Ligand-Based Molecular Modeling Study on a Chemically Diverse Series of Cholecystokinin-B/Gastrin Receptor Antagonists: Generation of Predictive Model

Madhu Chopra*,† and Anil K. Mishra‡

Laboratory of Molecular Modeling & Drug Design, Dr. B. R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110007, India, and Institute of Nuclear Medicine and Allied Sciences, Brigadier S. K. Mazumdar Road, Timar Pur, Delhi 110054, India

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Pharmacophore hypotheses were developed for six structurally diverse series of cholecystokinin-B/gastrin receptor (CCK-BR) antagonists. A training set consisting of 33 compounds was carefully selected. The activity spread of the training set molecules was from 0.1 to 2100 nM. The most predictive pharmacophore model (hypothesis 1), consisting of four features, namely, two hydrogen bond donors, one hydrophobic aliphatic, and one hydrophobic aromatic feature, had a correlation (*r*) of 0.884 and a root-mean-square deviation of 1.1526, and the cost difference between null cost and fixed cost was 81.5 bits. The model was validated on a test set consisting of six different series of 27 structurally diverse compounds and performed well in classifying active and inactive molecules correctly. This validation approach provides confidence in the utility of the predictive pharmacophore model developed in this work as a 3D query tool in the virtual screening of drug-like molecules to retrieve new chemical entities as potent CCK-BR antagonists. The model can also be used to predict the biological activities of compounds prior to their costly and time-consuming synthesis.

INTRODUCTION

The gastrointestinal peptides gastrin and cholecystokinin (CCK) have been implicated in various regulatory functions: as neurotransmitters in the brain and as regulators of various functions of the gastrointestinal tract, primarily, at the level of the stomach, pancreas, and gallbladder.[1] In addition, they can act as physiological growth factors in most parts of the gastrointestinal tract.[2−4] Gastrin and CCK possess the same five amino acids at their COOH terminus, which are key for activity; their actions are mediated by two different receptor types, CCK-A and CCK-B/gastrin.[5,6] CCK-B/gastrin receptors are present in the gut mucosa and in the brain,[1,7,8] while CCK-A receptors are present in the gallbladder, pancreas, and brain.[1,9]

In the central nervous system, these receptors have been associated with panic, anxiety, and pain, whereas in peripheral tissues, where they are mainly activated by gastrin, they stimulate gastric acid secretion[10] and gastrointestinal cell growth.[11] Several human tumors overexpress receptors for small regulatory peptides,[12] an observation that has led to a number of clinical applications in the diagnosis[13] and treatment of tumors.[14] Receptor autoradiograhic studies have shown that cholecystokinin CCK-B/gastrin receptors are expressed not only in more than 90% of medullary thyroid carcinomas[15] but also in a high percentage of small-cell lung cancer, some ovarian cancer, astrocytomas, and potentially a variety of adenocarcinomas, gastrointestinal tumor, and colorectal cell lines.[16]

Because of continued interest in developing novel therapeutic agents, which interact selectively with the CCK-B receptors for the treatment of anxiety, panic disorders, and so forth, several different classes of antagonists have been developed.[17,18] Antagonists of cholecystokinin-B receptors have been shown to alleviate CCK-4-induced panic attacks in humans and to potentiate opioid effects in animals. The clinical use of these compounds is critically dependent on their ability to cross the blood−brain barrier. To improve this property, new peptoid-derived CCK-B antagonists, endowed with high affinity, selectivity, and lipophilicity, have been developed.[19] A series of peptoid analogues of CCK-4 have been developed in recent years with excellent binding affinity and selectivity.[20] However, during the preclinical and clinical development of these compounds, their low bioavailability suggests a need to identify analogues with improved pharmacokinetic profiles.

In the absence of any three-dimensional (3D) structure for CCK-BR, a rational design of antagonists for this receptor using a structure-based approach is not feasible. Therefore, it is prudent to identify a series of structurally diverse CCK-BR antagonists with known binding affinities to understand the structural requirements for the potent and selective drugs against this target. We decided to exploit the wealth of information available, including the biological activity data, which covers almost a 4-log unit range, to develop pharmacophore hypotheses. A pharmacophore represents the 3D arrangements of chemical features in a molecule (ligand) that may be essential for important binding interactions with a receptor. In the absence of any knowledge of the 3D structure of a receptor, pharmacophores may provide such important information in the drug design process.

