Theoretical Studies to Estimate the Skin Sensitization Potential of Chemicals of the Schiff Base Domain.

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Abstract

Skin sensitization occurs when an exogenous chemical substance forms a covalent adduct with a dermal protein electrophile or nucleophile. This instigates an immune response which leads to inflammation. The local lymph node assay (LLNA) is an in-vivo model used in the assessment of relative skin sensitizing potency of chemicals. The method is time consuming and expensive, as well as poses ethical questions given that a number of mice must be sacrificed for each compound assessed. In this work we investigate the use of an inexpensive, rapid and ethical method to predict the skin sensitization potential of Schiff base chemicals. We employ quantum chemical methods to rationalize the sensitization potential of 22 compounds with a diverse range of activities. To this end we have evaluated the mechanistic profile associated with this type of reaction using gas-phase models. We subsequently use the predicted rate determining barriers and key physico-chemical parameters (such as logP) to establish SAR guidelines to predict the skin sensitization potential for new chemicals. We find that the predicted rate determining barriers for aldehydes, ketone and 1,2 and 1,3 diones generally decrease in the given order, which concurs with the overall trends in sensitization. We find that lipophilicity also plays a role, with those chemicals displaying both low barriers to reaction, and lower lipophilicity (i.e. diones), being more likely to display undesirable skin sensitization effects. These findings are in line with experimental based observations in the literature and point to the value 3D quantum chemical simulations can play in the combination of approaches used to estimate skin sensitization potential of chemicals.

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1.0 Introduction

Skin sensitization is a commonly observed occupational health issue which arises from an immunological allergic response. Skin sensitizers are chemical substances that elicit an allergic response after exposure to the skin, leading to allergic contact dermatitis (ACD).[1] It has been reported that between 15-20% of the general population will suffer sensitization over the course of their lives. The disease is a significant regulatory health concern and has resulted in European Union legislation in the form of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). This legislation requires that the skin sensitization potential of all chemical substances manufactured or imported at level of one ton per annum must be assessed. A further goal of REACH is to increase the use of nonanimal models for chemical assessment.[2, 3]

Skin sensitization arises from the reaction of chemical sensitizer with skin proteins triggering an immune response.[4, 5] A range of different techniques are available to assess the skin sensitization of chemicals. These including in-vivo, in-vitro, in-chemico and in-silico methods have been developed.[6-13] As a result of ethical standards set by REACH legislation there has been an increasing push away from *in vivo* models such as the gold standard *in vivo* murine Local Lymph Node Assay (LLNA),[7] towards *in vitro*methods, such as KeratinoSensTM assay,[14] and *in chemico*alternatives, such as peptide depletion assays.[13] Hoffman *et al.*analyzed 128 compounds with a range of sensitization endpoints find that the LLNA assay shows ˜75% concordance between human results and the LLNA assay, while that for the KeratinoSensTM assay

was roughly comparable.[15] Natsch *et al.* [8] reported that the latter*in vitro* approach showed a 60% concordance with the LLNA methods for a set of 312 chemicals. Hoffman[16] and others[8, 17] report that the most effective strategy to predict skinsensitization potential is to employ a multiple non-animal methods. The former reports that incorporation of essentially orthogonal test strategies comprising *in vitro* , *in chemico*and *in silico* inputs demonstrated the best overall performance, equivalent or superior to the LLNA assay on their curated set of 128 datapoints.[16]

In silico methods are desirable alternatives to *in vivo*models since a prediction on an unknown chemical can be made from its chemical structure alone. While this means the methods generally cost resource and time efficient, they are generally of lower accuracy than their experimental alternatives. *In silico* models can range from similarity or substructural methods[5, 18, 19] that allow the identification of like-molecules with experimental data (read-across) or statistical models that can relate 2D chemical descriptors to a qualitative or quantitative prediction of activity. [20-22] Methods TIMES, Toxtree, Derek Nexus *etc.* [17, 23, 24] have proved useful in compound assessment in their own right,[21, 25] and as part of multi-tiered testing strategies with *in silico* models as the first tier approach.[21, 26]

A number of different statistical models that relate chemical properties to the degree of sensitization have been reported in the literature. Guidelines that all *in silico* models must meet are: (a) a defined endpoint, (b) an unambiguous QSAR model, which is (c), mechanistically interpretable. In addition the model must have (d) predictivity that is fit for purpose and (e) a defined domain of applicability.[5, 27] Notable models include the relative alkylation index (RAI) of Roberts *et al.*[28], models built on individual chemical domain basis[12, 29-32] or global basis.[21, 22, 33, 34] Global models are generally desirable due to their greater applicability domain,[35] however in many cases focusing a QSAR model on individual chemical classes (i.e. Schiff bases, Michael acceptors, SN1/SN2, SNAr, Acyl,*etc.*)[12] we can obtain better "local" performance.[36] These mechanistically interpretable models can offer increased confidence over black box models which may be important in a regulatory situation.

