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Three-Dimensional Common-Feature Hypotheses for Octopamine Agonist 2-(Arylimino)imidazolidines

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Abstract—Three-dimensional pharmacophore hypotheses were built from a set of 10 octopamine (OA) agonist 2-(Arylimino)imidazolidines (AIIs), 2-(Arylimino)thiazolidines (AITs) and 2-(Arylimino)oxazolidines (AIOs). Among the 10 common-featured models generated by program Catalyst/HipHop, a hypothesis including a ring aromatic (RA), a positive ionizable (PI) and three hydrophobic aliphatic (HpAl) features was considered to be important in evaluating the OA-agonist activity. Active OA agonist 2,6-Et_2 AII mapped well onto all the RA, PI and HpAl features of the hypothesis. On the other hand, less active compounds were shown to be difficult to achieve the energetically favorable conformation which is found in the active molecules in order to fit the 3-D common-feature pharmacophore models. Taken together, 2,6-Et_2-Ph and foramidine structures are important as OA agonists. The present studies on OA agonists demonstrate that a RA, a PI and three HpAl sites located on the molecule seem to be essential for OA-agonist activity.

Introduction

Quantitative structure–activity relationship (QSAR) modeling is an area of research pioneered by Hansch and Fujita.[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. Catalyst/Hypo is useful in building 3-D 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 4- and 5-feature hypothesis from a set of 17 octopamine (OA) antagonists[4] and 43 agonists,[5] in which binding assays were used, respectively. Three-dimensional pharmacophore hypotheses were built from a set of nine OA agonists responsible for the inhibition of sex-pheromone production in *Helicoverpa armigera*.[6] These sets 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 was appropriate. This tool Hypo builds hypotheses (overlays of chemical features) for which the

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fit of individual molecules to a hypothesis can be correlated with the molecule's affinity. For OA antagonists binding assays are good enough to evaluate their affinity. Meanwhile, for OA agonists, they are not enough. Thus, in order to evaluate OA-agonist activity, an adenylate-cyclase assay was used. 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 aim of this work is to derive feature-based 3-D models from a small set of 10 OA agonists using HipHop.

Results and Discussion

Assessment of 3-D hypothesis for OA-agonist activity

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). The activity as OA agonist was structure specific. AII **57** with 2,6-Et_2-Ph substituent was the only full agonist in this study and all other AIOs and AITs were partial agonists. A slight modification of structure of **57** decreased the OA-agonist activity dramatically: substituents at 2,6 positions of the phenyl, the introduction of substituents at 4 position of the phenyl and the introduction of a heteroatom O or S to the imidazolidine. Hypotheses were generated to explain the specificity of the OA agonists. A set of 10 molecules, including **57** and its derivatives, was selected as the target training set. Their experimental biological activities are listed in Table 1. Among the 10 molecules of the training set, **57** was chosen as a reference compound, which was allowed to map all features, and the other nine molecules were allowed to map partially on the hypotheses (Table 2). Except for this classification, the activities of the molecules were not used in the analysis. This tool 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 3-D hypothesis study was performed with the Catalyst (version 4.0) package. The geometry of each compound was built with a visualizer and optimized by using the generalized CHARMm-like force field implemented in the program. A preparative test was performed with hydrogen-bond acceptor (HBA), hydrogen-bond acceptor lipid (HBAl), hydrogen-bond donor (HBD), hydrophobic (Hp), hydrophobic aromatic (HpAr), hydrophobic aliphatic (HpAl), negative ionizable (NI), ring aromatic (RA) and positive ionizable (PI).[8] 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 conformatinal poling,[9] 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 RA, PI and HpAl.[8]

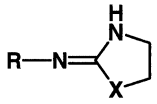
It was found that hypotheses contain good correlation with RA, PI and HpAl. The characteristics of 10 hypotheses are listed in Table 3. All the hypotheses contain five features with the ranking scores ranging from 109.823 to 97.566. Hypotheses 1 and 2 consist of the same common-feature functions of a RA, a PI and three HpAls. The second group composes of hypotheses 3–5 and 7–8 which are characterized by a RA, a PI, two HpAls and an Hp features. Another hypothese 6 is characterized by a PI, three HpAls and an Hp features. Hypotheses 9 and 10 consist of a PI, two HpAls and two Hps. The rank score range over the 10 generated hypotheses is 12.257. 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. Due to the relatively small range and owing, moreover, to the placement of the identified hypotheses within this range, special care was taken to test for chance correlation. The higher the ranking score, the less likely it is that the molecules in the training set fit the hypothesis by a chance correlation.

