MOLECULAR & CELLULAR TOXICOLOGY, Vol. 3, No. 2, 145-149, June 2007

CoMFA Based Quantitative Structure Toxicity Relationship of Azo Dyes

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Accepted 19 April 2007

Abstract

Studies of relationship between structure and toxicity of azo dyes have been performed with comparative molecular field analysis (CoMFA) techniques. 3D QSTR analyses indicate that the steric and electrostatic interactions are important. The steric field based model gives strong correlation (q^2=0.57, r^2= 0.92). The steric field in conjunction with electrostatic field give more strong correlation (q^2=0.57, r^2=0.95). All study indicates that a bulky and electronegative group at benzene ring and a small group at position 3 of aniline ring might be significant to reduce the mutagenicity.

Keywords: 3D QSTR, CoMFA, Azo Dyes, Toxicity

The Amino-azo-benzenes are a general dye. But they also possess some toxic effect. Several 4-aminoazobenzene, N-methyl-4-aminoazobenzene and N,N-dimethyl-4-aminoazobenzene derivatives are mutagenic and/or carcinogenic[1-4]. Interestingly, the toxic potency of these compounds is strongly dependent on the nature and position of substituents. 3-methoxy-4-aminoazobenzene (3-OMe-AAB) is a potent hepatocarcinogen in rats and a strong mutagen in Escherichia coli and Salmonella typhimurium, whereas 2-OMe- AAB is apparently a non-carcinogen and an extremely weak mutagen under similar conditions[5]. The biochemical mechanisms for such divergent behavior are not yet fully understood[6-9]. In order to have azo dyes with least or no toxicity it is necessary to establish a generalized relationship between toxicity values and molecular structure. For the purpose different QSAR techniques have been proposed and are in practice[10-14]. Recently QSAR[15] based techniques were applied to the variety molecules within framework of quantitative structure toxicity relationship (QSTR)[16]. In this paper we have taken 43 amino azo dyes with their observed mutagenicity[17]. 3D-QSAR based on comparative molecular field analysis (CoMFA)[18] used to explain the relationship between mutagenicity and structures.

Molecular Alignment

The CoMFA requires 3D alignment of all the structures according to a suitable conformational template[19]. In present study the molecule-43, the most active molecule used as template as shown in Figure 4 (Table 1). The molecule 43 drawn and minimized at Tripos force field[20] with MMFF94 charges by using conjugate gradient at convergence criterion of 0.005 kcal/mol. The full optimization of molecule 43 carried out at semi empirical PM3[21,22] level then by fixing the common moiety this structure was modified for further compounds. The all molecules aligned on

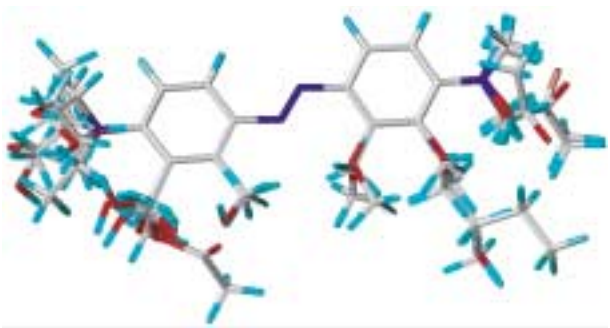

Figure 1. The aligned stricture of Dyes.

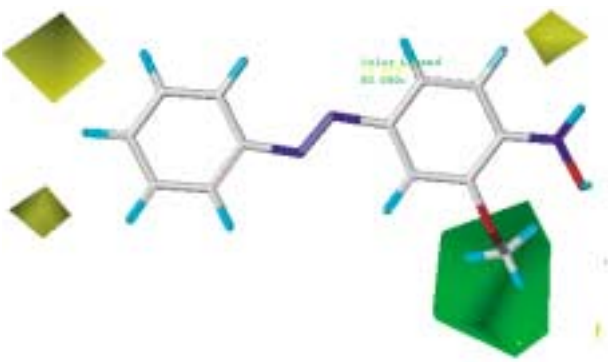

Figure 2. The CoMFA contour map by PA-1.

template by database alignment tool as shown in Figure 2.

