

Mutagenicity Studies of Benzidine and Its Analogs: Structure-Activity Relationships

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The Ames *Salmonella*/microsome assay was employed to test the mutagenicity of benzidine and its analogs using strains TA98 and TA100 in the presence and absence of Aroclor 1254-induced rat S9 mix. 3,3'-Dichlorobenzidine-2HCl and 4,4'-dinitro-2-biphenylamine were directly mutagenic to TA98, while 4,4'-dinitro-2-biphenylamine was directly mutagenic to both TA98 and TA100 in the absence of S9 mix. 2-Aminobiphenyl, 3-aminobiphenyl, and 3,3'-5,5'-tetramethylbenzidine were not mutagenic in either strains in the presence or absence of S9. In the presence of S9 mix, 4-aminobiphenyl, benzidine, 3,3'-dichlorobenzidine-2HCl, 3,3'-dimethoxybenzidine, 3,3'-4,4'-tetraaminobiphenyl, *o*-tolidine, N, N-N', N'-tetramethylbenzidine, and 4,4'-dinitro-2-biphenylamine were mutagenic to TA98; 4-aminobiphenyl, 3,3'-dichlorobenzidine-2HCl, 3,3'-dimethoxybenzidine, and 4,4'-dinitro-2-biphenylamine were mutagenic to TA100. Physicochemical parameters of these compounds including oxidation potentials, the energy difference between the lowest unoccupied molecular orbital and the highest occupied molecular orbital, ionization potentials, dipole moment, relative partition coefficient, and basicity did not correlate with their bacterial mutagenic activities.

Key Words: mutagenicity; oxidation potential; physicochemical properties.

Benzidine is used during the production of azo dyes for wide usage in the textile, paper, and leather industries (Haley, 1975). Benzidine can be generated from azo dyes through reduction by intestinal and environmental microorganisms (Chung *et al.*, 1992; Chung and Stevens, 1993). Although the production and use of benzidine have been banned in the United States since the mid-1970's, this compound is still present in wastewater effluent discharged from the dye industries as a result of microbial actions. Hazardous waste sites are common sources of environmental benzidine, capable of causing contamination of water sources (Choudhary, 1996).

Benzidine has been recognized as a human carcinogen by the U.S. Environmental Protection Agency, Occupational

Safety and Health Administration, National Institute for Occupational Safety and Health, and other health organizations (DHHS, 1991; IARC, 1982). Numerous studies indicate that occupational exposure to benzidine is responsible for the occurrence of cancers of the bladder and other organs in humans (Choudhary, 1996; Goldwater *et al.*, 1965; Meigs *et al.*, 1986; Piolatto *et al.*, 1991; Shinka *et al.*, 1991; You *et al.*, 1990; Zavan *et al.*, 1973).

Chung and Cerniglia (1992) reported that benzidine was a major mutagenic moiety of many azo dyes. Numerous studies have been conducted to determine the mutagenicity of benzidine and its analogs (Bos *et al.*, 1982; Lazear and Louie, 1978; Prival *et al.*, 1984; Savard and Josephy, 1986). However, only limited structure-toxicity data are available for the development of safer benzidine substitutes and to aid public officials in their risk assessment of the potential hazards of benzidine-based compounds (Messerly *et al.*, 1987).

Hatch and Colvin (1997) reported that the mutagenic potency of aromatic and heterocyclic amines was inversely related to the lowest unoccupied molecular orbital (LUMO) energy. Other physicochemical properties, such as oxidation potential, the energy difference (ΔE) between the LUMO and the highest occupied molecular orbitals (HOMO), ionization potential (I.P.), dipole moment (μ), partition coefficient (K_{HPLC}), and basicity (pKa) have been used to correlate the structure-activity relationships for various chemicals (Ford and Herman, 1992; Josephy, 1986; Lund, 1957; You *et al.*, 1993).