There has been a general trend towards more information rich 3D specific, or use of quantum chemical descriptors in QSAR modelling studies associated with ligand bioactivity.[37-45] This includes the incorporation of dynamical effects *via* descriptors derived from MD simulations[46-48] and interaction energies and conformational energies *via* quantum mechanics (QM).[49-53] and chemical reactivity.[54, 55] Indeed, these trends towards more information rich descriptors have been observed in studies related to skin sensitization prediction given that chemical reactivity can be encoded much effectively with quantum chemical derived descriptors than those that are empirical derived. For example, Miller *et al.* [34] have used semi-empirical HOMO-LUMO energies for their QSAR studies, Enoch *et al.* used density functional theory energies of key reaction intermediate as surrogates for the rate determining barriers of Michael acceptors [55] while Promkatkaew *et al.*fully profiled all intermediates and transition states in the reaction mechanism of SNAr chemicals.[54] Additional efforts have been spent investigating ligand conformational effects on sensitization - given that molecules are not perfectly described by a single conformation. Yu *et al.* have used 4D fingerprints in their studies[33] while Kostal *et al.* have incorporated Monte Carlo conformational sampling in their hybrid QSAR models with good results.[56]

In this work we apply quantum chemical methods to rationalize the sensitization potential of chemicals in the Schiff base (SB) domain (Scheme 1). Roberts *et al.* have previously reported a quantitative mechanistic model to predict the LLNA pEC3 using the Taft σ^* values and logP.[32] We were interested in expanding on this work by (a) employing a more diverse datasets to cover a wider range of SB functional groups as well as (b) investigate whether QM derived estimates of chemical reactivity could prove useful. In our previous work we showed that DFT derived barrier estimates did indeed perform comparably well for the SNAr domain.[54] A key advantage of such methods are that prediction can be made for functional groups where the experimental Taft σ^* values are not readily available. To this end we have collected 22 SB base chemicals covering aliphatic and aromatic aldehydes and ketones, 1,2 diones and 1,3 diones, expanding considerably the domain of applicability of the model over the previous study (11 out of 16 were aliphatic aldehydes). The full reaction energy profile leading to the formation of the 30 possible SB products for the

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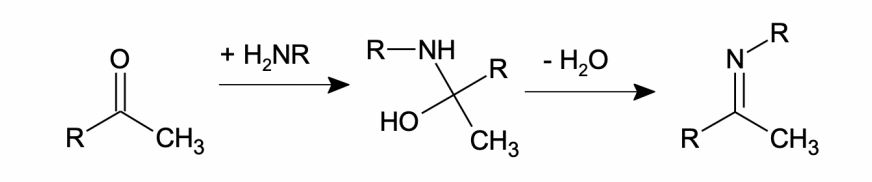
22 compounds, including 8 chemicals where more than one product is possible. The relationship between the rate determining (RDS) barrier to reaction and the LLNA pEC3 was then assessed. Finally we constructed a two parameter quantitative molecular model (QMM) using only the RDS barrier and the computed logP, the latter being another property identified as being important for skin sensitization of SBs.[32]

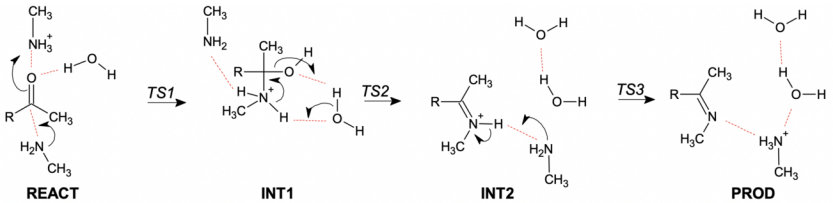


Scheme 1. Formation of the imine (Schiff base) from a carbonyl containing compound proceeds *via* a hemi-acetal/ketal-like intermediate.

2.0 Computational Methods

Twenty two molecules with the potential to react as Schiff bases (Scheme 1) were taken from data reported by ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)[57] and Kern *et al.* [58] as described previously.[54] All SB compounds with non-zero EC3 values were chosen, covering a different chemical substructures (Table 1). Some of the substrates contained chemical functionality associated with other chemical domains (SNAr, SN2 and Acyl) or had more than one position capable of forming a SB product. The compounds can be sub-classified as aliphatic and aromatic aldehydes, ketones, 1,2 diones and 1,3 diones. In the case of 1,2 and 1,3 diones, each molecule contains two carbonyl groups leading to two plausible points for nucleophilic attack.

Calculations were performed using a model consisting of the substrate, two water molecules and two methylamine molecules to approximate the biological reaction. The simulated reaction leading to the imine product (Schiff base) involves three distinct steps as shown in Scheme 2. The first step involves nucleophilic attack of the substrate by methylamine giving rise to a carbinolamine or hemiacetal-like intermediate (INT1). Proton transfer from the protonated intermediate, *via* water, leads to the elimination of water and the formation of the protonated imine product (INT2). The final step involves the deprotonation of INT2 to give the deprotonated imine (PROD).



Scheme 2 Individual sequences simulated in this study; (a) substitution by an amine nucleophile (b) elimination of water to form the imine and (c) deprotonation.

