ELSEVIER

Journal of Molecular Graphics and Modelling 21 (2002) 81–87

Journal of Molecular Graphics and Modelling

www.elsevier.com/locate/JMGM

Three-dimensional common-feature hypotheses for octopamine agonist arylethanolamines

Akinori Hirashima [a,*], Masako Morimoto [b], Eiichi Kuwano [a], Eiji Taniguchi [c], Morifusa Eto [d]

[a] *Department of Applied Genetics and Pest Management, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*

[b] *Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*

[c] *School of Agriculture, Kyushu Tokai University, Kumamoto 869-1404, Japan*

[d] *Kyushu Women's University, 1-1 Jiyugaoka, Yahatanishi-ku, Kita-Kyushu, Fukuoka 807-8586, Japan*

Accepted 30 March 2002

Abstract

Three-dimensional pharmacophore hypotheses were built from a set of 12 octopamine (OA) agonist arylethanolamines (AEAs). Among the 10 common-featured models generated by program catalyst/HipHop, a hypothesis including a hydrogen-bond donor (HBD) and a hydrogen-bond acceptor lipid (HBAl) features was considered to be important in evaluating the OA activity. OA mapped well onto all the HBD and HBAl features of the hypothesis. On the other hand, for some inactive compounds, their lack of affinity is primarily due to their inability to achieve an energetically favorable conformation shared by the active compounds. Taken together, structures of a 4-OH–Ph, α-OH, and a primary amine are important for OA activities. The present studies on OA agonists demonstrate that an HBD and an HBAl sites located on the molecule seem to be essential for OA activity.

Keywords: Octopamine agonist; Common-feature hypothesis; Catalyst; HipHop; *Periplaneta americana*

1. Introduction

Quantitative structure–activity relationship (QSAR) modeling is an area of research pioneered by Hansch and co-workers [1,2]. The QSAR study assumes that the difference of the molecules in the structural properties experimentally measured accounts for the difference in their observed biological or chemical properties [1–3]. The result of QSAR usually reflects as a predictive formula and attempts to model the activity of a series of compounds using measured or computed properties of the compounds. More recently, QSAR has been extended by including the three-dimensional information. In drug discovery, it is common to have measured activity data for a set of compounds acting upon a particular protein but not to have knowledge of the three-dimensional structure of the active site. In the absence of such three-dimensional information, one may attempt to build a hypothetical model of the active site that can provide insight on the nature of the active site. Such a model is known as a Hypo [4–6]. Catalyst/Hypo is useful in building three-dimensional pharmacophore models from the activity data and conformational structure. It can be used as an alternative for QSAR methods because of easy visualization and high prediction.

In a previous application, we described the use of Catalyst/Hypo to derive a four and five-feature hypothesis from a set of 17 octopamine (OA) antagonists [4] and 43 agonists [5], respectively. Three-dimensional pharmacophore hypotheses have been built from a set of nine OA agonists responsible for the inhibition of sex-pheromone production in *Helicoverpa armigera* [6]. These sets have included a variety of types of molecules, covering five orders of magnitude in activity. For these type of training sets, the use of the hypothesis-generation tool has been appropriate. This tool has built hypotheses (overlays of chemical features) for which the fit of individual molecules to a hypothesis can be correlated with the molecule's affinity. However, the high structural homology among the derivatives used in the current study combined with their smaller activity range makes this "quantitative" hypothesis-generation method inappropriate. For this type of training set, the common-feature hypothesis generation, also called HipHop [7], is more suitable. HipHop generates hypotheses consisting only of identification and overlay of common features (without the use of activity data). The present work shows how a set of activities of various OA agonists may be treated statis-

* Corresponding author. Tel.: +81-92-642-2856; fax: +81-92-642-2858.
E-mail address: ahirasim@agr.kyushu-u.ac.jp (A. Hirashima).

1093-3263/02/$ – see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S1093-3263(02)00124-9

tically to uncover the molecular characteristics which are essential for high activity. The aim of this work, is to derive feature-based three-dimensional models from a set of OA agonists using HipHop.