The CoMFA Analysis

The 3 different CoMFA analyses made by using steric, electrostatic and jointly both fields in conjunction with MMFF-94 charge. The model PA-1 based on steric field shows a strong correlation (q^2=0.57, r^2=0.92) with mutagenicity. The model PA-3 based on both the steric and electrostatic field (.69S/.31E) with a value of q^2=0.57 and r^2=0.95. The regression summary given under table-3 and predicted activities by PA-1 and PA-3 are presented under Table 2. The molecule 1, 2, 26 and 34 were not predicted well in initial step hence omitted from final model. The study reveals that steric and bulk interactions are important but the steric contribution is dominant in interaction as clear from regression summary.

The CoMFA Map

The 3D-CoMFA contour maps by model PA-1 shown in Figure 2 with highest mutagenic compound. In general the CoMFA green contours indicates the area in which steric bulk substituents might improve activity while yellow region favorable for small groups. Since this study is related to mutagenicity the

Table 1. The Structure and Observed mutagenicity of Azo dyes in terms of IC_{50} values.

No.	R	R1	Substituents	IC_{50}	logIC_{50}
1	H	H	4′-NEt2-3-OMe	0.007	−2.15
2	H	H	2-OMe	0.01	−2
3	H	H	4′-OH	0.053	−1.28
4	H	H	3′-Me-4′-OH	0.059	−1.22
5	H	H	4′-OH-2′,3-diMe	0.112	−0.95
6	H	H	-	0.204	−0.69
7	H	H	3′-Me	0.24	−0.62
8	H	H	3-OMe-4′-N $(CH_2CH_2OH)_2$	0.39	−0.41
9	H	H	3′-CH_2OH	0.596	−0.23
10	H	H	3-OH-AAB	0.687	−0.16
11	H	H	3-OCH_2CH_2OH-4′-N $(CH_2CH_2OH)_2$	1.052	0.02
12	H	H	3-OCH_2CH_2OH	1.348	0.13
13	H	H	2′-CH_2OH-3-Me	2.012	0.3
14	H	H	4′-OMe	2.3	0.36
15	H	H	2′,3-diMe	2.676	0.43
16	H	H	3-Obu	4.983	0.7
17	H	H	3-OEt	13.802	1.14
18	H	H	3-O-Pro	18.919	1.28
19	H	H	3-OMe	77.065	1.89
20	CH_3	H	3′-Me-4′-OH	0.071	−1.15
21	CH_3	H	3′-COOH	0.124	−0.91
22	CH_3	H	4′-OH	0.14	−0.85
23	CH_3	H	-	0.183	−0.74
24	CH_3	H	4′-Me	0.283	−0.55
25	CH_3	H	3′-Me	0.445	−0.35
26	CH_3	H	3′-CH_2OH	0.503	−0.3
27	CH_3	CH_3	3′-Me-40-OH	0.11	−0.96
28	CH_3	CH_3	-	0.14	−0.85
29	CH_3	CH_3	3′-COOH	0.201	−0.7
30	CH_3	CH_3	2-Me	0.22	−0.66
31	CH_3	CH_3	3′-Me	0.356	−0.45
32	CH_3	CH_3	3′-CHO	0.383	−0.42
33	CH_3	CH_3	3′-CH_2OAC	0.518	−0.29
34	CH_3	CH_3	3′-CH_2OH	0.601	−0.22
35	H	Ac	3′-Me	0.087	−1.06
36	H	OH	3′-Me-40-OH	0.089	−1.05
37	H	OH	2-OMe	0.11	−0.96
38	CH_3	Ac	3′-Me	0.524	−0.28
39	CH_3	OH	-	0.65	−0.19
40	CH_3	OH	3′-Me	1	0
41	H	OH	N-OH	1.03	0.01
42	CH_3	OH	4′-Me	1.132	0.05
43	H	OH	3-OMe	192	2.28

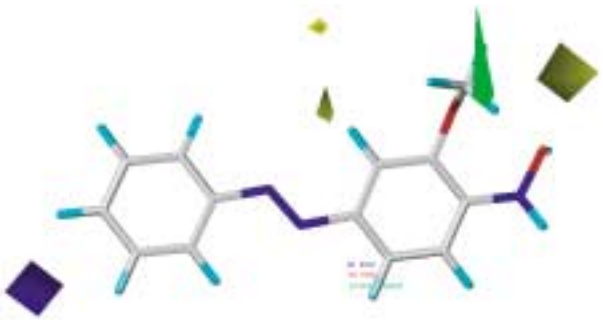

Figure 3. The CoMFA contour map by PA-3.