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All calculations were performed using DFT method implemented in the Gaussian G09. [59] All structures were optimized using hybrid meta exchange-correlation functionals, M06-2X with a 6-311+G(d,p) basis set.[60, 61] The reaction mechanism associated with SB formation was studied in the gasphase following the mechanism described in Scheme 2. In this model, a methylamine molecule acts as both the nucleophile and base in the reaction. Two explicitly modelled water molecules provide additional stabilization and facilitate proton transfer steps. The vibrational frequency calculations were performed to confirm all transition states (TS). TSs are characterized by having a single imaginary frequency and minima having none. The rate determining barrier were subsequently determined. Additional substrate properties (MWT and clogP) were obtained using the Chemaxon JChem.[62]

The correlation between independent and dependent variables was assessed using linear regression. The data was partitioned into a training (M=14) and test set (N=8) and a QMM was fitted using the RDS barrier and the logP using multiple linear regression. All statistical analyses were performed in Statistica 12.[63]

3.0 Results and Discussion

The reaction energetics for compounds ***1-22*** are displayed in Table 1. The compounds simulated include aldehydes, ketones, 1,2 diones and 1,3 diones. In addition, some molecules contain more than one position that can undergo Schiff base (SB) reaction and the results for these positions are noted.

Table 1: Structures, IDs and energetics associated with SB formation for compounds 1-22 . Energy reported relative to the reactant in kcal/mol. Absolute barriers are given in parenthesis.

ID	Name	Structure	CAS-No.	TS1	INT1	TS2	INT2	TS3	PI
1	6-methoxy-2,6-dimethyloctanal		929253-05-4	0.75	-12.75	15.39 (28.14)	-8.85	-7.60 (1.25)	-10
2	3-chloro-4-methoxy benzaldehyde		4903-09-7	2.42	-10.20	19.44 (29.63)	-8.45	-5.87 (2.58)	-9.
3	benzaldehyde		100-52-7	1.62	-11.38	18.68 (30.06)	-7.55	-6.72 (0.84)	-10
4	1-(3-chlorophenyl) propan-1-one		34841-35-5	6.14	-4.40	25.15 (29.55)	-1.35	-0.78 (0.57)	-2.
5	5-methylhexane-2,3-dione		13706-86-0	1.67	-13.01	20.63 (33.64)	-1.04	0.38 (1.42)	-9.
				3.09	-9.68	20.24 (29.92)	-4.61	-4.60 (0.01)	-8.
6	2-methyl-1-phenyl butane-1,3-dione		6668-24-2	3.07	-0.98	27.35 (28.33)	3.81	3.94 (0.12)	2.0
				14.58	-3.92	21.15 (25.07)	-2.42	-2.16 (0.26)	-4.
7	6-nonenal		2277-19-2	1.36	-11.14	15.33 (26.47)	-7.08	-6.67 (0.41)	-9.

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ID	Name	Structure	CAS-No.	TS1	INT1	TS2	INT2	TS3	PI
8	2,2,6,6-tetramethyl heptane-3,5-dione		1118-71-4	7.37	3.66	25.33 (21.66)	-2.56	-2.43 (0.13)	-6.
9	butane-2,3-dione		431-03-8	1.24	-14.24	19.82 (34.06)	-0.81	-0.68 (0.13)	-2.
10	4,4,4-trifluoro-1-phenylbutane-1,3-dione		326-06-7	-*	-	24.31 (24.31)	11.76	12.43 (0.67)	-5.
				25.86	14.56	37.24 (22.68)	7.93	7.96 (0.03)	5.5
11	2-methylundecanal		110-41-8	0.87	-12.32	14.87 (27.19)	-7.03	-6.08 (0.96)	-10
12	2-phenylpropanal		93-53-8	1.42	-11.88	17.25 (29.13)	-7.57	-2.20 (5.37)	-11
13	undec-10-enal		112-45-8	1.35	-11.24	15.55 (26.79)	-7.13	-6.65 (0.48)	-9.
14	1-(2,3,4,5-tetramethylphenyl)butane-1,3-dione		167998-73-4	7.65	0.02	20.70 (20.67)	2.51	5.40 (2.89)	-3.
				18.02	2.22	27.91 (25.69)	-6.23	-5.72 (0.52)	-6.
15	2-phenyl acetaldehyde		122-78-1	2.03	-9.57	18.34 (27.91)	-6.74	-0.98 (5.76)	-10
16	glyoxal		107-22-2	-*	-	31.54 (31.54)	-**	-	-2.
17	methyl 2-oxopropanoate		600-22-6	1.14	-3.64	17.85 (21.49)	0.45	2.21 (1.75)	-3.
				13.08	10.53	37.43 (26.90)	9.92	12.83 (2.91)	12.
18	2-bromo-5-hydroxy benzaldehyde		2973-80-0	1.71	-12.97	17.88 (30.85)	-6.30	-5.21 (1.10)	-11
19	N-(4-methoxyphenyl)-3-oxobutanamide		5437-98-9	4.10	-7.59	16.87 (24.45)	3.15	6.05 (2.91)	-2.
				16.10	10.46	39.45 (28.99)	-0.67	-0.62 (0.06)	-0.
20	acetyl benzoyl		579-07-7	3.83	-12.58	22.51 (35.09)	-1.83	-1.24 (0.59)	-1.
				8.35	-8.38	21.76 (30.14)	4.61	5.30 (0.69)	1.7
21	glutaraldehyde		111-30-8	7.56	-2.94	23.03 (25.97)	-5.28	-5.16 (0.12)	-6.

