

Three Dimensional Quantitative Structure-Activity Relationships of Sulfonamides Binding Monoclonal Antibody by Comparative Molecular Field

Analysis

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Abstract

The three-dimensional quantitative structure-activity relationship (3D-QSAR) model of sulfonamide analogs binding a monoclonal antibody (Mab_{SMR}) produced against sulfamerazine, was carried out by comparative molecular field analysis (CoMFA). The affinities of Mab_{SMR}, expressed as Log₁₀IC₅₀, for 17 sulfonamide analogs were determined by competitive fluorescence polarization immunoassay (FPIA). Removal of two outliers from the initial set of 17 sulfonamide analogs improved the predictability of the models. The 3D-QSAR model of 15 sulfonamides resulted in q^2_{cv} values of 0.600, and r^2 values of 0.995, respectively. This novel study combining FPIA with CoMFA demonstrates that multidisciplinary research can be used as a useful tool to investigate antigen-antibody interactions and provide information required for design of novel haptens, which may result in new antibodies with properties already optimized by an antibody-based immunoassay.

INTRODUCTION

Sulfonamides are widely used to control a number of diseases in the animal industry and aquaculture, as well as used for animal growth-promotion.¹ The presence of sulfonamide residues in foods of animal origin or the environment constitutes a potential hazard for humans due to the increasing incidence of microbial resistance and the risk of allergic reactions. The availability of reproducible, sensitive and rapid methods for screening sulfonamides in foodstuffs is essential. The antibody-based analytical methods, called immunoassays, have proven to be useful as simple, fast and sensitive tools for detecting and quantifying sulfonamides in a variety of matrices.^{2,3} The

antibody is key reagent in any format of immunoassay. However, the affinity and specificity of the generated antibodies often have un-uniform properties. The conventional process for antibody production, when carried out without careful theoretical considerations, primarily focuses on new hapten design and extensive screening protocols, is limited. There is considerable interest in understanding the structural basis of antibody-analyte complex interactions. A method that can provide useful information about the topological properties of a hapten can be very useful in producing an antibody with the desired affinity and specificity.

In this paper, the 3D-QSAR techniques based on CoMFA, were used to describe the quantitative binding affinities of sulfonamides towards Mab_{SMR}. The methods are useful in determining the most important features of antigen-antibody binding, and therefore, provide insights into the wide-range of variations in affinity values among sulfonamide structural analogs. This work may develop knowledge of interactions that govern drug/antibody binding, and may help in the design of novel, enhanced antibodies by recombinant techniques.⁴

EXPERIMENTAL SECTION

Fluorescence Polarization Immunoassay. The tracer used in binding affinity determinations, fluorescein isothiocyanate (FITC) labelled sulfamethazine (SMZ-FITC), was synthesized and purified by thin layer chromatography (TLC).⁵ The binding affinity (IC₅₀) values of Mab_{SMR} with 17 sulfonamides were determined by a previously developed FPIA.⁵

CoMFA analysis. Minimum energy conformations of all 17 sulfonamide analogs were calculated using the Minimize module of Sybyl 7.0 following the same process previously used for fluoroquinolone modeling.⁶ For CoMFA calculations⁷, the alignment molecules were placed in a 3D-cubic lattice with a 2 Å grid in x, y and z directions. The default sp³-hybridized carbon atom with +1 charge was selected as the probe atom for the calculation of the steric (Lennard-Jones 6–12 potential) and electrostatic fields (Coulombic potential) around the aligned molecules with a distance-dependent dielectric constant at all lattice points. Values of steric and electrostatic energy were truncated to 30 kcal mol⁻¹. PLS methodology was used for all 3D-QSAR analysis to determine the significance of the models. Quality of the final CoMFA models was measured by two statistical parameters: q^2_{cv} and r^2 .

RESULTS AND DISCUSSION

Determination of IC₅₀ Values Using FPIA.

The affinity of Mab_{SMR} for all sulfonamide analogs was expressed as IC₅₀ values.⁵ The structures, IC₅₀, and Log₁₀IC₅₀ values of all sulfonamides are summarized in Table 1. The only difference among the sulfonamide analogs lies in the diverse array of R-groups linked to the nitrogen at position 7 (Figure 1b, Table 1). Simple inspection of IC₅₀ values reveals that the R-group is of primary importance for Mab_{SMR} binding to the sulfonamide analogs (Table 1). Since Mab_{SMR} was produced to the sulfamerazine hapten, the sulfonamides with close structural similarity, such as sulfamethazine, demonstrated lower IC₅₀ values than that of other analogs. Even the addition of a methyl group at position 3 on the pyrimidine ring, as seen in sulfamethazine,