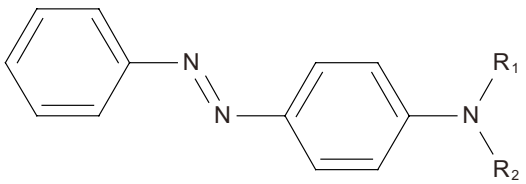

Figure 4. Basic skeleton of amino azo dyes.

higher activity is not desirable for most applications.

As clear from map a green contour appears around aniline ring at site 3 indicates that a bulky substituents will be favorable to improve the value of mutagenicity hence a small group required at this site while yellow contour appears around benzene ring indicates that a bulky group at this site will reduce the value of mutagenicity.

Model PA-3 based CoMFA contour map shown in Figure 3 with highest mutagenic compound. The CoMFA green contours indicates the area in which steric bulk substituents might increase the potency while yellow region favorable for small groups. The blue contour indicates the region where positive group required for high value while red zone indicates the place favorable for negative groups for high value. The small blue contour appears around benzene ring indicates that a electropositive group around this region will enhance the value of mutagenic activity and will be harmful hence a electronegative group is desired around benzene ring while a green contour appears around aniline ring at site 3 indicates that a small substituents is desired to reduce the mutagenicity of molecule.

Discussion

The ligand based both 3D-QSAR model is significant, a more prominent conclusion can be made by

Table 2. Observed and predicted Activities of azo dyes by CoMFA models.

No.	A	PA-1	Resid.	PA-3	Resid.
1[a]	−2.15	1.415	2.21	1.21	−3.36
2[a]	−2	−0.721	−0.733	−0.733	−1.267
3	−1.28	−1.094	−1.306	−1.306	0.026
4	−1.22	−1.032	−1.03	−1.03	−0.19
5	−0.95	−0.317	−0.706	−0.706	−0.244
6	−0.69	−0.568	−0.477	−0.477	−0.213
7	−0.62	−0.533	−0.537	−0.537	−0.083
8	−0.41	−0.253	−0.227	−0.227	−0.183
9	−0.23	−0.332	−0.236	−0.236	0.006
10	−0.16	−0.308	−0.27	−0.27	0.11
11	0.02	−0.119	0.127	0.127	−0.107
12	0.13	0.501	0.226	0.226	−0.096
13	0.3	0.398	0.301	0.301	−0.001
14	0.36	0.228	0.175	0.175	0.185
15	0.43	0.19	0.123	0.123	0.307
16	0.7	0.6	0.663	0.663	0.037
17	1.14	1.217	1.268	1.268	−0.128
18	1.28	0.886	1.072	1.072	0.208
19	1.89	2.034	1.713	1.713	0.177
20	−1.15	−1.04	−1.109	−1.109	−0.041
21	−0.91	−0.683	−0.687	−0.687	−0.223
22	−0.85	−1.048	−1.336	−1.336	0.486
23	−0.74	−0.397	−0.382	−0.382	−0.358
24	−0.55	−0.577	−0.373	−0.373	−0.177
25	−0.35	−0.363	−0.442	−0.442	0.092
26[a]	−0.3	−0.208	−0.302	−0.302	0.002
27	−0.96	−1.03	−1.079	−1.079	0.119
28	−0.85	−0.574	−0.522	−0.522	−0.328
29	−0.7	−0.743	−0.586	−0.586	−0.114
30	−0.66	−0.902	−0.935	−0.935	0.275
31	−0.45	−0.529	−0.574	−0.574	0.124
32	−0.42	−0.628	−0.454	−0.454	0.034
33	−0.29	−0.204	−0.338	−0.338	0.048
34[a]	−0.22	−0.395	−0.407	−0.407	0.187
35	−1.06	−1.074	−1.053	−1.053	−0.007
36	−1.05	−1.033	−1.03	−1.03	−0.02
37	−0.96	−0.879	−0.863	−0.863	−0.097
38	−0.28	−0.328	−0.377	−0.377	0.097
39	−0.19	−0.121	−0.066	−0.066	−0.124
40	0	−0.086	−0.091	−0.091	0.091
41	0.01	−0.604	−0.369	−0.369	0.379
42	0.05	−0.297	0.007	0.007	0.043
43	2.28	2.268	2.409	2.409	−0.129