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ID	Name	Structure	CAS-No.	TS1	INT1	TS2	INT2	TS3	PI
22	1,3-butanedione, 1-phenyl-		93-91-4	9.30	-3.04	23.87 (26.91)	5.35	11.70 (6.35)	-0.
				21.30	9.88	28.62 (18.74)	3.35	3.51 (0.16)	1.6

* no barrier to formation of INT1 from REACT, ** no barrier to formation of PROD from INT2

3.1 General Reaction Scheme

The reaction simulated is described in Scheme 1 where methylamine is used to simulate a generic amine nucleophile. The reaction involves an acid catalyzed process through the inclusion of a methylammonium cation and two water molecules. The first step sees the SN2 based substitution of the carbonyl of the substrate by the methylamine, the breaking of the carbonyl double bond with concomitant proton transfer from the methylammonium cation to give the corresponding carbinolamine. As can be seen from Figure 1, the transition state (TS1) associated with the nucleophilic attack at position-a of compound 22 has a short C-N nucleophilic distance of 2.0 Å. However, it does not involve noticeable proton transfer to the oxyanion being formed, this occurring after the barrier has been traversed. Furthermore, it is found that nucleophilic attack of the carbonyl directly connected to the phenyl ring (position-b) results in a dramatically higher barriers to reaction (9.3 vs 21.3 kcal/mol) as a result of the loss of resonance effects with the carbonyl bond not observed at the other position. This also helps to explain why the corresponding intermediate at position-a is considerably lower in energy (-3 vs 9.9 kcal/mol).

Despite position-a having the lowest barrier to substitution, it is found that position-b has the lowest barrier associated with the elimination of water and the formation of the imine. This can be explained by the resonance stabilization afforded by the phenyl ring attached to position-b. The reaction proceeds with shuttling of a proton from the amine of the carbinolamine intermediate to its hydroxy group*via* a water molecule. The proton transfer step is essentially complete at the transition state with the C-O carbinolamine bond being 1.5 Å. The barrier to reaction for position-a is found to be 26.9 kcal/mol compared to 18.7 kcal/mol for position-b. The final step in the reaction requires the abstraction of the remaining proton on the imine nitrogen to generate the neutral product. This step is found to be a low energy process (˜0.1-10 kcal/mol) and sees the regeneration of the methylammonium catalyst.

All compounds simulated follow the same general trends as compound 22 . Two exceptions are compounds 10 and 16 which do not display a barrier to substitution (TS1) for positions containing strongly electron withdrawing groups. Furthermore, 16 forms product directly from INT2 without the formation of INT3. More detailed analysis of the complete reaction profiles of all molecules in Table 1 showed clearly that the barrier to water elimination (TS2) was observed to be the rate determining step for the conversion of the substrates to the corresponding SB. The TS2 barrier heights for the main chemical classes present ranged from ˜ 18-25 kcal/mol for 1,3 diones (6), ˜21-34 kcal/mol for 1,2 diones (5), ˜25-32 kcal/mol for aliphatic aldehydes (7), ˜29-30 kcal/mol for aromatic aldehydes and ketones (4).

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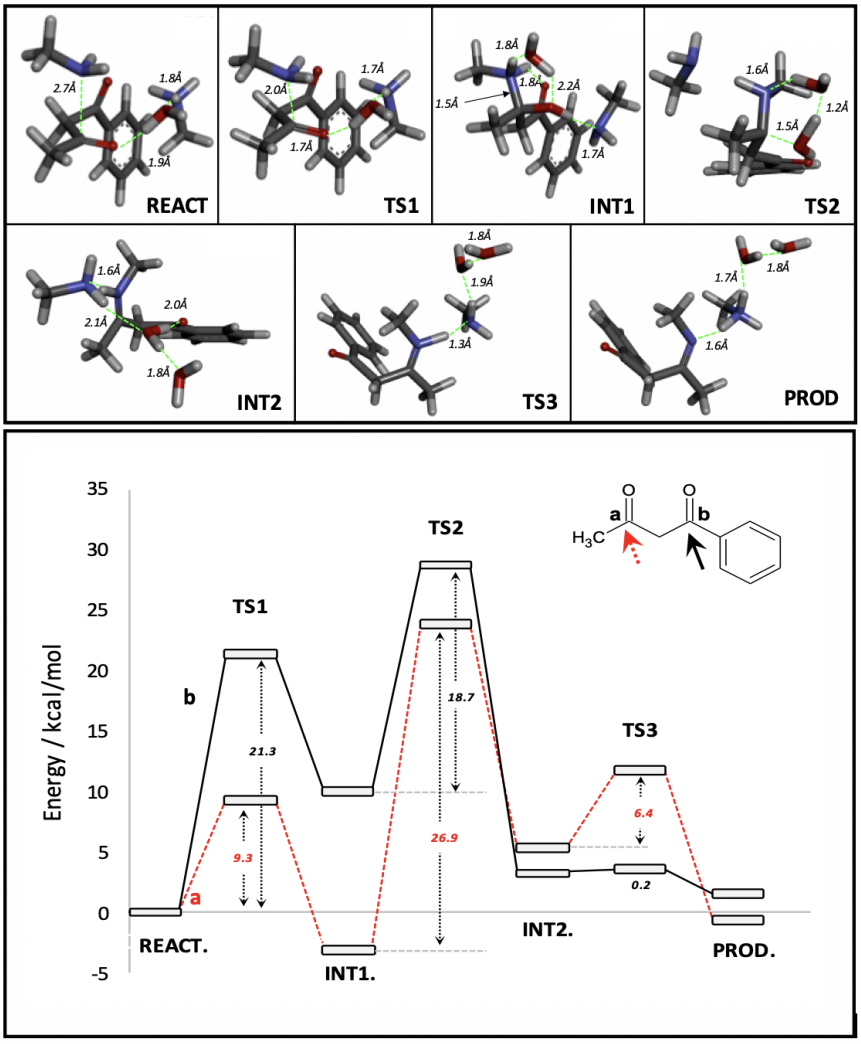