* Author to whom correspondence should be addressed. Phone: 91-011-27666272. Fax: 91-011-27666248. E-mail: mchopra@acbr.du.ac.in, madhu_india04@rediffmail.com.

† Dr. B. R. Ambedkar Center for Biomedical Research.

‡ Institute of Nuclear Medicine and Allied Sciences.

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The pharmacophores may be used in several ways, for example, as a 3D query in searching 3D databases containing "drug-like"[21,22] small organic molecules to identify active and specific inhibitors or in evaluating a new compound for mapping on a known pharmacophore. The concepts of pharmacophores, their development techniques, and their applications have been elegantly compiled in a recently published book.[23] This approach is powerful and has found wide application in drug design.[23−28] A drug discovery cycle, to identify, optimize, and eventually take a compound to the market, is generally a long process (approximately 12−15 years) and is very expensive (approximately $500 million R&D expense).[29] Therefore, there is a pressing need to reduce the cost of drug discovery steps. Pharmaceutical companies are taking more rational approaches than trial and error to identify new chemical entities. The hypothesis generation methods (HipHop and HypoGen) of the Catalyst software[30] have been successfully used in drug discovery research[31−55] and toxicology[56] (for a more comprehensive reference list, see http://www.accelrys.com/references/rdd_pub.html). Kaminski et al. reported the development of pharmacophore models from a series of farnesyl protein transferase (FPT) inhibitors.[35] The best-derived pharmacophore model was used to search a 3D database from the Schering−Plough Research Institute and successfully identified several low micromolar FPT inhibitors with varied structures compared to the structures used in the training set to develop the pharmacophore.

Sprague[36] used this method in developing pharmacophores for inhibitors against angiotensin converting enzymes, protein farnesyl transferase, human immunodeficiency virus (HIV) protease, and HIV reverse transcriptase. Recently, Kurogi and Güner have used Catalyst/HipHop-generated pharmacophores in searching 3D databases to identify novel mesangial cell proliferation inhibitors.[52] These studies suggest that the Catalyst-generated pharmacophores can be effectively used for rational drug design.

We present, in this report, the development of pharmacophores of a data set of antagonists for cholecystokinin-B/gastrin receptors by using the Catalyst/HypoGen module. Because there is, so far, no report on developing pharmacophores using antagonists for cholecystokinin-B/gastrin receptors, this study is expected to provide useful knowledge for developing new drugs targeted to CCK-B receptors.

MATERIALS AND METHODS

Molecular Modeling. All molecular modeling works were performed on a Silicon Graphics Octane2 computer running Irix 64 6.5, 600 MHz (SGI, 1600 Amphitheatre Parkway, Mountain View, CA 94043). Catalyst 4.7 software[30] was used to generate pharmacophore models.

