

Chirality index, molecular overlay and biological activity of diastereoisomeric mosquito repellents†

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Abstract: Both 1-methylisopropyl 2-(2-hydroxyethyl)piperidine-1-carboxylate, (Picaridin®) and cyclohex-3-enyl 2-methylpiperidin-1-yl ketone (AI3-37220; 220) have two asymmetric centers, and the four diastereoisomers of each compound are known to have differing degrees of mosquito-repellent activity according to quantitative behavioral assays conducted at the United States Department of Agriculture. Computational chemistry was used to identify the structural and configurational basis for repellent activity. Molecular overlay of the optimized geometries of the lowest energy conformers of the diastereoisomers was investigated to elucidate the role of chiral centers in 220 and Picaridin. It was found that the presence of a chiral carbon alpha to the nitrogen with the *S* configuration in the piperidine ring is essential to the three-dimensional arrangement of the atoms of the pharmacophore for effective repellent activity.

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Keywords: mosquito repellency; molecular overlay; Picaridin; AI3-37220

1 INTRODUCTION

Because of the enantioselectivity of biological systems, enantiopure compounds are replacing racemates that have been used as agrochemicals¹ and therapeutic agents in pharmaceuticals. The use of enantiomerically pure chemicals is much more common in pharmaceuticals than in agrochemicals, the reason being that medically adverse effects of chemicals² can result from some stereoisomers present in the racemic mixtures. There are a limited number of commercially available chiral compounds used as agrochemical insecticides or herbicides. However, advancements in chiral synthesis and separation technology may enable development of new chiral agricultural pesticide agents. Equally important is that regulatory authorities are advocating the use of pure stereoisomers in the case of chiral compounds as a way to reduce the pesticide burden placed on the environment. Consider the example of the insecticidal activity of four isomers of fenvalerate against house flies; the (2*S*, α -*S*) isomer has the highest activity (the relative insecticidal activities are: 2*S*, α -*S*: 1000; 2*R*, α -*S*: >5; 2*S*, α -*R*: 40; 2*R*, α -*R*:

>5).¹ The higher the activity difference, the lower the amount of active enantiomer which needs to be used to produce a specific activity. If one uses the mixture of the diastereoisomers, then the other three diastereoisomers that are comparatively less toxic to the flies will still be loaded into the environment. The scenario is still worse if even one of these three is toxic to other non-target organisms or possesses another different kind of undesirable bioactivity.

In the case of mosquito repellents, the United State Department of Agriculture (USDA) is trying to develop alternatives to DEET (*N,N*-diethyl-3-methylbenzamide) which has been the most commonly used topical repellent for nearly five decades, despite the relatively high dose required for activity, its tendency to dissolve many plastics, perceived toxicity and uncomfortable dermal-cosmetic stickiness.³ Klun *et al.*⁴ recently carried out bioassays of the four diastereoisomers of cyclohex-3-enyl 2-methylpiperidin-1-yl ketone (AI3-37220; 220) against the yellow fever mosquito, *Aedes (Ae.) aegypti* (L.) and found that the diastereomer with

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the absolute configuration 1*S*, 2'*S* had the highest mosquito repellency. Another compound that has been in use as a topical mosquito repellent in some commercial products is 1-methylisopropyl 2-(2-hydroxyethyl)piperidine-1-carboxylate (Picaridin®).

In order to understand the stereochemical selectivity of these repellents and the importance of chiral centers, one has to use the stereochemical structure–activity relationship (SSAR) approach instead of general structure–activity relationship (SAR) modeling. The results of such a study are of paramount importance in guiding the synthesis of new insect repellents because the preferred absolute configuration at any one chiral center in the molecules can be an essential condition to reveal new chemical structures that enhance bioactivity. The assignment of a hierarchy in significance among the absolute configuration of chiral center sites could also be valuable in discovering and in designing more potent and more effective repellent activity.

To carry out SSAR, descriptors capable of handling the stereochemical nature of compounds are needed. Compared with the large number of topological descriptors⁵ available to describe the molecular structure, indices that can address the stereochemistry are very limited in number because these indices are derived from molecular graphs that reflect only on the two-dimensional topology. Schultz *et al.*⁶ developed a set of indices that could handle polychiral diastereoisomerism. In this paper we used two different approaches for the characterization of diastereoisomers of insect repellents:

- (1) Use of Schultz indices as the numerical quantifier of the chirality of molecules.
- (2) Application of molecular overlay to compare the various diastereoisomers of 220 and Picaridin.

2 MATERIALS AND METHODS

2.1 Bioassay

The comparative mosquito-repellent effectiveness of AI3-37220 and Picaridin diastereoisomers against *Ae. aegypti* were determined by applying the compounds to the skin of human volunteers and using so-called Klun and Debboun (K&D) modules to quantify repellent efficacy, as described by Klun and Debboun.⁷ Data presented for the AI3-37220 diastereoisomers are from Klun *et al.*⁴ Details for the Picaridin diastereoisomer isolation, absolute conformation determination and replicated bioassay will be published elsewhere (Klun JA and Schmidt WF, unpublished). The combined results of the bioassays of the diastereoisomers of AI3-37220 and Picaridin are presented in Table 1.

