

Molecular Structure Optimization and Molecular Dynamics Using Hamiltonian Algorithm: Structure of Benzodiazepine Minor Tranquilizers—Towards Non-Empirical Drug Design—

Hiroyuki Teramae,*[1] Kazushige Ohtawara,[2,†] Takayoshi Ishimoto,[3,4] and Umpei Nagashima[3,4]

[1]Department of Chemistry, Faculty of Science, Josai University, 1-1 Keyakidai, Sakado 350-0295

[2]ATR Adaptive Communication Research Laboratories, 2-2-2 Hikaridai, Seika-cho, Soraku-gun, Kyoto 619-0288

[3]Research Institute for Computational Science, National Institute of Advanced Industrial Science and Technology, 1-1-1 Umezono, Tsukuba 305-8568

[4]Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi 332-0012

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We have studied the molecular dynamics of the benzodiazepine and thienodiazepine minor tranquilizers using a Hamiltonian Algorithm (HA) combined with ab initio molecular orbital methods. The HA utilizes classical dynamics for optimization of the complicated systems. We show that the HA gives an effective search of the potential energy surfaces and we can find an energy minimum even if we start from another energy local minimum. The conformers of these minor tranquilizers are calculated after computation of the molecular dynamics. Relations between electronic states and strength of tranquilizers are studied for about 17 species of benzodiazepines and thienodiazepines which are sold in the Japanese market as minor tranquilizers. The orbital energy levels of the next highest occupied molecular orbital (next-HOMO or HOMO−1) are found to be strongly related to the strength of the tranquilizers. The simple structure–activity relationship is obtained by considering just one-electron properties, i.e., the molecular orbital energies.

From the last two decades of the 20th century, energy gradient methods combined with ab initio molecular orbital theory have been widely used and have enabled the optimization of calculated molecular structures through electronic structure calculations. In these optimizations, several optimization methods such as the Newton–Raphson method, the steepest descent method, or the conjugate gradient method are usually applied as an algorithm of the optimization itself. These methods, however, have two difficulties that need to be overcome in searching for the global minimum.

(1) It is necessary to start from the vicinity of minima or the optimization process sometimes diverges.

(2) Starting from one minimum point, it is impossible to locate another minimum point, i.e., it is impossible to escape from a local-minimum.

The Monte Carlo (MC) method as well as the simulated annealing method is usually applied to escape from a local-minimum. In the MC method, a large part of the computational time is wasted in calculating unstable structures of higher energy. In the simulated annealing method, it is difficult to control the temperature of the critical points. Optimization using the above two methods combined with ab initio molecular orbital calculations is considered difficult and has not been reported to the authors' best knowledge.

† Present address: Technology Development Division, Victor Company of Japan, Ltd., 58-7 Shinmei-cho, Yokosuka 239-8550

The Hamiltonian Algorithm (HA)[1] is proposed as a general optimization method and it has been applied to many problems, such as the optimization of packet routing, the optimization of axis adjustment of optical fibers, and the optimization of the quantum table used by JPEG.[2] We recently applied the HA to quantum chemistry problems,[3] i.e., the optimization of molecular structures. We showed that the HA gives an effective search of the potential energy surface and we can find an energy minimum even if we start from another energy local minimum through the search of the optimized structure of an HCN molecule starting from an HNC molecule.

For more advanced application of the HA, we choose electronic structure calculations with geometry optimization of benzodiazepines and thienodiazepines. Small molecule pharmaceuticals are of relatively large size and generally show no symmetry. It is, therefore, difficult to obtain even a local optimized geometry for them, and requires much effort to find the global minimum.

The first benzodiazepine (BZP) compound as a minor tranquilizer or sedative was chlordiazepoxide (Librium[4]) which has been sold by Roche in the United States since 1960. After the great success of chlordiazepoxide, many kinds of BZP compounds have been synthesized. Nowadays, 17 minor tranquilizers which have the BZP or thienodiazepine (TZP) backbone are sold in Japan.

The action mechanism of BZP and TZP minor tranquilizers are as follows; γ-amino-butyric acid (GABA) interacts with a GABA-A receptor at the postsynaptic membrane. The GABA-

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A receptor then opens a chloride ion channel and the chloride ion passing through the channel results in sedation. The interaction of the BZP or TZP on the β-unit of the chloride ion channel just accelerates the interaction of the GABA and GABA-A receptor, resulting in sedation. The BZP and TZP minor tranquilizers do not directly control the chloride ion channel and therefore are very safe drugs.

The study of these BZP and TZP minor tranquilizers is interesting because many drugs have been synthesized with different substituents and a different substituent gives different potency. In addition, there are conformers for certain substituents. The substituents play an important role in both the potency of the drug and the differences in molecular structure, which are closely correlated with each other. Ab initio calculation of molecules of such size has become relatively easy with today's parallel processing computers. Therefore, drug design by ab initio molecular orbital theory is considered to be an interesting target.

In the present study therefore, we investigate the optimized geometries and electronic structures of the 17 BZP and TZP minor tranquilizers sold in Japan using the Hamiltonian algorithm, which will be a prototype of ab initio drug design.