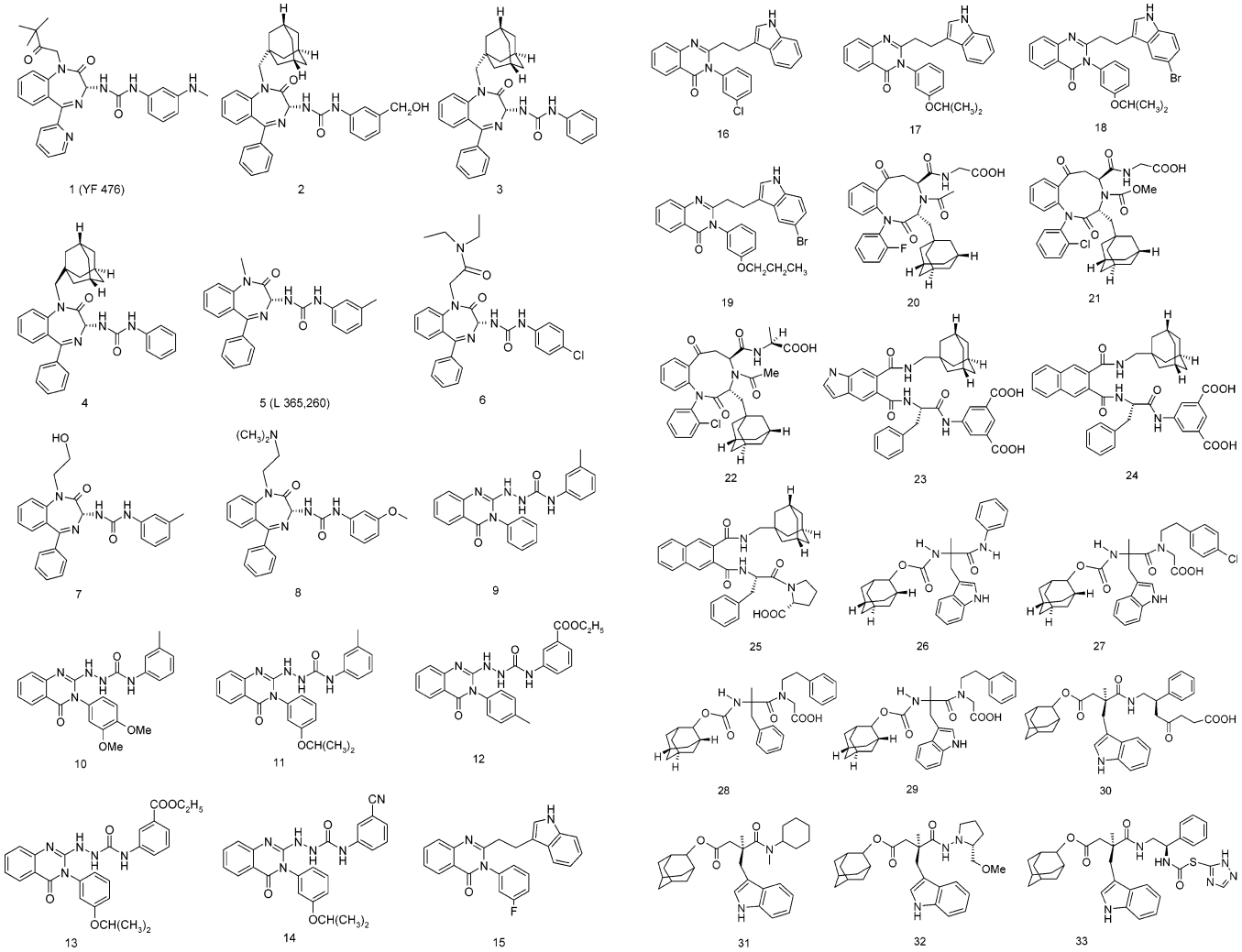
Selection of the Training Set. The most important aspect of the hypothesis generation in HypoGen is the selection of the training set of molecules. The selection has to follow some basic requirements, such as a minimum of 16 structurally diverse compounds should be selected to avoid any chance correlation.[52] The activity data should have a range of 4−5 orders of magnitude. The selected compounds should provide clear and concise information. Any redundancy should be avoided in terms of structural features or activity range. A compound that is considered to be inactive because of steric factors should not be included because current catalyst features in the Catalyst software cannot handle such cases.

Biological Data. The sources of the biological activity data, represented as K_i in nM (compounds 2−8, 20−40, and 48−60) and IC_{50} in nM (compounds 1, 9−19, and 41−47), were from the literature,[58−67] and the chemical structures of the antagonists are listed in Charts 1 and 2. The data sets are divided into a training set and a test set. The most active compounds were included so that they would provide critical information for pharmacophore requirements. Several moderately active and inactive compounds were also included to spread the activity ranges as wide as possible. The important aspect of this selection scheme is that each active compound should teach something new to the HypoGen module to help it uncover as much critical information as possible for predicting biological activity. In case of CCK-BR, a training set of 33 compounds with the above criteria has been selected; the other 27 compounds were used as the test set. An uncertainty value of 3 (default) was used for compound activity, which is a ratio range of uncertainty in the activity value. The activities against CCK-BR have been classified as follows: highly active (<30 nM), moderately active (>30−200 nM), and poorly active (>200 nM). These activities are classified, somewhat arbitrarily, on the basis of the lowest and the highest activity ranges predicted (fit values) by the hypothesis for selected training set molecules.