OA agonists–receptor interaction

Comparison of the procedure and regression studies shows that hypotheses 1, 3, 6 and 9 are the best models among the four groups and are selected for further evaluation. Figures 1–3 depict AII **57**, the most active compound, AII **56** and AIO **49**, which is an analogue of **57**, mapped onto hypothesis 1, respectively. The molecule **57** maps well onto the five features of hypothese 1 (Fig. 1), whereas two HpAls do not map on the iPr and Me in **56** (Fig. 2). A PI of hypothesis 1 does not fit to either **49** (Fig. 3) nor its AIT derivative **27** (data not shown). The methyl group at 4 position of phenyl of **59** does not map to none of 5 features (Fig. 4), suggesting that substituents at 4 position of phenyl are not favorable. It is, however, still to be clarified, preferably using 2,4,6-Et_3 derivative which showed a high binding affinity in a previous report[5] but was not available in this study. Taken together, 2,6-Et_2-Ph and foramidine structures are important as OA agonists. Generally, more active molecules map well onto all the features of the hypothesis (Fig. 1), and compounds that have low activity map poorly to the hypothesis (Figs 2–4). Other compounds in Table 1 with low activity also do not fit to these features.

A HpAl of hypothesis 1 is replaced by a Hp, leading to hypothesis 3 and a RA of hypothesis 3 is replaced by a HpAl, leading to hypothesis 6. A HpAl of hypothesis 5 is replaced by a Hp, leading to hypothesis 9. The small range of rank score suggests that these hypotheses were homogenous. Roughly speaking, hypotheses 1, 3, 6 and 9 have the good similarity in 3-D spatial shape and therefore these hypotheses are considered to be equivalent.

Table 1. OA agonists AITs, AIOs and AIIs used in this study



Compd[a]	R	X	Mp (°C)	Adenylate-cyclase activity (relative to OA,%)[b]
1	Ph	S	174–176	3.7±0.1
2	2-Br-Ph	S	166–168	5.2±0.3
3	2-Et-Ph	S	59–61	12.3±2.5
4	3-NO_2-Ph	S	124–126	4.8±1.0
5	4-Cl-Ph	S	164–166	7.4±1.4
6	4-CF_3-Ph	S	163–165	6.8±0.2
7	4-Me-Ph	S	145–147	3.0±0.2
8	4-MeS-Ph	S	105–107	2.2±0.2
9	4-EtO-Ph	S	108–111	2.0±0.4
10	4-Et_2N-Ph	S	106–108	1.1±0.4
11	4-$(Bz)_2N$-Bz	S	156–157	2.5±1.2
12	2,3-Cl_2-Ph	S	168–170	3.8±0.5
13	2-Cl,4-Br-Ph	S	171–173	4.2±1.0
14	2,4-F_2-Ph	S	146–148	0.6±0.2
15	2-Br,4-Me-Ph	S	148–150	25.0±2.5
16	2-Me,4-Cl-Ph	S	139–141	36.7±3.8
17	2,4-Me_2-Ph	S	66–68	55.0±3.9
18	2,4-$(MeO)_2$-Ph	S	130–132	3.4±0
19	2,5-Cl_2-Ph	S	171–173	1.2±0.1
20	2-MeO,5-Cl-Ph	S	136–138	8.0±0.7
21	2-MeO,5-Me-Ph	S	144–146	14.0±2.6
22	2,5-$(MeO)_2$-Ph	S	155–157	18.5±2.2
23	2,6-Cl_2-Ph	S	171–173	1.8±0.9
24	2-Cl,6-Me-Ph	S	141–143	4.8±1.1
25	2,6-Me_2-Ph	S	109–111	7.9±0.9
26	2-Me,6-Et-Ph	S	Oil	30.4±4.7
27	2,6-Et_2-Ph	S	72–74	50.2±2.2
28	2-Et,6-iPr-Ph	S	115–117	24.4±3.5
29	3,4-Cl_2-Ph	S	137–139	8.4±0.5
30	3-Cl,4-Me-Ph	S	107–109	5.5±0.3
31	3-CF_3,4-Cl-Ph	S	116–117	0.2±0.2
32	3,5-Cl_2-Ph	S	197–199	3.4±0.2
33	2,3,4-Cl_3-Ph	S	194–196	9.1±1.0
34	2,4,5-Cl_3-Ph	S	188–190	9.1±0.7
35	2,4,6-Br_3-Ph	S	176–178	2.7±0.1
36	2,4,6-Cl_3-Ph	S	142–144	5.3±0.4
37	2,6-Me_2,4-Br-Ph	S	42–44	19.3±2.3
38	2,4,6-Me_3-Ph	S	99–101	33.3±2.9
39	2,3,4,5-Cl_4-Ph	S	185–187	4.8±0.1
40	Ph	O	132–134	2.7±0.4
41	2-Me-Ph	O	Oil	5.2±0.7
42	2-Et-Ph	O	61–63	9.1±0.4
43	2-iPr-Ph	O	89–91	5.9±0.3
44	2,6-Cl_2-Ph	O	175–176	11.5±0.1
45	2,6-F_2-Ph	O	147–148	−0.3±0.6
46	2,6-Me_2-Ph	O	Oil	7.9±0.6
47	2-Me,6-Et	O	102–104	10.3±1.2
48	2-Me,6-iPr	O	Oil	15.6±0.4
49	2,6-Et_2-Ph	O	103–105	50.5±7.1
50	2-Et,6-iPr-Ph	O	172–174	42.5±0.8
51	2,6-iPr_2-Ph	O	169–170	13.9±1.4
52	2-Me-Ph	N	144–145	6.1±0.4
53	2-Et-Ph	N	103–104	33.0±14.8
54	2,6-Me_2-Ph	N	159–160	47.7±7.6
55	2-Me,6-Et-Ph	N	149–150	47.6±2.7
56	2-Me,6-iPr-Ph	N	200–201	18.8±8.7
57	2,6-Et_2-Ph	N	168–169	104.0±7.2
58	2,6-iPr_2-Ph	N	234–235	10.9±3.3
59	2,4,6-Me_3-Ph	N	174–175	59.1±10.2

