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ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · JANUARY 2014

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Skin Sensitization Prediction Using Quantum Chemical Calculations: A Theoretical Model for the S_NAr Domain

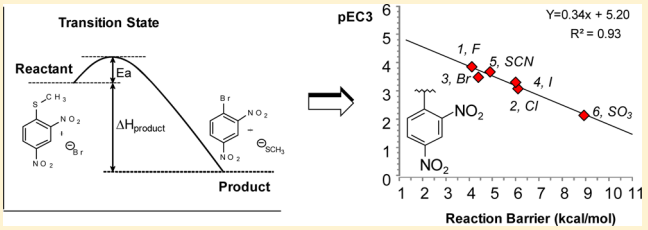
Malinee Promkatkaew,[†] Duangkamol Gleeson,[‡] Supa Hannongbua,[†] and M. Paul Gleeson*[,†]

[†]Department of Chemistry, Faculty of Science, Kasetsart University, 50 Phaholyothin Road, Chatuchak, Bangkok 10900, Thailand

[‡]Department of Chemistry, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

Supporting Information

ABSTRACT: It is widely accepted that skin sensitization begins with the sensitizer in question forming a covalent adduct with a protein electrophile or nucleophile. We investigate the use of quantum chemical methods in an attempt to rationalize the sensitization potential of chemicals of the S_NAr reaction domain. We calculate the full reaction profile for 23 chemicals with experimental sensitization data. For all quantitative measurements, we find that there is a good correlation between the reported pEC3 and the calculated barrier to formation of the low energy product or intermediate (r^2 = 0.64, N = 12) and a stronger one when broken down by specific subtype (r^2 > 0.9). Using a barrier cutoff of ∼10 kcal/mol allows us to categorize 100% (N = 12) of the sensitizers from the nonsensitizers (N = 11), with just 1 nonsensitizer being mispredicted as a weak sensitizer (9%). This model has an accuracy of ∼96%, with a sensitivity of 100% and a specificity of ∼91%. We find that the kinetic and thermodynamic information provided by the complete profile can help in the rationalization process, giving additional insight into a chemical's potential for skin sensitization.



1.0. INTRODUCTION

Contact dermatitis is a common environmental and occupational health concern that arises from exposure to certain chemical substances. Contact dermatitis can be caused by the physical effects of chemical irritants on tissue directly (irritant contact dermatitis, ICD), which includes solvents, acids, or bases. An irritation may also result from a more extreme allergic response (allergic contact dermatitis, ACD), a complex phased response of the immune system to an allergen.[1] Experimental methods for the detection of sensitizers include the guinea pig maximization test (GPT) and the more recent murine local lymph node assay (LLNA).[2] The LLNA assay is now the method of choice following extensive validation and has been adopted by the OECD as a standard protocol.[3] The assay works by identifying compounds with the capacity to provoke a T lymphocyte proliferative response within the lymph nodes. Chemicals are classified as sensitizers if they show a 3-fold or greater proliferative response in the induced draining in lymph nodes compared with controls.[3] While the EC3 is not an absolute response, it can be used to rank order compounds in terms of their relative toxicity. EC3 can be subclassified into strong, weak, and moderate sensitizers as shown in Table 1. According to the European Union's Registration, Evaluation, Authorization and Restriction of Chemical Substances Regulations (REACH), greater effort is needed to reduce the numbers of animals and the costs associated with toxicity testing. This requires the greater use of chemical assay surrogates[4,5] and theoretical methods such as QSAR models and read-across methods.[6,7]

Table 1. EC3 Cut-Offs Used to Classify the Sensitization Potential of Chemicals in the LLNA Assay

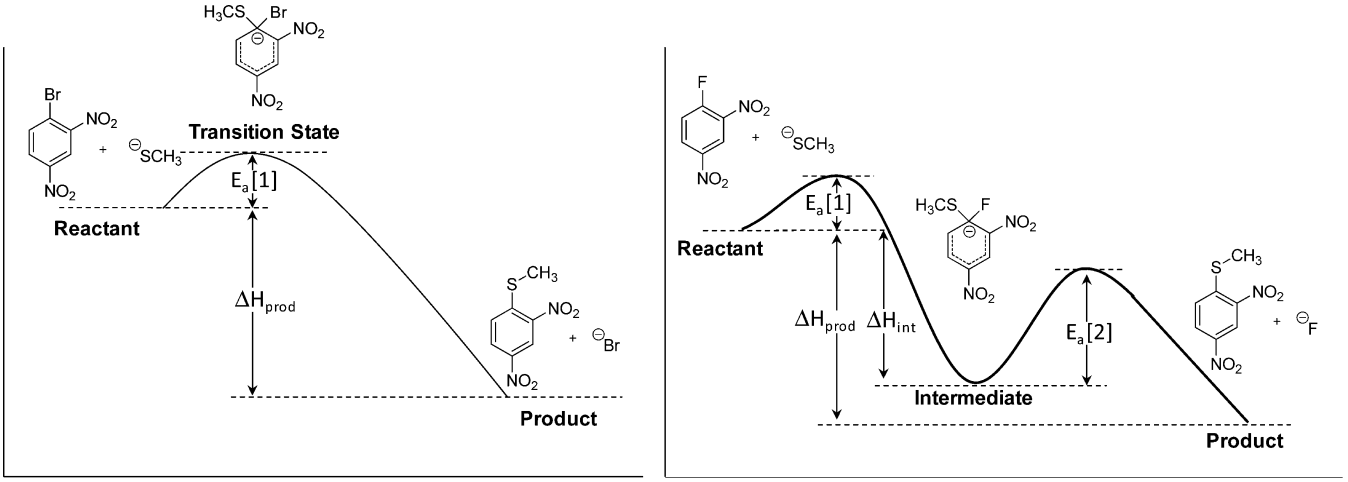
potency classification	EC3 value (% weight)
nonsensitizer	NR
weak	≥10 to ≤100
moderate	≥1 to <10
strong	≥0.1 to <1
extreme	≤0.1

