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Actions between neonicotinoids and key residues of insect nAChR based on an ab initio quantum chemistry study: Hydrogen bonding and cooperative π–π interaction

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Abstract—Neonicotinoid insecticides show selective actions on insect nicotinic acetylcholine receptor (nAChR). Two key residues (Trp and Arg/Lys) have been identified as contributing to the neonicotinois binding. To investigate the selective mechanism, a computational model was set up to simulate the interaction between residues (Trp and Arg) of insect nAChR and neonicotinoids by quantum chemistry method. Three analogues of neonicotinoid derivatives without the chloropyridinyl moiety and 3-methyl-indole (3MI), guanidinium (Gua) were used to mimic the neonicotinoids and the side chain of key residues Trp and Arg accordingly. Interaction features of 3MI-analogues, analogues-Gua and 3MI-analogues -Gua complexes were analyzed comparatively. Hydrogen bonding between the nitro group of analogues and Gua was found to be the most important for binding. Moreover, the cooperative π–π interaction between analogues and the indole ring, which is strengthened by the existence of Gua, also contributes to the binding. The alternative binding model of neonicotinoids proposed here, although slightly different from others, might be close to the actual.

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1. Introduction

Nicotinic acetylcholine receptors (nAChR), the prototypical member of the Cys-loop ligand-gated ion channel (LGIC) superfamily, contribute to a wide range of brain activities and influence a number of physiological functions, and serve as the targets for treatments of Alzheimer's disease, Parkinson's disease, schizophrenia, stroke, learning deficits, and drug addiction.[1,2] Furthermore, nAChRs are also the targets of neonicotinoids, the novel class insecticides including the most widespread pesticide of pest control, imidacloprid (IMI).[3–5] However, insect resistance for neonicotinoids has emerged recently,[6–10] which increases the necessity for understanding the binding details of neonicotinoids. Compared to the mammalian nAChR selective compounds with the quaternary ammonium group, neonicotinoids have distinct structure features and selectively bind to insect nAChRs.[11–13] Now, the binding modes of mammalian selective compounds are clear. The cation–π interaction[14–18] between the positive center of the ligand and the aromatic residue of nAChR is the prominent feature in the recognition of agonists by nAChR.[1,19] However, the binding mode of neonicotinoids is not clear now.

Pioneering mutation and bioinformatics studies identified two key residues are critical for the neonicotinoid binding: the aromatic residue Trp and positively charged residue Arg/Lys.[1,20] By ^{15}N NMR research, Yamamoto et al.[21] proposed that the unshared electron pair on the nitrogen atom (N1, labeled in Fig. 1, the same below) is delocalized by the presence of strong electron-withdrawing group (–NO_2, –CN) and becomes partially positive, forming cation–π interaction with Trp in insect nAChR.[22] Tomizawa and Casida et al. suggested that the electronegative group (–NO_2, –CN) of neonicotinoids selectively interacs with the unique positively charged residue (Arg/Lys) of insect nAChR.[13,23,24] The sp^2 hybridized nitrogen atom (N1) may undergo a complementary interaction with the π-electrons of the

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Keywords: Neonicotinoids; Nicotinic acetylcholine receptor; Binding model.

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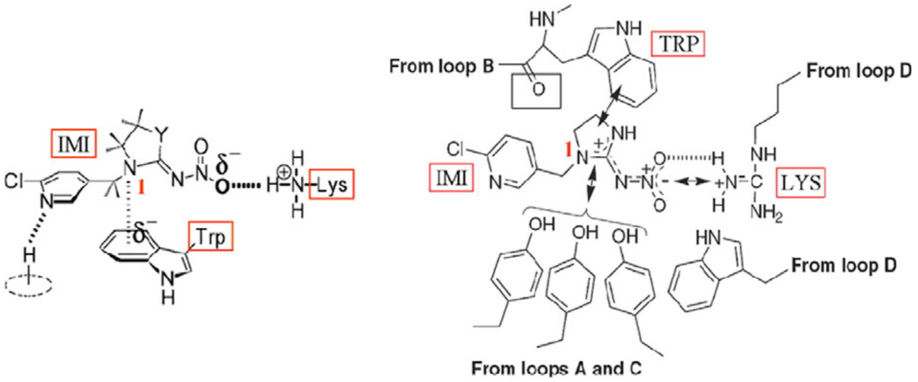