Computations and modeling

Picaridin and AI3-37220 are piperidine analogs and each has two chiral centers in its structure (Fig. 1). They exhibit polychiral diastereoisomerism and each has four diastereoisomers, i.e. two pairs of

Table 1. Mosquito bioassay of diastereoisomers of AI3-37220 and Picaridin

Diastereoisomer	Proportion biting ^a	
	AI3-37220	Picaridin
1 <i>R</i> , 2' <i>S</i>	0.32 b	0.18 a
1 <i>R</i> , 2' <i>R</i>	0.56 c	0.22 a
1 <i>S</i> , 2' <i>R</i>	0.51 c	0.40 b
1 <i>S</i> , 2' <i>S</i>	0.18 a	0.44 b
Racemate	0.45 bc	0.22 a
Control	0.83 d	0.72 c

^a Proportions followed by the different letters are significantly different from one another at $P = 0.05$.

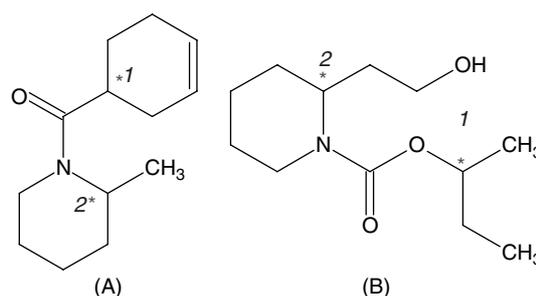


Figure 1. Structures of (A) AI3-37220 and (B) Picaridin. The diastereoisomeric centers are denoted with asterisks.

enantiomers. The stereoisomers are represented as 1*R*, 2'*S*; 1*R*, 2'*R*; 1*S*, 2'*R* and 1*S*, 2'*S*. The geometry of each stereoisomer was optimized by MM2 method using the Chem3DPro⁸ interface. The MM2 force field for geometry optimization uses first derivatives of energy and can result in a geometry that is near a saddle point. Hence, energy minimization routine performed using MM2 results in local energy minimization, and not necessarily in a global minimum. In order to have the starting geometry close to the global minimum, molecular dynamics simulation was performed⁸ prior to optimization. Carrying out molecular dynamics heats the molecule, thereby crossing transition states. In addition to this, the dihedral angle about the N–C=O plane was changed by 5° every time and the optimization was repeated until no further lowering of total energy could be achieved. This again ensured that the final geometry obtained was close to a global minimum. It may be noted that the global minimum conformation need not be the conformation that binds to the target receptor. Since the active conformations of the diastereoisomers of 220 and of Picaridin, and the precise structure of the receptors are not known, the minimum energy conformations were considered for molecular overlay so that different diastereoisomers could be compared on similar conformations. Moreover, the global minimum is a single conformation and every higher energy state has multiple conformations with equal energy. Without a receptor structure, it is impossible to select one of the multiple higher energy conformations in preference to another.

2.3 Molecular overlay

The optimized geometries were superimposed using the overlay command in the Chem3D Ultra 8.0 from CambridgeSoft.⁷ Three points (atoms) common in the two structures were considered as the points of superimposition, and the distances between them were set to 0.001 Å. Once the two structures had been superimposed with respect to the three points, a minimization routine was not applied to avoid the two structures being superimposed at a mean distance with respect to all the atoms in them. If we had used a minimization route, the importance of a substructure or fragment and its effect on the putative pharmacophore could not have been understood. Hence, after superimposition with respect to three points, the interatomic distances of five atom-pairs were obtained and used as a measure of match/mismatch.

2.4 Calculation of chirality indices

Schultz *et al.*⁶ developed topological indices that can handle both polychiral diastereoisomerism and diastereomerism due to the carbon–carbon double bond (*E*, *Z* isomers). In their approach chiral corrections are added to topological indices calculated from distance matrices. Calculation of chiral indices is outlined below:

In a molecular graph vertices correspond to atoms and edges represent the covalent bonds between them. Molecular geometry is not usually considered in depicting a molecule as a graph. Adjacency matrix and distance matrix are the most commonly used matrix representations of a graph. The adjacency matrix $A = A(G)$ of a graph with N vertices is a square symmetric matrix whose elements are $[A]_{ij}$. The entry for a matrix element $[A(G)]_{ij}$ is 1 when the two vertices v_i and v_j are connected by an edge, and is 0 when the two edges are not connected. The distance matrix $D = D(G)$ of a graph is also an $N \times N$ square symmetric matrix and the elements of the matrix are the graph distance d_{ij} between the vertices v_i and v_j . A molecular topological index (*MTI*) was defined by Schultz⁹ as the sum of the elements obtained from the product, $v(A + D)$, where v is the row matrix obtained from the vertex sum of the adjacency matrix $A(G)$. Mihalić *et al.*¹⁰ and Müller *et al.*,¹¹ in contrast, used only the distance matrix instead of $(A + D)$ and the molecular topological index *MTI'* is therefore, the sum of the elements of the product, $v(D)$. Gutman¹² preferred to call the new index *MTI'* the Schultz index (*S*) after the discoverer of *MTI*. Another graph invariant product of row sum (*PRS*) is also computed from the distance matrix.

In the case of molecules with stereocenters, the Schultz index *S* and *PRS* are calculated from a valence–vertex-weighted distance matrix $D(G_{Vvw})$ instead of from the distance matrix $D(G)$. In order to calculate $D(G_{Vvw})$, the vertices of a graph are weighted following two schemes namely, vertex weight V_i and

valence weight v_i . The vertex weight V_i is:

$$V_i = 1 + (\text{atomic number of heteroatom} \\ - \text{atomic number of carbon}).$$

For example, vertex weights V_i for C, N, O and F are 1, 2, 3 and 4, respectively. The valence weight v_i is obtained from the number of covalent bonds in a hydrogen-suppressed graph and the unshared pairs of electrons:

$$v_i = (\text{number of bonds} \\ + \text{unshared pairs of electrons}).$$

It should be noted that these valence weights are different from those advocated by Kier and Hall¹³ in the calculation of valence connectivity indices. Diagonal matrices V and v are formed from the vertex weights V_i and the valence weights v_i , and the two matrices have the corresponding weights as the diagonal elements while all the other elements are zero.