Since oxidation of aromatic amines to form N-hydroxyamine derivatives is considered the primary step in the conversion of amines to their ultimate mutagenic forms (Kadlubar *et al.*, 1990; Lai *et al.*, 1996), an amine that can be oxidized more easily is expected to be more mutagenic. Therefore, we hypothesized that the mutagenicity of aromatic amines would correlate with the potential of the electrochemical oxidation of these amines to their N-hydroxy derivatives.

In this study, we examine whether the mutagenic activities of benzidine and its analogs in the Ames *Salmonella*/microsome assay correlate with their physicochemical properties. The ultimate goal is to predict the genotoxicity of benzidine

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FIG. 1. Chemical structures of benzidine and its analogs.

and its analogs by understanding their structure-activity relationships.

MATERIALS AND METHODS

Chemicals. 2-Aminofluorene, 2-aminobiphenyl, 4-aminobiphenyl, benzidine, 3,3'-dichloro-benzidine-2HCl, 3,3'-dimethoxybenzidine, 3,3',-4,4'-tetraaminobiphenyl, *o*-tolidine, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Company (St. Louis, MO). 4,4'-Dinitro-2-biphenylamine, N-methyl-N-nitro-N'-nitrosoguanidine (MNNG), 2-nitrofluorene, 3,3'-5,5'-tetramethylbenzidine, and N,N,N',N'-tetramethylbenzidine were purchased from Aldrich Chemical Company (Milwaukee, WI), and 3-aminobiphenyl was obtained from NCI Chemical Carcinogen Reference Standard Laboratory (Kansas City, MO). The chemical structures of tested compounds are shown in Figure 1. Test solutions were freshly prepared by dissolving the chemicals in water or DMSO and kept in the dark. In some cases, chemicals were further purified by sublimation techniques in our laboratory.

Bacterial cultures. *Salmonella typhimurium*. TA98 and TA100 were provided by Dr. B. N. Ames of the Department of Biochemistry, University of California, Berkeley, California. The tester strains were stored at -80°C . The frozen stocks were prepared from overnight cultures to which DMSO was added as a cryoprotective agent. The tester strains were checked routinely to confirm their genetic features using the procedures outlined by Maron and Ames (1983).

Mutagenicity tests. Mutagenicity tests were performed using standard preincubation procedures and in the presence or absence of the liver S9 mix (Maron and Ames, 1983). S9 preparation in 0.154 M KCl (Aroclor 1254-induced) was purchased from Molecular Toxicology, Inc. (Annapolis, MD). Concurrently, 2-aminofluorene, 2-nitrofluorene, and MNNG were included in all assays. Except under special conditions, 0.5 ml of S9 mix was used. The same amount of DMSO (25 μl) was delivered to each plate. All operations were conducted under yellow light to avoid photooxidation of the compound. The mutagenic potency was calculated by taking the slope of the dose-response curve.

Measurement of oxidation potentials. Voltametric measurements were conducted with a Model 660 Electrochemical Workstation with a PC computer loaded with electrochemical software. The voltammograms were plotted using a Hewlett Packard 7475A plotter. A three-electrode cell with a platinum counter electrode, an Ag/AgCl reference electrode, and a graphite working electrode were used for all electrochemical experiments. The electrolytic solution was 0.1 M KCl solution. In a typical cyclic voltametry measurement,

a sample was pre-reduced at -0.2 V for 1 min, and the potential scanned down to -0.40 V ; after pausing for 40 s, the anodic potential was scanned up to 1.0 V, then the cathodic potential was scanned down to -0.20 V . In some cases, the anodic potential was scanned up to higher than 1.0 V.

Semiempirical approximation calculations. The optimization of molecular geometry was carried out by the modified neglect of diatomic overlap (MNDO) approximation method for each of these molecules (Dewar and Thiel, 1977). The resultant molecular structure was then used to calculate the ΔE between the LUMO and the HOMO, I.P., and μ using Austin Model 1 (AM1) method (Dewar *et al.*, 1985). In setting up initial structural parameters for the calculations, the experimental structure of anilines (Lister *et al.*, 1974), *p*-nitroaniline (Sadova *et al.*, 1976), and *p*-phenylenediamine (Calapietro *et al.*, 1987) were used as references.