Figure 1. (a) The binding model of Casida et al.[22] (b) The binding model of Sattelle et al.[24]

aromatic residue by p–π interaction, which is different from the binding mode of N protonated nicotinoids with nAChR (cation–π interaction).[23] They also emphasized the importance of the coplanarity between the heterocyclic ring and the guanidine or amidine moiety of neonicotinoids (Fig. 1a). Fig. 1b presents the other binding model that proposed by Sattelle et al.[3,25] In this model, the interaction between positively charged residue (Arg/Lys) and the nitro group of neonicotinoids makes the nitrogen atom (N1) more positive. Therefore, it is possible that the positive imidazolidine moiety comes into contact with the aromatic residues (Trp) through cation–π interactions. According to these models, questions related to the binding mode of neonicotinoids include the following:

1. Which model is more close to the actual feature?
2. Roles of nitrogen atom (N1) in the five-membered ring for the neonicotinoid binding.
3. Roles of key residues (Trp and Arg/Lys) for the neonicotinoid binding.

In the current study, the possible binding mode of the neonicotinoids was investigated by ab initio computational approach. Three neonicotinoid derivatives (Fig. 2, **I**–**III**) with different binding affinities ($pIC_{50(II)} > pIC_{50(I)} > pIC_{50(III)}$) to insect nAChR were selected.[5,26] Considering the computational efficiency reasons, analogue molecules **M1**–**3**, 3-methyl-indole (3MI), and guanidinium (Gua) were used to represent the neonicotinoids derivatives **I**–**III**, aromatic residue Trp, and positively charged residue Arg, respectively (Fig. 2).

Here, the complexes of 3MI–M_x (x = 1–3; the same below), M_x–Gua and 3MI–M_x–Gua were studied, along with the influence of Gua on the interaction of M_x and 3MI. A positive correlation was found between the binding affinities of compounds **I**–**III** to insect nAChRs and the interaction energies of both M_x–Gua and 3MI–M_x–Gua complexes, which indicated that this computational model could represent the binding features of neonicotinoids well. Based on the results, an alternative binding model was built up to picture the binding mode of neonicotinoids to nAChRs.

2. Results and discussion

2.1. The rationality for the structure simplification

In consideration of computational efficiency, the chloropyridinyl moiety of neonicotinoids derivatives was removed because all three derivatives own this moiety. The rationality of such simplification was verified by the NPA atom charge population analysis. Using imidacloprid (I) and the corresponding simplified molecular

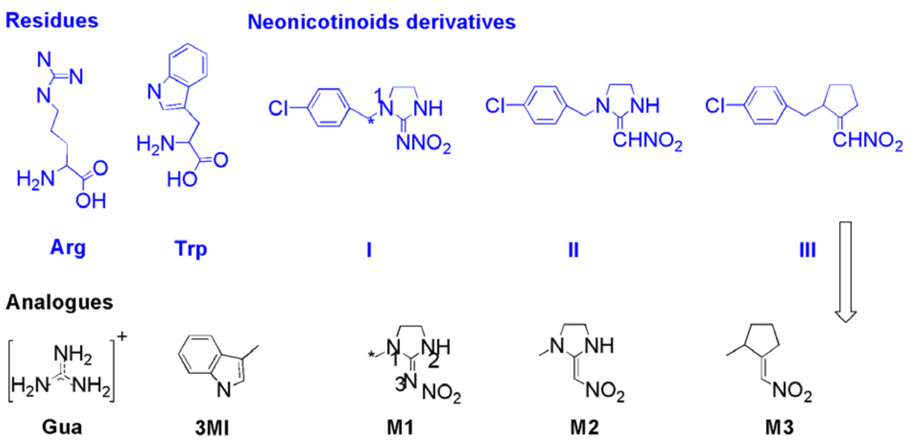


Figure 2. Chemical structures of three neonicotinoids derivatives, key residues in nAChR, and their analogues simplified for computation.