Method of Calculations

The details of the HA have been discussed elsewhere and we will just repeat the part which is necessary for the present study.

In the HA, we consider the virtual motion of particles x (in this case, the atomic nuclei) of the cost function $V(x)$, which is the energy value calculated by ab initio molecular orbital methods in the present case, and write a Hamiltonian of the motion

$$H(p,x) = \frac{1}{2}\sum_{i,j}\frac{p_i b_{ij} p_j}{\sqrt{m_i m_j}} + V(x) \tag{1}$$

where (b_{ij}) is a positive definite symmetric matrix, p_i is momentum, and m_i is mass of the particles. The first term of the right-hand side of the equation is the virtual kinetic energy term, and we allow the off-diagonal term of the kinetic energy. The off-diagonal term reveals mixing of the motion of each particle, and the coefficient b_{ij} represents the degree of this mixing. This mixing procedure provides an effective search of the energy surface.[5]

By including the off-diagonal term of the kinetic energy, the randomness of motion would be expected to increase and the dependency on the initial structure would be expected to decrease. The possibility to reach the global minimum thus becomes larger, which is clearly shown in the optimization of axis adjustment of optical fibers.[1] It is equivalent to ab initio molecular dynamics simulation when the off-diagonal terms are zero. The usual molecular dynamics (MD) calculations depend on the initial structures and require starting from several initial structures, increasing the total computational time.

The following is used to define the coefficient b_{ij}. Let **B** denote the positive definite symmetric matrix (b_{ij}).

$$\mathbf{D} = \mathbf{I} + \lambda\mathbf{A} \tag{2}$$

where **I** is an identity matrix, **A** is a symmetric matrix whose elements are given by a random number, and λ represents suitable constants. The matrix **C** is obtained from matrix **D** by using the Gram–Schmidt process.[6] The positive definite symmetric matrix **B** is given by using the non-constant eigenvalue $\boldsymbol{\varepsilon}$:

$$\mathbf{B} = \mathbf{C}\boldsymbol{\varepsilon}C^{\mathrm{T}} \tag{3}$$

Here, we just note that the degree of mixing is represented by the eigenvalues of the matrix. We define the maximum difference between the eigenvalues of matrix **B** and 1 as the mixing coefficient. Finally the equation of the motion is presented as

$$\ddot{x}_i = \sum_j \frac{b_{ij} f_j}{\sqrt{m_i m_j}} \tag{4}$$

where f_i is the force acting on atom i, which can be calculated by the energy gradient method. Under a certain initial structure and initial kinetic energy, we can successively solve the equation of motion by the Verlet method[7,8] while keeping the total energy of the system constant. Within the framework of the Verlet method, the velocity at a time t is represented as

$$v_i(t) = (x_i(t) - x_i(t-\Delta t))/\Delta t + \ddot{x}_i(t)\Delta t/2 \tag{5}$$

This velocity and the previous geometry can then be used to calculate the next geometry,