2. Synthesis of test compounds

All compounds were prepared using published methods. Agonist arylethanolamine (AEA) **1** was obtained by reducing mandelic acid via its ester and amide with lithium aluminum hydride (LAH) [8] and other AEAs were synthesized from trimethylsilyl cyanide and the corresponding aldehyde in the presence of catalytic amount of anhydrous zinc iodide, followed by reduction with LAH[9]. The structures of the compounds were confirmed by ^{1}H and ^{13}C NMR measured with a JEOL JNM-EX400 spectrometer at 400 MHz, tetramethyl silane (TMS) being used as an internal standard for ^{1}H NMR, and elemental analysis.

2.1. Chemicals

OA (2-amino-1-(4-hydroxyphenyl)ethanol), theophylline (1,3-dimethylxanthine), and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were purchased from Nacalai Tesque (Kyoto, Japan); GTP was from Sigma (St. Louis, USA); ATP disodium salt was from Kohjin Co. (Tokyo, Japan); LAH was from Chemetall GmbH (Frankfurt, Germany); DL-norepinephrine (NE) hydrochloride were from Janssen Chimica (Beerse, Belgium); DL-epinephrine (E) hydrochloride from Tokyo Chem. Ind. Co. Ltd. (Tokyo, Japan); DL-synephrine (SN) was from Sigma (St. Louis, MO).

2.2. Radiochemical

The cAMP radioimmunoassay (RIA) kit (cord RPA 509) was purchased from Amersham International (Buckinghamshire, England).

3. Biological assay

3.1. Insects

Males and females of *Periplaneta americana* were used indiscriminately, as their nervous systems exhibited no gross structural or neurochemical differences. The insects were reared under crowded conditions in this laboratory at 28 °C with a photoperiod of 12 h light, and 12 h dark and at a relative humidity of 65–70% for more than 7 years; they were provided with an artificial mouse diet (Oriental Yeast Co., Chiba, Japan) and water ad libitum.

3.2. Adenylate-cyclase assay

OA-agonist activities of test compounds at several concentrations were examined using the adenylate-cyclase assay which was conducted on adult American cockroaches (*P. americana* L.) as shown in previous report [10–13]. Thoracic nerve cords of *P. americana* were homogenized (15 mg/ml) in a 6 mM Tris–maleate buffer (pH 7.4) by using a chilled microtube homogenizer (S-203, Ikeda Science, Tokyo, Japan) as shown in previous report. The homogenate was diluted (1 mg/ml) in 6 mM Tris–maleate, and then centrifuged at 120,000 × *g* and 4 °C for 20 min. The supernatant was discarded, the pellet being resuspended by homogenizing (1 mg/ml) in the buffer, and again centrifuged at 120,000 × *g* and 4 °C for 20 min. The resulting pellet (P2) resuspended in the buffer was equivalent to the starting amount (15 mg/ml). The adenylate-cyclase activity was measured according to Nathanson's procedure under optimal conditions [12–15] in a test tube containing 200 μl of 120 mM Tris–maleate (pH 7.4, including 15 mM theophylline, 12 mM $MgCl_2$, and 0.75 mM EGTA), 60 μl of the P2 fraction, and 30 μl of each synthesized compound solution in polyethylene glycol. An appropriate solvent control was run in parallel. The enzyme reaction (5 min at 30 °C) was initiated by adding 10 μl of a mixture of 3 mM GTP and 60 mM ATP, stopped by heating at 90 °C for 2 min, and then centrifuged at 1000 × *g* for 15 min to remove the insoluble material. The cAMP level in the supernatant was measured by RIA [10–13]. Protein concentration was determined by the Lowry's method, using bovine serum albumin (Sigma, St. Louis, USA) as the standard. Enzyme activity in each assay was corrected using OA as a reference [14]. The maximal stimulatory activity (mostly at 0.1 mM) was calculated relative to OA (100%) and control (0%).