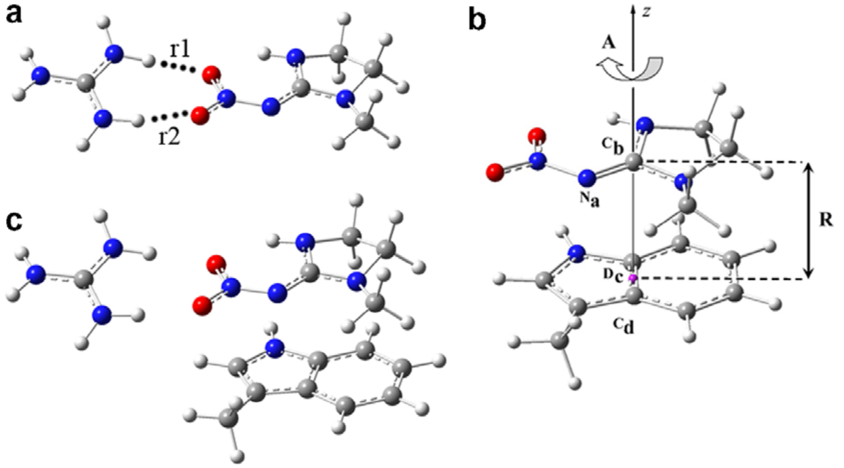


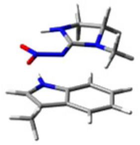
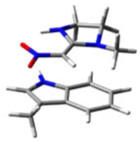
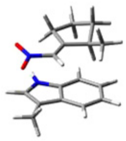
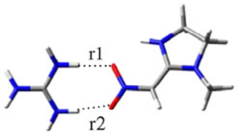
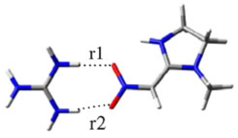
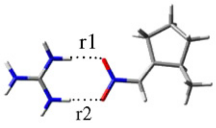
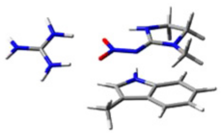
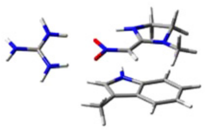
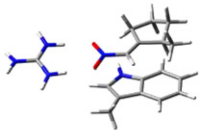
Figure 3. The geometries of models (Using M1 as example). (a) M1–Gua complex; (b) 3MI–M1 complexes. The dummy atom, D_c, is in purple color; (c) 3MI–M1–Gua complexes.

M1 as a reference, the NPA atomic charges were calculated at the MP2/6-31G* level. All calculated NPA atom charge differences were quite small, with an exception of the atom C (with * labeled in Fig. 2). It is because that the chloropyridinyl group directly attachs on this atom. Considering the limiting roles of this atom, the simplification in this work is reasonable.

2.2. The Geometry parameters

The geometry properties of 3MI–M$_x$, M$_x$–Gua and 3MI–M$_x$–Gua complexes are given in Table 1. For all 3MI–M$_x$ complexes, the final geometry parameters are similar. The interaction distance R is 3.4 Å in 3MI–M3 complex, about 0.1 Å larger than that in other two complexes, which showed that 3MI–M3 is slightly less stable compared to the other two complexes. For the M$_x$–Gua complexes, the hydrogen bond distance of M3–Gua is about 0.1 Å longer than that of the other two complexes, indicating that the hydrogen bond of M3–Gua is slightly weaker than that of the other two complexes. For the 3MI–M$_x$–Gua complexes, the geometry parameters R and A were rescanned but remain coincident with that of 3MI–M$_x$.

Table 1. The geometries of 3MI–M$_x$, M$_x$–Gua, and 3MI–M$_x$–Gua complexes[a]

	M1	M2	M3
3MI–M$_x$			
R	3.30 Å	3.30 Å	3.40 Å
A	20.0°	30.0°	20.0°
M$_x$-Gua			
$r1$	1.89 Å	1.84 Å	1.93 Å
$r2$	1.84 Å	1.81 Å	1.91 Å
3MI–M$_x$–Gua			
R	3.30 Å	3.30 Å	3.40 Å
A	20.0°	30.0°	20.0°

[a] R and A were defined in Figure 3b.

2.3. The energy properties

The BSSE corrected binding energies for all complexes are listed in Table 2. For the 3MI–M_x complexes, the binding energies ranged from −6.88 to −7.63 kcal/mol, at the MP2/6-311++G** level. Both geometries and energy data of 3MI–M_x showed that there is no obvious difference among 3MI–M_x complexes, indicating that the binding of 3MI–M_x complexes is not sensitive to the chemical structures of three analogues. M1 and M2 own two sp^2 nitrogen atoms in the five-membered ring, whereas M3 does not. Clearly seen from Table 2, this structure difference has little influence on the interaction between 3MI and M_x. Therefore, the sp^2 nitrogen atom does not influence the 3MI–M_x interaction. Meanwhile, large difference between MP2 and HF binding energies of 3MI–M_x complexes indicates that the electronic correlation is essential for the 3MI–M_x binding. Since all analogues have a conjugate part, the π–π stacking between the conjugate part and the indole ring should dominate in the 3MI–M_x complexes. Similar interaction energies for three complexes also indicated that the conjugation in M1–3 does not exert much effect on the interaction.