The vertex weighted distance matrix $D(G_{Vw})$ is

$$D(G_{Vw}) = V \times D(G)$$

The valence–vertex-weighted distance matrix, $D(G_{Vvw})$ is obtained by the multiplication of the v and $D(G_{Vw})$ matrices:

$$D(G_{Vvw}) = v \times D(G_{Vw})$$

$D(G_{Vvw})$ thus obtained is used to compute the Schultz index and *PRS*. A correction term is applied to these invariants to take care of configuration around stereocenter(s). The following operations are carried out to calculate the correction term:

- 1) $D(G_{Vvw})$ is converted into a matrix for chiral correction D_s which is nothing but the sum of $D(G_{Vvw})$ and its transpose.
- 2) $D_s = D(G_{Vvw}) + D(G_{Vvw})^T$
- 3) The chiral correction matrices are formed from the chiral factors (CF) of the vertices. A stereocenter with *R* configuration is assigned a chiral factor +1; while the *S* configuration is assigned –1. All the other vertices are assigned chiral factor values of zero. The number of chiral correction matrices thus formed is equal to the number of diastereoisomers.
- 4) The chiral modifier is obtained from the sum of the element of a resultant matrix obtained by premultiplying D_s with $D(CF)$ for a particular stereoisomer.

The chiral modifier is then used to alter the value of the topological indices *S* and *PRS*. In the present study we used the Schultz index (*S*) and the *MTI* calculated from distance matrix. However, we did not use the product of row sum (*PRS*) because its magnitude is several times that of the other indices, and Schultz⁹ used the same correction factor across the board for

Table 2. Chirality indices calculated for the diastereoisomers of AI3-37220 and Picaridin

	<i>CM</i>	<i>MTI</i>	<i>S(D_V)</i>	<i>S(D(G_{V_{vw}))}</i>	<i>MTI_{KH}</i>	<i>S(D_{V_{KH})}</i>	<i>S(D(G_{V_{vw}-KH))}</i>
<i>AI3-37220</i>							
Racemate		1568	1823	7177	2044	1952	10 627
<i>RR</i>	486	2054	2309	7663	2530	2438	11 113
<i>SR</i>	40	1608	1863	7217	2084	1992	10 667
<i>RS</i>	-40	1528	1783	7137	2004	1912	10 587
<i>SS</i>	-486	1082	1337	6691	1558	1466	10 141
<i>Picaridin</i>							
Racemate		1802	2227	11 601	2726	2628	22 215
<i>RR</i>	686	2488	2913	12 287	3412	3314	22 901
<i>RS</i>	88	1890	2315	11 689	2814	2716	22 303
<i>SR</i>	-88	1714	2139	11 513	2638	2540	22 127
<i>SS</i>	-686	1116	1541	10 915	2040	1942	21 529

all indices. A computer program was developed to calculate the chirality indices, and the values obtained for diastereoisomers of Picaridin and AI3 = 37 220 are given in Table 2.

In computing the Schultz index both from the distance matrix $D(G)$ and the vertex–valence-weighted distance matrix $D(G_{V_{vw}})$, the valence weights were taken according to the formulation by Schultz discussed above. However, we used the valence δ suggested by Kier and Hall¹³ to handle the chemical nature of the atoms and the bond multiplicity. The indices thus calculated are indicated in Table 2 with the subscript KH to differentiate them from the similar indices calculated according to Schultz *et al.*⁶

3 RESULTS AND DISCUSSIONS

The energies obtained by MM2 routine of energy optimization for the lowest energy conformers of 220 and Picaridin are given in Table 3. It can be seen that the enantiomers have almost the same energy. This is quite obvious because optical antipodes absorb the same amount of energy and do not differ in their electronic properties. The physical and optical properties which are similar among chiral compounds, however, are factors that would incorrectly predict that repellency activities among the chiral compounds were identical. The optimized geometries of the diastereoisomers are given in Fig. 2.

The stereochemical structure–activity studies were carried out using the chirality indices listed in Table 2

and, for the reasons mentioned above, energies of the conformers and the electronic parameters such as E_{HOMO} (energy of the highest occupied molecular orbital), E_{LUMO} (energy of the lowest unoccupied molecular orbital), HOMO–LUMO gap ($E_{\text{LUMO}} - E_{\text{HOMO}}$) were ruled out. Comparison of proportion of biting and the chirality indices of the diastereoisomers of AI3-37220 shows a trend (Fig. 3) and indicates that decrease in any one of the chirality indices decreases the proportion of biting. In contrast, the plot of the repellencies of the diastereoisomers of Picaridin against the chirality indices used in this study is a scatter plot (Fig. 4). One of the possible reasons could be the nature of the chirality indices used in the study. Schultz indices calculated from the chirality measure do not seem to be a good quantitative measure of chirality because binary entries, +1 or -1, were used in computing the chiral modifier (*CM*). It may be recalled that the chiral correction matrices for *RR* and *SS* isomers, $D(CF, RR)$ and $D(CF, SS)$ have two +1 and two -1 entries, respectively. Hence, the chiral modifier for the *RR* diastereomer becomes the largest whereas that for the *SS* diastereomer becomes the smallest. In the case of *RS* and *SR*, there is something of an equivocal situation and their correction term is always intermediate in value. Irrespective of the groups or the atoms attached to the stereocenter, the order of numerical values of the diastereoisomers will be $R > S$ for a compound with one chiral center, and $RR > RS/SR > SS$ for a compound with two chiral centers. In the case of