$$x_i(t+\Delta t) = x_i(t-\Delta t) + 2\Delta t \cdot v_i(t) \tag{6}$$

Only the second geometry cannot be calculated by this scheme, and we utilize

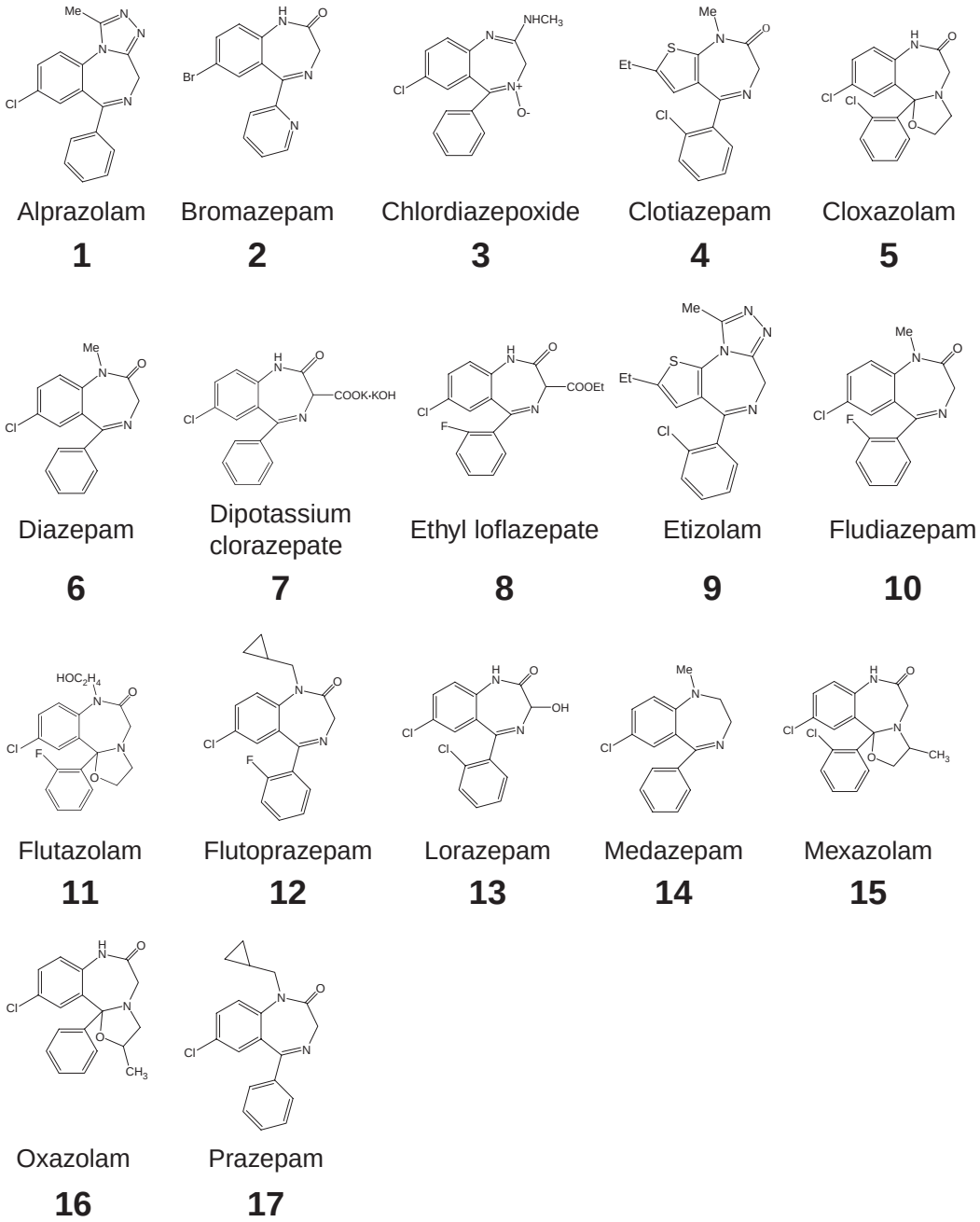
$$x_i(0+\Delta t) = x_i(0) + v_i(0)\Delta t + \ddot{x}_i(0)\Delta t^2/2 \tag{7}$$

When the constraints of the kinetic energies for the translations of the center of the gravity are zero,

$$\sum_i m_i v_i(0) = 0 \tag{8}$$

The initial geometry and velocity should be given as the parameters. We examined initial kinetic energy in 0.20 au in this study. The time interval Δt is 40 au throughout the present study. We keep all of the geometry and potential energy (=cost function) of the molecule during the motion period and determine the minimum point after all of the calculations were finished. We can find the potential energy minimum and perform the optimization if the motion has mixing and we observe the motion for a sufficient period of time.

The optimization procedure utilizes classical dynamics and therefore, we call it a "Hamiltonian Algorithm." We wrote a program based on the HONDO5 package[9] running on a LINUX operating system with MPI parallel library on a PC cluster system. The RHF calculation with the 3-21G basis set is used throughout. The time step to solve the Verlet method is 40 atomic units as denoted above and the iterative calculations are repeated 1000–3000 times. All of the calculations start from one of the MM2 optimized structures using Chem3D.[10] We obtain snapshots at every 100 calculations, and further optimize with conventional optimization procedures to obtain rigorous structures. We obtain therefore, 10 optimized structures for 1000 iterative calculations including duplicated structures. We usually obtain several isomers, and we use the structure having the lowest energy for the next analysis. All the figures of the molecular structures and the molecular orbitals in the present work are obtained by using Molekel program.[11]



Scheme 1.

Results and Discussion

The 15 BZP and 2 TZP minor tranquilizers calculated in the present work, are, alprazolam, bromazepam, chlordiazepoxide, clotiazepam, cloxazolam, diazepam, dipotassium clorazepate, ethyl loflazepate, etizolam, fludiazepam, flutazolam, flutoprazepam, lorazepam, medazepam, mexazolam, oxazolam, and prazepam which are shown in Scheme 1.

Table 1 shows the number of isomers of these 17 compounds found through the HA computational procedures described in the last part of the method and calculations. All the optimized structures have just C_1 symmetry and generally it is difficult to find these structures. We find five isomers for flutoprazepam, four isomers for fludiazepam and oxazolam, three isomers for clotiazepam, ethyl loflazapate, and mexazolam, two isomers for dipotassium clorazepate, etizolam, medazepam, and prazepam. The other compounds are calculated to have just one structure. These compounds are alprazolam, bromazepam, chlordiazepoxide, cloxazolam, diazepam, flutazolam, and lorazepam.