Generation of Pharmacophores. Details of the pharmacophore development procedures have been described in the literature.[23,36] In brief, conformational models of all training set molecules for CCK-BR were generated using the "best quality" conformational search option in Catalyst using a constraint of a 20 kcal mol^{-1} energy threshold above the global energy minimum and Charmm force field parameters.[57] A maximum of 250 conformations were generated using the best-fit method to ensure maximum coverage in the conformational space. All other settings were kept as a default. Instead of using just the lowest energy conformation of each compound, all conformational models for molecules in each training set were used in Catalyst for pharmacophore hypothesis generation. An initial analysis revealed that four chemical feature types such as hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and hydrophobic (aliphatic) (HY-ALI) and hydrophobic (aromatic) (HY-AR) features could effectively map all critical chemical features of all molecules in the training and test sets. The minimum and maximum counts for HBA and HBD were set to 0 and 3, whereas for HY-ALI and HY-AR, they were set to 0 and 2, respectively. These four feature types were used to generate 10 pharmacophores from the training set. The uncertainty value was defaulted to 3, and MinPoints and MinSubsetPoints were 4 (default value). The MinPoints parameter controls the minimum number of location constraints required for any hypothesis. The MinSubsetPoint parameter defines the number of chemical features that a hypothesis must match in all the compounds set. The Catalyst software can generate pharmacophore hypotheses consisting of a maximum of five features.

Important Output Parameters That Determine the Quality of the Pharmacophore Hypothesis. 1. The Cost Function Analysis. The algorithm employed for the catalyst automatic hypothesis generation (HypoGen) optimizes hy-

Chart 1. Chemical Structures of 33 Training Set Compounds[a]

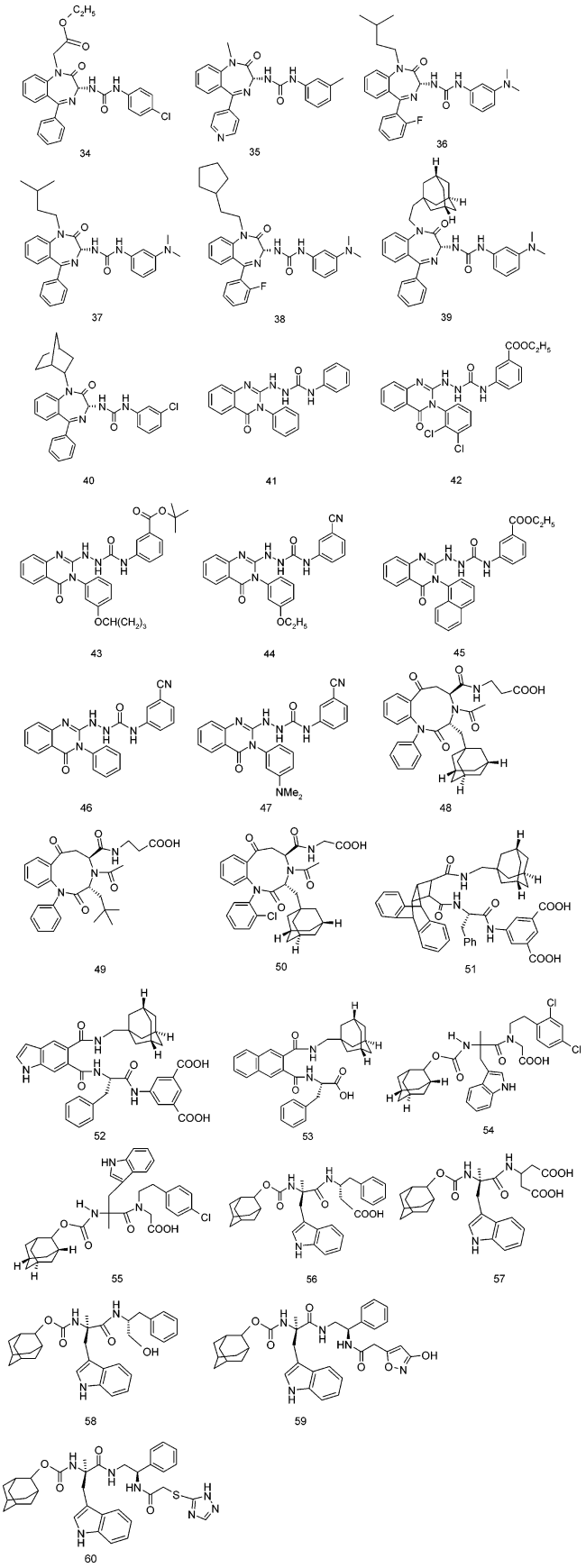


[a] All structures were drawn using ISIS Draw 2.4 (MDL Information Systems, Inc., San Leandro, CA). Numbers represent the chemical numbers.

potheses that are common to the active compounds in the training set but not shared by the inactive compounds. This is done in three phases. In the constructive phase, hypotheses common to all actives are defined. The subtractive phase removes hypotheses common to the inactive compounds. The third phase optimizes the resultant hypotheses from phase 2 that have survived the subtractive phase.