reduced the affinity compared to sulfamerazine by about 3.5-fold (Figure 1b, Table 1). The favorable Mab_{SMR} binding requirement for a methyl group at position 5 on the pyrimidine ring was illustrated by the IC₅₀ value of sulfadiazine (137 ng mL⁻¹), which does not have a methyl group at position 5 and has a reduced affinity by 7.2-fold compared to the hapten, sulfamerazine. The size influence of the groups at positions 3 and 5 on binding affinity can be observed by comparing the IC₅₀ value of sulfamethazine to that of sulfadimethoxine (Table 1). It is interesting that the methoxy substituted analog, sulfameter, binds Mab_{SMR} with a 40-fold lower affinity than sulfamerazine. However, the effect of the methoxy oxygen atom at position 4 of the pyrimidine ring on Mab_{SMR} binding is not understood. But the importance of the pyrimidine ring can be shown based on the affinity of Mab_{SMR} for sulfamerazine (IC₅₀ = 19 ng mL⁻¹), which is 2–4 orders of magnitude higher than the affinity for other sulfonamide analogs where the pyrimidine ring was substituted with a different heterocyclic ring. Mab_{SMR} has a higher binding affinity for sulfamerazine, sulfamethazine and sulfadiazine, all which contain a pyrimidine ring at position 7, and the inhibition curves of these three sulfonamides including sulfadimethoxine are shown in Figure 2.

It is well known that antigen-antibody formation is mainly dependent on molecular shape, defined by the geometry and low-energy interactions.⁸ In an effort to determine which structural and electronic effects were primarily important for Mab_{SMR}-sulfonamide binding, studies using advanced molecular modeling techniques were undertaken. The contribution of R-group structure to Mab_{SMR}-sulfonamide complex formation was analyzed by using CoMFA.

CoMFA Analysis.

The CoMFA model was used to correlate variability in Mab_{SMR} binding affinities to variations in sulfonamide analog molecular structure. An improved CoMFA model (M4) was obtained with 15 sulfonamide analogs but without sulfanilamide and sulfaphenazole. The Model exhibited a satisfactory predictive ability with a cross-validate q^2_{cv} value of 0.600, non-cross-validated r^2 value of 0.995 and standard error of the estimate of 0.071. The contributions of the steric and the electrostatic fields to binding affinity were 55.8% and 44.2%, respectively, by PLS analysis, indicating a strong relationship between the sulfonamide analogs structure and binding affinity. In the case of sulfonamide-Mab_{SMR} complexes, steric interactions dominated the contribution toward the observed binding variations. There is a very good agreement between the experimental and predicted values. Table 1 lists experimental binding affinities, predicted binding affinities and residual values (defined as the difference between experimental and predicted binding affinity) by CoMFA.

Figures 3 and 4 feature steric and electrostatic contour plots, respectively, of CoMFA that show where the changes in steric and electrostatic fields are associated with Mab_{SMR} binding to sulfonamide analogs. Greater values were correlated with more bulk near the green contours and with less bulk near the yellow contours (Figure 3); whereas, more positive charge was correlated with the blue contours, and more negative charge with the red contours (Figure 4). Some of the most noticeable features in Figure 3 are the presence of large green areas that indicate improved binding affinity with increased steric tolerance in the region of sulfamerazine near the NH₂ group at position 17 and the NH group at position 7 (see Figure 1). However, even more attention was focused on the R-group (see Figure 1a), since all sulfonamide analogs had differences in the R-group. The green steric contours for CoMFA shown in Figure 3 localized near position 3 and 4 of the pyrimidine ring indicate that bulky substituents in this region of the R-group will enhance

binding affinity. However, the introduction of the methoxyl group in the pyrimidine ring at position 4 decreased the binding affinity as seen with sulfameter. This may be due to the effects of other fields like electrostatic, hydrophobic or hydrogen-bonding that may play an important role in the interactions of the sulfonamide analogs and Mab_{SMR}. Yellow-colored and green-colored regions near position 5 (the pyrimidine ring methyl), in respect to the proportion of the two colors, show that small groups at position 5 of the R-group increases binding affinity.

The electrostatic field of the CoMFA model is shown in Figure 4 with sulfamerazine as a reference. The large blue area above the molecule from position 13 to position 18 represents where a positive Gasteiger-Hückel charge is favored (see Figure 1b). Red-colored regions near positions 3 and 5 show that suitable electronegative groups in these areas of the R-group are favored to bind Mab_{SMR}. This interpretation is born out by the greater binding affinity of sulfadimethoxine, having methoxy groups at positions 3 and 5, compared with sulfameter, sulfamethoxypyridazine, or sulfachloropyridazine, which are substituted with an electronegative atom at position 4.