4. Computational details

4.1. Hypothesis generation

All computational experiments were conducted on a Silicon Graphics O2, running under the IRIX 6.5 operating system. Hypotheses generation, whose functionality was available as part of Accelrys's catalyst/HipHop (version 4.0) modeling environment (San Diego, USA), was applied against OA agonists. Molecules were edited using the catalyst two- or three-dimensional visualizer. Catalyst was used to automatically generate conformational models for each compound using the poling algorithm [16–18]. The number of conformations needed to produce a good representation of a compound's conformational space depends on the molecule. Conformation-generating algorithms were adjusted to produce a diverse set of conformations, avoiding repetitious groups of conformations all representing local minima. The characteristics, which are essential for high activity, are expressed as common features disposed in three-dimensional space and are collectively termed a hypothesis. The HipHop builds hypotheses (overlays of common features) for which the fit of individual molecules

to a hypothesis can be correlated with the molecule's activity. The conformations generated were used to align common molecular features and generate pharmacophoric hypotheses. HipHop used conformations generated to align chemically important functional groups common to the molecules in the study set. A pharmacophoric hypothesis then was generated from these aligned structures.

The models emphasized conformational diversity under the constraint of 20 kcal/mol energy threshold above the estimated global minimum based on use of the CHARMm force field [16–19]. Molecular flexibility was taken into account by considering each compound as a collection of conformers representing a different area of conformational space accessible to the molecule within a given energy range. Catalyst provides two types of conformational analysis: fast and best quality. Best option was used, specifying 250 as the maximum number of conformers. The molecules associated with their conformational models were submitted to catalyst hypothesis generation. Hypotheses approximating the pharmacophore were described as a set of features distributed within a three-dimensional space. This process only considered surface accessible functions such as hydrogen-bond acceptor (HBA), hydrogen-bond acceptor lipid (HBAl), hydrogen-bond donor (HBD), hydrophobic (Hp), Hp aromatic (HpAr), Hp aliphatic (HpAl), negative ionizable (NI), and positive ionizable (PI) [20].

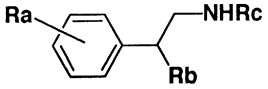
HipHop provides feature-based alignment of a collection of compounds without considering activity. It matches the chemical features of a molecule, against drug candidate molecules. HipHop takes a collection of conformational models of molecules and a selection of chemical features, and produces a series of molecular alignments in a variety of standard file formats. HipHop begins by identifying configurations of features common to a set of molecules. A configuration consists of a set of relative locations in three-dimensional space and associated feature types. A molecule matches the configurations if it possesses conformations and structural features that can be superimposed within a certain tolerance from the corresponding ideal locations. HipHop also maps partial features of molecules in the alignment set. This provision gives the option to use partial mapping during the alignment. Partial mapping allows one to identify larger, more diverse, more significant hypotheses, and alignment models without the risk of missing compounds that do not map to all of the pharmacophore features. Hyposes were allowed, for which some compounds needed not to map to any feature and the following settings controlled how many molecules in the training set could map incompletely to a hyothesis. Misses, the number of molecules which do not have to map to all features in generated hypotheses, feature misses, the number of maximal molecules which do not have to map to each feature in generated hypotheses, and Complete misses, the number of molecules which do not have to map to any feature in a given hypothesis, were set as 3, 2, and 2,respectively.

5. Assessment of three-dimensional hypothesis for OA activity

The OA activity was structure specific as indicated in Tables 1 and 2. OA **7** with 4-OH–Ph substituent had the highest activity in this study and all other AEAs were partial OA agonists [21]. A slight modification of structure of **7** decreased the OA-agonist activity dramatically: the introduction of substituents at the phenyl instead of 4-OH, the elimination of an α-OH, leading to TA **6**, and the introduction of a methyl to the primary amine, leading to SN **8**. Hypotheses were generated to explain the specificity of OA and the OA agonists. A set of 12 molecules, including **7** and its derivatives, was selected as the target training set. The experimental biological activities are listed in Table 1. Among the 12 molecules of the training set, **7** was chosen as a reference compound, which was allowed to map all features, and the other 11 molecules were allowed to map partially on the hypotheses (Table 3). Except for this classification, the activities of the molecules were not used in the analysis. The addition of 10 compounds into the training set followed by regeneration of common feature pharmacophores did not make any big differences of the models.