Catalyst uses bits for language and assigns costs to hypotheses in terms of number of bits required to describe them fully. The HypoGen module in Catalyst performs two important theoretical cost calculations (represented in bit units) that determine the success of any pharmacophore hypothesis. One is known as the "fixed cost", which represents the simplest model that fits all data perfectly (all compounds fall along a line of slope = 1), and is calculated by adding the minimum achievable error and weight cost and the constant configuration cost. The second one is known as "null cost", which represents the highest cost of a pharmacophore with no features and which estimates activity to be the average of the activity data of the training set molecules. Its absolute value is equal to the maximum occurring error cost. A meaningful pharmacophore hypothesis may result when the difference between these two values (total cost − fixed cost) is large; a value of 70−100 bits suggests an excellent chance (>90%) for a true correlation; if the difference is between 40 and 70 bits for a pharmacophore hypothesis, it may indicate that it has a 75−90% probability of correlating the data (Catalyst 4.7 documentation). The total cost of any pharmacophore hypothesis should be close to the fixed cost to provide any useful models. The total cost value is the sum of three costs: a weight, an error, and a configuration cost value. These three cost components could be described as follows: Each feature of a hypothesis represents certain orders of magnitude of the compounds' activity. With the default setting of 0.302, the represented orders of magnitude are kept as close to 2 as possible. The weight component is a value that increases in a Gaussian form as these function weights in a model deviate from the ideal value of 2. The error cost is dependent on the root-mean-square (rms) differences between the estimated and actual activities of the training set molecules. The rms deviations represent the quality of the correlation between the estimated and actual activity data. The configuration cost is also known as the entropy cost and depends on the complexity of the pharmacophore hypothesis space. Mathematically, the configuration cost is expressed as $\log_2 P$, where P is the number of initial hypothesis created in the constructive phase and that survived in the subtractive phase. It should not be greater than 17 (corresponds to a number of 217 pharmacophore models). Higher values would lead,

Chart 2. Chemical Structures of 27 Test Set Compounds[a]



[a] All structures were drawn using ISIS Draw 2.4 (MDL Information Systems, Inc., San Leandro, CA). Numbers represent the chemical numbers.

more likely, to a chance correlation of the generated hypothesis, since Catalyst cannot consider more than 217 models in the optimization phase, and so, the rest is left out of the process. Any number greater than 17 suggests that some attention has to be given in selecting the training set molecules. Limiting the minimum and maximum features can reduce the entropy cost.

2. Fisher's Cross-Validation Test. To evaluate the statistical relevance of the model, the Fischer's randomization test was applied. The Fisher's randomization test is used to validate the strong correlation between chemical structures and biological activity. The purpose of this technique is to randomize the activity data associated with the training set compounds, and the randomized training sets are used to generate pharmacophore hypotheses using the same features and parameters to develop the original pharmacophore hypothesis. If the randomized data set results in the generation of a pharmacophore with similar or better cost values, rms, and correlation, the original hypothesis is considered to have been generated by chance.[68] The statistical significance is given by the equation significance $= [1 - (1 + x)/y]100$, where $x =$ total number of hypotheses having a total cost lower than a best significant hypothesis and $y =$ the number of initial HypoGen runs + random runs. With the aid of the CatScramble program available in Catalyst/HypoGen module, the experimental activities in the training set were scrambled randomly, and the resulting training set was used for a HypoGen run. Thereby, all parameters were adopted from the initial HypoGen calculation. The number of such random trials depend on what level of statistical significance is to be achieved. For a 95% confidence level, 19 spreadsheets were created. For 98% and 99% confidence levels, 49 and 99 spreadsheets, respectively, are created. This procedure was reiterated 19 times to achieve a 95% confidence level.

RESULT AND DISCUSSION

Pharmacophore Generation. A pharmacophore model has been generated using a set of 33 training set compounds representing six series of structurally diverse compounds existing in the literature. Sets of 10 hypotheses were generated using the data from 33 training set compounds. Different cost values, correlation coefficients (r), rms deviations, and pharmacophore features are listed in Table 1.