Table 3. Energies (kcal mole⁻¹) calculated from Chem3D-Pro (MM2 energy minimization)

Energy	AI3-37220				Picaridin			
	<i>1R, 2'R</i>	<i>1S, 2'S</i>	<i>1R, 2'S</i>	<i>1S, 2'R</i>	<i>1R, 2'R</i>	<i>1S, 2'S</i>	<i>1R, 2'S</i>	<i>1S, 2'R</i>
Stretch	0.7464	0.7394	1.4568	1.1429	1.3025	1.3283	1.2247	1.2197
Bend	4.1748	4.2322	4.7885	4.7739	7.6120	7.6205	5.2840	5.3832
Stretch–bend	0.4542	0.3932	0.3583	0.3555	0.4357	0.4492	0.4449	0.4479
Torsion	0.4191	0.4288	5.1933	5.1835	1.6861	1.6147	1.6590	1.6490
Non-1,4 VDW	-4.8266	-4.4674	-1.1250	-1.1250	-3.8729	-3.7377	-4.7405	-4.7793
1,4 VDW	9.4959	9.5766	11.3358	11.3471	12.8129	12.7371	12.6547	12.6185
Dipole/dipole	0.7318	0.7446	-4.2932	-4.2923	-4.5236	-4.5525	-4.4767	-4.5280
Total	11.1957	11.2472	17.4144	17.4025	15.4527	15.4597	12.0501	12.0112

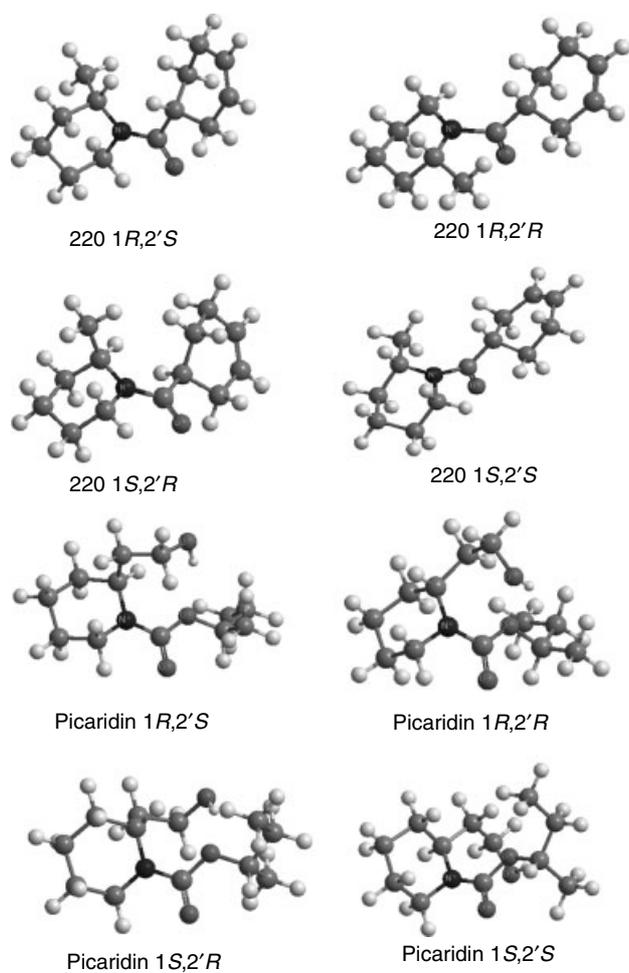


Figure 2. Optimized geometries of the diastereomers of AI3-37220 and Picaridin.

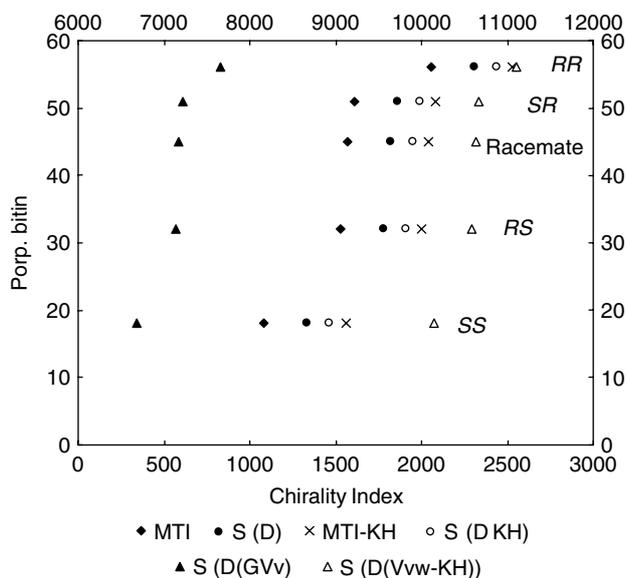


Figure 3. Comparison of repellency of diastereomers of AI3-37220 and chirality indices.