Skin sensitization begins with the sensitizer in question forming a covalent adduct with a protein electrophile or nucleophile. From the pioneering work in this field by Roberts and Aptula, skin sensitizing chemicals can be assigned to 5 separate chemical classes (or domains) capable of causing protein adducts: aromatic nucleophilic substitution (S_NAr), Schiff base formation (SB), Michael-type addition (MA), aliphatic nucleophilic substitution (S_N2), and acylation (Acyl).[8,9] The presence of structural or reactive features alone are not reliable indicators of toxicity,[10,11] which is perhaps unsurprising given that a classification scheme neglects the overall molecular and local electronic characteristics of a molecule and the fact that a degree of target recognition may be present.

Attempts to develop truly global (i.e., covering a wide diversity of sensitizer types) quantitative structure−activity

Received: September 5, 2013
Published: January 10, 2014

Scheme 1. Two Possible Nucleophilic Aromatic Substitution Reaction (S_NAR) Profiles for Chemicals in This Study[a]



[a]Addition–elimination via concerted (right) and stepwise processes.

relationships (QSAR), either by relative alkylation index (RAI) approaches[12] or by theoretical descriptor-based QSAR approaches,[13,14] have not yet met with sufficient success to conform with the complete set of OECD QSAR guidelines.[6,15] These guidelines are (a) a defined end point and (b) an unambiguous QSAR model, which is (c) mechanistically interpretable. In addition, the model must have (d) predictivity that is fit for the purpose and (e) a defined domain of applicability for which the model can be used. QSAR models that currently best fit the OECD principles are termed quantitative mechanistic models (QMM). These are restricted to chemicals from an individual reaction domain and thus resemble the simple but very functional QSARs first reported by Hansch and Fujita in the 1960s.[16] These QSAR methods typically make use of experimentally derived physicochemical descriptors and are generally accurate for the particular chemical series under investigation.[5,8,9,17,18] However, given that these methods rely on experimentally derived descriptors (i.e., σ electronic and π steric parameters), novel compounds cannot be predicted without first determining these parameters directly if they are not already known. Thus, a QMM-like approach based on purely theoretical methods would therefore be desirable if it could match the performance of that obtained with experimentally derived descriptors.

In a recent paper, Enoch and Roberts reported the development of a theoretically based QMM. This method relied on quantum chemical (QC) and an empirically based molecular descriptor to derive an LLNA QSAR for Michael acceptors.[19] The authors approximated the rate determining barrier to reaction by using the energy of the high energy intermediate formed following the attack of a substrate by the negatively charged nucleophile (i.e., relying on the Hammond postulate to estimate the barrier),[20] and included an additional solvent accessible surface area term in their equation. This QC based protocol appears to be a significant improvement over the HOMO-LUMO estimate often used as a surrogate for reactivity.[14] The model led to good discrimination between sensitizers and nonsensitizers for 26 compounds, with only 4 outliers.

In this article, we investigate the use of QC methods to rationalize the sensitization potential of chemicals. We start by collating the LLNA data in the literature to assess the prevalence of skin sensitizers within the different reaction domains. On the basis of this analysis, we then focus on the most problematic domain. The electrophilic reactivity of the S_NAr domain, which was identified as the most toxic of all the five domains, is determined by a combination of the effects of the leaving group X and the activating groups Y. The reaction can occur when X is any halogen or pseudohalogen or a range of other groups which are not usually considered as good leaving groups (NO_2, SO_2Ph, SOPh, and SO_3^-). We calculate the full reaction profile for 23 chemicals reported in the QMM study of Roberts et al.,[21] providing complete details of the reaction kinetics and thermodynamics using a model sulfur nucleophile (Scheme 1). This is because it is not clear how the reactivity of the chemicals are influenced by kinetic and thermodynamic factors. Thus, computing the complete energy profile is preferable to estimate reactivity. We then use the kinetic and thermodynamic data to try and rationalize the experimentally reported sensitization results for the compounds in question. We also compare the discriminating potential of an approximated barrier to the S_NAR domain, as used by Enoch and Roberts for the MA domain.[19] In addition, we assess the performance of the commonly used HOMO–LUMO band gap descriptor. We are particularly interested in determining whether the extra cost of the detailed profile is in any way beneficial over the latter two more approximate representations of reactivity.