[a]AITs **1**–**39** were synthesized by cyclization of the corresponding *N*-arylthioureas with concentrated hydrogen chloride.[10] AIOs **40**–**51** were obtained by cyclodesulfurizing the corresponding *N*-arylthioureas with yellow mercuric oxide.[11] AIIs **52**–**59** were prepared according to a reported method by refluxing the corresponding substituted anilines and 1-acetyl-2-imidazolidone in phosphoryl chloride followed by hydrolysis.[12]

[b]The adenylate-cyclase assay of test compounds was conducted at several concentrations on adult American cockroaches as shown in previous report.[10–13] 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%).

Table 2. Characteristics for the common feature hypothesis run

Compd	Confs[a]	Features/confs[a]	Principal[b]	MaxOmitFeat[c]
27	30	10.93	1	1
49	30	11.00	1	1
52	4	9.00	1	1
53	20	9.30	1	1
54	11	10.64	1	1
55	20	11.65	1	1
56	18	11.83	1	1
57	36	12.00	2	0
58	15	11.27	1	1
59	9	12.44	1	1

[a]Abbreviations: 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.

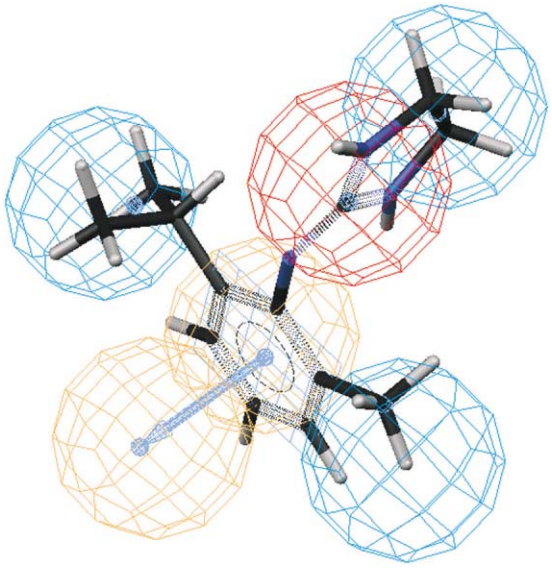


Figure 2. Mapping of **56** onto hypothesis 1, which contains a RA (orange), a PI (red) and three HpAls (blue).