For Gua–M_x complexes, significant interaction energy differences were found among M_x–Gua complexes. Gua interacts more favorably with M1 (−28.73 kcal/mol) and M2 (−33.40 kcal/mol) than with M3 (−23.11 kcal/mol), at the MP2/6-311++G** level. In the M1–Gua and M2–Gua complexes, both M1 and M2 have a guanidine or amidine moiety, thus the conjugated electronic system facilitates electron clouds flowing toward $-NO_2$, which enhances the hydrogen bond strength, as pointed out by Casida et al.[13] In contrast to the former two complexes, M3 in M3–Gua complex only contains a C═C double bond, whose conjugation degree is lower than those of M1 and M2, and thus shows less potency to interact with Gua. The NPA (natural population analysis) atom charges were calculated here to prove the impact of conjugation and the coplanarity on inter-molecular interaction. An uncoplanar molecular M4 (non-coplanar) was included here to examine the impacts of coplanarity and conjugation on the intermolecular interaction (Fig. 4). After interacting with Gua, more negative charges flow toward the $-NO_2$ in M1, M2 (higher conjugate status) than those of M3 (lower conjugate status) and M4 (lower conjugate status and non-coplanar), agreeing well with the viewpoint of Casida et al. that coplanarity is essential for the activity of neoicotinoids. Moreover, the conjugation also influences the binding strength of ligand with Arg.

Table 2. Calculated energies for 3MI–M_x, M_x–Gua, 3MI–M_x–Gua, and 3MI–[M_x–Gua] after BSSE correction (Δ*E* in kcal/mol) at 6-311++G**

	3MI–M1	3MI–M2	3MI–M3
MP2	−7.02	−6.88	−7.63
HF	7.97	8.85	6.67
	M1–Gua	M2–Gua	M3–Gua
MP2	−28.73	−33.40	−23.11
HF	−27.77	−32.14	−23.70
	3MI–M1–Gua	3MI–M2–Gua	3MI–M3–Gua
MP2	−37.94	−42.90	−32.47
HF	−20.63	−24.81	−18.11
	3MI–[M1–Gua][a]	3MI–[M2–Gua]	3MI–[M3–Gua]
MP2	−12.13	−12.68	−11.58
HF	6.05	6.21	4.69

[a] Concerning the distance and the orientation of 3MI and Gau in the three-body systems, 3MI have little interaction with Gua, so the energy value of 3MI–[M_x–Gua] (x = 1–3) is the interaction energy between 3MI and M_x, with the existence of Gua.

Figure 4. The charge changes of analogues when interacting with Gua. The structure of M4 shown in the right. The bars in graph present the changes of the sum of charges in two oxygens after the molecular interacting with Gua.

Similar to the M_x–Gua complexes, there are distinct differences in interaction energies between 3MI–M_x–Gua complexes. At the MP2/6-311++G** level, the BSSE corrected interaction energy of 3MI–M3–Gua complex is 5.47 kcal/mol smaller than that of M1, and 10.43 kcal/mol smaller than that of M2, indicating that the 3MI–M3–Gua complex is less favorable than 3MI–M1–Gua and 3MI–M2–Gua complexes. The binding energy of 3MI–M_x–Gua complexes decreases in the sequence of $\Delta E_{M2} > \Delta E_{M1} > \Delta E_{M3}$, which are well correlated with the experimental binding affinities of neonicotinoids derivatives I–III ($pIC_{50(II)} > pIC_{50(I)} > pIC_{50(III)}$).[5,26]

Of course, IC_{50} is not a binding constant and both entropic effects and other residues in the binding site should be considered. In our study, we are not assuming that entropic effects and other residues are unimportant; we are only assuming that variation in IC_{50} is related to the interaction energies between ligand and two key residues. This is reasonable in this case study. Three analogues are very similar, all that the changing is one atom N/C to C/N. It is hard to imagine the dramatic changes in Δ*S* would result from such a change. Similarly, ignoring other residues is based on the assumption that the impacts of other residues are relatively constant for three similarly neonicotinoids derivatives, especially compared with two key residues Trp and Arg/Lys.