2.0. COMPUTATIONAL PROCEDURES

Three different data sets were extracted in order to determine whether the chemical application affects the prevalence of chemical sensitizers. Topical drugs were obtained from ChEMBL,[22] and the top 200 drugs (primarily oral) were taken from Stepan et al.[10] The LLNA data set was created from 3 sources. Four hundred forty-three unique chemicals with LLNA test information were obtained from (1) ICCVAM (Interagency Coordination Committee on the Validation of Alternative Methods),[23] (2) Kern et al.,[24] and (3) Enoch et al.[25] The data was merged and cleaned using the following protocol: CAS numbers and/or smiles were rechecked; LLNA data for smiles duplicates were averaged; and compounds with contradictory measurements were excluded. The EC3 values of the 296 compounds with quantitative EC3 values were converted to the molar logarithmic parameter pEC3 (−log (MWT/EC3)). Compounds were assigned to a reaction domain using SMARTs rules created by Enoch et al.[25] recoded in Pipeline Pilot 6.1,[26] as well as manually for the purpose of

dx.doi.org/10.1021/tx400323e | Chem. Res. Toxicol. 2014, 27, 51–60

comparison. Manual assignment was only performed in cases where it was not previously reported.[25] Compounds that were unambiguously assigned to a single reaction class were used for categorization analyses (~83%).

The S_NAr domain chemicals under QC investigation consist of the 23 halo- and pseudohalobenzenes from the publication of Roberts et al.[21] Of these, 12 are reported skin sensitizers and 11 are nonsensitizers. An $^-SCH_3$ model of a typical cysteine nucleophile was used to simulate the reaction profile for these 23 compounds in line with others.[19] We evaluate the following states for each chemical: (a) the isolated reactants, (b) the nonbonded reactant complex, (c) the bonded intermediate complex (if stable), (d) the nonbonded product complex, and (e) the isolated products, as well as (f) the transitions state(s) connecting the different states. The reaction coordinates of each chemical were modeled using a density functional theory (DFT) based QC model in Gaussian 09, revision C01.[27] In this case, we use the extensively validated M062X functional developed by Truhlar and co-workers,[28−30] in conjunction with the 6-31+G(d,p) basis set. Stationary points were confirmed as such using vibrational frequency analysis. Transition states were confirmed as having a single negative frequency, while minima were confirmed to have none. Calculated properties (MWT and clogP) were obtained using the Chemaxon JChem software package.[31]

3.0. RESULTS AND DISCUSSION

We were interested in assessing whether chemicals used in a particular application (consumer vs pharmaceutical for example) are more likely to display differences in reactivity alerts. For example, it might be expected that drugs will be more carefully screened for reactive features (e.g., potentially leading to drug−drug interactions[32]) than chemicals used in manufacturing or consumer products due to the high systemic concentration generally achieved.[33] All compounds were therefore assigned to their chemical domains using the SMARTs developed by Enoch et al.[25] We first assessed the concordance between manual and in-silico assignment of all chemicals in the LLNA data set, which had been assigned using both methods. For cases where a single, unambiguous assignment can be made by both methods (~70% of all 443 compounds), it was observed that the in-silico assignment was correct ~82% of the time. While lower than the reported statistics for the original 210 compounds used to generate the SMARTs patterns,[25] the performance of the method has still remained high.

Compounds in the oral drug data set are expected to achieve higher systemic exposure than the topically applied drug data set. The LLNA data set differs from the former two as it contains primarily nondrug-like compounds used in consumer products. Indeed, Figure 1 shows that the three different data sets are subtly different in terms of the proportion of nonreactive chemicals present (NR = 55%, 50%, and 39% for oral drugs, topical drugs, and consumer chemicals, respectively). This might have been expected given the extensive development testing of the latter set and also because the LLNA data set is likely artificially enriched with sensitizers. For example, many known sensitizers identified from other assays will have been used to validate the LLNA assay. Nevertheless, almost 50% of oral and topical drugs are members of one or more reactive domains. This confirms that the presence of a reactive structural alert in a molecule should not simply be taken as meaning a compound is high risk and should only be used in a weight of evidence approach.

Analysis of the compiled LLNA measurements broken down by the reaction domain reveals significant differences. The mean pEC3 (quantitative measures only) of each reaction

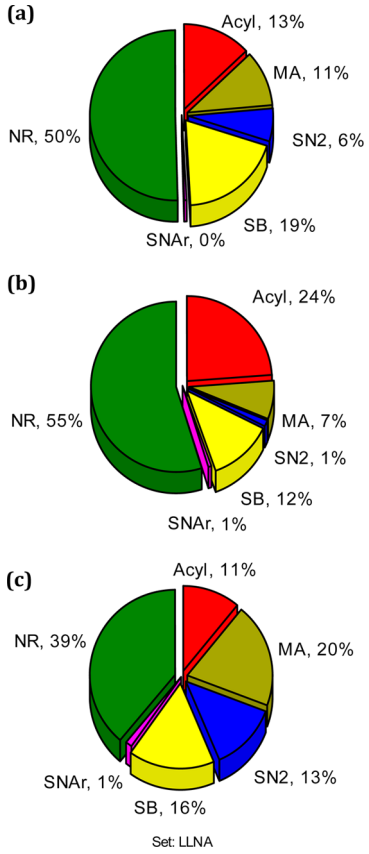


Figure 1. Pie chart showing the distribution of chemical domains within three different compound sets: (a) topical oral drugs, (b) the top-200 reported oral drugs, and (c) all compounds reported with LLNA measurements.