Relative partition coefficient (K_{HPLC}). High performance liquid chromatography with a C-18 reverse phase column (Bondapak $3.9 \times 300\text{ mm}$, Waters, Milford, MA) was used to determine the value of the relative partition coefficient (K) for each compound. The procedures were based on the protocol of You *et al.* (1993). The mobile phase was the mixture solution of water and methanol (45:55 with 0.1% ammonium formate) at a flow rate of 1 ml/min. Samples in methanol were injected for determination of retention time.

The partition coefficients were calculated by the methods of Carlson *et al.* (1975) using the relationship $K_{\text{HPLC}} = \log K'_{\text{substituted}} - \log K'_{\text{parent}}$, where $K' = (\text{tr-to})/\text{to}$.

pKa values were from Perrin (1965) and You *et al.* (1993); some values were obtained from SPARC's chemical reactivity model (Hilal and Karickhoff, 1995).

Statistical analyses. The SAS suite of statistical programs for regression analysis was used (Cody and Smith, 1997).

RESULTS

The Ames mutagenicity assay showed that 3,3'-dichlorobenzidine-2HCl was directly mutagenic to TA98, while 4,4'-dinitro-2-biphenylamine was directly mutagenic to both TA98 and TA100 (Table 1). In the presence of S9 mix, 4-aminobiphenyl, benzidine, 3,3'-dichlorobenzidine-2HCl, 3,3'-dimethoxybenzidine, 4,4'-dinitro-2-biphenylamine, 3,3'-4,4'-tetraaminobiphenyl, N, N-N', N'-tetramethylbenzidine and *o*-tolidine were mutagenic to TA98, and 4-aminobiphenyl, 3,3'-dichlorobenzidine-2HCl, 3,3'-dimethoxybenzidine, and 4,4'-dinitro-2-biphenylamine were mutagenic to TA100. 2-Aminobiphenyl, 3-amino-biphenyl, and 3,3'-5,5'-tetramethylbenzidine were not mutagenic to either TA98 or TA100. The mutagenic potencies of 4-aminobiphenyl, 3,3'-dichlorobenzidine(-2HCl), 3,3'-dimethoxybenzidine, and 4,4'-dinitro-2-biphenylamine, as expressed by the slope (number of revertants/ μmol) of the linear portion of the dose-response curve derived from the data of Table 1, are shown in Table 2.

The experimental oxidation potentials, K_{HPLC} , and pKa of these compounds are shown in Table 3. Deamination of benzidine (becoming aminobiphenyl) tends to increase the oxidation potential, while the addition of methoxy and/or methyl groups at the 3,3' or 5, 5' position of the benzene ring tends to decrease their oxidation potentials. Nevertheless, no apparent proportional correlation exists between these values and their mutagenicity (Table 2). Benzidine had the least value for K_{HPLC} , while N,N,N',N'-tetramethylbenzidine had the greatest K_{HPLC} value among all the benzidine analogs tested. The pKa of

TABLE 1
Mutagenicity Responses of *Salmonella* Tester Strains to Benzidine and Its Analogues