The geometry of each compound was built with a visualizer and optimized by using the generalized CHARMm [16–19] force field implemented in the program of the catalyst package. A preparative test was performed with HBA,

Table 1
OA agonist AEAs used in this study



Compound[a]	Ra	Rb	Rc	Melting point (°C)	Adenylate-cyclase activity (relative to OA, %)[b]
1	H	OH	H	61–62	34.2 ± 1.1[c]
2	2-MeO	OH	H	115–118	45.8 ± 3.2[c]
3	4-Br	OH	H	110–112	45.2 ± 2.7[c]
4	4-Cl	OH	H	94–95	72.1 ± 1.2[c]
5	4-F	OH	H	65–68	44.0 ± 0.9[c]
6 (TA)	4-OH	H	H	–	22.4 ± 5.6
7 (OA)	4-OH	OH	H	–	100.0 ± 5.5[c]
8 (SN)	4-OH	OH	Me	–	57.6 ± 5.6
9	4-Me	OH	H	75–76	52.0 ± 1.4[c]
10	2,4-F_2	OH	H	1.5059 n_D	41.7 ± 0.2[c]
11 (NE)	3,4-$(OH)_2$	OH	H	–	31.5 ± 8.7
12 (E)	3,4-$(OH)_2$	OH	Me	–	21.7 ± 3.5

[a] AEA **1** was obtained by reducing mandelic acid via its ester and amide with LAH and other AEAs were synthesized from trimethylsilyl cyanide and the corresponding aldehyde in the presence of catalytic amount of anhydrous zinc iodide, followed by reduction with LAH.

[b] The adenylate-cyclase assay of test compounds was conducted at several concentrations on adult American cockroaches as shown in previous report [9–12]. The basal (control) and maximal adenylate-cyclase activities stimulated by OA (0.1 mM) were 26.2 ± 5.6 and 612.2 ± 127.5 pmol cAMP/min/mg of protein, respectively. The maximal stimulatory activities (mostly at 0.1 mM) of test compounds were calculated relative to OA (100%) and control (0%).

[c] Cited from [21].

Table 2
Characteristics for the common-feature hypothesis run

Compound	Confs[a]	Features/confs[a]	Principal[b]	MaxOmitFeat[c]
1	24	26.50	1	1
2	27	25.56	1	1
3	24	26.42	1	1
4	24	26.50	1	1
5	24	26.50	1	1
6	104	32.62	2	0
7	52	24.79	1	1
8	40	16.65	1	1
9	25	26.68	1	1
10	32	25.78	1	1
11	16	38.56	1	1
12	45	30.62	1	1

[a] Confs, number of conformers; features/confs, total number of features divided by the number of conformers (summed over the entire family of conformers).

[b] Principal = 1 means that this molecule must map onto the hypotheses generated by the search procedure. Partial mapping is allowed. Principal = 2 means that this is a reference compound. The chemical feature space of the conformers of such a compound is used to define the initial set of potential hypotheses.

[c] MaxOmitFeat = 1 means a feature of a compound may not be mapped on a hypothesis model. MaxOmitFeat = 0 means all features of a compound are mapped on a hypothesis model.

HBAl, HBD, Hp, HpAr, HpAl, NI, and PI [20]. NI and PI were used rather than negative charge and positive charge in order to broaden the search for deprotonated and protonated atoms or groups at physiological pH. Using conformational poling [16], a representative family of conformers was generated, within a 20 kcal/mol range of the computed minimum, for each molecule. Potential hypothesis models were produced with the minimum permitted interfeature spacing of 2.00 Å generating alignments of common features [7], which included the projected point of HBA and Hp [20].