domain clearly shows that some are more likely to lead to severe skin sensitization than others (Figure 2). It is notable that the least represented type of compound in Figure 1 is that of the S_NAr domain. This class on average leads to the highest sensitization response of all of the chemical classes. MA, Acyl, and S_N2 are shown to display a comparable risk, with SB being

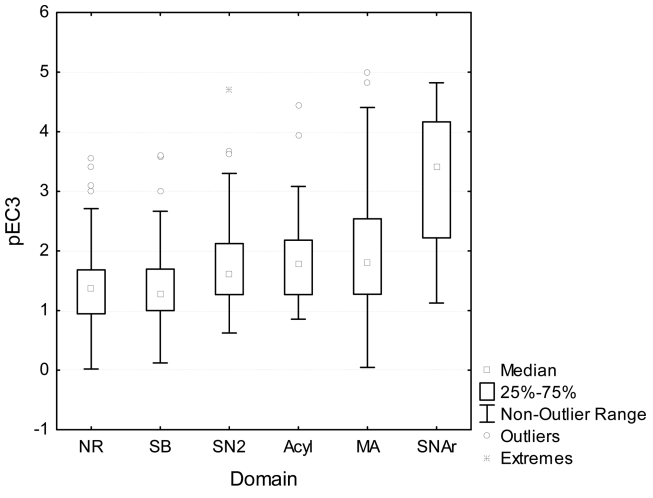


Figure 2. Relationship between skin sensitization potential (pEC3) and chemical domain for 275 chemicals with both absolute pEC3 values and a single defined chemical domain: SB, S_NAr, S_N2, Acyl, MA, and nonsensitizer (NR).

dx.doi.org/10.1021/tx400323e | Chem. Res. Toxicol. 2014, 27, 51−60

Table 2. Predicted QC Reaction Profiles for 23 Chemicals of the S_NAr Domain[a]

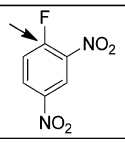
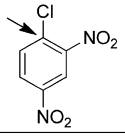
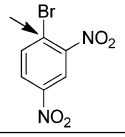
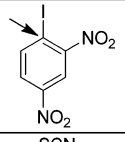
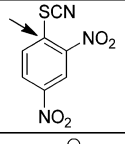
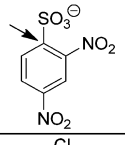
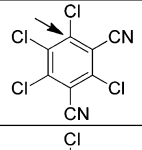
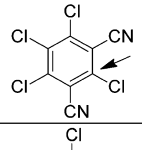
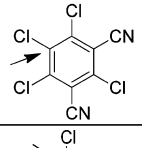
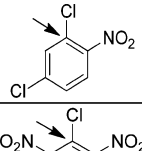
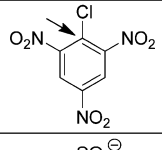
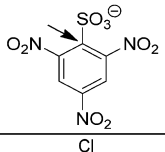
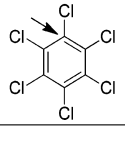
	Structure	X	Class	*pEC3*	Relative energy (kcal/mol)						Homo-Lumo E	clogP
					Isolated Reactant	TS1	Inter	TS2	Product	Isolated Product		
1		F	Extreme	3.76	4.80	4.27	-14.04	-3.67	-12.29	-11.85	3.99	1.81
2		Cl	Extreme	3.42	5.25	5.84	-11.84	15.97	-42.36	-39.76	3.85	2.33
3		Br	Extreme	3.46	5.66	4.41	-	-	-41.75	-31.76	3.81	2.50
4		I	Strong	3.24	3.24	5.99	-	-	-48.60	-50.31	4.02	2.81
5		SCN	Extreme	3.68	5.26	4.90	-12.29	-12.28	-37.02	-33.44	3.70	2.13
6		SO_3	Moderate	2.12	2.17	8.91	-9.37	9.13	-1.35	98.75	3.74	0.97
7		Cl	Extreme	4.88	5.37	5.37	-	-	-37.35	-34.52	4.38	3.88
						4.36			-37.92	-35.00		
						12.29			-37.25	-34.45		
8		Cl	Weak	0.98	4.13	9.93	-	-	-41.74	-39.68	4.11	3.07
9		Cl	Extreme	3.69	8.99	1.88	-27.36	-26.12	-41.52	-36.99	3.55	2.20
10		SO_3	Strong	2.99	3.32	5.89	-24.55	1.56	0.44	103.98	3.70	0.85
11		Cl	NR	NR	4.28	15.29	-	-	-36.67	-34.61	5.41	5.68

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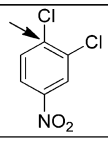
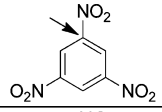
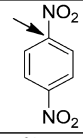
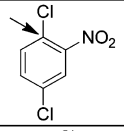
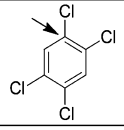
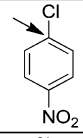
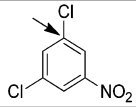
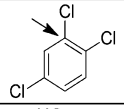
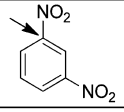
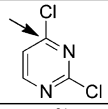
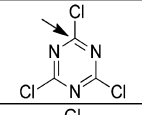
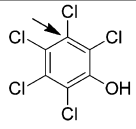
Table 2. continued

