17.0 g, 0.094 mol) in ligroine (17 ml, boiling range 90–110°C) at 25°C, zinc (18.4 g, 0.28 mol) was added, and the mixture was heated to 70–80°C. This was followed by a dropwise addition of 50% (w/w) NaOH (13.5 g; 16.9 mmol) over 3 h. Water (12 ml, 0.66 mol) was then added dropwise over 1 h and the mixture was stirred for 7 h, first becoming orange, then red. More zinc (3.6 g, 5.64 mmol) was added and the mixture stirred until the organic layer turned colorless. TLC (PhMe:Hx 3:1) indicated complete conversion to the hydrazo intermediate (a colorless product that oxidized on heating to become orange).

Ligroine (17 ml) was added to the cooled reaction mixture, and the mixture was filtered. The organic layer was washed twice with 1% (w/w) HCl (2×20 ml), then twice with water (2×20 ml). The organic layer was collected, and stirred at 10–15°C as 20% (w/w) HCl (56 ml) was added dropwise over 30 min. After stirring for a further 90 min, the gray solid was filtered, washed repeatedly with acetone, and dried at 40°C. The crude product was purified by recrystallization (MeOH/EtOAc) to provide **IXc**.2HCl (12.9 g).

To a stirred suspension of the above diamine dihydrochloride (9.3 g; 0.025 mol) in water (50 ml) and EtOAc (100 ml) at 15°C, 5% (w/w) NaOH was added dropwise until a constant pH value of 8 was obtained. After stirring a further 30 min, the organic layer was separated, dried (Na_2SO_4) and the solvent was removed by rotary evaporation. The pale brown solid was purified by flash chromatography (PhMe:EtOAc, 3:1) to provide diamine **IXc** as a pale brown solid (7.1 g, yield 51%), mp 141–142°C. Elemental analysis, calculated for $C_{18}H_{14}N_2O_2$: C, 71.97; H, 8.02; N, 9.32. Found: C, 72.08; H, 7.99; N, 9.29. Cl mass spectrum: *m*/*z* free (rel. int.) 229 (28%) 300 ($M^{+\cdot}$ 100%); ^{1}H NMR (d_6-DMSO): δ 0.99–1.04 (6H, t, j = 7.33), δ 1.77 (4H, m), δ 3.95–3.99 (4H, t, *j* = 6.6), δ 4.62 (4H, s), δ 6.63–6.66 (2H, d, *j* = 8.06), δ 6.86–6.94 (4H, m).

3.2.8. *Preparation of 2,2′-dimethylbenzidine* (**IXa**)

The procedure described above for **IXc** was employed, with 3-methylnitrobenzene used as the starting compound. In this case, the temperature was maintained at 70°C, and zinc and 50% (w/w) NaOH were added alternately over 3 h. Flash column chromatography and recrystallization were not required. Compound **IXa** was obtained as an off-white solid (yield 62%). Elemental analysis, calculated for $C_{14}H_{16}N_2$: C, 79.20; H, 7.59; N, 13.19. Found: C, 79.17; H, 7.62; N, 13.17. ^{1}H NMR ($CDCl_3$) δ 6.9 (2H, d, *j* = 7.34), δ 6.6 (2H, d, *j* = 2.20), δ 6.5 (2H, dd, 8.06, 2.16) δ 3.6 (4H, s), δ 1.9 (6H, s).

3.2.9. *Preparation of 3,3′-di*-n-*propylbenzidine* (**IXb**)

The procedure described above for **IXc** was employed, with 2-nitropropylbenzene as the starting compound. In this case, the temperature was maintained at 70°C, and zinc and 50% (w/w) NaOH were added alternately over 3 h. Flash column chromatography was not required. Compound **IXb** was obtained as a pale brown semi-solid (3.57 g, yield 46%). CI mass spectrum: *m*/*z* (rel. int.) −239 (63%), 268 ($M^{+\cdot}$ 100%); ^{1}H NMR (d_6-DMSO): δ 0.92–0.96 (6H, t, *j* = 7.33), δ 1.55 (4H, m), δ 2.41–2.46 (4H, t, j = 7.7), δ 4.74 (4H, s), δ 6.59–6.62 (2H, d, *j* = 8.8), δ 7.04–7.06 (4H, m).

3.2.10. *Preparation of 3,3′-di*-n-*butoxybenzidine* (**IXd**)

The procedure described above for the synthesis of **IXc** was employed, using 2-*n*-butoxynitrobenzene as the starting compound. Flash column chromatography was not required. Compound **IXd** was obtained as a pale brown solid (6.94 g, yield 45%), mp 69–71°C. Cl mass spectrum: *m*/*z* (rel. int.) −329 ($[M+H]^+$, 100%). ^{1}H NMR (d_6-DMSO): δ 0.93–0.95 (6H, t, *j* = 7.33), δ 1.49 (4H, m), δ 1.73 (4H, m), δ 3.99–4.03 (4H, t, *j* = 6.6), δ 4.61 (4H, s), δ 6.62–6.65 (2H, d, *j* = 8.06), δ 6.87–6.89 (2H, d, *j* = 9.07), δ 6.94 (2H, s).

