



SKIN SENSITIZATION STRUCTURE-ACTIVITY RELATIONSHIPS FOR PHENYL BENZOATES

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Abstract—The key to determining whether a chemical has the ability to behave as a contact allergen must reside ultimately in the structure and properties of that chemical rather than in the immune system. Quantitative structure-activity relationships (QSARs) have been demonstrated for a small number of series of chemicals in the past, using the relative alkylation index model based on this principle. In the present work, a carefully chosen range of phenyl benzoate esters has been synthesized such that they would have a single mechanism of action but otherwise would span important areas of parameter space—reactivity, skin penetration characteristics and biological response. Computer-based methods of analysis were used to generate a QSAR. The model showed that molecular volume and particularly the calculated logarithm of the partition coefficient (ClogP) were key parameters. Surprisingly, while chemical reactivity is a requirement for skin sensitization, it was not found to be an important variable in the QSAR. In conclusion, the study confirms that within a series of related chemicals it is possible to derive a useful QSAR.

INTRODUCTION

The key to determining whether a chemical has the ability to behave as a contact allergen must reside in the structure and properties of that chemical rather than in the immune system. Thus it should be possible to relate parameters describing chemical structure and structure-dependent physicochemical properties with skin sensitization potential.

One approach to establishing such relationships is the Relative Alkylation Index (RAI) model (Roberts and Williams, 1982), which has been used to evaluate data on various sets of chemicals (Basketter *et al.*, 1992; Roberts, 1987; Roberts and Basketter, 1990; Roberts *et al.*, 1983; Roberts and Williams, 1982). However, potential problems have also been noted (Basketter and Roberts, 1990) including modification of allergen on or in skin (Barratt and Basketter, 1992; Basketter and Goodwin, 1988; Basketter and Lidén, 1992). We have begun to extend the applicability of the RAI approach to different test models, wider types of chemical reaction, and to compounds that undergo (bio)chemical transformation in order to act as contact allergens. This has involved refinements of the mathematical treatment (Fraginals *et al.*, 1991) and experimental determination of key parameters

(Franot *et al.*, 1994). A complementary approach is to search for empirical quantitative structure-activity relationships (QSARs) by application of statistical methods to sets of biological data and structural descriptors. The QSAR and mathematical modelling methods approach the problem from different perspectives but may often converge to the same outcome, particularly when the selection of descriptors is guided by mechanistic considerations.

In the present work, a range of 12 phenyl benzoates has been evaluated and a QSAR defined. It is important to note that, as in previous studies, the compounds were designed in such a way as to have only one mode of action—benzoylation of skin protein.

MATERIALS AND METHODS

Test chemicals. 12 phenyl benzoates with the general structure $\text{XC}_6\text{H}_4\text{OCOC}_6\text{H}_5$ were synthesized using the method of Vogel (1956) for phenyl benzoate. The synthesis involved dissolution of phenol, or the appropriate derivative, into 75 ml 10% aqueous NaOH, adding 0.078 mol benzoyl chloride and stirring at ambient temperature until the reaction was complete. If the product was solid it was taken into ether. The organic liquid was then washed three times with 10% aqueous Na_2CO_3 to remove benzoic acid and dried using MgSO_4 . Solid product was recrystallized from industrial methylated spirit. Purity was assessed by 360 MHz HNMR and infra red analysis.

Skin sensitization assay. Each of the chemicals was evaluated using the modified single injection adjuvant test (MSIAT; Goodwin and Johnson, 1985).

QSAR analysis. Key factors were judged to be those relating to skin penetration of the chemical,

Abbreviations: ClogP = logarithm of the partition coefficient; ΔHF = difference between the heat of formation of the anionic leaving group and that of the parent compound; MSIAT = modified single injection adjuvant test; MV = molecular volume; QSAR = quantitative structure-activity relationship; RAI = relative alkylation index; TES = total erythema score; TSAR = Tools for Structure Activity Relationships.