Figure 1: Optimized 3D structures for 22 (position a only, top) and the corresponding energetic profile.

A subset of molecules contain additional SNAr functionality (2 , 4 & 18)[54] and Michael acceptor groups (7 & 13).[55] We also predicted the SNAr reactivity of compounds 2 , 4 and 18 using our previously reported QM-QSAR method (Figure 4).[54] All 3 compounds were re-simulated using the methodology previously described and it was observed that only compound 18 was predicted to have a barrier to reactivity likely to result in a significant LLNA response. Compound 18 , which contains bromide leaving group was predicted to have a barrier of 10.5 kcal/mol while compounds 2 and 4 , the chloride leaving groups, have predicted barriers of >16 kcal/mol. Compounds 7 and 13 were not simulated as they consist of Michael acceptors connected to aliphatic carbons and couples with their small solvent accessible surfaces are not predicted to be sensitizers.

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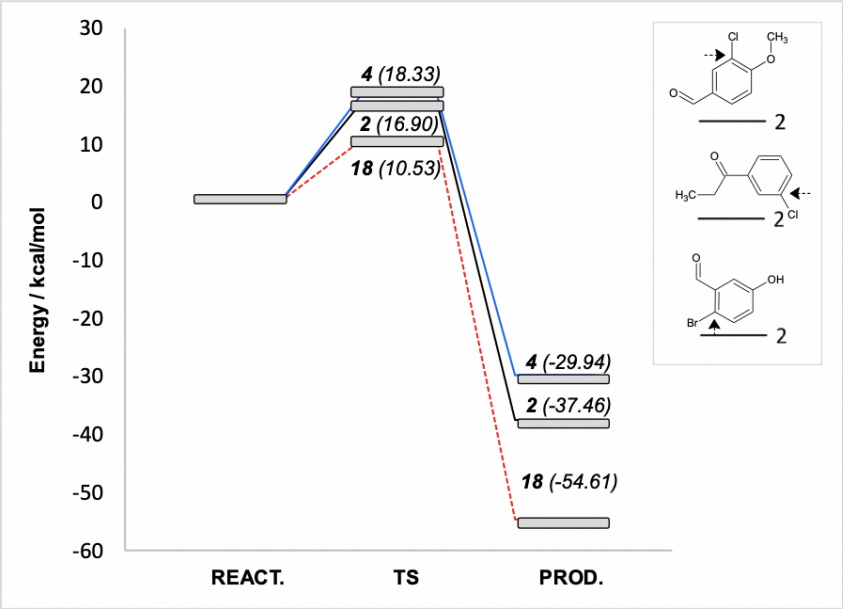

Figure 2. Plot of the SNAr reaction profiles for compounds 2 , 4 and 18 .

It should be acknowledged that such analyzes are further complication by the fact that the LLNA response is not always a result of direct sensitization the molecule itself, rather an effect of reactive metabolites. This issue further complicates the construction and validation of computational methodologies such as these reported here.[21, 64] In particular, it is known that 1,2 diones such as compound 16 , glyoxal are not commonly encountered because it can form a hydrate, which can then undergo oligomerization.