	Structure	X	Class	*pEC3*	Relative energy (kcal/mol)						Homo-Lumo E	clogP
					Isolated Reactant	TS1	Inter	TS2	Product	Isolated Product		
12		Cl	NR	NR	3.07	11.12	-	-	-39.48	-36.98	4.08	3.07
13		NO_2	NR	NR	5.52	10.77	-	-	-23.06	-18.46	3.69	1.58
14		NO_2	NR	NR	3.64	7.31	-0.67	0.90	-21.71	-18.57	3.59	1.71
15		Cl	NR	NR	4.41	10.59	-	-	-40.59	-39.08	4.09	3.07
16		Cl	NR	NR	2.86	19.03	-	-	-38.76	-37.47	5.61	4.44
17		Cl	NR	NR	2.11	13.06	-	-	-38.31	-37.15	4.19	2.45
18		Cl	NR	NR	3.07	18.96	-	-	-37.63	-36.37	4.06	3.07
19		Cl	NR	NR	2.51	20.43	-	-	-38.57	-37.39	5.81	3.82
20		NO_2	NR	NR	3.93	18.03	-	-	-19.71	-16.31	3.97	1.71
21		Cl	Strong	2.33	3.16	8.69	-	-	-38.34	-37.43	5.18	2.20
22		Cl	Extreme	3.31	4.93	2.35	-	-	-42.88	-39.48	4.75	3.53
23		Cl	NR	NR	3.82	16.83	-	-	-36.41	-34.68	5.54	4.68

[a]Also reported are the measured skin sensitization pEC3 values (or corresponding NR results from the GPA assay). Also shown are the predicted HOMO-LUMO and clogP.

the least problematic on average. It is also worth noting that some chemicals classified as containing no reactive functionality also show sensitization potential. This, however, is overestimated since most NR compounds do not have quantitative pEC3 measures so are excluded from the analysis (i.e., class based result only).

Attempts to develop trends with simple molecule properties were unsuccessful, which is consistent with reports by others.[21] This may be due to the relatively small size and limited chemical diversity of the LLNA data sets currently available.[34] Nevertheless, Figures 1 and 2 clearly show the need for additional methods, on top of the reaction domain scheme, to help discriminate sensitizers from nonsensitizers more

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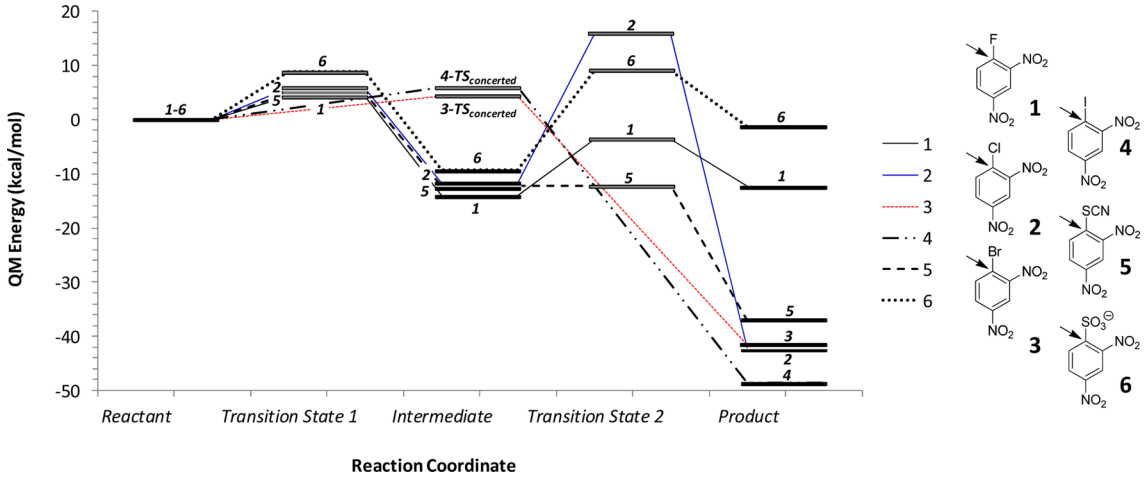

Figure 3. Reaction profiles obtained for compounds 1−6. Compounds 1, 2, 5, and 6 show a stepwise reaction profile, while for compounds 3 and 4, it is concerted.

effectively. It is also notable that the S_NAr domain represents a particularly significant threat. Nevertheless, such compounds make up 13% of oral drugs, 2% of topical drugs, and 2% of all compounds tested in the LLNA assay. In the next section, we discuss a purely theoretical QC based method suitable for use in the ranking of the sensitization potential of chemicals.

3.1. QC Model of Skin Sensitization. The addition−elimination reaction of the S_NAr domain chemicals modeled here are summarized in Scheme 1. On the basis of the gas-phase QC calculations, the displacement of the halogen or pseudohalogen group was found to proceed in either one or two steps. Addition of the nucleophile to the aromatic ring leads to the expected resonance-stabilized carbanion intermediate[35] in 7 out of the 23 cases. These intermediates are primarily found where the leaving group is less bulky (F, SCN, SO_3^-, NO_2, and Cl in 2 out of 14 cases). For the Cl leaving group, it is also found that the ring system and substituents also play an important role. The majority of reactions are predicted to proceed via an S_N2-like process that lacks the resonance stabilized intermediate. In this case, the resonance stabilized structure is found to be the transition state. From these calculations, it is clear that the nature of the leaving group (bulk and electronics), the stabilizing effect of the ring substituents, and the resonance effects can lead to dramatically different barrier heights, as well as the profile.[21] We note that these calculations lack solvent effects, which may lead to the reaction profile changing from the expected S_NAR process to an S_N2 process. Nevertheless, we still expect the computed barriers to be a reasonable reflection of the relative reactivity of each of the chemicals under investigation here.