We are able to find the third isomers of dipotassium clorazepate, etizolam, and fifth and sixth isomers of flutoprazepam, if we allow extra iterations after 1000 iterative calculations, also shown in Table 1. We find the third isomers of dipotassium clorazepate and etizolam after 1500 and 1100 iterations, respectively. This fact shows that the 1000 iterative calculations are not sufficient for certain compounds, however, it should be noted that the global minimum structures for most of the study

Table 1. The Number of Isomers Found through the HA Calculations

Compounds	Number of isomers
Alprazolam	1
Bromazepam	1
Chlordiazepoxide	1
Clotiazepam	3
Cloxazolam	1
Diazepam	1
Dipotassium clorazepate	2[a)]
Ethyl loflazapate	3
Etizolam	2[b)]
Fludiazepam	4
Flutazolam	1
Flutoprazepam	4[c)]
Lorazepam	1
Medazepam	2
Mexazolam	3
Oxazolam	4
Prazepam	2

a) Found the 3rd isomer at 1500 iterations. b) Found the 3rd isomer at 1100 iterations. c) Found the 5th and 6th isomer at 1200 and 1300 iterations, respectively.

compounds are calculated within 1000 iterations. The only exception is dipotassium clorazepate whose global minimum is obtained after 1500 iterations.

Figure 1 shows the results of clotiazepam. We obtain three conformers of clotiazepam. The rotation of the ethyl group attached to the thiophene ring makes the three isomers. Figure 2 shows the three optimized structures of dipotassium clorazepate. These isomers result from proton transfer. As denoted before, the third isomer is the global minimum among the three isomers and cannot be obtained within 1000 iterative calculations.

Figure 3 shows the optimized structure of ethyl lofrazepate. These isomers are obtained from the rotation of the phenyl and methyl groups. We think that there are yet up to six isomers, however, we terminate the calculations at 1000 iterations because it is easy to calculate the remaining isomers combining two rotations. Figure 4 shows the optimized structures of etizolam. The rotation of the ethyl group attached to the thiophene ring makes the three isomers and this is identical to the case of clotiazepam. The third isomer is found after 1100 iterative calculations.

Figure 5 shows the optimized structures of flutoprazepam, while Figure 6 shows those of prazepam. The six isomers of flutoprazepam are obtained from rotations of the phenyl group and the cyclopropyl group and two isomers of prazepam are obtained from rotation of the cyclopropyl group. The difference between flutoprazepam and prazepam is just a fluorine substituent on the phenyl group. It is very interesting that the introduction of the fluorine on the phenyl ring allows the isomers with the rotation of the phenyl ring. The fifth and sixth isomers of flutoprazepam are found after 1200 and 1300 iterative calculations, respectively. We consider yet other isomers should be found for these prazepam compounds. For example, a similar isomer of flutoprazepam to the second isomer of prazepam shown in Figure 6b is not found. The isomer in Figure 6b has, however, much higher energy than that of Figure 6a and is not important.

Figure 7 shows the three optimized structures of mexazo-

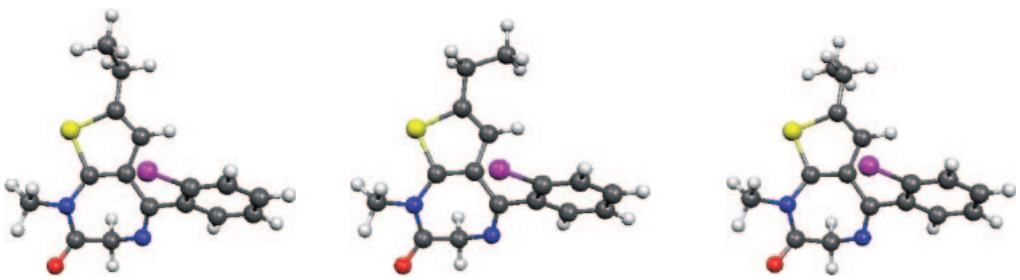


Figure 1. The optimized stuructures of clotiazepam.

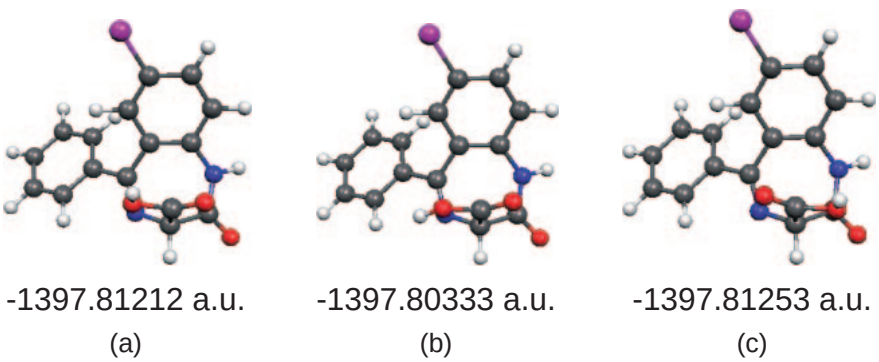


Figure 2. The optimized structures of dipotassium clorazepate.