3.2 Reaction Barriers vs LLNA pEC3

The goal of the present work is to determine whether it is possible to attribute the skin sensitization to the predicted chemical reactivity of the functional groups present the molecules ***1-22*** . We first assessed whether a relationship existed between the predicted barriers to reaction and the skin sensitivity. The expectation was that TS2 should show the most significant correlation with the LLNA pEC3 given that this was almost exclusively the rate determining barrier for the molecules studied here. Indeed we did see the strongest correlation between the TS2 (lowest value of the reported positions if more than one) and the pEC3. However, when all 22 compounds were included, the observed correlation coefficient squared (r2) was just 0.16. This prompted us to re-evaluate the data by excluding compound 18 , which is a predicted to be a strong sensitizer *via* the SNAr domain, and the 1,2 dione compound (***5, 9, 16, 17, 20***), which potentially exist in their hydrated form. This is similar to the approach of Roberts *et al.* who focused on compounds with a single reactive group consisting primarily of aldehydes and ketones and may point to a need for a more restricted domain of applicability.[32]

Re-analysis of the correlation between the 16 remaining compounds and the pEC3 reveals an improved, albeit weak correlation, with an r2 of 0.36. It is found as the predicted barrier to reaction decreases, the experimental sensitization is found to increase in line with expectation (Figure 2). It can also be seen from Figure 3 there is a lipophilicity effect operating within the dataset. Compounds, which have been colored by logP, show a noticeable trend in that, those with a higher logP generally have lower pEC3 for a given barrier. For example, the 1,3 dione 8 has a predicted barrier of approximately 22 kcal/mol compared to 19 which has a barrier of ˜25 kcal/mol (19 a). This would suggest that 8 should be a stronger sensitizer than

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19 , whereas in fact the opposite is observed (pEC3 of 0.84 vs 1.97, respectively). The predicted logP of 8 is 3.93 while that of 19 is 1.28 suggesting that a lower barrier combined with a low logP leads to increased sensitization for this set of diverse compounds.

This observation apparently contradicts the findings of the earlier QSAR study of Roberts *et al.* on Schiff bases.[32] However, since this publication, the authors have reported an extensive analysis of 525 substances and note that logP is not simply a surrogate for permeability and that higher logP values do not result in higher sensitization on a diverse set of compounds.[65] The result is also consistent with observations that the Schiff base, formaldehyde (logP=-0.51), which is known to be highly sensitizing in sensitization based-assays.[66] It should be noted that we are using a somewhat larger (22 vs 16 compounds), and more diverse set of chemicals (70% were aldehydes in the latter study). Indeed evidence for similar series specific-logP behavior can be seen from the non-linear relationship between logP and pEC3 for azlactones of the acyl domain.[5]

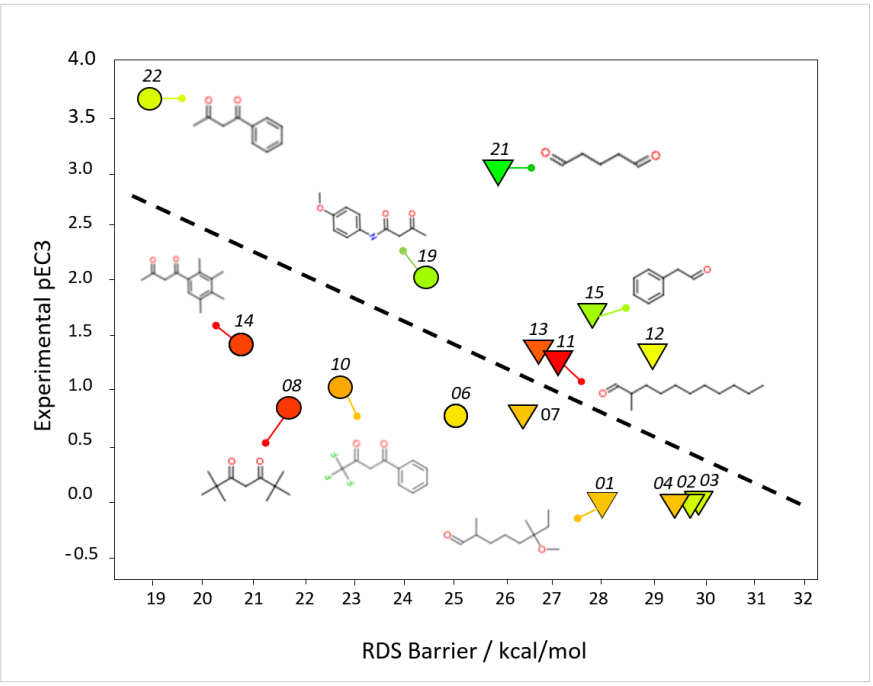

Figure 3: Plot of pEC3 versus the predicted rate determining barrier for aldehydes (triangle), ketones and 1,3 diones (circles). Data is colored by logP (red=high, green=low). R2=0.36, n=16.

3.3 Quantitative molecular model

Other factors such as clogP have been included in a number of QSAR models related to LLNA. Given that the parameter also appeared possible in further differentiating between active and inactive compounds as shown in Figure 3, an additional 2-parameter linear regression model was constructed on the data. The goal here is to produce a conservative model using small numbers of descriptors that have known relevance to skin sensitization. This is in part due to the REACH requirements for simple, robust and interpretable QSAR models for use within a regulatory framework, but also due to the small numbers of datapoints available for such modelling exercises.