It was found that hypotheses contain good correlation with an HBD and an HBAl. The characteristics of 10 hypotheses are listed in Table 3. All the hypotheses contain five features with the ranking scores ranging from 34.8368 to 37.2368. Hypotheses 1, 2, 6, and 8 consist of the same common-feature functions of an HBD and an HBAl. The second group composes of hypotheses 3 and 4 which are characterized by two HBAl features. Other hypotheses 5 and 7 are characterized by two HBD features. Hypotheses 9 and 10 consist of an HBAl and an HBA. The rank score range over the 10 generated hypotheses is 2.4. The small rank score range observed here may be due to two factors, namely molecules in the training set are fairly rigid and have a high degree of structural homology. The small range of rank score suggests that these hypotheses were homogenous. Roughly speaking, all hypotheses have the good similarity in three-dimensional spatial shape and therefore these hypotheses are considered to be equivalent. All hypotheses that meet the user specified criteria in the setup (i.e. maximum number of hypotheses) will be returned in order of rank. Hypotheses are ranked based on the portion of training set members that fit the proposed pharmacophore and the rarity of the pharmacophore. The higher the ranking, the less likely it is that the molecules fit the hypothesis by a chance correlation.

6. OA agonist-receptor interaction

Generally, more active molecules map well to all the features of the hypothesis (Fig. 1), and a compound that has low activity maps poorly to the hypothesis (Figs. 2–4). Figs. 1–4 depict the most active compound OA **7**, its 4-Cl analog **4**, *N*-Me derivative **7**, and compound **8** without α-OH which have a low OA-agonist activity, mapped to hypothesis 1, respectively. The molecule **7** maps well to the two features of hypothesis 1 (Fig. 1); an HBD to 4-OH and a nitrogen to HBAl, whereas an HBD maps to the α-OH and an HBAl does not map to **4** at all that has intermediate agonist activity (Fig. 2). Besides, the elimination of an α-OH group of **7** nullified the activity (Table 1), leading to TA **6**. The least active compound **6** maps onto the pharmacophore in which an HBD maps to NH and an HBAl to the oxygen of 4-OH (Fig. 3). However, this dose not make sense at all, since NH should be more basic than 4-OH and therefore, HBAl is

Table 3
Results of the common-feature hypothesis run

Hypotheses	Feature[a]		Rank score	Direct hit[b]	Partial hit[b]
1	HBD	HBAl	37.2368	111111111111	000000000000
2	HBD	HBAl	37.2368	111111111111	000000000000
3	HBAl	HBAl	37.2368	111111111111	000000000000
4	HBAl	HBAl	37.2368	111111111111	000000000000
5	HBD	HBD	37.2368	111111111111	000000000000
6	HBD	HBAl	37.1974	111111111111	000000000000
7	HBD	HBD	37.1974	111111111111	000000000000
8	HBD	HBAl	37.1974	111111111111	000000000000
9	HBAl	HBA	34.8368	111111111111	000000000000
10	HBAl	HBA	34.8368	111111111111	000000000000

[a] HBD, hydrogen-bond donor; HBAl, hydrogen-bond acceptor lipid; HBA, hydrogen-bond acceptor.

[b] Direct hit, all the features of the hypothesis are mapped. Direct hit = 1 means yes and direct hit = 0 is no; partial hit, partial mapping of the hypothesis. Partial hit = 1 means yes and partial hit = 0 means no. Each number refers to a molecule in Table 2 (same order).

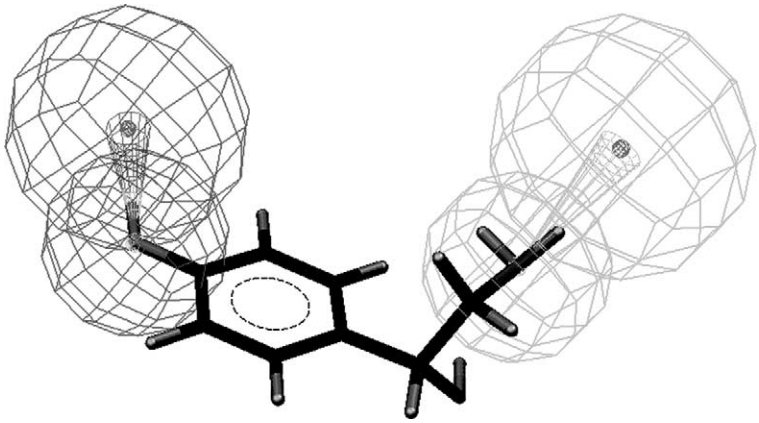


Fig. 1. Mapping of OA **7** to hypothesis 1, which contains an HBD (left) and an HBAl (right).