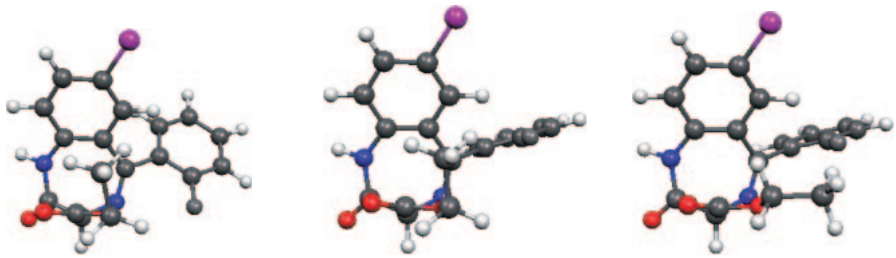


Figure 3. The optimized structures of ethyl lofrazepate.

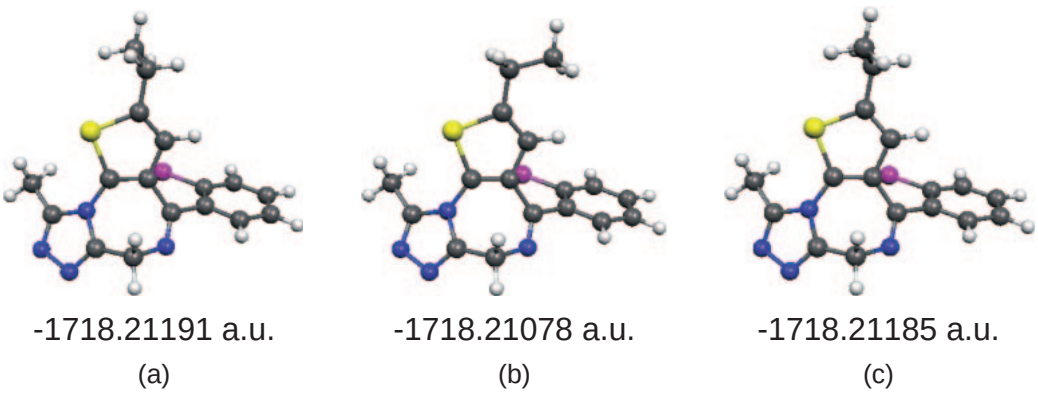


Figure 4. The optimized structures of etizolam.

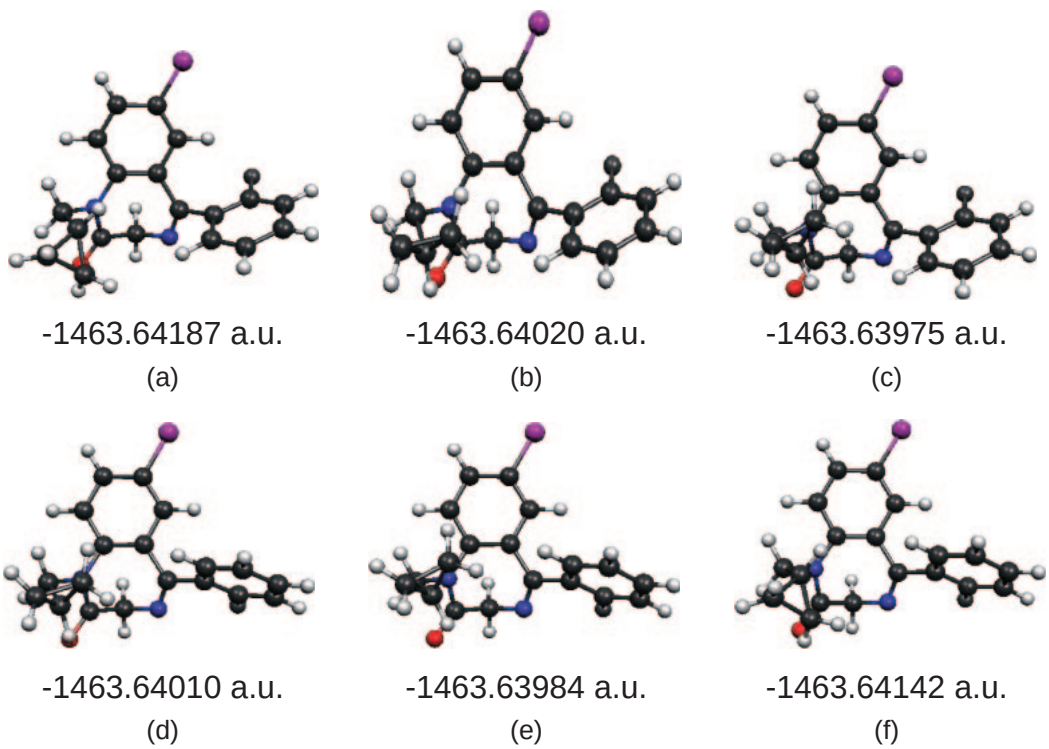
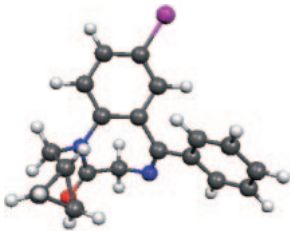
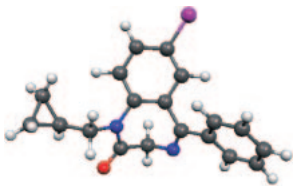


Figure 5. The optimized structures of flutoprazepam.