Table 1. Results of MSIAT and parameter calculations

Chemical	Sensitization score*		ClogP†	MV‡ (Å ³)	ΔHF§ (kcal/mol)
	% positive	Total erythema score			
Phenyl benzoate	80	25.75	3.62	158.6	-15.1
3-(Trifluoromethyl)phenyl benzoate	100	54.00	4.75	197.3	-25.5
3-Methoxyphenyl benzoate	88	17.50	3.71	181.9	-17.6
4-Methoxyphenyl benzoate	90	12.50	3.71	192.1	-15.1
4-Chlorophenyl benzoate	67	40.00	4.47	184.4	-22.9
3-Chlorophenyl benzoate	100	39.50	4.47	175.5	-21.1
3-Nitrophenyl benzoate	50	7.75	3.66	194.2	-32.6
4-Nitrophenyl benzoate	50	6.50	3.66	204.2	-42.6
3-Acetylphenyl benzoate	100	32.0	3.36	193.0	-20.5
4-Acetylphenyl benzoate	88	16.00	3.36	195.4	-24.6
4-Butylphenyl benzoate	100	40.0	5.45	225.6	-15.3
3-Butylphenyl benzoate	100	52.00	5.45	227.9	-14.3

*Presented as the % positive and as the total erythema score derived by summing individual erythema scores at both time points on guinea pigs judged to show a positive response.

†Logarithm of the partition coefficient.

‡Molecular volume.

§Difference between the heat of formation of the anionic leaving group and that of the parent compound.

including its partitioning into the epidermal compartment where it would react with skin protein(s), and the properties that would determine its chemical reactivity. Important determinants of partitioning into skin are the octanol-water partition coefficient, usually represented as logP, and molecular volume (Hinz *et al.*, 1991). For this series of chemicals, logP was calculated (ClogP) using MedChem software (Daylight). Molecular volume was calculated using TSAR (Tools for Structure Activity Relationships) software (Oxford Molecular, Oxford, UK).

Parameters calculated using Sybyl software (Tripos Associates, Bracknell, UK) to provide a measure of the ability of the chemicals ability to behave as

benzoylating agents included sigma⁻ values, partial charges on the ester carbonyl, molecular orbital energies, and the difference between the heats of formation of the anion leaving group and that of the parent compound (ΔHF).

The data set was then subjected to multiple regression analysis using TSAR.

RESULTS

Each of the 12 phenyl benzoates caused a skin sensitization response (Table 1). As previously (Roberts, 1987), total erythema score (TES) was taken as the index of biological activity since it

