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Molecular Orbital Calculation for the Model Compounds of Kainoid Amino Acids, Agonists of Excitatory Amino Acid Receptors. Does the Kainoid C4-Substituent Directly Interact with the Receptors?

Kimiko Hashimoto,[a,*] Takatoshi Matsumoto,[b] Kensuke Nakamura,[c] Shu-ichi Ohwada,[a] Tatsuro Ohuchi,[a] Manabu Horikawa,[a] Katsuhiro Konno[d] and Haruhisa Shirahama[a,†]

[a]*Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan*
[b]*Department of Synthetic Organic Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan*
[c]*Institute of Medical Molecular Design, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[d]*Institute of Biosciences of Rio Claro, São Paulo State University, Rio Claro, SP 13506-900, Brazil*

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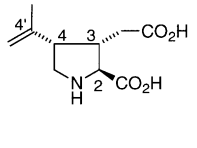
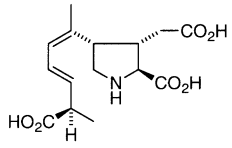
Abstract—Kainoid amino acids are agonists of the AMPA/kainate receptors and exhibit highly potent neuroexcitatory activity. From the results of extensive structure–activity relationship studies, we previously postulated that the C4-substituent of the kainoid amino acids interacts with an allosteric site of the glutamate receptor with electron-donating character. In order to investigate the mode of action in more detail, molecular orbital calculation for model compounds of the kainoid were performed. The results indicated that the HOMO energy level of the C4-substituent is involved in the potent neuroexcitatory activity, thus supporting our hypothesis.

Introduction

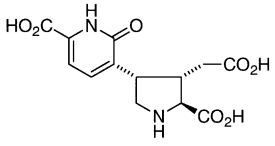
Excitatory amino acid (EAA) receptors play a central role in memory and learning in mammalian central nervous systems and are also involved in neurodegenerative disorders such as epilepsy and stroke. These receptors are classified into two major subtypes, ionotropic and metabotropic. The former is further classified by selective representative agonists into two groups: the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)/kainate (kainic acid) group and the NMDA (*N*-methyl-D-aspartic acid) group. The function and pharmacology of the NMDA receptors have been well documented because selective antagonists are available, but those of the AMPA/kainate receptors have been hampered due to the lack of selective ligands. However, selective antagonists have developed in recent years, which would accelerate the studies of these receptors.[1–4]



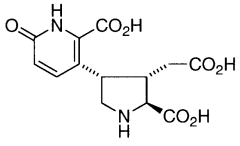
kainic acid (**1**)



domoic acid (**2**)



acromelic acid A (**3**)



acromelic acid B (**4**)

Kainoid amino acids including kainic acid (**1**), domoic acid (**2**), and acromelic acids A (**3**) and B (**4**) are the agonists of the AMPA/kainate receptors and exhibit

*Corresponding author at present address: Lab. of Biochemical Resources, Plant Science Center, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama, 351-0198, Japan. Tel.: +81-48-462-111; fax: +81-48-467-5407; e-mail: kimikoh@postman.riken.go.jp
†Second corresponding author at present address: 2-16, Minami 18 Nishi 8, Chuo-ku, Sapporo, 064-0918, Japan.

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highly potent neuroexcitatory activity. Due to their remarkable biological properties, the chemistry of these compounds have attracted much attention over the last decade.[5,6] We have synthesized a number of kainoid analogues in order to investigate the structure–activity relationships on depolarization in the rat spinal motoneuron, which revealed that the depolarizing potency depends on the kainoid structures as depicted in Figure 1.[7]

These studies by us and others have provided the following findings; (a) modification of the C4-substituent significantly alters its depolarizing potency, and without the C4-substituent, the compound is no longer the agonist of the AMPA/kainate receptors but of the NMDA-type agonist, (b) the C4′-carbon attached to C4 should have sp^2 hybridization for potent activity, (c) stereochemical arrangement of the side chains in the pyrrolidine ring is favored to be the same as that of the natural products, and (d) the spatial arrangement of the C4′–π-plane should be almost orthogonal to the pyrrolidine ring for potent activity. Taking all these results together, we postulated a model for the interaction of kainoid with its receptors; the C4-substituent of the kainoid interacts with an allosteric site of the receptor in addition to the three binding groups of the glutamic acid moiety (two carboxyl and one amino groups) as schematically shown in Figure 2.[7e] This hypothesis prompted a new synthesis of 4-arylkainoids.[8,9]