-1365.32206 a.u.

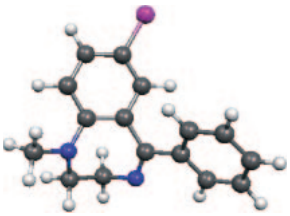
(a)



-1365.31973 a.u.

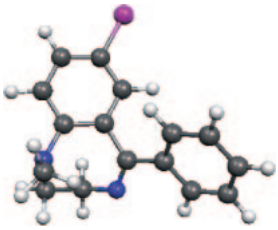
(b)

Figure 6. The optimized structures of prazepam.



-1176.76452 a.u.

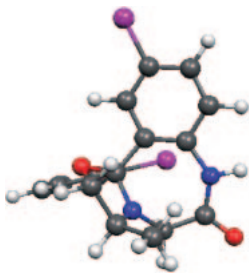
(a)



-1176.76071 a.u.

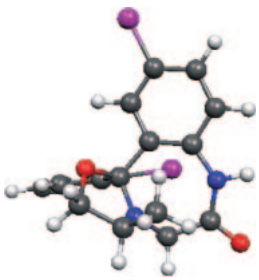
(b)

Figure 8. The optimized structures of medazepam.



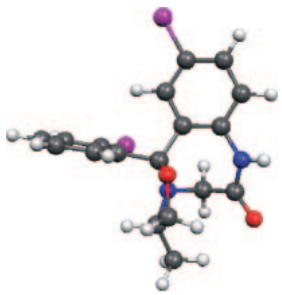
-1858.86747 a.u.

(a)



-1858.86602 a.u.

(b)



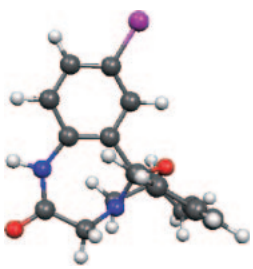
-1858.87397 a.u.

(c)

Figure 7. The optimized structures of mexazolam.

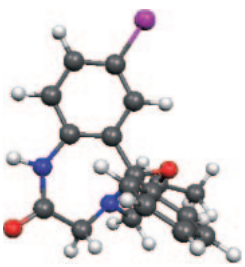
-1402.17034 a.u.

(a)



-1402.16715 a.u.

(b)



-1402.16630 a.u.

(c)

-1402.16996 a.u.

(d)

Figure 9. The optimized structures of oxazolam.

lam. The isomers result from the rotation of the five-membered ring. Figure 8 shows the two optimized structures of medazepam. Two isomers are obtained by inversion of the methyl group attached to a nitrogen atom. Figure 9 shows the four optimized structures of oxazolam, which result from rotation of the phenyl group and inversion of the five-membered ring. Finally, Figure 10 shows the four optimized structures of fludiazepam, which are obtained by rotation of the fluorine-substituted phenyl group. As is the case for prazepam as discussed above, diazepam has only one structure. It is very interesting that both fludiazepam and flutoprazepam have strong potency as tranquilizers, as shown in the following. The rotation of the phenyl group could be a factor in efficacy.

We do not show the other compounds that have only one structure. We believe that we have found all global minimum structures and the most important structures of all 17 compounds. The unstable isomers having higher total energies may not be obtained because the residence time in such a local minimum is very small and requires longer simulation time. There is yet a possibility for other isomers which have much higher energies, however, these structures are considered not to play an important role on the potency of the tranquilizer. The following discussions are based on the structures obtained here.

We almost certainly consider the lowest energy structure of the isomers as the global energy minimum and use them in the following discussions concerning with the activity of the tranquilizer. This would be the most important merit of the HA calculations. We need not struggle to find the minimum structure in almost all cases. We believe the HA calculations rarely fail to find the minimum, although no one can prove it. In almost all the cases in our experience, these candidates are the

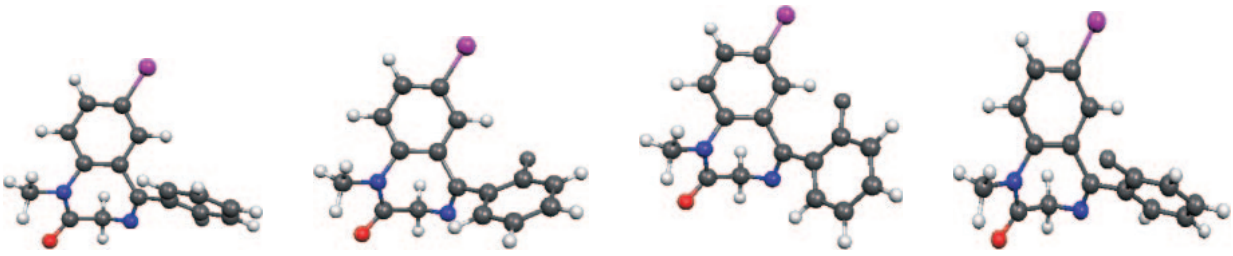


Figure 10. The optimized structures of the fludiazepam.