CONCLUSIONS

This paper provides a QSAR of CoMFA, studies on 17 sulfonamide analogs binding Mab_{SMR} produced to sulfamerazine. The developed CoMFA models had excellent agreement with 15 of the 17 sulfonamides studied. Results from this multidisciplinary research can also provide insights into key structural elements required to design new haptens for development of more desirable antibodies.

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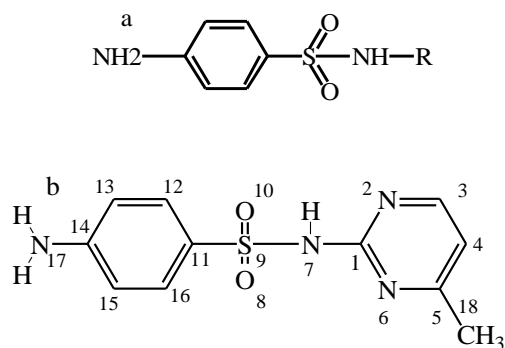


Figure 1 Schematic a) backbone of sulfonamides and b) pharmacophore of sulfamerazine.

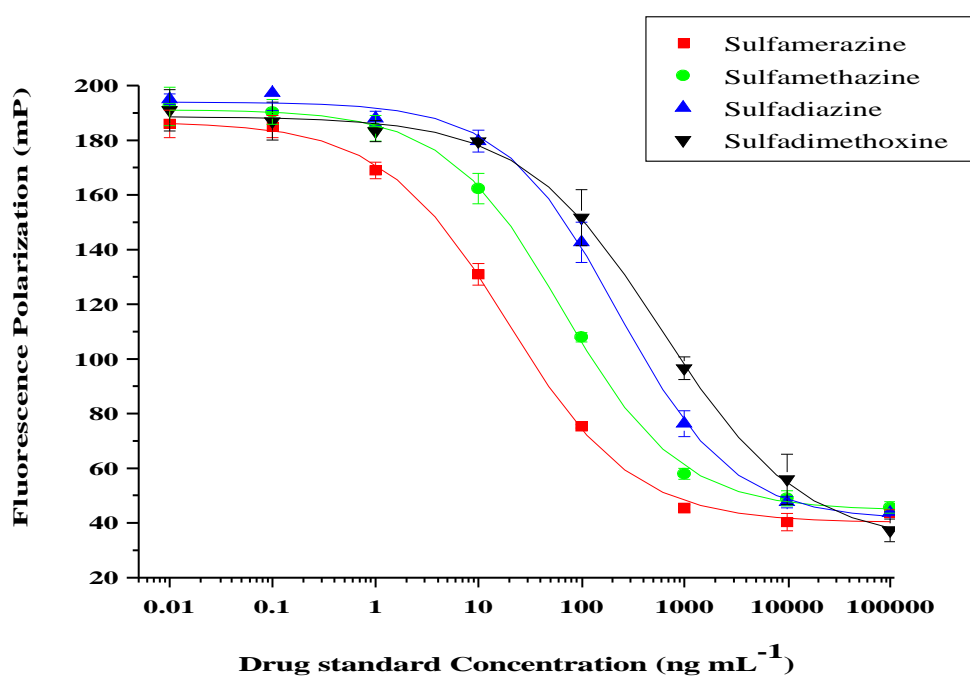


Figure 2 Representative examples of competition ELISA determination of the binding specificities of Mab. The calibration curves show the ability of sulfamerazine, sulfamethazine, sulfadiazine and sulfadimethoxine to inhibit SMZ-FITC binding to Mab. Each point is the average of three determinations.

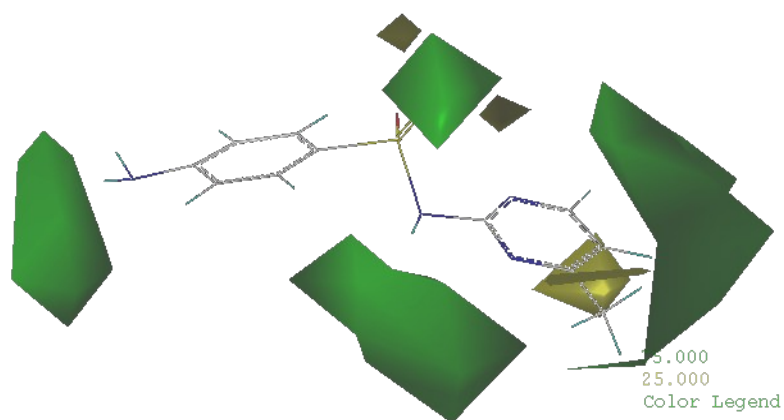


Figure 3 CoMFA contour plots of steric field contributions of sulfonamides binding to Mab. Green contours indicate regions where bulky groups increase antibody affinity, and yellow contours indicate regions where bulky groups decrease antibody affinity.