This allosteric interaction should arise from the electronic state of the C4′–π-system since the depolarization activity is variably altered within the analogues having the C4′–π-system. Moreover, it seems that the compounds show a high potency when the C4′–π-system has an electron-donating character. Therefore, we performed a molecular orbital calculation for the model compounds in order to estimate the electronic effect of the C4-substituent. As a consequence, we found the HOMO (highest occupied molecular orbital) energy level is related to the potent depolarization activity. Reported herein are the results and discussion of this model study.[10]

Results and Discussion

The molecular orbital calculation was performed for the model compounds in which the pyrrolidine ring was replaced by the methyl group. The calculated values of the HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy levels, and atomic orbital coefficients are separately shown

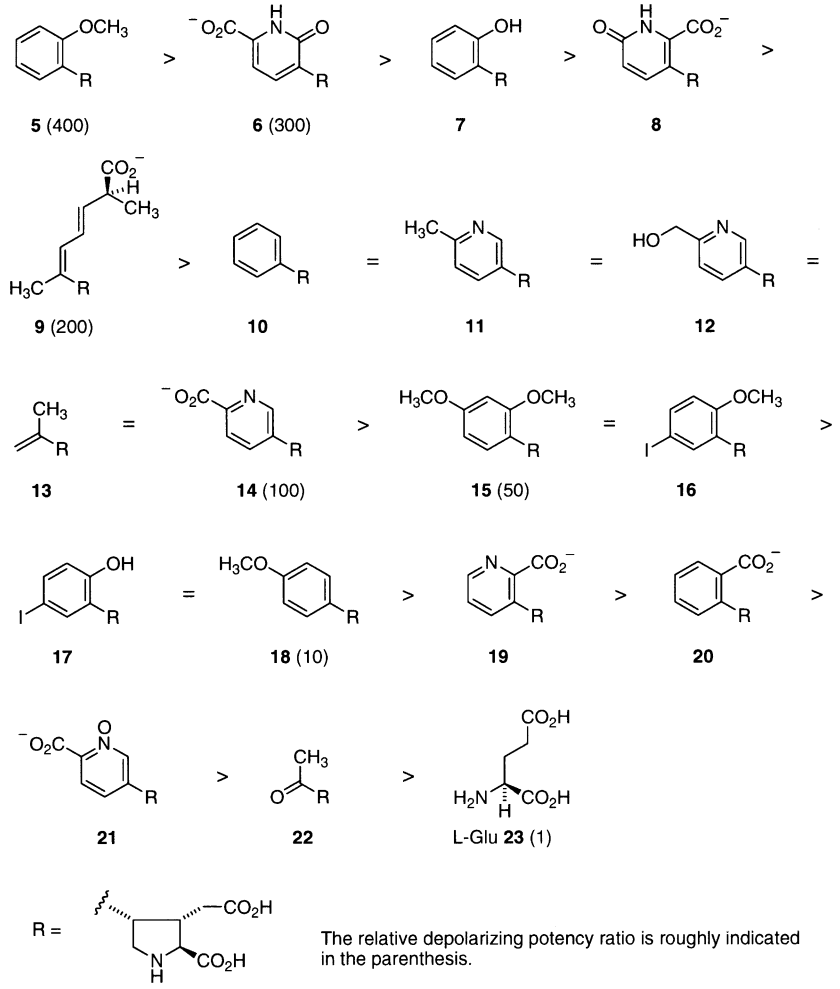


Figure 1. The precise potency ratio cannot be obtained, because the dose–response curve of the each molecule shows different shape.

in Tables 1 and 2 for the undissociated and dissociated compounds, respectively, according to the dissociation state in an aqueous solution. The electronic states in these two cases are far different from each other, and therefore, they can hardly be considered together. In each table, the compounds are listed following the order of the depolarizing potency of the parent compound. For the undissociated compounds (Table 1), the atomic orbital coefficients of each compound are confined to the three atoms corresponding to the smallest molecules, compounds **13** and **22**. These three atoms are numbered as shown, that is, the C4′-atom (kainoid numbering) is numbered as C1, and the more bulky position is numbered as C2. For the dissociated compounds (Table 2), the atomic orbital coefficients of HOMO are not always located at C1–C3; in these cases, the listed HOMO energy means 'the highest orbital having a meaningful coefficient at C1–C3'.[11] The atomic orbital coefficients of the entire molecule are shown in Figures 3 and 4.