The complete reaction profile for the 23 chemicals studied here are reported in Table 2. The first step in the reaction is expected to be the rate determining step due to the loss of aromaticity. If the reactivity of the sensitizing chemicals is under kinetic control, we would expect the RDS to correlate well with the experimental pEC3s. Alternatively, should the process depend on the overall thermodynamics, we would expect the exothemicity of the products to be important. The reaction profiles of compounds 1−6 are shown pictorially in Figure 3. It can be seen for those chemicals with halogen or pseudohalogen leaving groups and common core (i.e., 2,4-dinitrobenzene), that the profile can vary substantially. It is apparent that compounds with more effective leaving groups, Br- (3) and I- (4), display the expected stepwise S_NAR reaction and result in highly exothermic products. In contrast, compounds 1, 2, 5, and 6 follow a concerted pathway. For compounds 1 and 6, the products are equivalent or somewhat higher in energy than the corresponding intermediates or reactants, and the second step is rate determining for the full addition−elimination process. Compound 2 displays a higher barrier for the second step. While this result might suggest that the intermediate can also lead to the sensitization response, rather than the elimination product, it may be a subtle artifact of the method due to the lack of solvent stabilization.

For the 2,4-dinitrobenzene series of compounds, analysis of the correlation between the quantitative pEC3 value and the computed barrier to intermediate/product shows a rather strong correlation (r^2 = 0.93) (Figure 4). Plotting the rate determining step to product formation (i.e., for the stepwise process it may correspond to transition state 2) does not improve the correlation. As mentioned above, the lack of solvent in the simulation may in some cases make the carbanion intermediate appear more stable than it is in reality (see Figure 2). Analysis of a more diverse set of chemicals that contain a common Cl leaving group, shows a moderate correlation between the pEC3 and the barrier (r^2 = 0.58). More importantly, the line of best fit for both relationships in Figure 4 are remarkably similar suggesting that a single QC descriptor is needed to explain the sensitization potential, irrespective of ring substituents or leaving group.

Compound 7 appears to be an outlier. Like compounds 11, 16, and 23, compound 7 has substituents at all six phenyl positions. However, compound 7 has both more strongly electron withdrawing CN substituents and three distinct positions for nucleophilic attack. To try and understand whether this was the cause of this compound being an outlier, we investigated the reaction profile associated with the two other distinct addition−elimination positions (Figure 5). However, the other two positions of attack, not initially considered, were as expected considerably higher in energy and were thus not the reason for compound 7 being an outlier. We note that the r^2 obtained from a plot of the observed pEC3 vs QC barrier for the chemicals with Cl leaving groups (Figure 4) would increase from 0.58 to 0.76 were compound 7 to be removed. However, no good reason exists for excluding this compound, and we do not consider it prudent to include an

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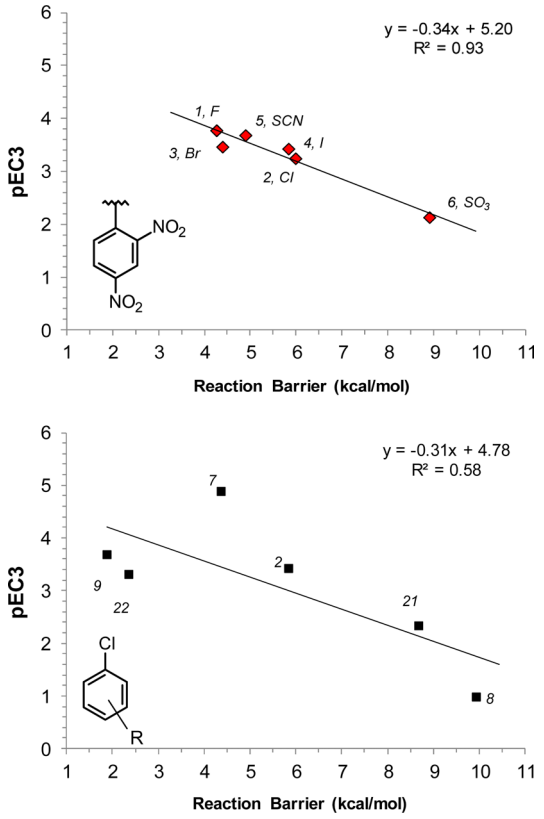

Figure 4. Plot of the pEC3 vs the predicted barrier to reaction for chemicals with a common dinitro-phenyl core but different halogen or pseudohalogen leaving groups (top). A more diverse set of chemicals with a common chloro leaving group (bottom).