3.2.11. Preparation of 3,3′-di-(β-methoxyethoxy) benzidine (**IXe**)

The procedure described above for the synthesis of **IXc** was employed, using 2-(β-methoxyethoxy)-nitrobenzene as the starting compound. Flash column chromatography was not required. Compound **IXe** was obtained as a pale brown solid (6.87 g, yield 44%), mp 116–118°C. CI mass spectrum: m/z (rel. int.) –333 ($[M+H]^+$, 100%). ^{1}H NMR (d_6-DMSO): δ 3.33 (6H, s), δ 3.67–3.70 (4H, d, $j=3.67$, 4.4), δ 4.13–4.15 (4H, t, $j=3.66$, 4.4), δ 4.65 (4H, s), δ 6.67 (2H, d, $j=8.07$), δ 6.91–6.93 (2H, d, $j=8.07$), δ 7.00 (2H, s).

3.2.12. Preparation of 2,2′-dimethyl-5,5'-dipropoxybenzidine (**IXf**)

The procedure described above for the synthesis of **IXc** was employed, using 4-methyl-2-nitropropoxybenzene as the starting compound. In this case the hydrazo intermediate was diluted with more ligroine prior to treatment with aqueous acid, to prevent precipitation prior to rearrangement. Flash column chromatography and recrystallization were not required. Compound **IXf** was obtained as an off-white solid (yield 63%), mp 136°C. Elemental analysis, calculated for $C_{20}H_{28}N_2O_2$: C, 73.14; H, 8.59; N, 8.53. Found: C, 73.29; H, 8.65; N, 8.48. EI mass spectrum: m/z (rel. int.): 328 (100%). ^{1}H NMR (d_6-DMSO): δ 6.58 (2H, s), δ 6.42 (2H, s), δ 4.60 (4H, bs), δ 3.80 (4H, t, $j=7.2$), δ 1.82 (6H, s), δ 1.7 (4H, sx, $j=7.2$), δ 1.05 (6H, t, $j=7.33$).

3.2.13. Preparation of 2,2′-dimethoxy-5,5′-dipropoxybenzidine (**IXg**)

The procedure described above for the synthesis of **IXc** was employed, using 4-methoxy-2-nitropropoxybenzene as the starting compound. Flash column chromatography was not required. Compound **IXf** was obtained as an off-white solid (yield 53%), mp 136 °C. Elemental analysis, calculated for $C_{20}H_{28}N_2O_4$: C, 66.64; H, 7.83; N, 7.77. Found: C, 66.74; H, 7.86; N, 7.83. CI mass spectrum: m/z (rel. int.): 361 ($[M+H]^+$, 100%). ^{1}H NMR (d_6-DMSO): δ 6.58 (2H, bs), δ 6.38 (2H, bs), δ 4.64 (4H, bs), δ 3.80 (4H, t, $j=7.2$), 3.58 (6H, s), δ 1.80 (4H, sx, $j=7.2$), δ 1.00 (6H, t, $j=7.33$).

3.2.14. Preparation of 2,2′-dichloro-5,5′-dipropoxybenzidine (**IXh**)

The procedure described above for the synthesis of **IXc** was employed, using 4-chloro-2-nitropropoxybenzene as the starting compound. Flash column chromatography and recrystallization were not required. Compound **IXh** was obtained as an off-white solid (yield 4.6%), mp 136°C. Elemental analysis, calculated for $C_{18}H_{22}N_2$: C, 58.54; H, 6.00; N, 7.59; Cl, 19.2. Found: C, 58.47; H, 5.95; N, 7.44; Cl, 18.56. CI mass spectrum: m/z (rel. int.).

4. Conclusions

2,2′-dimethylbenzidine was mutagenic in the standard mammalian mutagenicity assay in strains TA98 and TA100 with S9 metabolic activation. Hence, the incorporation of a high dihedral angle across the biphenyl linkage does not appear to significantly reduce the genotoxicity of benzidine-type compounds. However, tetrasubstituted benzidines incorporating $>C_3$ alkyl and alkoxy groups in positions *ortho, ortho′* to the amino groups were all nonmutagenic irrespective of the incorporation of chloro, methyl and methoxy groups in positions *ortho, ortho′* to the biphenyl linkage. The synthesis of tetrasubstituted benzidines, 2,2′-dimethyl-5,5′-dipropoxybenzidine and 2,2′-dimethoxy-5,5′-dipropoxybenzidine, were obtained in good yield with minimal purification. Hence, the nongenoxicity and ease of synthesis of the latter two intermediates makes these two compounds ideal candidates as intermediates in the synthesis of dyes and pigments.

Acknowledgements

The authors are grateful to Dr. Larry Claxton of the US EPA Genetic Toxicology Laboratory for providing assistance with mutagenicity studies, and to Dr. Laura Edwards and Ms. Julie Clemmons for undertaking the mutagenicity testing. The study was supported through a grant from Dainippon Ink and Chemicals, Inc.

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