Starting with all 22 compounds, a multiple linear regression model was constructed with the two variables resulting in equation 1. The model results in an explained variance of 66% with a p value of 0.008, consider-

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ably better than that observed with the energy barrier associated with TS2 alone. The model predicts that as the barrier to reaction decreases, pEC3 will increase. In addition, as the logP decreases, the barrier to reaction will concomitantly decrease.

pEC3=-0.377(±0.138)*clogP – 0.127(±0.0436)*ETS2 + 5.69 (±1.30) ***Equation 1***

n=22, r2=0.66, r2adj=0.38,. p=0.008)

Subsequently, the known SNAr compound 18 , was excluded from the analysis and the logP of 20 was corrected due to large difference between the clogP value used here (1.82) and two other commonly used methods (0.58 for clogP[67] and 0.82 for ACD[68]). The latter value, intermediate between the two other value was used. SNAr compounds 2 and 4 were not excluded as they are not predicted to function *via* an SNAr mechanism according to the predicted QSAR methodology of Promkatkaew*et al.* [54] The updated multiple regression model was developed for the 21 compounds as shown in equation 2. The model has slightly improved prediction statistics with an r2=0.71 and a p-value of 0.002. Again, it is found that both increasing logP and barrier height lead to decreased sensitization.

pEC3=-0.425(±0.124)*clogP – 0.152(±0.0412)*ETS2 + 6.17 (±1.22) ***Equation 2***

n=21, r2=0.71, r2adj =0.46, p=0.002

As a final exercise, the dataset of 22 compounds was partitioned into a training (N=14) and test set (N=8). As with the work of Roberts *et al.* , we generated the QSAR model on a set consisting primarily of nonfunctional aldehydes and ketones to avoid confounding effects. A small number of additional exemplars that help cover the full range in pEC3 were also included (Table 2). All compounds were predicted using equation 3. Compounds **2** , **4** and 18 were also predicted using our previously reported model for SNAr domain.[54] Again, a two-parameter model was fitted using the training data resulting in equation 3. The training set explained variance is somewhat lower than observed with the larger combined set (r2=0.40), however the descriptor coefficients are qualitatively similar. Prediction on the test set of compounds show the compounds are quite well ranked (r2=0.49). A noticeable outlier in figure 3 is compound 17 which on further analysis of the structure can also potentially function *via* the acyl reaction domain.[5] This could account for its low predicted activity from this Schiff-base derived model. When compounds 20 (SNAr domain) and 17 (Acyl domain) are excluded from the test set, r2 of 0.62 is observed.

pEC3 = -0.388(±0.150)*clogP – 0.172(±0.061)*ETS2 + 6.671 (±1.841) ***Equation 3***

Training set (n=14, r2=0.49, r2adj=0.40, p=0.02),

Test set (n=8, r2=0.49), Test set (n=6 (ex ***17*** & ***20***), r2=0.62)

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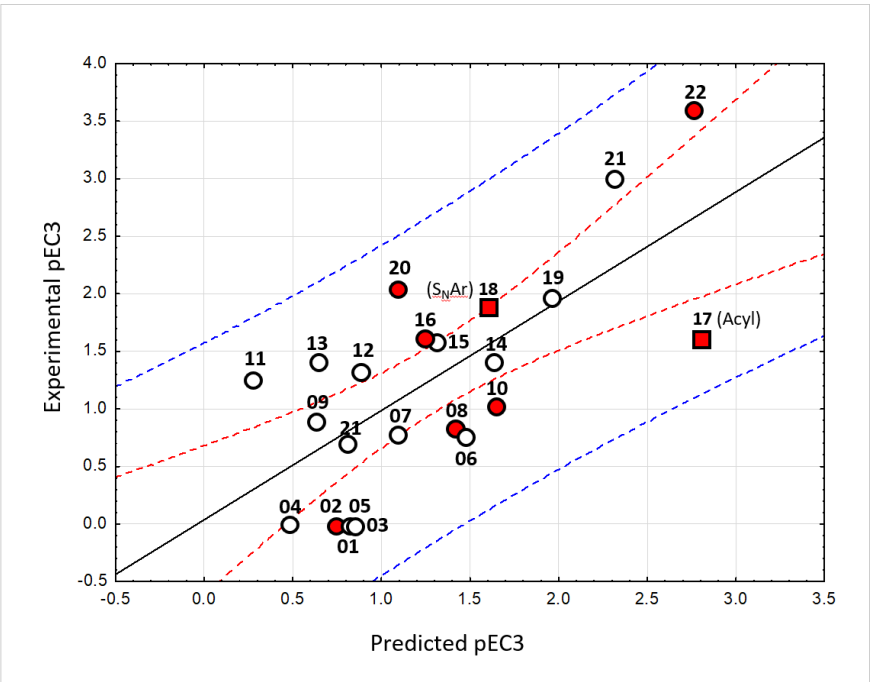

Figure 4: Plot of the observed vs predicted pEC3 using equation 3 for the training set (white circles) and test set (red circles). Value for test set compound 20 using previously reported SNAr model.[54]

Table 2: Descriptors, observed and predicted pEC3 values from equation 3.