2.4. The impact of the Gua on the 3MI–M_x interaction

To investigate the influence that Gua has on the interaction between 3MI and M_x, the interaction energies of 3MI–M_x and 3MI–[M_x–Gua] complexes were compared. The 3MI–[M_x–Gua] complex stands for the interaction between 3MI and M_x in the presence of Gua. For 3MI–[M_x–Gua] complexes, the binding energy difference between MP2 and HF level remains very large.

Therefore, the electron correlation energies are still substantial, which indicated that the dispersion interaction is significantly important for the π–π interaction between 3MI and M_x, not only in 3MI–M_x complexes but also in 3MI–M_x–Gua complexes. As shown in Table 2, interaction energy between 3MI and M_x increased about −3.95 to −5.80 kcal/mol with the existence of Gua, which indicated that Gua could strengthen the π–π interaction between M_x and 3MI, a similar phenomenon has also been revealed by Narahari Sastry et al.[27] that the cations could assist the π–π interaction strengths greatly. The enhanced π–π interaction between 3MI and M_x appears to be an important factor for the binding of neonicotinoids to nAChR.

2.5. The atom charge population analysis

The NPA atomic charges were calculated at the MP2/6-31G* level. The sums of the atom charge on each part of the complexes are given in Table 3. For 3MI–M_x–Gua ($x = 1$–3) complexes, the total amount of charge transfer is the sum of the atom charges on 3MI and M_x ($x = 1$–3), which are 0.096, 0.111, and 0.086, respectively, for the three complexes. Similar to the energy properties, the total atom charge variations are also coinciding with the binding activities. The charge transfer values between 3MI and M_x ($x = 1$–3) are quite small no matter with or without Gua, comparing to that of M_x–Gua ($x = 1$–3) complexes, which also indicated that the hydrogen bond between M_x and Gua is more important than the π–π stacking for ligand's binding to insect nAChR.

By semi-empirical PM3 method, it was suggested that the nitrogen atom of IMI can possess enough positive atom charge (positive by 0.11e) after interacting with Arg/Lys, and then this positive nitrogen atom can mimic the $–CH_2N^+(CH_3)_3$ group of ACh to form similar cation-π interaction with Trp.[3] However, at the MP2/6-31G* level in this case, the sum of the atom charges on M1 and M2 in 3MI–M_x–Gua ($x = 1,2$) complexes is no more than 0.002e. It is difficult to say that cation-π interaction could contribute to the binding of neonicotinoids to nAChR, which is different from the other previous prediction.

Table 3. The sum of the atom charges on each monomer of the complexes (Q/e)

	3MI–M1	M1–Gua	3MI–M1–Gua
M1	0.001	0.093	0.094
3MI	−0.001	/	0.002
Gua	/	0.907	0.904
	3MI–M2	M2–Gua	3MI–M2–Gua
M2	0.001	0.108	0.109
3MI	−0.001	/	0.002
Gua	/	0.892	0.889
	3MI–M3	M3–Gua	3MI–M3–Gua
M3	−0.014	0.083	0.066
3MI	0.014	/	0.020
Gua	/	0.917	0.914

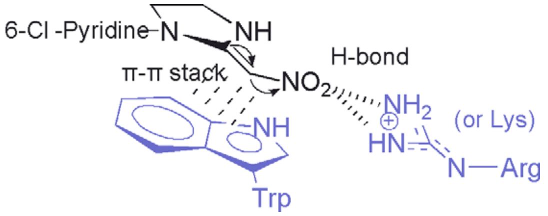


Figure 5. The alternative binding model between neonicotinoids and nAChR.

From the geometry, energy, and charge transfer analysis, a modified model including hydrogen bonding and cooperative π–π interaction between neonicotinoids and nAChR is depicted here (Fig. 5). The positively charged side chain of Arg/Lys interacts with the nitro group of neonicotinoids through hydrogen bonding, which is most important for the binding of neonicotinoids to nAChR. The conjugation and coplanarity of the guanidine or amidine moiety greatly influence this hydrogen bonding strength. Nitrogen atoms in the five-membered ring affect the conjugation and thus contribute to hydrogen binding, instead of affecting the interaction between neonicotinoids and Trp. The conjugation part interacted with the aromatic residue Trp through π–π stacking. The strength of π–π interaction could be enhanced when neonicotinoids interacting with Arg/Lys. This cation enhanced π–π interact is also important for the binding of neonicotinoids to nAChR.