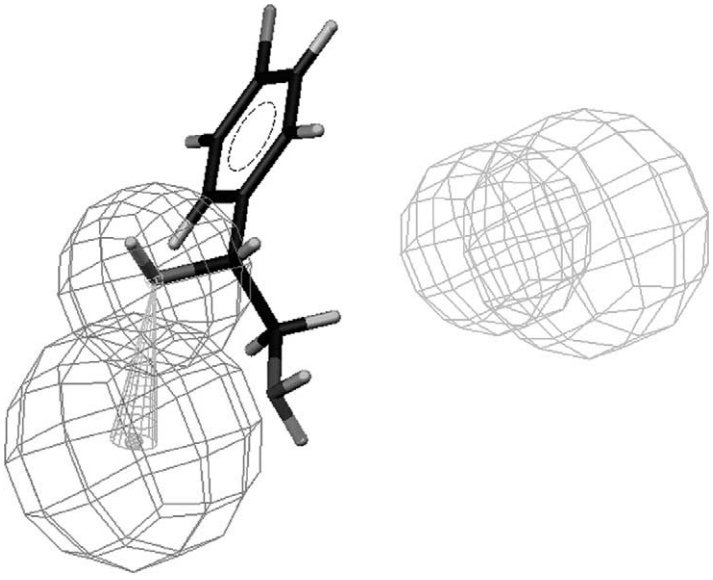


Fig. 2. Mapping of **4** to hypothesis 1, which contains an HBD (left) and an HBAl (right).

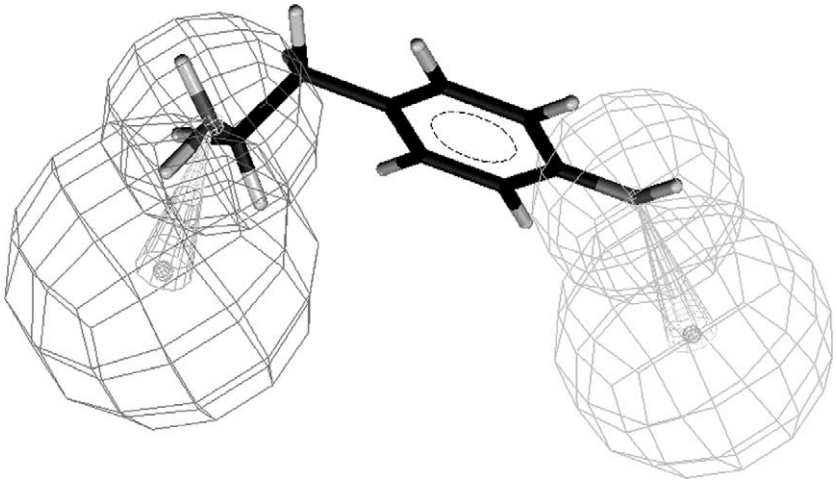


Fig. 3. Mapping of TA **6** to hypothesis 1, which contains an HBD (left) and an HBAl (right).