Compound	Dose/plate (μg)	Revertant colonies per plate (mean \pm SD)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
DMSO	100	20 \pm 4	22 \pm 3	101 \pm 10	110 \pm 4
2-Nitrofluorene	4	349 \pm 20	-	-	-
MNNG	1	-	-	2112 \pm 142	-
2-Aminofluorene	10	-	4625 \pm 30	-	2110 \pm 135
2-Aminobiphenyl	3	19 \pm 8	28 \pm 6	121 \pm 4	111 \pm 21
	10	17 \pm 5	17 \pm 6	142 \pm 23	126 \pm 14
	30	15 \pm 3	19 \pm 6	155 \pm 3	117 \pm 12
	100	20 \pm 3	25 \pm 11	153 \pm 13	168 \pm 28
	300	18 \pm 10	21 \pm 1	93 \pm 2	277 \pm 72
3-Aminobiphenyl	3	29 \pm 17	18 \pm 2	133 \pm 14	141 \pm 65
	10	18 \pm 8	18 \pm 4	102 \pm 20	141 \pm 29
	30	16 \pm 2	32 \pm 6	84 \pm 35	103 \pm 16
	100	18 \pm 2	27 \pm 3	111 \pm 11	117 \pm 14
4-Aminobiphenyl	300	15 \pm 6	25 \pm 3	76 \pm 6	100 \pm 32
	3	12 \pm 3	91 \pm 24	83 \pm 6	577 \pm 46
	10	10 \pm 2	205 \pm 32	70 \pm 12	2154 \pm 464
	30	11 \pm 1	551 \pm 183	93 \pm 4	3499 \pm 478
Benzidine	100	16 \pm 4	1479 \pm 222	97 \pm 17	3640 \pm 1175
	300	11 \pm 4	2062 \pm 88	94 \pm 3	4579 \pm 192
	3	26 \pm 12	22 \pm 3	61 \pm 9	88 \pm 3
	10	27 \pm 11	30 \pm 5	64 \pm 2	99 \pm 1
	30	-	45 \pm 9	75 \pm 2	103 \pm 9
	100	22 \pm 5	81 \pm 13	60 \pm 3	121 \pm 14
	300	-	131 \pm 20	60 \pm 9	138 \pm 11
3,3'-Dichlorobenzidine-2HCl	1000	32 \pm 11	221 \pm 58	88 \pm 6	140 \pm 1
	3	47 \pm 3	169 \pm 14	92 \pm 5	111 \pm 7
	10	94 \pm 18	1735 \pm 198	81 \pm 29	125 \pm 14
	30	110 \pm 6	3776 \pm 879	85 \pm 2	210 \pm 29
	100	221 \pm 18	10517 \pm 1515	86 \pm 12	465 \pm 32
3,3'-Dimethoxybenzidine	300	249 \pm 3	11520 \pm 1008	93 \pm 8	1739 \pm 221
	1000	246 \pm 26	14805 \pm 132	82 \pm 17	1756 \pm 421
	3	27 \pm 13	29 \pm 9	66 \pm 4	101 \pm 9
	10	27 \pm 4	70 \pm 5	62 \pm 6	155 \pm 18
	30	-	107 \pm 14	72 \pm 11	219 \pm 31
	100	26 \pm 13	231 \pm 46	68 \pm 9	224 \pm 33
4,4'-Dinitro-2-biphenylamine	300	-	578 \pm 77	60 \pm 1	405 \pm 44
	1000	35 \pm 8	469 \pm 100	61 \pm 7	230 \pm 14
	3	152 \pm 2	26 \pm 9	74 \pm 5	113 \pm 4
	10	1344 \pm 392	64 \pm 25	312 \pm 51	147 \pm 21
	30	1696 \pm 437	191 \pm 10	1387 \pm 37	206 \pm 4
	100	2558 \pm 805	445 \pm 32	1408 \pm 128	276 \pm 11
3,3'-4,4'-Tetraaminobiphenyl	300	3542 \pm 30	651 \pm 77	331 \pm 10	1047 \pm 300
	1000	4170 \pm 590	2866 \pm 382	474 \pm 54	1648 \pm 248
	3	14 \pm 2	65 \pm 6	76 \pm 5	104 \pm 23
	10	12 \pm 1	124 \pm 12	74 \pm 12	104 \pm 14
	30	10 \pm 1	212 \pm 10	75 \pm 25	116 \pm 14
	100	11 \pm 3	387 \pm 68	73 \pm 11	105 \pm 39
3,3'-5,5'-Tetramethylbenzidine	300	12 \pm 1	499 \pm 16	84 \pm 11	129 \pm 8
	1000	11 \pm 3	460 \pm 13	100 \pm 14	163 \pm 16
	3	14 \pm 3	20 \pm 3	74 \pm 15	107 \pm 15
	10	11 \pm 4	20 \pm 5	66 \pm 3	92 \pm 13
	30	12 \pm 7	20 \pm 2	90 \pm 8	88 \pm 8
	100	14 \pm 3	17 \pm 5	65 \pm 6	115 \pm 12
	300	10 \pm 1	17 \pm 3	73 \pm 20	76 \pm 2
1000	10 \pm 4	16 \pm 1	76 \pm 4	86 \pm 9	