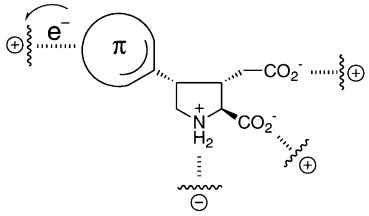
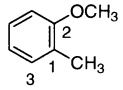
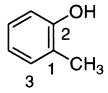
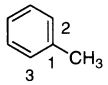
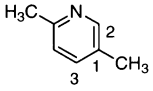
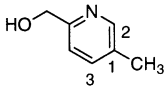
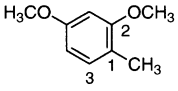
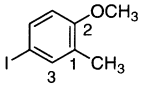
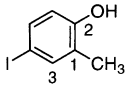
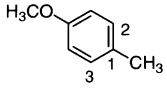


Figure 2. A schematic model for interaction of kainoid with its receptor.

Table 1. HOMO and LUMO orbital energy and atomic orbital coefficient of HOMO concerning several substituents at C4 of kainoids (undissociated fragments in aqueous solution[a]

		Orbital energy/hartree		Atomic orbital coefficient of HOMO		
		HOMO	LUMO	C1	C2	C3
	**5**	−0.242	0.271	0.387	0.426	−0.070
	**7**	−0.245	0.269	0.387	0.441	−0.089
	**10**	−0.270	0.272	0.522	0.287	0.287
	**11**	−0.277	0.248	0.507	0.390	0.135
	**12**	−0.287	0.234	0.493	0.384	0.129
	**13**	−0.302	0.331	0.578	−0.110	−0.646
	**15**	−0.231	0.275	0.495	0.301	0.117
	**16**	−0.232	0.257	0.259	0.362	−0.146
	**17**	−0.234	0.254	0.249	−0.201	0.365
	**18**	−0.239	0.271	0.483	0.178	0.257
	**22**	−0.397	0.297	0.530	−0.155	0.670

[a]The atomic orbital coefficients of each compound are confined to the three atoms corresponding to the smallest molecules, the compounds **13** and **22**. These three atoms are numbered as shown, that is, the C4′-atom (kainoid numbering) is numbered as C1, and the more bulky position is numbered as C2. The atomic orbital coefficients of the entire molecule are figured in Figures 2 and 3.

Comparing the rank order of the potency and the energy level of HOMO and LUMO in Table 1, there may be a positive relationship between the potency and the HOMO energy level. Thus, the HOMO energy levels of **5**, **7**, **10**, **11**, **12**, **13** and **22** decrease in this order and it parallels the rank order of the potency of these compounds. Compounds **15**, **16**, **17** and **18** are not potent despite having the high HOMO energy level comparable to those of **5** and **7**. This can be rationalized by a steric reason; that is, the *p*-methoxy group of **15** and **18**, and the *m*-iodo group of **16** and **17** are so bulky that they are not acceptable to the binding site. In contrast, no relations were observed between the activity and the LUMO energy level nor the atomic orbital coefficient of HOMO. These results supported our hypothesis that the higher electron-donating character of the C4′–π-system, the more potent the activity.

In Table 2, a similar relationship between the potency and the HOMO energy level was observed. The order of potency of **6**, **8**, **9** and **14** parallels their HOMO energy levels. Compounds **19** and **20** exhibit only a poor activity despite having comparable HOMO energy levels to those of **9** and **14**. This might be attributable to the change in their conformation and/or charge distribution that disturbs the interaction with the receptor. The lower activity of the *N*-oxide **21** might be due to the small atomic orbital coefficient of HOMO at C1 relative to the other compounds.[11] The indicated HOMO for **21** is the HOMO-3 and a meaningful coefficient value on