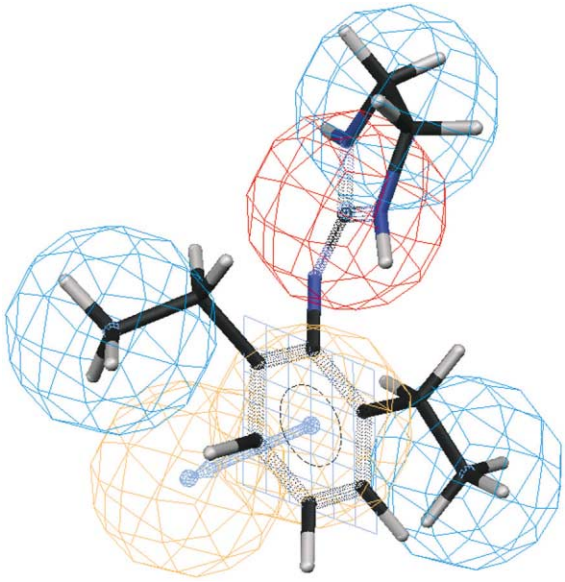


Figure 1. Mapping of **57** onto hypothesis 1, which contains a RA (orange), a PI (red) and three HpAls (blue).

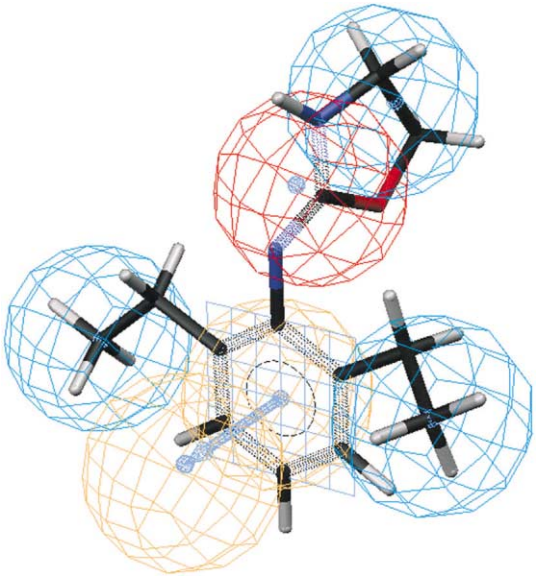


Figure 3. Mapping of **49** onto hypothesis 1, which contains a RA (orange), a PI (red) and three HpAls (blue).

Table 3. Results of the common feature hypothesis run

Hypotheses	Feature[a]					Rank Score	Direct Hit[b]	Partial Hit[b]
1	RA	PI	HpAl	HpAl	HpAl	109.823	0111111101	1000000010
2	RA	PI	HpAl	HpAl	HpAl	106.505	0111111101	1000000010
3	RA	PI	HpAl	HpAl	Hp	103.423	0111111101	1000000010
4	RA	PI	HpAl	HpAl	Hp	103.352	0111111101	1000000010
5	RA	PI	HpAl	HpAl	Hp	103.352	0111111101	1000000010
6	PI	HpAl	HpAl	HpAl	Hp	103.269	0111111101	1000000010
7	RA	PI	HpAl	HpAl	Hp	100.105	0111111101	1000000010
8	RA	PI	HpAl	HpAl	Hp	100.043	0111111101	1000000010
9	PI	HpAl	HpAl	Hp	Hp	98.988	0111111101	1000000010
10	PI	HpAl	HpAl	Hp	Hp	97.566	0111111101	1000000010

[a]Abbreviations: RA, ring aromatic; PI, positive ionizable; HpAl, hydrophobic aliphatic; Hp, hydrophobic.
[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).