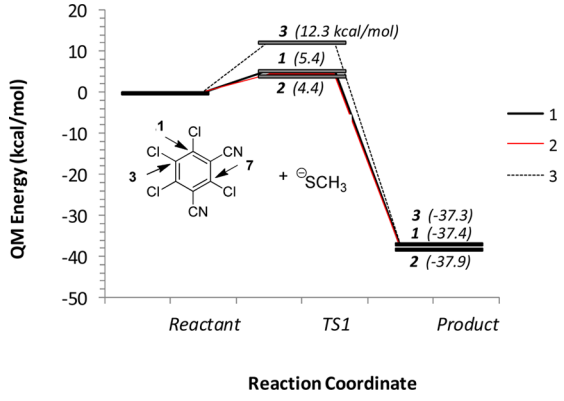

Figure 5. Three distinct concerted reaction profiles observed for compound 7.

additional term in this model to account for lipophilicity or other factors that might also contribute. This is because the small number of data points available are not sufficient to reliably test any hypothesis or to fit a multiparameter QSAR equation which can conform to the OECD QSAR guidelines. We cannot of course rule out contributory factors including (a) the use of a suboptimal QC description in this study, (b) the fact that confounding factors associated with molecular recognition between a protein and sensitizer are lacking, or (c) that a lack of concordance is due to experimental error.[3]

In Figure 6, we plot the predicted QC barrier to formation of the stable product or intermediate vs the pEC3 (or class) for all 23 chemicals investigated here. The correlation of pEC3 vs the

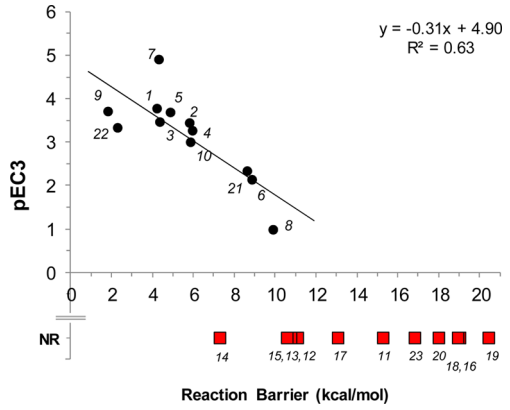

Figure 6. Plot of the LLNA pEC3 vs the predicted barrier for all chemicals investigated in this study. NR compounds are those determined as nonreactive in the GPA assay and are included for the purpose of comparison.

barrier for the 12 chemicals with quantitative pEC3s is reasonably strong (r^2 = 0.63). Barriers to reaction below ~10 kcal/mol indicate a sensitizer, and the absolute value can be related directly to the strength of sensitization response. Compound 7 still appears to be an outlier as discussed above, being more potent than predicted. This could be in part due to its higher than average clogP.[17] Again, discarding this outlier would result in an r^2 = 0.74.

The least potent sensitizer has a predicted barrier of 9.93 kcal/mol. A cutoff of ~10 kcal/mol could be used to separate the sensitizers from nonsensitizers with 100% accuracy (N = 12), with just 1 nonsensitizer out of 11 being mispredicted as a sensitizer (9%). This corresponds to an overall accuracy of ~96%, with a sensitivity of 100% and a specificity of ~91%. For quantitative measurements, we find that there is a high correlation between the experimental pEC3 and the calculated barrier to formation of the low energy product or intermediate. The nonsensitizer mispredicted is compound 14. However, analysis of the QC data shows that compound 14 has a rather unstable product compared to that of the others chemicals (only 1, 6, 10, and 20 have higher product energies). The relatively low barrier to reaction but also the only moderately stable product appears to act as a counter balance.

The S_NAr QMM model reported by Roberts et al.[21] consists of a QSAR equation that takes into account both the electron withdrawing effect of the ring substituents (i.e., electronegative inductive effects of substituents and resonance effects) and the strength of the leaving group (i.e., electronegative inductive effects). Their 2 descriptor model was also effective at discriminating between chemical sensitizers and nonsensitizers as can be seen in Supporting Information, Figure S1 and Table S1. They obtained an r^2 of 0.41 for the 12 compounds with quantitative pEC3 values, although it should be noted that some of these compounds were also used to fit the model. The correlation is lower than the 0.63 observed for the QC model developed here. In addition, using the suggested cutoff of ~1 for their model, 100% of the sensitizers are correctly classified, but 5 of the nonsensitizers would also be misclassified (i.e., 45%). This suggests the 2 descriptor QMM is somewhat inferior to the 1 descriptor QC model developed here. The key difference between the two methods is that the QC based model accounts for the same electronic terms used in the QMM but also accounts for interaction terms (steric and electrostatic) between the ring, substituents, and leaving and

dx.doi.org/10.1021/tx400323e | Chem. Res. Toxicol. 2014, 27, 51−60

attacking groups implicitly. The QC model is also unambiguous since QMMs require fitting and can also require user modification of standard physicochemical substituent parameters to give optimal results.[21] Nevertheless, an advantage of the QMM approach is that estimations take seconds to minutes when substituent parameters are available. In contrast, simulations to obtain the complete reaction profile can take 1 day per compound per computer (i.e., Intel i7).