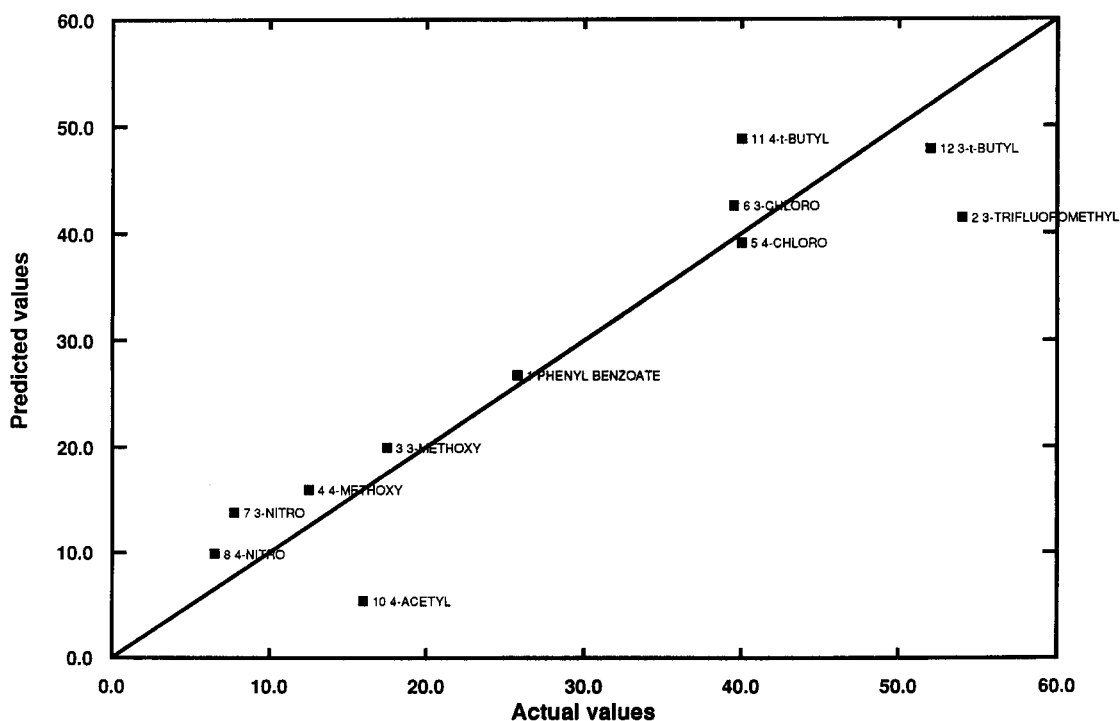


Fig. 1. QSAR plot of actual *v.* predicted values of sensitization scores for 11 phenyl benzoates.

reflects the incidence of positive reactions and their severity. Also in Table 1 are the important physicochemical parameters calculated for each compound.

The data were then subjected to multiple regression analysis on TSAR using TES as the dependent variable and ClogP, molecular volume (MV) and the various reactivity parameters as the independent variables. Surprisingly, none of the reactivity parameters made a significant contribution to the determination of sensitization potential. The following relationship was obtained based on data from 11 of the 12 compounds (see below):

$$\text{TES} = 26.48 \text{ ClogP} - 0.394 \text{ MV} - 6.673$$

$$r^2 = 0.855$$

$$t \text{ prob. (ClogP)} = 1.759\text{E-}04; t \text{ prob. (MV)} = 0.031$$

The *t* probabilities of the independent variables indicate that ClogP is substantially more important than molecular volume.

The results are presented in graphical form in Fig. 1.

DISCUSSION

Publication of the theoretical basis for QSARS for skin sensitizers (Roberts and Williams, 1982) provides a route of exploration in the search for alternatives to animal assays. In the present work, the sensitization potential of a series of 12 phenyl benzoates has been evaluated. These were designed and synthesized to provide a span of chemical reactivities, but in the knowledge that their mode of action would be the same (i.e. benzylation). All of the chemicals evaluated gave a sensitization response with the data spanning the biological range. A good QSAR was derived using parameters that would be expected to model skin penetration and relative benzylation activity. Of these parameters, those modelling skin penetration proved the most important, particularly logP. Of these 12 compounds, 3-acetylphenyl benzoate was a clear outlier, with an unexpectedly high sensitization response (Table 1) and therefore was omitted from the QSAR. On the basis of the physicochemical parameters, there was no reason to expect that it would react significantly differently from the 4-isomer. However, it was the least pure of all the phenyl benzoate esters tested and a potential impurity (3-methylene-(1'-benzyloxy)phenyl benzoate) would be anticipated to be a potent sensitizer. From its computed logP (5.98) and molecular volume (246.6 Å) the predicted sensitization score of this potential impurity would be 50.7.

The most surprising aspect of this work was the absence of any clear dependence of sensitization on the relative reactivities of the chemicals. The ΔHF values listed in Table 1 show that all of the chemicals tested are reactive, with the nitrophenyl benzoates having the highest reactivity.

The high reactivity of these compounds is, however, negated by their low ClogP values. One conclusion from the present study is that although chemical reactivity is of course a requirement for skin sensitization, it does not determine the response in the tests performed on the present series of compounds.

In conclusion, the present study confirms that within a homologous series of chemicals and where the mode of action can be predicted to be common, it is possible to derive a usable and testable QSAR. It also underlines the importance of skin penetration as a key parameter in determining skin sensitization potential. An important challenge for the future will be to extend the series of compounds and investigate the factors that might allow disparate QSARs for sensitizers to be harmonized.

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