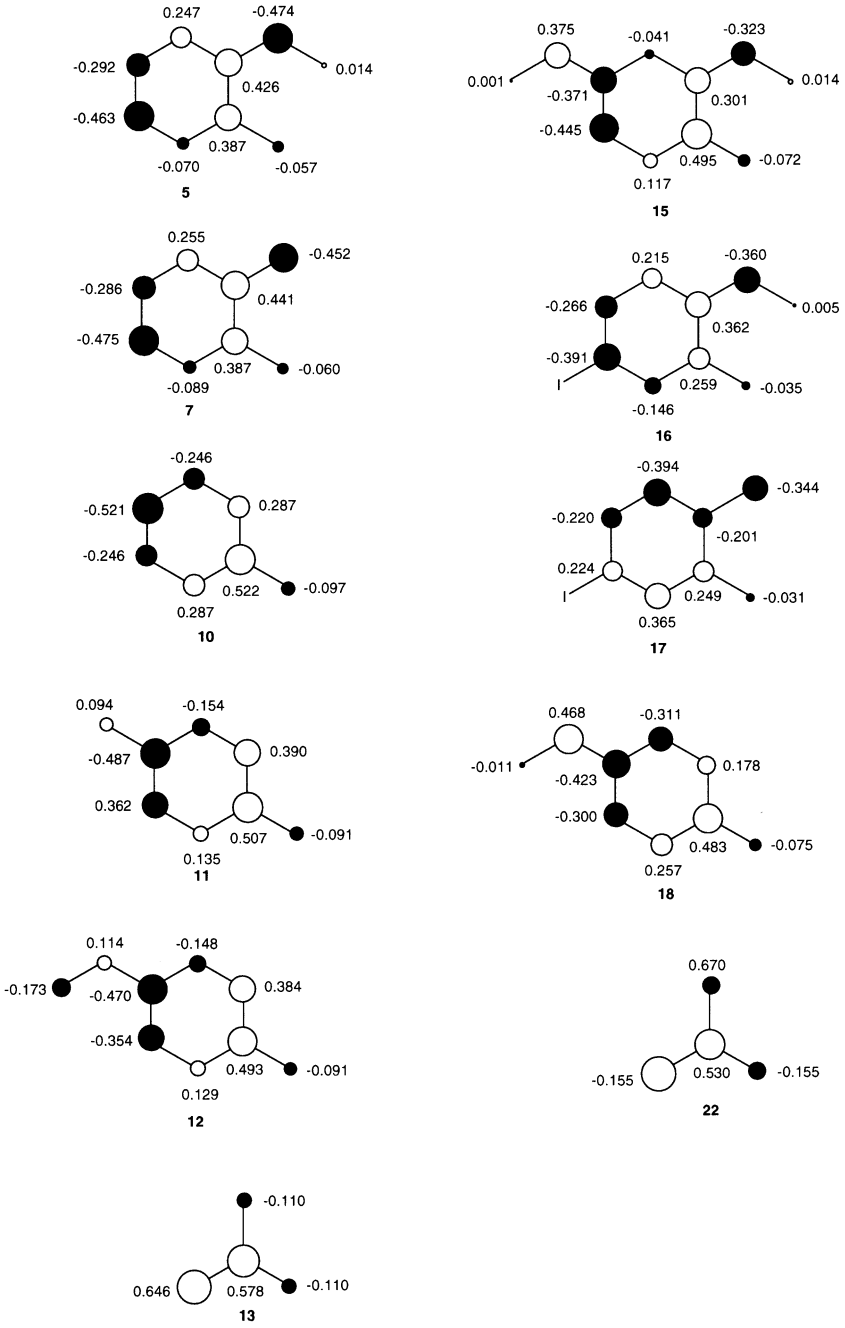


Figure 3. The atomic orbital coefficients of the entire molecule for undissociated compounds.