[a]: data point not used in deriving equation

Table 3. The regression summery of CoMFA models.

	PA-1	PA-2	PA-3
Fields	S	E	S+E
n	6	4	6
q^2	0.57	0.164	0.57
r^2	0.92	0.87	0.95
F value	65.6	36.73	98.24
SE	0.245	0.319	0.2
$r^2_{boot\ strap}$	0.96	–	0.97
SD	0.016	–	0.012
SE	0.175	–	0.14
SD	0.095	–	0.078

considering steric and electrostatic field together. The model PA-3 has strong correlation (q^2=0.57, r^2=0.95) suggest that, to reduce the mutagenic activity of azo dyes an electronegative and bulky group required at benzene ring while small group at aniline ring will be favorable to reduce the mutagenicity.

Methods

The basic skeleton of amino azobenzene is shown in Figure 1, the 43 amino azobenzene dyes with mutagenicity values taken from literature[17].

Computational Details

The comparative molecular field analysis (CoMFA) was used for 3D QSAR. Structures were drawn and assigned with MMFF-94 charge using Sybyl 7.3 software running on Linux cluster. All the structures minimized at Tripos force field[20] level with distance -dependent dielectric and conjugate gradient method. The convergence criterion was 0.01 kcal/mol.

CoMFA

The steric and electrostatic potential fields for CoMFA were calculated at each lattice intersection of a regularly spaced grid of 2.0. The lattice was defined automatically and is extended 4 units past van der waals volume of all molecules in X, Y, and Z directions. The vander waals potential and columbic term, which represent steric and electrostatic fields, respectively, were calculated using Tripos force field. A distance-dependent dielectric expression $\varepsilon = \tilde{\varepsilon}Rij$ with ε_0=1.0 was used. A sp3 carbon atom with a vander waals radius of 1.52 and +1.0 charges served as the probe atom to calculate steric and electrostatic fields. The steric and electrostatic contributions were truncated to $\pm$30 kcal/mol and electrostatic contributions were ignored at lattice intersections with maximum steric interactions. The CoMFA steric and electrostatic fields generated were scaled by CoMFA standard option given in Sybyl.

Statistics

The partial least square analysis (PLS) performed by Sybyl 7.3 and leaves one out method used to calculate q^2 values of each set with 2.0 column filtering. The molecules not predicted well in initial step of regression have been omitted from final model.