AI3-37220, it was quite accidental that the order of activity happened to follow the order of the numerical values of the chirality indices, but this was not so for the diastereoisomers of Picaridin. Thus,

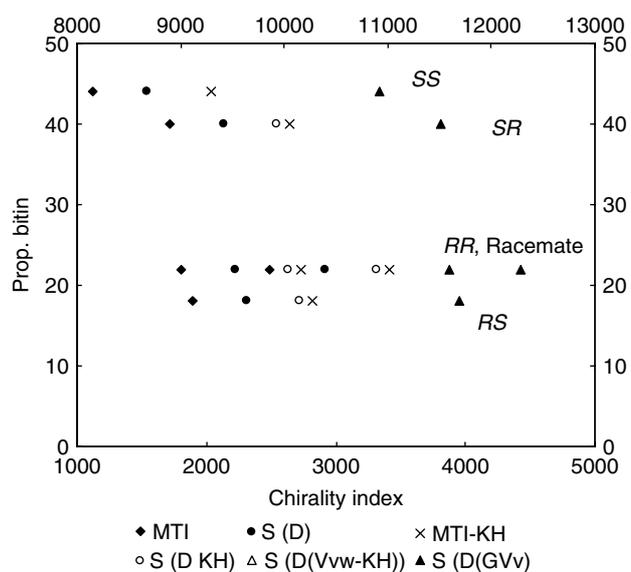


Figure 4. Comparison of repellency of diastereomers of Picaridin and chirality indices.

the two examples of insect repellency considered in the current study brought out the limitations of the applicability of the type of indices introduced by Schultz in SSAR. An additional limitation is that the site of one chiral center could be of primary importance and another of secondary importance, whereas the Schultz calculations arbitrarily assumes equal importance to each chiral center. To overcome this, one must consider the concept of continuous measure of chirality advocated by Zabrodsky and Avnir,¹⁴ and chiral indices of this type have yet to be developed.

In the present study, lowest energy conformers of different diastereoisomers of the same compound as well as of different compounds were compared. As mentioned earlier, the complete knowledge of interaction between the repellent (ligand) and the receptor is not known in this situation, and the superimposition of energy-optimized conformers seems to be an appropriate tool. This approach had already been used by Warthen *et al.*¹⁵ in modeling the activities of *trans*-trimeclure enantiomers. They used the Chem-X program to get the staggered fit and superimposed fit and then calculated the differences in van der Waals volume, surface area and their ratio as independent parameters to model the activities of the enantiomers. We used the overlay option in Chem3D Ultra by CambridgeSoft because it enables comparison of structural similarities of different compounds as well as the conformers of the same compound.

The other objective of the study using molecular overlay is to bring out the relative importance of the chiral centers and their positions. The common feature among DEET, Picaridin and AI3-37220 is the presence of the $-\text{NC}(=\text{O})-$ moiety, and it is interesting that DEET is an effective repellent without having any stereocenter. This indicates that the $-\text{NC}(=\text{O})-$ group

may be the putative pharmacophore. These facts raise the questions:

- (1) Is one or both of the chiral centers in AI3-37220 and Picaridin essential for enhanced repellency?
- (2) Is the correct absolute configuration at one chiral center more important than the other in determining its efficacy?
- (3) Which other molecular sites in common between the achiral DEET and the chiral compounds are critical components in the repellency?

It was thought that molecular overlay might provide answers to the above questions. Overlays of molecules facilitate the simultaneous comparison of similarities of the structures overlaid and include both their correct absolute configuration and their lowest energy conformation. Three schemes of overlays were carried out initially to find out the importance of chiral center(s) and they are:

- Scheme 1: The common atoms, namely N, C and O of the $-N-C(=O)-$ group, as the points of contact
- Scheme 2: The chiral carbon of the piperidine ring, the N atom and the other carbon atom alpha to the N atom as the points of contact
- Scheme 3: The chiral carbon of the cyclohexene ring, and the C and O atoms of the $C=O$ group while superimposing diastereoisomers of AI3-37220, and the chiral carbon of the *sec*-butyl group, alkoxy-O atom and the C atom of the $C=O$ group in the case of Picaridin, as the points of contact.

Each of the above three schemes was used to overlay (A) the diastereoisomers of 220 over the most active diastereoisomer (1*S*, 2'*S*) (B) the diastereoisomers of Picaridin on its 1*R*, 2'*S* isomer, which is the most active Picaridin diastereoisomer. The overlays according to Scheme 1 and Scheme 3 did not show any distinctly observable trend between the degree of matching and the order of repellency of the configurational isomers. In contrast, the overlays according to Scheme 2, that is superimposing on the chiral carbon of the piperidine ring, N and the other carbon alpha to N as the points of contacts showed very clear trends in the degree of matching of the overlaid structures and the order of repellency. The overlays are shown in Figs 5 and 6, and the atom-pair distances for the five common atom-pairs of the overlaid structures are given in Table 4. The sum of atom-pair distances is considered as a measure of degree of match or closeness of a diastereoisomer to the most active diastereoisomer on which it was overlaid. The lower the sum of the atom-pair distances the greater the structural similarity between the two superimposed structures or the structural moiety under consideration.