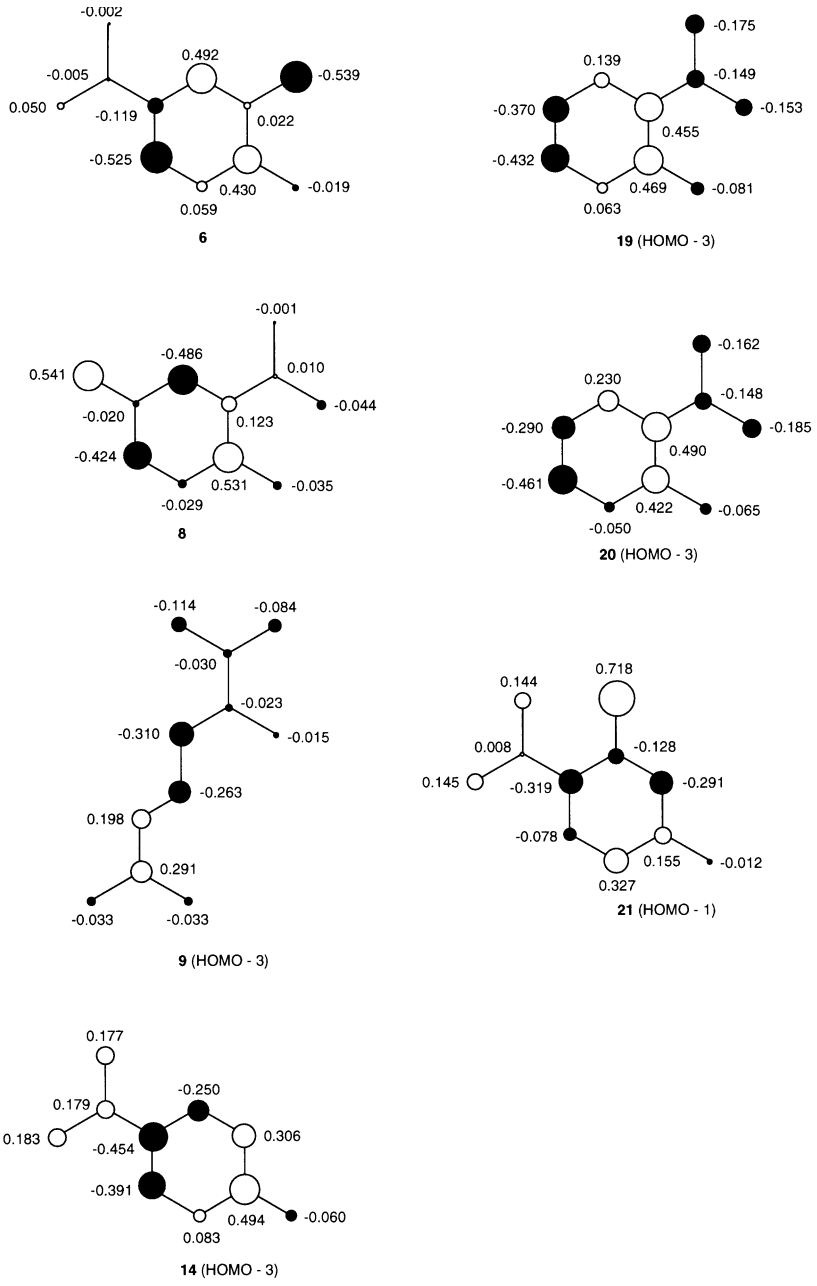


Figure 4. The atomic orbital coefficients of the entire molecule for dissociated compounds.

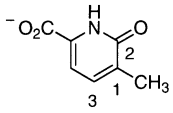
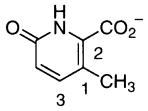
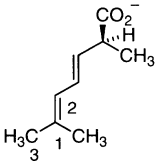
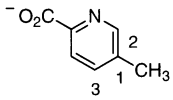
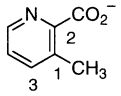
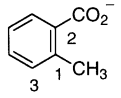
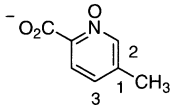
C1 was not observed even at the -4 level under the HOMO.

As we postulated that the C4′–π-plane should be almost orthogonal to the pyrrolidine ring, the conformation around C4–C4′, in other words, the arrangement of the C2 and C3 atoms (shown in Tables 1 and 2) may be considered in this allosteric interaction. However, it is not clear because the differentiation between C2 and C3 by spectral analysis is not always possible. In some of the kainoids, the less bulky substituent tends to locate inside or beneath the pyrrolidine ring based on the NMR and X-ray analyses.[12] The conformation of the pyrrolidine ring can also be involved because it affects the spatial disposition of the functional groups that interact with the receptor. However, no conformation–activity relationship has been found; for example, kainic acid (**1**) and acromelic acid B (**4**) adopt the same conformation in aqueous solution, but the latter shows a far greater activity than the former, and this is also the case for domoic acid (**2**) and acromelic acid A (**3**).[13] Elucidation of the conformational behavior of kainic acid by molecular modeling and NMR spectra revealed that kainic acid is a complex mixture of conformers with comparable energies in aqueous solution.[14]

Conclusions

The above described results supported our hypothesis that the C4-substituent of kainoid directly interacts with its receptors, donating electrons to the receptors, and suggested that the HOMO energy level of the C4′–π-system is related to their potent neuroexcitatory activity.