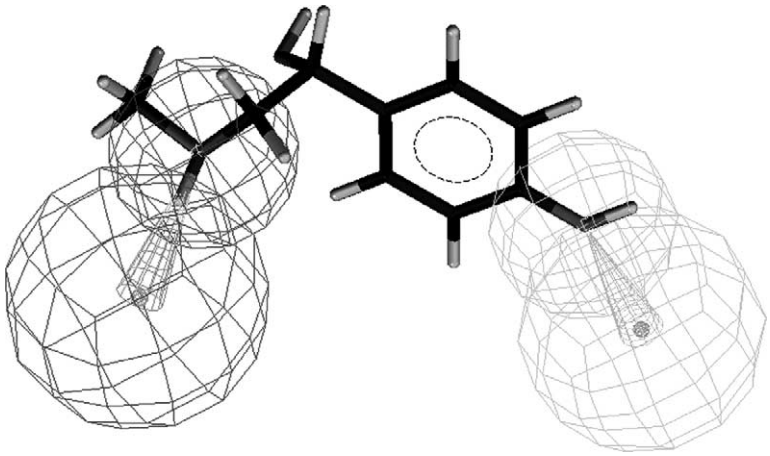


Fig. 4. Mapping of SN **8** to hypothesis 1, which contains an HBD (left) and an HBAl (right).

supposed to map to NH and an HBD to the oxygen of 4-OH as in the case of OA **7** in Fig. 1. In physiological conditions, nitrogen would be protonated, which is not suitable for an HBD. Additionally, the introduction of a methyl group at the primary amine of OA **7** lowered the activity dramatically, leading to SN **8** (Fig. 4), in which an HBD again maps to NH and an HBAl to the oxygen of 4-OH. A similar tendency was observed for NE **11** and E **12**. Taken together, 4-OH–Ph, an α-OH, and the primary amine without any substituents are important for OA activity. Other compounds in Table 1 with low activity also do not fit to these features.

7. Conclusions

In rational drug-design process, it is common that the biological activity data of a set of compounds acting upon a particular protein is known, while information of the three-dimensional structure of the protein active site is absent. A three-dimensional pharmacophore hypothesis that is consistent with known data should be useful and predictive in evaluating new compounds and directing further synthesis. A pharmacophore model postulates that there is an essential three-dimensional arrangement of functional groups that a molecule must possess to be recognized by the active site. It collects common features distributed in three-dimensional space that is intended to represent groups in a molecule that participates in important interactions between drugs and their active sites. Hence, a pharmacophore model provides crucial information about how well the common features of a subject molecule overlap with the hypothesis model. It also informs the ability of molecules to adjust their conformations in order to fit an active site with energetically reasonable conformations. Such characterized three-dimensional models convey important information in an intuitive manner.

Hypotheses were obtained and applied to map the active or inactive compounds. Important features such as an HBD and an HBAl in hypothesis 1 of the surface-assessable models were found for OA activity. They are the minimum components of a hypothesis for effective OA activity, as shown in Fig. 1, since no hypotheses were obtained by adding any features to them. Graphical examination of the 10 hypotheses shows that there are four major families of models depending mainly on the location and the orientation of the projected point of the HBD and HBAl. It was found that OA maps well to all the features of the hypotheses. For some inactive compounds, their lack of affinity is primarily due to their inability to achieve an energetically favorable conformation shared by the active compounds, as shown in Figs. 2–4. Taken together, an HBD and an HBAl located on the molecule seem to be essential for OA activity.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References

[1] C. Hansch, A. Leo, in: Exploring QSAR: Fundamentals and Applications in Chemistry and Biochemisry, American Chemical Society, Washington, DC, 1995.
[2] C. Hansch, T. Fujita, ρ–σ–π analysis: a method for the correlation of biological activity and chemical structure, J. Am. Chem. Soc. 86 (1964) 1616–1626.
[3] V.E. Golender, E.R. Vorpagel, in: H. Kubinyi (Ed.), 3D-QSAR in Drug Design: Theory, Methods, and Applications, ESCOM Science Publishers, The Netherlands, 1993, p. 137.
[4] C. Pan, A. Hirashima, E. Kuwano, M. Eto, Three-dimensional pharmacophore hypotheses for the locust neuronal octopamine receptor (OAR3). Part 1. Antagonists, J. Mol. Model. 3 (1997) 455–463.
[5] A. Hirashima, C. Pan, E. Kuwano, E. Taniguchi, M. Eto, Three-dimensional pharmacophore hypotheses for the locust neuronal octopamine receptor (OAR3). Part 2. Agonists, Bioorg. Med. Chem. 7 (1999) 1437–1443.