It can be seen from the figures of overlay (Figs 5 and 6) that an increasing degree of mismatch (increases in the sum of atom-pair distances) correlates with decreasing orders of repellency. In the case of Picaridin, the increasing order of repellency ($SS <$

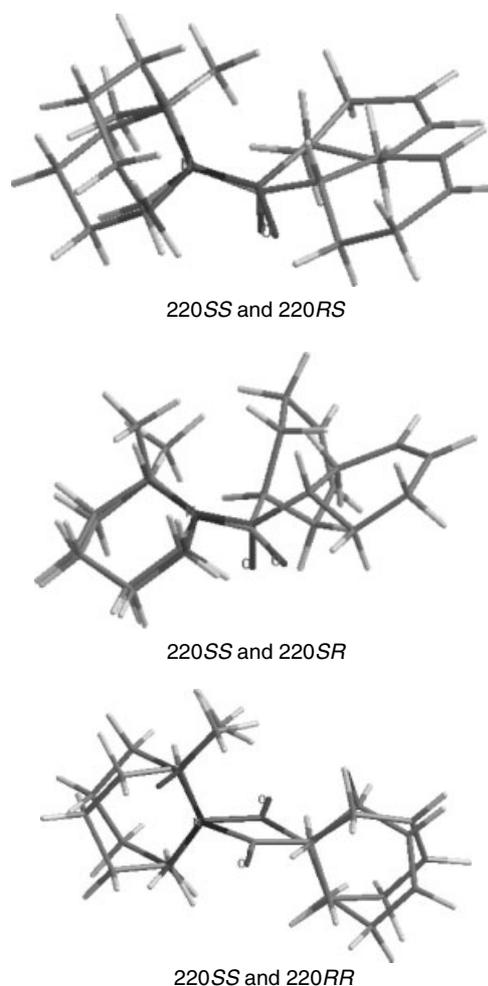


Figure 5. Overlay of diastereoisomers of AI3-37220 over the most active isomer, IA3-37220SS.

$SR < RR < RS$) parallels the decreasing order of the sum of atom-pair distances. The order of repellency of 220 isomers is $RR < SR < RS < SS$: the order of sums of atom-pairs for 220*RS* and 220*SR* are reversed, and this might be due to the percentage of error in overlay and bringing the atoms to the closest for superimposition. Looking at the overlays (Fig. 5) of diastereoisomers of AI3-37220 over 220*SS*, it can be seen that the conformer of *RS* superimposes better with *SS* than *SR*. The striking feature of the overlays of diastereoisomers of AI3-37220 is that the most active and the least active have the oxygen atoms in the anti-periplanar position, and this explains why AI3-37220*RR* is the least active while AI3-37220*SS* is the most active among the diastereoisomers of AI3-37220. Molecular overlay provides an answer not only to the difference in the activity of the most active and the least active, but also shows that the atoms of the pharmacophore are almost coplanar. This reveals why the difference between the biological activity of the most active and the least active is less for the diastereoisomers of Picaridin than that between the diastereoisomers of AI3-37220. In the case of Picaridin the proportion of biting for most active (*RS*) and the least active (*SS*) are 0.18 and 0.44, respectively. For

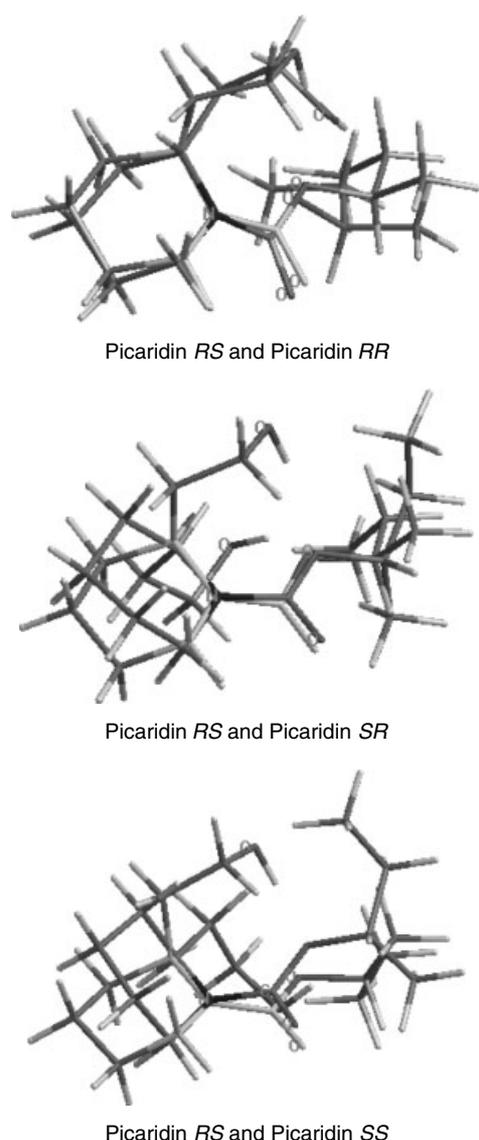


Figure 6. Overlay of diastereomers of Picaridin over Picaridin *RS*, the most active Picaridin isomer.

AI3-37220, it varied from 0.18 for most active (*SS*) to 0.56 for the least active (*RR*). Diastereoisomers of Picaridin do not seem to vary significantly in the orientation of the putative pharmacophore with respect to the most active isomer, which is observable from the overlays and might be due to the alkoxy group on the side of the $-NCO-$ group. The near self-similarity among the diastereoisomers of Picaridin is revealed by molecular overlay, at least qualitatively.