Continued

TABLE 1—Continued

Compound	Dose/plate (μg)	Revertant colonies per plate (mean \pm SD)			
		TA98		TA100	
		-S9	+S9	-S9	+S9
N,N-N',N'-Tetramethylbenzidine	3	15 \pm 2	34 \pm 4	89 \pm 13	132 \pm 11
	10	21 \pm 8	101 \pm 13	83 \pm 16	120 \pm 11
	30	17 \pm 8	108 \pm 21	84 \pm 3	112 \pm 5
	100	14 \pm 2	122 \pm 27	93 \pm 21	130 \pm 13
	300	17 \pm 1	139 \pm 10	104 \pm 6	115 \pm 6
	1000 ^a	15 \pm 9	268 \pm 57	117 \pm 71	125 \pm 7
o-Tolidine	3	22 \pm 4	34 \pm 4	86 \pm 14	121 \pm 3
	10	24 \pm 6	47 \pm 2	75 \pm 12	81 \pm 19
	30	—	71 \pm 8	84 \pm 12	135 \pm 28
	100	23 \pm 5	72 \pm 5	81 \pm 5	100 \pm 16
	300	—	75 \pm 1	69 \pm 16	125 \pm 2
	1000	26 \pm 3	79 \pm 8	66 \pm 19	143 \pm 5

Note. Values are means of revertants/plate \pm standard deviations of quadruplicate runs, each with 3 plates/dose. Dash indicates not tested.

^a N,N-N',N'-Tetramethylbenzidine at 1000 μg was barely soluble in DMSO; some precipitate occurred.

2-amino-biphenyl was lower than that of 3-aminobiphenyl or 4-aminobiphenyl, although these were isomers. N,N-N',N'-tetramethylbenzidine had the highest pKa value compared to the other compounds (Table 3).

Other calculated physicochemical properties of these compounds such as ΔE , I.P., and μ were calculated using the MNDO method (Table 4). 3,3'-Dimethoxybenzidine had the greatest ΔE value among all the chemicals tested. The greatest value of I.P. among the 11 chemicals tested belonged to 4,4'-dinitro-2-biphenyl-amine. 3,3'-Dichlorobenzidine-2HCl and 3,3'-5,5'-tetramethylbenzidine had lower μ values than the other compounds.

DISCUSSION

The mutagenicity results of benzidine and its analogs are generally in agreement with the literature except for 2-aminobiphenyl (Messerly *et al.*, 1987; You *et al.*, 1993). 2-Aminobiphenyl was not mutagenic to both TA98 and TA100 with or without metabolic activation. Messerly *et al.* (1987) reported that 2-aminobiphenyl was mutagenic to TA98 with S9 activation, but failed to show a dose-related response. However, Ioannides *et al.* (1989) showed that 2-aminobiphenyl was not mutagenic to TA98 and TA100 with or without S9. We carefully purified the compound by the sublimation method and found consistent results of negative mutagenic activity with TA98 in repeated testings.