References

1. Kimura, T., Kodama, M. & Nagata, C. A correlation of the rate of N-hydroxylation of aminoazo dyes with their carcinogenic activity in the rat. *Carcinogenesis* **3**(12):1393-1396 (1982).
2. Miller, J. A. & Miller, E. C. The carcinogenicity of 3-methoxy-4-aminoazobenzene and its N-methyl derivatives for extrahepatic tissues of the rat. *Cancer Res.* **21**:1068-1072 (1961).
3. Mori, Y. *et al*. Carcinogenicity of 3'-hydroxymethyl-N, N-dimethyl-4-aminoazobenzene in rat liver. *Carcinogenesis* **1**(6):533-535 (1980).
4. Sugiura, K., Halter, C. R., Kensler, C. J. & Rhoads, C. P. Observations on Rats Fed with Compounds Related to Dimethylaminoazobene. *Cancer Research* **5**(4):235-238 (1945).
5. Kojima, M., Degawa, M., Hashimoto, Y. & Tada, M. Different effects of DNA adducts induced by carcinogenic and noncarcinogenic azo dyes on in vitro DNA synthesis. *Biochem. Biophys. Res. Commun.* **179**(2): 817-823 (1991).
6. Ashby, J., Lefevre, P. A. & Callander, R. D. The possible role of azoreduction in the bacterial mutagenicity of 4-dimethylaminoazobenzene (DAB) and 2 of its analogues (6BT and 5I). *Mutat. Res.* **116**(3-4): 271-279 (1983).
7. Chung, K. T. The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes. *Mutat. Res.* **114**(3):269-281 (1983).
8. Hashimoto, Y., Degawa, M., Watanabe, H. K. & Tada, M. Amino acid conjugation of N-hydroxy-4-aminoazobenzene dyes: a possible activation process of carcinogenic 4-aminoazobenzene dyes to the ultimate mutagenic or carcinogenic metabolites. Gann. **72**(6):937-943 (1981).
9. Lin, J. K., Miller, J. A. & Miller, E. C. Structures of hepatic nucleic acid-bound dyes in rats given the carcinogen N-methyl-4-aminoazobenzene. *Cancer Res.* **35**(3):844-850 (1975).
10. Singh, P. P., Srivastava, H. K. & Pasha, F. A. DFT-based QSAR study of testosterone and its derivatives. *Bioorganic & Medicinal Chemistry* **12**(1):171-177 (2004).
11. Pasha, F. A., Srivastava, H. K. & Singh, P. P. QSAR study of estrogens with the help of PM3-based descriptors. *International Journal of Quantum Chemistry* **104**(1):87-100 (2005).
12. Pasha, F. A., Srivastava, H. K. & Singh, P. P. Comparative QSAR study of phenol derivatives with the help of density functional theory. *Bioorganic & Medicinal Chemistry* **13**(24):6823-6829 (2005).
13. Ashek, A. & Cho, S. J. A combined approach of docking and 3D QSAR study of beta-ketoacyl-acyl carrier protein synthase III (FabH) inhibitors. *Bioorganic & Medicinal Chemistry* **14**(5):1474-1482 (2006).
14. Ashek, A., Lee, C., Park, H. & Cho, S. J. 3D QSAR studies of dioxins and dioxin-like compounds using CoMFA and CoMSIA. *Chemosphere* **65**(3):521-529 (2006).

15. Hansch, C. The QSAR paradigm in the design of less toxic molecules. *Drug Metab. Rev.* **15**(7):1279-1294 (1984).
16. Pasha, F. A., Srivastava, H. K., Srivastava, A. & Singh, P. P. QSTR study of small organic molecules against Tetrahymena pyriformis. *Qsar & Combinatorial Science* **26**(1):69-84 (2007).
17. Garg, A., Bhat, K. L. & Bock, C. W. Mutagenicity of aminoazobenzene dyes and related structures: a QSAR /QPAR investigation. *Dyes and Pigments* **55**(1):35-52 (2002).
18. Cramer, R. D., Patterson, D. E. & Bunce, J. D. Comparative Molecular-Field Analysis (Comfa) .1. Effect of Shape on Binding of Steroids to Carrier Proteins. *Journal of the American Chemical Society* **110**(18): 5959-5967 (1988).
19. Kim, K. H., Greco, G. & Novellino, E. A critical review of recent CoMFA applications. *Perspectives in Drug Discovery and Design* **12**:257-315 (1988).
20. Clark, M., Cramer, R. D. & Vanopdenbosch, N. Validation of the General-Purpose Tripos 5.2 Force-Field. *Journal of Computational Chemistry* **10**(8): 982-1012 (1989).
21. Stewart, J. J. P. Optimization of Parameters for Semiempirical Methods. 2. Applications. *Journal of Computational Chemistry* **10**(2):221-264 (1989).
22. Stewart, J. J. P. Optimization of Parameters for Semiempirical Methods. 1. Method. *Journal of Computational Chemistry* **10**(2):209-220 (1989).