Only in the second scheme out of the three schemes of overlay did order of repellency correlate to the degree of similarity of the orientation of pharmacophore atoms. This indicates that chiral carbon with *S* configuration in the piperidine ring is an essential condition for a piperidine analog to be an effective repellent, and this seems to be necessary in orienting the pharmacophore in a particular three-dimensional disposition for a molecule to be an effective repellent. The correct absolute configuration at one of two chiral centers enhances

Table 4. Sum of atom-pair distances between overlaid structures

Overlay	Atom-pair distances of the five atoms (N, αC^* , $\alpha C2$, C of C=O and O of C=O)					Sum
	αC^*	αC	N	C=O	C=O	
<i>AI3-37220SS</i> (the most active) versus other diastereoisomers of <i>AI3-37220</i>						
<i>220SS-220RS</i>	0.099	0.226	0.082	0.374	1.913	2.694
<i>220SS-220SR</i>	0.078	0.179	0.131	0.104	1.406	1.898
<i>220SS-220RR</i>	0.231	0.093	0.100	0.231	3.901	4.556
<i>Picaridin RS</i> (the most active) versus other diastereoisomers of <i>Picaridin</i>						
<i>Picaridin RS-RR</i>	0.088	0.187	0.100	0.212	0.410	0.997
<i>Picaridin RS-SR</i>	0.304	0.207	0.100	0.433	0.602	1.646
<i>Picaridin RS-SS</i>	0.132	0.360	0.102	0.842	1.034	2.470
<i>220SS</i> (the most active) versus diastereoisomers of <i>Picaridin</i>						
<i>220SS-Picaridin RS</i>	0.252	0.172	0.243	0.010	1.661	2.338
<i>220SS-Picaridin RR</i>	0.615	0.001	0.011	0.600	0.670	1.897
<i>220SS-Picaridin SR</i>	0.058	0.522	0.230	1.396	2.897	5.103
<i>220SS-Picaridin SS</i>	0.162	0.196	0.367	1.691	3.369	5.785
<i>220SS-DEET</i>	0.100	0.347	0.080	0.074	0.142	0.743
<i>DEET</i> versus diastereoisomers of <i>220</i> and <i>Picaridin</i>						
<i>DEET-220SS</i>	0.022	0.723	0.001	0.076	0.095	0.917
<i>DEET-220SR</i>	0.100	0.977	0.086	0.532	1.626	3.321
<i>DEET-220RS</i>	0.080	0.265	0.05	0.307	0.941	1.643
<i>DEET-220RR</i>	0.348	0.205	0.234	0.880	2.336	4.003
<i>DEET-Picaridin SS</i>	0.370	0.353	0.010	0.616	1.250	2.599
<i>DEET-Picaridin SR</i>	0.311	0.268	0.148	0.080	1.272	2.079
<i>DEET-Picaridin RS</i>	0.128	0.410	0.010	0.087	1.208	1.843
<i>DEET-Picaridin RR</i>	0.243	0.514	0.010	0.683	1.944	3.394

the activity by a factor of about two; the absolute configuration of the second chiral center did not matter much. If the influence of both chiral centers mattered in the efficacy of this compound, the sum enhancement effect could have been cumulative. Molecular overlay study enabled assignment as to which chiral center was most important for repellency activity. The current study demonstrates that chirality can be important in identifying the sites within lead compounds that happen to be chirally responsible for a specific biological activity. The effect for some sets of compounds could be less than a factor of 2; for others the effect could be more than a factor of 2. The value of such studies is not to explain why some compounds are less active, but to find why any lead compound has enhanced activity. Without chirality as a factor, there would be no way to explain why one of the eight chiral compounds was most active.

We also compared the structural similarities of the diastereoisomers of Picaridin with *AI3-37220SS*, the most active diastereomer. The diastereoisomers of Picaridin were overlaid on *AI3-37220SS* according to Scheme 2, discussed earlier. The superimposed structures are shown in Fig. 7 and the atom-pair distances are listed in Table 4. The self-similarity between *RR* and *RS*, and *SR* and *SS*, is brought out well and this is also reflected by the narrow range in their insect repellencies (*RS*: 0.18, *RR*: 0.22, *SR*: 0.40, *SS*: 0.44). The increasing order of repellency

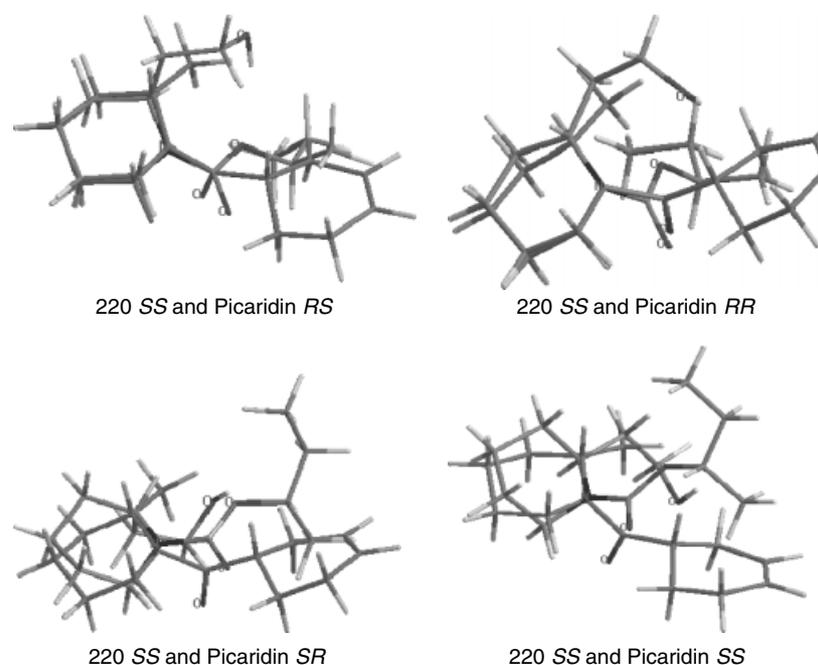


Figure 7. Overlay of diastereomers of Picaridin over the most active isomer, AI3-37220 SS.