The addition of 4 methyl groups to the benzidine molecule (to form 3,3'-5,5'-tetramethylbenzidine) abolished the mutagenic activity completely. 3,3'-5,5'-Tetramethylbenzidine is not a carcinogen and is currently used as an industrial substituent for benzidine (Ashby *et al.*, 1982). The addition of a

methoxy group to the benzidine molecule increases its mutagenicity. Chung *et al.* (1996) discovered that introduction of a nitro group to the benzene ring would greatly affect its mutagenicity. This is also true for the direct mutagen, 4,4'-dinitro-2-biphenylamine, after introduction of two nitro groups to the 2-aminobiphenyl ring.

3,3'-Dichlorobenzidine was the most potent mutagen tested toward TA98 in the presence of S9 mix. Both benzidine and 3,3'-dichlorobenzidine are potent carcinogens (Stula *et al.*, 1978). The addition of a halogen group to the benzidine molecule converts it into a direct mutagen to TA98, but not to TA100. This is in agreement with the other reported findings

TABLE 2
Mutagenic Potency of Benzidine and Its Analogs
in *Salmonella typhimurium*

Compound	TA98	TA100
2-Aminobiphenyl	0	94
3-Aminobiphenyl	0	0
4-Aminobiphenyl	1122	1930
Benzidine	32	0
3,3'-Dichlorobenzidine (-2HCl)	4419	590
3,3'-Dimethoxybenzidine	454	229
4,4'-Dinitro-2-biphenylamine	721	409
3,3'-4,4'-Tetraaminobiphenyl	292	0
3,3'-5,5'-Tetramethylbenzidine	0	0
N,N-N',N'-tetramethylbenzidine	51	0
o-Tolidine	7	0

Note. Values are the calculated slopes (number of revertants/ μmol) of the linear portion of the dose-response curve derived from data in Table 3. Only data with S9 were used.

TABLE 3
Measured Potentials (Volts), K_{HPLC} , and pKa
of Benzidine and Its Analogs

Compound	Oxidation potential	K_{HPLC}	pKa
2-Aminobiphenyl	0.79	0.51	3.83 ^b
3-Aminobiphenyl	0.81	0.49	4.25 ^b
4-Aminobiphenyl	0.69	0.54	4.22 ^b
Benzidine	0.36, 0.63	0	4.80 ^c
3,3'-Dichlorobenzidine-2HCl	0.60	0.77	2.16 ^d
3,3'-Dimethoxybenzidine	0.34, 0.54	0.20	4.20 ^d
4,4'-Dinitro-2-biphenylamine ^a	—	0.66	1.05 ^d
3,3'-4,4'-Tetraaminobiphenyl	0.48	0.20	4.34 ^d
3,3'-5,5'-Tetramethylbenzidine	0.34, 0.52	0.59	4.17 ^d
N,N-N',N'-tetramethylbenzidine	0.47	1.46	5.35 ^d
<i>o</i> -Tolidine	0.35, 0.60	0.30	5.16 ^c

Note. Each chemical (10 mg) was dissolved in 1 ml DMSO and the solution was added to 20 ml of 0.1 M KCl solution. K_{HPLC} , partition coefficient; pKa, basicity.

^aThe compound was not soluble in 0.1M KCl. The oxidation potential could not be detected.

^bValues were from Perrin, 1965.

^cValues were from You *et al.*, 1993.

^dValues were from SPARC's chemical reactivity model (Hilal and Karickhoff, 1995).

(Messerly *et al.*, 1987; You *et al.*, 1993). The addition of two nitro groups to the molecule (i.e., 4,4'-dinitro-2-biphenylamine) converts it into a direct mutagen to both TA98 and TA100. Therefore, the halogen molecule is different from the nitro group in affecting the binding of these test compounds with bacterial DNA to form mutagenic DNA adducts. The halogen benzidine caused only frameshift mutations (TA98), whereas the nitro compounds caused both frameshift (TA98) and base-pair substitution mutations (TA100). Yet most of these amines require metabolic activation for their mutagenicities. The in-depth mechanism of mutagenesis of this group of compounds requires further investigation.