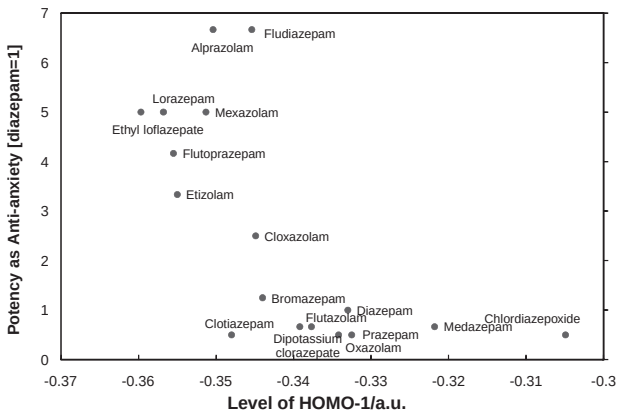


Figure 11. Plots of orbital energy and potency of anti-anxiety.

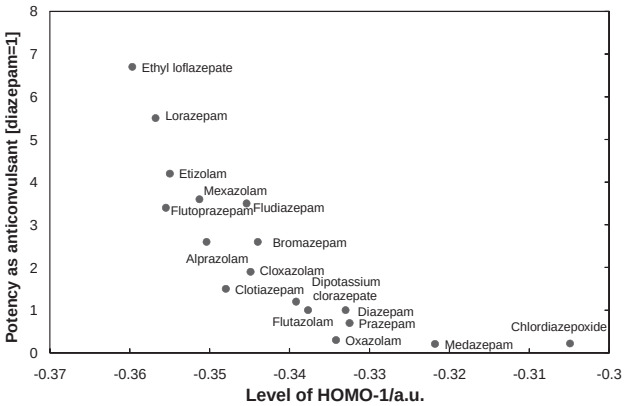


Figure 12. Plots of orbital energy and potency as an anticonvulsant.

Table 2. Correlation Coefficients between Molecular Orbital Energies and the Potency of the Anti-Anxiety, Anti-Convulsant, and Muscle-Relaxing, Respectively[a)]

	Anti-anxiety	Anti-convulsant	Muscle-relaxing
HOMO−2	−0.672701	−0.853517	−0.658609
HOMO−1	−0.660981	−0.814359	−0.538766
HOMO	−0.485885	−0.501642	−0.314232
LUMO	−0.216179	−0.296146	−0.440491
LUMO+1	−0.518712	−0.380488	−0.476319

a) HOMO and LUMO represents the highest occupied molecular orbital and the lowest unoccupied molecular orbital, respectively.

true minimum structures. Unfortunately, we do not have a certain measure when we can terminate further iterations, however, it should be an important subject to show a measure in the near future.

Generally, the relationship between structure and activity is studied by quantitative structure–activity relationships (QSAR), however, we will show that a simpler method can be applied in the case of the BZP and TZP tranquilizers as shown below. Table 2 shows the correlation coefficients[12] between the orbitals and potency as drugs. Figure 11 shows the plots of orbital energies and potency of anti-anxiety and Figure 12 shows plots of orbital energy and potency as an anticonvulsant. Both orbital energies are those of the next highest occupied molecular orbital (next-HOMO; HOMO−1 and HOMO−2). Figure 11 shows a weak relation between the HOMO−1 energy level and the potency of anti-anxiety proposed by Tooru.[13] Figure 12 as well as Table 2 shows the strong relation between the HOMO−1 energy level and the potency of the anticonvulsant, which is also an important factor of the tranquilizer. The figures for HOMO−2 are almost the same and are omitted here. For muscle-relaxing the correlations is a little small but is not negligible. It suggests that a more complex scheme is necessary for muscle-relaxing and it requires further study for this potency.

Figure 13 shows the HOMO−1 of 15 BZP and 2 TZP minor tranquilizers. The HOMO−1 and HOMO−2 are originally degenerate orbitals of the benzene ring, except for chlordiazepoxide, dipotassium clorazepate, diazepam, and prazepam whereas the HOMO and HOMO−1 are swapped as also shown in Figure 13. If we took these MO exchanges into consideration, the correlation coefficients shown in Table 2 would be even a little bit larger. We consider here just the correlation at the global minimum, because the contribution from the global minimum is expected to be largest in the first approximation. It should be interesting, however, to discuss the correlation at the local minimum rather than the global minimum for future study.