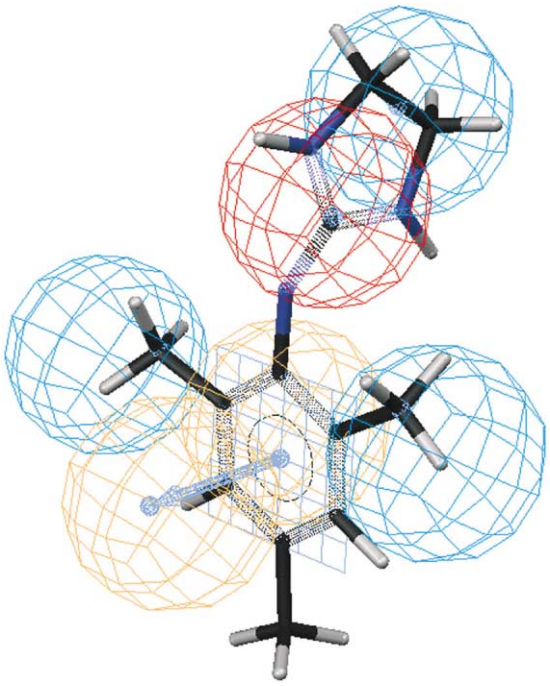


Figure 4. Mapping of **59** onto hypothesis 1, which contains a RA (orange), a PI (red) and three HpAls (blue).

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 3-D 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 3-D models convey important information in an intuitive manner.

The present work shows how a set of activities of various OA agonists may be treated statistically to uncover the molecular characteristics which are essential for high activity. These characteristics are expressed as common features disposed in three-dimensional space and are collectively termed a hypothesis. Hypotheses were obtained and applied to map the active or inactive compounds. Important features such as a RA, a PI and three HpAls of the surface-assessable models were found for OA agonists. They are the minimum components of a hypothesis for effective OA agonists. 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 RA, PI, HpAl and Hp. It was found that more active OA agonists map well onto 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. Taken together, a RA, a PI and three HpAls located on the molecule seem to be essential for activity of OA agonists.

Experimental

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 Chemical Co. (St. Louis, USA); ATP disodium salt was from Kohjin Co. (Tokyo, Japan); lithium aluminum hydride (LAH) was from Chemetall GmbH (Frankfurt, Germany).

Radiochemical

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

Methods

Synthesis of test compounds. All compounds were prepared using published methods. 2-(Arylimino)thiazolidines (AITs) **1**–**39** were synthesized by cyclization of the corresponding thiourea with concd hydrogen chloride.[10] 2-(Arylimino)oxazolidines (AIOs) **40**–**51** were obtained by cyclodesulfurizing the corresponding thiourea with yellow mercuric oxide.[11] 2-(Arylimino)imidazolidines (AIIs) **52**–**59** were prepared according to a reported method by refluxing the corresponding substituted anilines and 1-acetyl-2-imidazolidone in phosphoryl chloride followed by hydrolysis.[12] 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.

Insects. Males and females of *P. 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: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.

Adenylate-cyclase assay. The adenylate-cyclase assay 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 Sci., 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[10–13] 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.[10,11,13] Protein concentration was determined by the Lowry method,[14] using bovine serum albumin (Sigma, St. Louis, USA) as the standard. Enzyme activity in each assay was corrected using OA as a reference. The maximal stimulatory activity (mostly at 0.1 mM) was calculated relative to OA (100%) and control (0%).

Hypothesis generation

All experiments were conducted on a Silicon Graphics O2, running under the IRIX 6.5 operating system. Hypotheses generation was applied against previously described data sets and their functionality is available as part of Molecular Simulations Incorporated's Catalyst/HipHop (version 4.0) modeling environment (Burlington, USA). Molecules were edited using the Catalyst 2-D/3-D visualizer. Catalyst automatically generated conformational models for each compound using the Poling Algorithm.[9,15,16] 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 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 a conformational diversity under the constraint of 20 kcal/mol energy threshold above the estimated global minimum based on use of the CHARMm force field.[9,15–17] 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. The best option was used, specifying 250 as the maximum number of conformers. The molecules associated with their conformational models was submitted to Catalyst hypothesis generation. Hypotheses approximating the pharmacophore were described as a set of features distributed within a 3-D space. This process only considered surface accessible functions such as HBA, HBAl, HBD, Hp, HpAr, HpAl, RA, NI and PI.

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 3-D 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 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. Misses, the number of molecules which do not have to map to all features in generated hypotheses, FeatureMisses, the the number of maximal molecules which do not have to map to each feature in generated hypotheses, and CompleteMisses, 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.

Acknowledgements

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