ID	CAS No.	Chemical Class	pEC3	RDS Barrier	clogP	Predicted pEC3	Set
01	929253-05-4	Aliphatic aldehyde	0.00	28.14	2.62	0.81	Train
02	4903-09-7	Aromatic aldehyde	0.00	29.63	2.13	0.74 (0.00)a	Test
03	100-52-7	Aromatic aldehyde	0.00	30.06	1.69	0.84	Train
04	34841-35-5	Aromatic Ketone	0.00	29.55	2.84	0.48 (0.00)a	Train
05	13706-86-0	1,2 dione	0.69	29.92	1.83	0.81	Train
06	6668-24-2	1,3 dione	0.78	25.07	2.30	1.46	Train
07	2277-19-2	Aliphatic aldehyde	0.80	26.47	2.62	1.09	Train
08	1118-71-4	1,3 dione	0.84	21.66	3.93	1.42	Test
09	431-03-8	1,2 dione	0.89	34.06	0.40	0.65	Train
10	326-06-7	1,3 dione	1.03	22.68	2.89	1.64	Test
11	110-41-8	Aliphatic aldehyde	1.27	27.19	4.42	0.27	Train
12	93-53-8	Aliphatic aldehyde	1.33	29.13	2.00	0.88	Train
13	112-45-8	Aliphatic aldehyde/MA	1.39	26.79	3.57	0.67	Train

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ID	CAS No.	Chemical Class	pEC3	RDS Barrier	clogP	Predicted pEC3	Set
14	167998-73-4	1,3 dione	1.42	20.67	3.81	1.63	Train
15	122-78-1	Aliphatic aldehyde	1.60	27.91	1.45	1.30	Train
16	107-22-2	1,2 dione	1.62	31.54	0.00	1.24	Test
17	600-22-6	1,2 dione	1.63	21.49	0.45	2.80	Test
18	2973-80-0	Aldehyde / SNAr	1.89	30.85	2.15	1.60a (0.52)	Test
19	5437-98-9	1,3 dione	1.97	24.45	1.28	1.96	Train
20	579-07-7	1,2 dione	2.06	30.14	1.82 (0.82)b	1.10b (0.77)	Test
21	111-30-8	Aliphatic aldehyde	3.00	25.97	-0.27	2.30	Train
22	93-91-4	1,3 dione	3.61	18.74	1.75	2.76	Test

(a) pEC3 predicted based on its SNAr potential (pEC3 = -0.31*SNAr barrier + 4.90)[54] b pEC3 predicted using ACD logP

As highlighted by other researchers the prediction of skin sensitization is a highly challenging process and that the concordance between different *in vivo* , *in vitro* and *in chemico* based methods is often no more than 60-80% in agreement with gold-standard methods such as the LLNA assay.[8, 15, 16] This is in part due to the fact that the LLNA response is not always a result of direct sensitization the molecule itself, potentially arising as a function of reactive metabolites.[21, 64] In this case we report a purely theoretical approach to predict a diverse set of Schiff base chemicals – using just two relatively simple and interpretable descriptors. The observation that the model can explain ˜50-60% of the variance in the dataset is therefore not surprising.

4.0 Conclusions

In this paper we have reported the use of a quantum chemical-based approach to assess the skin sensitization potential of chemicals from the Schiff base domain. We have evaluated the mechanistic profile associated with 22 SB substrates using a model consisting of two methylamine and two water molecules. We find that calculating the full reaction profile for the chemicals is important as the substrates can often react in more than one position, while also allowing for mechanistic exceptions to be uncovered. We find that the use of a single computed descriptor, namely the rate determining barrier to formation of the SB product can help us to separate sensitizer and non-sensitizer. A RDS barrier of ˜28 kcal/mol indicate that a molecule is unlikely to act as a sensitizer. We also observed that compounds with low barriers, but higher logP values show reduced sensitization prompting us to generate a 2 parameter QMM.

A QMM equation established suggests that SB of lower logP have a greater propensity to react resulting in r2 of 0.50-0.60. The predicted RDS and logP establish SAR guidelines to rationalize the skin sensitization potential. The RDS barriers for aldehydes, ketone, 1,2 and 1,3 diones broadly decrease in that order, in line with their increasing experimental sensitivity. These findings agree with experimental based observations in the literature and point to the value computational methods can play in skin sensitization predictions. We find that the rate determining barrier and the computed lipophilicity can be used to estimate the skin-sensitization of unknown compounds. This orthogonal source of information could prove useful in consensus based predictions of likely sensitization potential.[21, 26]

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The results presented here show that 3D quantum chemical simulation of SB chemicals, while useful, will lead to the mischaracterization of some compounds. This is not so different to the variation observed between the different types of *in vivo* , *in vitro* and *in silico* methods reported to date which show predictions accuracies of no more than 70-80%. This is simply a reflection of the complex event being simulated, a multitude of potential protein targets, and the fact that the molecules may function in the form of a metabolite rather than the dosed substrate. The utility of such simulations is that physical insight and understanding can be garnered which could prove useful, especially when combined in the so–called weight of evidence approach with other methods.

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