It should be noted that there is a strong correlation between the π-orbital on the benzene ring and potency of the drugs. These π-orbitals seem to be keys of a key and a key–hole pair. As noted in the previous paragraph, flutoprazepam and fludiazepam show stronger potency than prazepam and diazepam, respectively. The π-orbitals of the latter compounds have

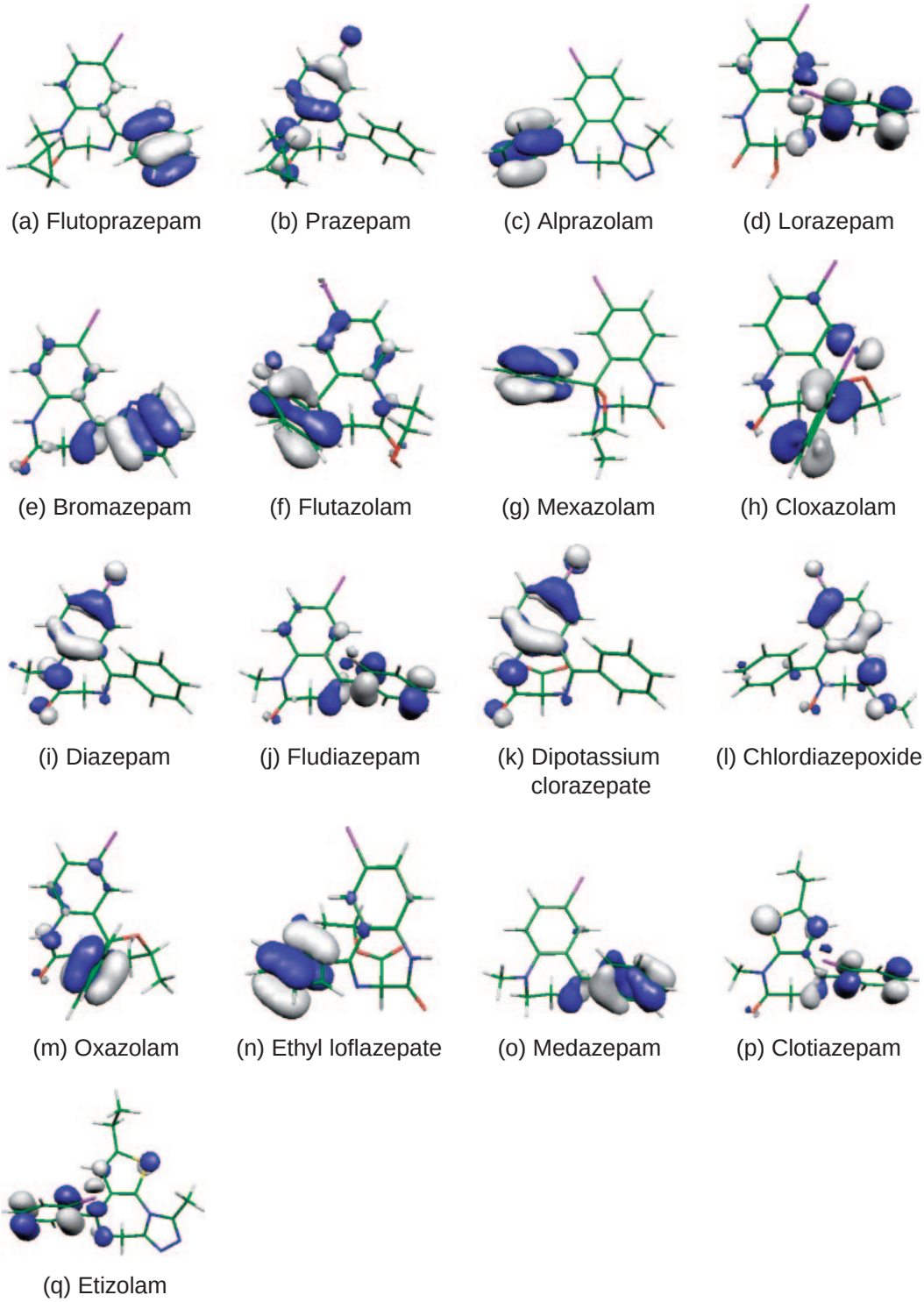


Figure 13. Orbital patterns of next-HOMO of (a) flutoprazepam, (b) prazepam, (c) alprazolam, (d) lorazepam, (e) bromazepam, (f) flutazolam, (g) mexazolam, (h) cloxazolam, (i) diazepam, (j) fludiazepam, (k) dipotassium clorazepate, (l) chlordiazepoxide, (m) oxazolam, (n) ethyl loflazepate, (o) medazepam, (p) clotiazepam, and (q) etizolam.

higher energies than the former compounds. Indeed, the HOMO−1s' of prazepam and diazepam are not π-orbitals on the benzene as shown above.

Conclusion

In the present paper, we have examined the molecular structure optimization of 17 BZP and TZP minor tranquilizers through molecular dynamics calculations based on the HA. We have found that the procedure is very useful to obtain possible minimum structures of the complicated molecules. After obtaining the optimized molecular structure, we examined them to analyze the relation between the orbital energies and the potency of the drugs. The energy levels of HOMO−1 and HOMO−2 are found to correlate with anticonvulsant aci-

tivity. It is interesting that the simple structure–activity relationship is obtained by considering just one-electron properties, i.e., the molecular orbital energies after structure survey by HA.

We will apply these methods of structure analysis to many kinds of drugs and to folding simulation of the proteins as well as application to the analysis of chemical reactions such as searching for the transition states of reactions. We also consider that the extension of treating canonical ensembles could be important for future application of the HA to drug design. These calculations are in progress and will be published elsewhere.

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