The recent study by Enoch and Roberts[19] on the MA reaction domain is worth comparing and contrasting to the approaches used here. The authors in the former study approximated the barrier by calculating the energy of the intermediate, minus the isolated energies of the reactants (i.e., Michael acceptor and $^{-}SCH_3$), which we term E_{int}. This approximation assumes that the barrier heights are directly proportional to the energy of the intermediates. This approach will not work in situations where no stable intermediate is formed. The authors found that the correlation between the approximated barrier and the pEC3 for 26 compounds (4 outliers removed) was sizable at $r^2 = 0.43$. Addition of a single additional descriptor (solvent accessible surface area) and removal of an additional outlier led to a much better correlation ($r^2 = 0.79$). In our study of the S_NAr domain, only 6 of the compounds react via a stepwise process, thus using the E_{int} measure is limited, leading to a rather poor correlation with the pEC3 ($r^2 = 0.08$, $N = 6$ for compounds 1, 2, 5, 6, 9, and 10). It is also apparent from Figure 3 that in our case the barrier heights do not necessarily correlate well with either the intermediate or product energy, helping to explain the poor correlation between pEC3 with E_{int}. Finally, it is also worth noting that the correlation between the HOMO−LUMO energy and pEC3 for all 12 compounds with quantitative pEC3 measurements is negligible ($r^2 = 0.02$). This suggests that the latter parameter is not such a good surrogate for the barrier height of the S_NAr domain compounds assessed here.

4.0. CONCLUSIONS

In this article, we have reported the use of a QC based approach to assess skin sensitization potential. We have used a model $^{-}SCH_3$ nucleophile to predict kinetic and thermodynamic parameters associated with the addition−elimination reaction for a set of 23 chemicals from the S_NAr domain. We find that calculating the full reaction profile for the chemicals is important since, as highlighted in Scheme 1, the reactions can proceed by either concerted or stepwise addition−elimination processes depending on the activating substituents, ring resonance effects, and the nature of the leaving group. It does not appear to be suitable to approximate the transition state with either the HOMO−LUMO energy or the energy of the high energy intermediate.

We find that the use of a single computed descriptor, namely, the barrier to formation of the stable product or intermediate can help us to separate sensitizers and nonsensitizers. Barriers to reaction below ∼10 kcal/mol indicate a sensitizer, and the absolute value can be related directly to the strength of sensitization. The use of a cutoff of ∼10 kcal/mol allows us to categorize 100% ($N = 12$) of the sensitizers from the nonsensitizers ($N = 11$), with just 1 nonsensitizer being mispredicted as a weak sensitizer (i.e., 9%). This corresponds to a sensitivity of 100% and a specificity of ∼91%. For quantitative measurements, we find that there is a high correlation between the experimental pEC3 and the calculated barrier to formation of the low energy product or intermediate. We find an $r^2 = 0.64$ for all 23 chemicals, compared to $r^2 = 0.41$ for the comparable QSAR based approach reported elsewhere.[21] The one nonsensitizer found to be an outlier can be rationalized by a consideration of the reaction thermodynamics. In the case of compound 14, it has a low barrier to reaction but forms a less stable product than most other sensitizers and nonsensitizers..

Physical chemistry approaches such as QSARs[5,8,9,17,18] based on physicochemical parameters and substituent constants, or QC calculations[19] have proved useful in helping discriminating sensitizers from nonsensitizers. The physical insight and understanding that can be garnered from QC methods could prove useful for skin sensitization assessment, especially when combined in the so-called weight of evidence approach with other methods. QC calculations by necessity must employ surrogate nucleophiles for what is a complex biological process, and that is a key limitation. However, we postulate that the experimental identification of the most prevalent nucleophiles or indeed the precise proteins that cause skin sensitization would provide an additional means to help improve the performance of such atomic simulations.

■ ASSOCIATED CONTENT

Supporting Information

Optimized coordinates and the full list of LLNA data compiled for this study. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: paul.gleeson@ku.ac.th.

Funding

M.P.G. would like to acknowledge the financial support from the Thailand Research Fund (TRF) (RSA5480016). M.P. is grateful to the TRF for her Royal Golden Jubilee (RGJ) Ph.D. scholarship (TRF RTA5380010, PhD/0009/2552).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the help of Dr. Sandeep Modi and Dr. Aynur Aptula who helped with the classification of chemicals into reaction domains. We also thank Professor David Roberts for providing insight into the QMM developed for the S_NAR domain.

■ ABBREVIATIONS

ChEMBL, Chemistry at European Bioinformatics Institute (database); clogP, calculated logarithm of the octanol−water partition coefficient; DFT, density functional theory; EC3, concentration needed to produce a 3-fold increase response compared to that of vehicle-treated controls; GPT, guinea pig maximization test; HOMO, highest occupied molecular orbital; ICCVAM, Interagency Coordination Committee on the Validation of Alternative Methods; ICD, irritant contact dermatitis; LLNA, local lymph node assay; LUMO, lowest unoccupied molecular orbital; M062X, a DFT methodology; MA, Michael acceptor; MWT, molecular weight; NR, nonreactive; OECD, Organization for Economic Cooperation and Development; PCM, polarizable continuum model; pEC3, the log of the molar EC3; QC, quantum chemical; QMM, quantitative mechanistic model; QSAR, quantitative structure−activity relationship; RAI, relative alkylation index;

REACH, registration, evaluation, authorization and restriction of chemical substances regulations; SB, Schiff base; S_N1, substitution nucleophilic 1 (unimolecular); S_N2, substitution nucleophilic 2 (bimolecular); S_NAr, aromatic nucleophilic substitution

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