Our results showed that the mutagenicity of benzidine and its analogs do not correlate with their oxidation potentials (Table 3). This result did not support the hypothesis that an amine that can be oxidized more easily is more mutagenic. However, this might only indicate that the N-oxidation of aromatic amines to form N-hydroxyamine derivatives is not the limiting step of the generation of the mutagenic molecules.

As oxidation involves the removal of an electron from the HOMO, it is expected that a compound with a higher HOMO has a lower oxidation potential. A linear relationship of oxidation potentials with the energies of HOMOs for some aromatic compounds has been reported (Lund, 1957). Results obtained from the present study on MNDO calculations of benzidine and its analogs (Table 4) indicated that the ΔE values of these compounds between LUMO and HOMO did not correlate with their bacterial mutagenic potencies. The calculated values of I.P. or μ did not have any positive correlation with the bacterial mutagenicity of these compounds.

You *et al.* (1993) found that the mutagenicity of benzidine derivatives in TA98, TA98/1,8-DNP₆ and TA100 strains increased when their pKa values decreased. Messerly *et al.* (1987) also reported that the mutagenicity of 3,3'-disubstituted compounds (dimethoxybenzidine, diaminobenzidine, and dichlorobenzidine) were inversely linearly proportional to their pKa values in both TA98 and TA100 strains. Meanwhile, they examined the mutagenicity data of benzidine and 3,3'-disubstituted compounds (3,3'-difluorobenzidine, 3,3'-dichlorobenzidine, and 3,3'-dibromobenzidine) in TA98 with S9 mix, as reported by Savard and Josephy (1986), and found a negative correlation between the pKa values and mutagenicity. The pKa of a substituted aromatic amine is influenced by the electron donating/withdrawing ability of its substituent. You *et al.* (1993) indicated that pKa might influence the mutagenic ability of nitrenium ions, the ultimate products of benzidine metabolism (Josephy, 1986). Maynard *et al.* (1986) had discussed the effects of substituents on nitrenium ion formation and the need in some cases for *o*-acetylation of hydroxamic acid intermediates to diminish the energy of activation associated with formation of such ion. Ford and Herman (1992) pointed out that the stability of nitrenium ions would increase the mutagenicity of polycyclic aromatic amines. This warrants further study.

The present findings indicate that different analogs of benzidine vary in their ability to cause bacterial mutagenicity. The introduction of a halogen group to the molecules converts them into direct mutagens. With the method used in this study and the limited number of compounds tested, the mutagenic potency of the compounds does not correlate with their oxidation potentials and other physicochemical properties when these parameters are compared individually. However, better and/or higher-level calculation methods and the inclusion of more compounds might improve correlation. Physicochemical parameters, when considered collectively, may also correlate to their genotoxicities. Further structure-activity relationships studies are still necessary.

TABLE 4
Calculated Properties of Benzidine and Its Analogs by Means
of Semiempirical Approximate Method (AM1)

Compound	ΔE (electrovolt)	I.P. (electrovolt)	μ (Debye)
2-Aminobiphenyl	5.15	11.68	1.45
3-Aminobiphenyl	5.83	11.92	1.55
4-Aminobiphenyl	4.54	11.76	1.79
Benzidine	4.43	10.80	1.86
3,3'-Dichlorobenzidine	5.09	11.61	0.17
3,3'-Dimethoxybenzidine	5.89	11.72	2.17
4,4'-Dinitro-2-aminobiphenyl	4.16	13.75	1.56
3,3'-4,4'-Tetraaminobiphenyl	3.83	10.18	2.93
3,3'-5,5'-Tetramethylbenzidine	3.88	10.34	0.42
N,N-N',N'-tetramethylbenzidine	3.93	10.42	1.11
<i>o</i> -Tolidine	4.34	10.43	2.26

Note. ΔE , the energy difference between LUMO and HOMO; I.P., ionization energy; μ , dipole moment.

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