3. Conclusions

This study provides an alternative binding model between neonicotinoids and nAChR. The hydrogen bonds between neonicotinoids and the positively charged side chains of Arg/Lys play essential roles in the binding, as well as the π–π interaction between the conjugate guanidine/amidine moiety and the indole ring of Trp.

The conjugation of guanidine/amidine moiety exerts great influence on the strength of hydrogen bond to Arg and then affects the activity and selectivity of neonicotinoids. According to the binding energies, the impact of the sp^2 hybridized nitrogen atom (N1) on the interaction between 3MI and M_x is very weak, which is different from previous prediction.[23] The sp^2 N may contribute to the hydrogen bonding through the conjugation effect.

The atom charges transfer analysis showed that it is difficult to form cation–π interaction between neonicotinoids and the indole ring of Trp, which is different from the other opinion. Both energy and charge calculations proved that the π–π stacking dominates in the 3MI–M_x interaction, which is also enhanced by the hydrogen bond between neonicotinoids and Arg.

Our results coincide well with the experimental activities. So, the binding model proposed here might provide an alternative way close to the actual binding features of neonicotinoids.

4. Computational details

4.1. Geometries of monomer and M_x–Gua complexes

The initial geometries of analogues of neonicotinoids were taken from the crystal structure of imidacloprid.[28] The geometries of all monomers and M_x–Gua complexes (Fig. 3a. M1–Gua as an example) were fully optimized at the MP2/6-31G* level of theory. Based on the optimized geometries, the vibrational frequency analysis was performed at the same level to make sure that all structures are genuine minimums.

4.2. Geometries of 3MI–M_x complexes

According to the common features of Sattelle's and Casida's models, 3MI and M_x were parallelly placed in the initial structures. Figure 3b shows the 3MI–M1 complex as an example, of which the C_b atom and D_c (the dummy atom) were located along the vertical axes z. At the MP2/6-311++G** level of theory, the final geometries were determined by the potential energy curves scan against the interaction distance R and the dihedral angle A (N_a-C_b-D_c-C_d). Method adopted here is similar to that proposed by others.[29,30] The distance R was scanned by moving the analogues from 6.0 to 2.8 Å along the axes z at an interval of 0.2 Å, and then at 0.1 Å in the range around the minimum (range: minimum ±0.5 Å). The dihedral angle A was scanned by rotating the analogues around the axes z at an interval of 30° and then at 10° in the range around the minimum (range: minimum ±20°). Then the final geometries were defined by the two parameters: $R_{\min}$ and $A_{\min}$.

4.3. Geometries of 3MI–M_x–Gua complexes

Similarly, the initial geometries of 3MI–M_x–Gua complexes are depicted in Figure 3c (using 3MI–M1–Gua as the reference). The M_x–Gua parts were taken from the former MP2/6-31G* optimized structure. The values of R and A were obtained by the former potential energy curves scan of 3MI–M_x complexes. Taking into account the influence of Gua on the geometries of 3MI with M_x, the geometry parameters R and A (as defined in Fig. 3b.) were rescanned at the MP2/6-311++G_{**} level.

4.4. Energy calculations

All interaction energies of complexes were calculated at the MP2/6-311++G** level of theory. The basis set superposition errors (BSSE) were corrected by the counterpoise method at the same level. The interaction energy ΔE in complex A–B was defined as:

$$\Delta E = E_{\text{A–B}} - E_{\text{A}} - E_{\text{B}} + \text{BSSE}.$$

The interaction energy ΔE in three-body system was defined as:

$$\Delta E = E_{\text{A–B–C}} - E_{\text{A}} - E_{\text{B}} - E_{\text{C}} + \text{BSSE}.$$

The electron correlation energy (ΔE_{ec}) was roughly estimated by the ΔE difference between the MP2 and HF level:

$$\Delta E_{\text{ec}} = \Delta E_{\text{MP2}} - \Delta E_{\text{HF}}.$$

4.5. Atom charge computation

The NPA (natural population analysis) atomic charges were calculated at the MP2/6-31G* level using the MP2 density.

All calculations were performed using the Gaussian03 package.[31] The chemical structures are drawn by using ISIS/Draw (http://www.bio-soft.net/chemdraw/ISISDRAW.htm).

Acknowledgments

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