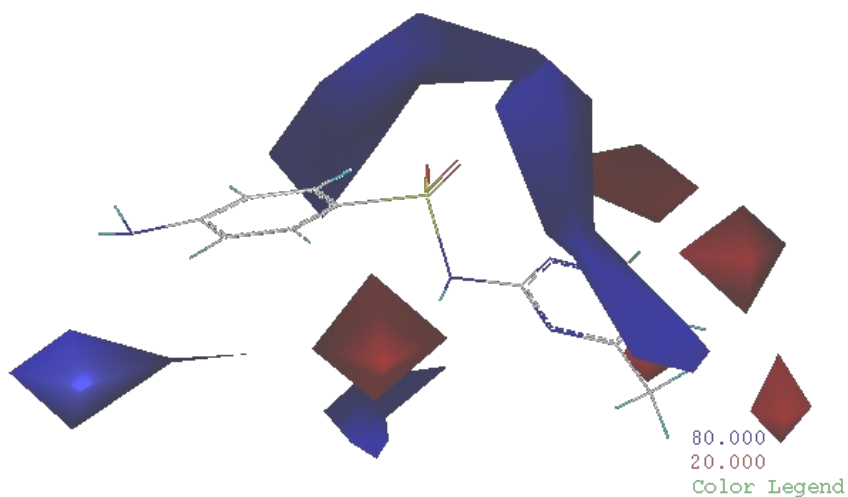
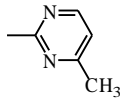
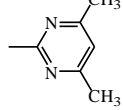
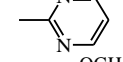
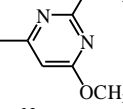
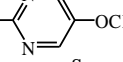
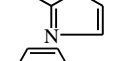
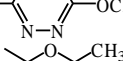
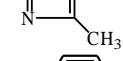
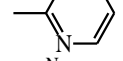
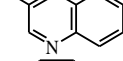
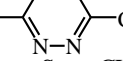
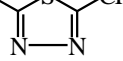
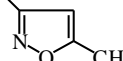
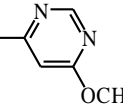
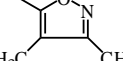
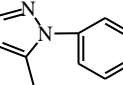


Figure 4 CoMFA contour plots of electrostatic field contributions of sulfonamides binding to Mab. Blue contours indicate regions where positive charged groups increase antibody affinity, and red contours indicate regions where negatively charged groups increase antibody affinity.

Table 1. Experimental and Predicted Sulfonamides Binding Affinity to Mab_{SMR}

drugs ^a	R	IC ₅₀	Log ₁₀ IC ₅₀ (exp.)	CoMFA	
				Log ₁₀ IC ₅₀ (pre.)	residual
SMR		19	1.28	1.24	0.04
SMZ		65.7	1.82	1.87	-0.05
SDZ		137	2.14	2.10	0.04
SDM		528	2.72	2.77	-0.05
SME		760	2.88	2.87	0.01
STZ		1000	3.00	3.03	-0.03
SMP		1056	3.02	3.01	0.01
SMO		1118	3.05	3.01	0.04
SPY		1189	3.07	3.10	-0.03
SQX		1538	3.20	3.20	0.00
SCP		3800	3.58	3.55	0.03
SMT		6333	3.80	3.83	-0.03
SMX		9048	3.96	3.94	0.02
SMM		12667	4.10	4.09	0.01
SFX		19000	4.28	4.27	0.01
SPA		>190000	-	-	-
SAM	H	>190000	-	-	-

^a The abbreviations are as follows: Sulfamerazine (SMR), sulfamethazine (SMZ), sulfadiazine (SDZ), sulfadimethoxine (SDM), sulfameter (SME), sulfathiazole (STZ), sulfamethoxypyridazine (SMP), sulfamoxole (SMO), sulfapyridine (SPY), sulfaquinoxaline (SQX), sulfachloropyridazine (SCP), sulfamethizole (SMT), sulfamethoxazole (SMX), sulfamonomethoxine (SMM), sulfisoxazole (SFX), sulfaphenazole (SPA), and sulfanilamide (SAM).