Table 2. HOMO and LUMO orbital energy and atomic orbital coefficient of HOMO concerning several substituents at C4 of kainoids (dissociated fragments in aqueous solution[a]

		Orbital energy/hartree		Atomic orbital coefficient of HOMO		
		HOMO	LUMO	C1	C2	C3
	**6**	0.020	0.405	0.430	0.022	0.059
	**8**	0.021	0.405	0.531	0.123	−0.029
	**9**	−0.120 (HOMO − 3)	0.389	0.291	0.198	−0.033
	**14**	−0.112 (HOMO − 3)	0.402	0.494	0.306	0.083
	**19**	−0.113 (HOMO − 3)	0.403	0.469	0.455	0.063
	**20**	−0.099 (HOMO − 3)	0.426	0.422	0.490	−0.050
	**21**	−0.018 (HOMO − 1)	0.364	0.155	−0.291	0.327

[a]In this case, the atomic orbital coefficients of HOMO are not always located to the C1–C3; in these cases, the listed 'HOMO energy' means 'the highest orbital having meaningful coefficient on the C1–C3'.

Therefore, there should be an allosteric site in the AMPA/kainate receptors with an electron withdrawing character. Studies on the AMPA/kainate receptors have made great progress in recent years; that is, selective agonists and antagonists have been developed[3,15] and X-ray diffraction analyses have identified several amino acid residues involved in the ligand–receptor interaction.[16] In addition to these results, the results of this study would be useful to precisely reveal the ligand–receptor interaction and to design new and selective AMPA/kaninate receptor agonists and antagonists.

Experimental

Input coordinates were built with the Chem3D Pro Ver. 4.0 (Cambridge Soft Corporation) on a Macintosh Power Book 2400 Personal computer. All calculations were performed with MOPAC 93 Rev. 2 (Fujitsu Ltd., Tokyo, Japan; available from Quantum Chemistry Program Exchange) and Gaussian 94 Rev. B.3. (Gaussian, Inc., Pittsburgh PA, USA, 1995) on a DEC Alpha Station 5/333 workstation.

The geometry optimization was fully carried out by MOPAC 93 Rev. 2-AM1[17] with using the PRECISE option in order to decrease the gradient norm. We selected the minimal basis set to calculate the orbital coefficient of bigger molecules. The orbital coefficient calculation was carried out by Gaussian 94 Rev. B.3.-RHF/STO-3G[18] since these methods required no significant calculation time.

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11. The HOMO and some under HOMO level energies and the corresponding atomic orbital coefficients for the compounds **9**, **14**, **19**, **20**, and **21**.

	Orbital energy/hartree		Atomic orbital coefficient		
			C1	C2	C3
9	HOMO	0.031	0.002	0.000	0.000
	−1	0.012	0.091	0.027	−0.001
	−2	−0.018	−0.001	0.000	0.000
	−3	−0.120	0.291	0.198	−0.033
14	HOMO	0.038	0.001	0.007	−0.008
	−1	−0.004	0.000	0.000	0.000
	−2	−0.015	0.000	0.000	0.000
	−3	−0.112	0.494	0.306	0.083
19	HOMO	0.017	0.014	0.004	−0.003
	−1	−0.013	0.001	0.001	0.002
	−2	−0.028	0.006	0.002	−0.005
	−3	−0.113	0.469	0.455	0.063
20	HOMO	0.021	0.011	0.008	−0.007
	−1	−0.012	0.000	0.000	0.000
	−2	−0.026	0.000	0.000	0.000
	−3	−0.099	0.422	0.490	−0.050
21	HOMO	0.014	−0.004	0.000	0.000
	−1	−0.018	0.155	−0.291	0.327
	−2	0.024	0.000	0.001	0.000
	−3	−0.042	0.003	−0.169	0.164
	−4	−0.112	0.001	−0.001	0.000

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