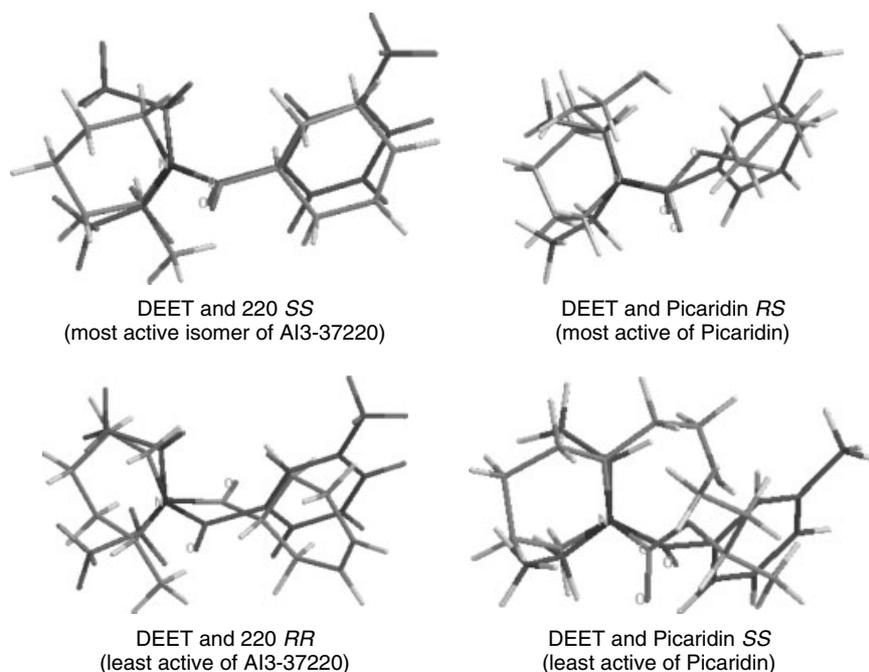


Figure 8. Overlay of the most active and the least active diastereomers of AI3-37220 and Picaridin over DEET.

of Picaridin diastereoisomers is $RS > RR > SR > SS$, and we compared this to the sum of atom-pair distances (shown in last column of Table 4) for the overlay of Picaridin isomers over AI3-37220SS. The sum of atom-pair distances of *RR* was found to be an exception, i.e. it was lower than that for *RS*. If we take a closer look at the atom-pair distances, this exception is due to the closeness of the carbonyl oxygen atoms in the overlaid structures, and is clearly observable in Fig. 7. The presence of the oxygen atom ($-\text{OCH}(\text{CH}_3)\text{CH}_2\text{CH}_3$) in Picaridin seems to put the carbonyl oxygen closer through space.

In comparing the similarity of the diastereoisomers of AI3-37220 and Picaridin with the lowest energy conformer of DEET, (Fig. 8) we found that the most active diastereomer of all, that is 220SS, has a closer similarity than even the other diastereomer of AI3-37220 and Picaridin. The atom pair distances are given in Table 4. In the case of Picaridin the $1R,2'R$ isomer was a discrepancy. Molecular overlay approach could not differentiate the best among the three most active repellents, DEET, AI3-37220SS and Picaridin *RS*. This stereochemical structure–activity relationship was based on data from nine compounds,

eight of which are chiral. Inclusion of an even larger data base of active chiral compounds could enhance the quantitative accuracy of these predictions. Hence, the overlay procedure is useful to predict the order of repellencies among a given set of diastereoisomers only when data on the activity of chiral compounds is collected and compared with a known, most active, (chiral) reference compound. Given a set of structures, molecular overlay, as with any other structure–activity relationship, could predict similarity/dissimilarity among them but could not independently identify the most active structure. In the absence of information about the identity and structure of the appropriate receptor binding site, SARs can be developed on the basis of structural similarity/dissimilarity of the molecules with the active chemical. When the most active isomer has a chiral center critical for enhanced activity, no theoretical calculation available can assign whether one isomer or its mirror image is most active. When no data on differences in activity among chiral compounds are collected, computational chemistry cannot evaluate molecular based hypotheses that explain why some are more active and some are less active. Once a most active three-dimensional configuration has been experimentally identified, the absolute configuration of all the active isomers tested must be known for the comparison to be valid. Then, on the basis of the valid three-dimensional structural overlay criteria, new compounds can be designed and synthesized (and/or existing compounds can be selected). Screening of large libraries of compounds using the overlay method may lead to the discovery of chemicals with even greater enhanced arthropod repellency, some or many of which will be chiral.

4 CONCLUSION

In this paper we used molecular overlay to investigate the stereochemical structure–activity relationship among Picaridin and IA3-37220 diastereoisomers and DEET. It is clear from the study that most active compounds, namely Picaridin *RS*, IA3-37220*SS* and DEET, have very similar structural motifs which lead to high degree of matching of the relevant parts of the molecule. A sharp contrast to this is the

stereochemical similarity/dissimilarity between these three active structures vis-à-vis less active isomers of IA3-37220 and Picaridin. SSAR suggests that the critical bio-macromolecule responsible for the recognition of the repellent is highly sensitive to the dispositions of the atoms in space. Commonalities in the vicinity of structures within the active space defined by Picaridin *RS*, IA3-37220*SS* and DEET may be useful in the computer-assisted design and synthesis of novel molecules from overlay studies.

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