[6] A. Hirashima, A. Rafaeli, C. Gileadi, E. Kuwano, Three-dimensional pharmacophore hypotheses of octopamine receptor responsible for the inhibition of sex-pheromone production in *Helicoverpa armigera*, J. Mol. Graphics Mod. 17 (1999) 43–50.
[7] D. Barnum, J. Greene, A. Smellie, P. Sprague, Identification of common functional configurations among molecules, J. Chem. Inf. Comput. Sci. 36 (1996) 563–571.
[8] S.Y. Wu, A. Hirashima, R. Takeya, M. Eto, Synthesis and insecticidal activity of optically active 2-methoxy-5-phenyl-1,3,2-oxazaphospholidine-2-sulfide, Agric. Biol. Chem. 53 (1989) 165–174.
[9] A. Hirashima, Y. Yoshii, K. Kumamoto, K. Oyama, M. Eto, Structure–activity studies of insecticidal 2-methoxy-1,3,2-oxazaphospholidine 2-sulfides against *Musca domestica* and *Tribolium castaneum*, J. Pesticide Sci. 15 (1990) 539–551.
[10] A. Hirashima, K. Shinkai, E. Kuwano, E. Taniguchi, M. Eto, Synthesis and octopaminergic-agonist activity of 3-(substituted phenyl)imidazolidine-2-thiones and related compounds, Biosci. Biotech. Biochem. 62 (1998) 1179–1184.
[11] A. Hirashima, H. Tarui, M. Eto, Synthesis and octopaminergic-agonist activity of 2-(arylimino)thiazolidines, 2-(aralkylamino)-2-thiazolines, and related compounds, Biosci. Biochem. Biotech. 58 (1994) 1206–1209.
[12] A. Hirashima, C. Pan, Y. Katafuchi, E. Taniguchi, M. Eto, Synthesis and octopaminergic-agonists activity of 2-(arylimino)oxazolidines and 2-(substituted benzylamino)-2-oxazolines, J. Pesticide Sci. 21 (1996) 419–424.
[13] A. Hirashima, Y. Yoshii, M. Eto, Action of 2-aryliminothiazolidines on octopamine-sensitive adenylate cyclase in the American cockroach nerve cord and on the two-spotted spider mite *Tetranychus urticae* Koch, Pesticide Biochem. Physiol. 44 (1992) 101–107.
[14] J.A. Nathanson, G. Kaugars, A probe for octopamine receptors: synthesis of 2-[(4-azido-2,6-diethylphenyl)imino]imidazolidine and its tritiated derivative, a potent reversible-irreversible activator of octopamine-sensitive adenylate cyclase, J. Med. Chem. 32 (1989) 1795–1799.
[15] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[16] A. Smellie, S.L. Teig, P. Towbin, Poling-promoting conformational variation, J. Comp. Chem. 16 (1995) 171–187.
[17] A. Smellie, S.D. Kahn, S.L. Teig, Analysis of conformational coverage. Part 1. Validation and estimation of coverage, J. Chem. Inf. Comp. Sci. 35 (1995) 285–294.
[18] A. Smellie, S.D. Kahn, S.L. Teig, Analysis of conformational coverage. Part 2. Application of conformational models, J. Chem. Inf. Comp. Sci. 35 (1995) 295–304.
[19] B.R. Brooks, R.E. Brucolleri, B.D. Olafson, D.J. States, S. Swaminathan, M. Karplus, A program for macromolecular energy, minimization, and dynamics calculations, J. Comput. Chem. 4 (1983) 187–217.
[20] J. Greene, S. Kahn, H. Savoj, P. Sprague, S. Teig, Chemical function queries for 3D databse search, J. Chem. Inf. Comput. Sci. 34 (1994) 1297–1308.
[21] A. Hirashima, Y. Yoshii, M. Eto, The agonist action of substituted phenylethylamine analogs on octopamine receptors in cockroach ventral nerve cords, Comp. Biochem. Physiol. 103C (1992) 321–325.