The value of the total cost of each hypothesis was close to the fixed cost values, which is expected for good hypotheses. The entropy (configuration cost) value of the hypotheses was 16.28, within the allowed range. The difference between the null hypothesis and the fixed cost and between the null hypothesis and the total cost of the best hypothesis (hypothesis 1) was 81.59 and 57.21 bits, respectively. Nine out of 10 hypotheses consist of four features. The first 3 of the best 10 hypotheses have two HBD features, one HY-ALI feature, and one HY-AR feature; three have one feature each of HBD and HY-ALI and two HY-AR; and three hypotheses have one HBA, one HBD, one HY-ALI, and one HY-AR feature. There was one three-feature hypothesis with one feature each for HBD, HY-ALI, and HY-AR. The best pharmacophore (hypothesis 1), which has the highest cost difference (81.5 bits), lowest error cost, lowest rms difference, and the best correlation coefficient,

Table 1. Results Obtained from Pharmacophore Hypothesis Generation Using the Training Set Molecules[a]

hypothesis number	total cost	error cost	rms	correlation (r)	features[b]
1	152.78	132.92	1.153	0.884	HBD, HBD, HY-ALI, HY-AR
2	156.57	136.39	1.241	0.865	HBD, HBD, HY-ALI, HY-AR
3	156.84	137.79	1.274	0.855	HBD, HBD, HY-ALI, HY-AR
4	158.19	140.21	1.331	0.839	HBD, HY-ALI, HY-AR, HY-AR
5	158.83	138.26	1.285	0.854	HBD, HY-ALI, HY-AR
6	165.29	147.85	1.494	0.792	HBA, HBD, HY-ALI, HY-AR
7	165.46	147.21	1.482	0.797	HBA, HBD, HY-ALI, HY-AR
8	166.72	148.82	1.514	0.786	HBA, HBD, HY-ALI, HY-AR
9	167.09	149.39	1.525	0.782	HBD, HY-ALI, HY-AR, HY-AR
10	167.78	150.24	1.542	0.777	HBD, HY-ALI, HY-AR, HY-AR

[a] Null cost = 209.99. Fixed cost = 128.404. Configuration cost = 16.28. All costs are in units of bits. [b] HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; HY-ALI, hydrophobic aliphatic; HY-AR, hydrophobic aromatic.

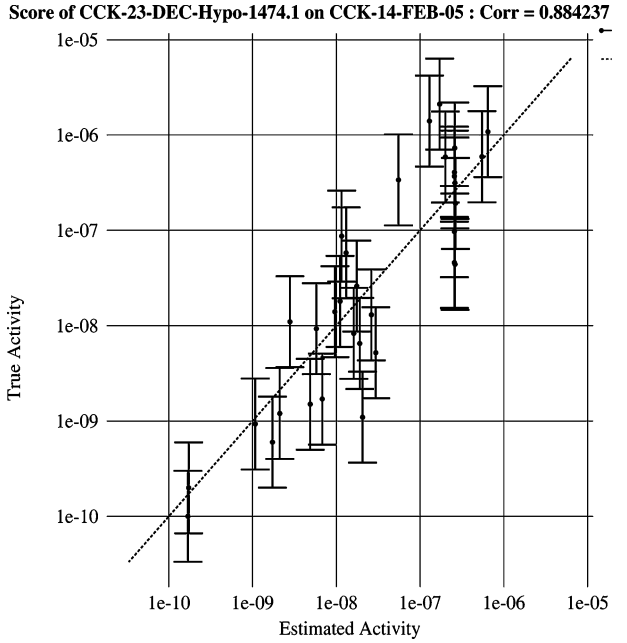


Figure 1. Regression of actual versus predicted activities by hypothesis 1 for the training set inhibitors. The training set includes 33 structurally diverse CCK-B receptor antagonists.

has two HBDs, one HY-ALI feature, and one HY-AR feature. A regression of the predicted activities for the training set by the best hypothesis versus the actual activities results in the same relationship for those predicted by hypothesis 1. The graph of estimated activities against actual activities for the training set is shown in Figure 1.

Table 2 shows the actual and estimated antagonistic activities of training set molecules for cholecystokinin-B/gastrin receptors. In the training set compounds, all highly active compounds (<30 nM) were predicted correctly as highly active. Two moderately active compounds (30−200 nM) were predicted to be poorly active, whereas one moderately active compound was predicted to be highly active. One poorly active compound (>200 nM) was incorrectly predicted to be moderately active, and one was predicted as highly active.

Validation of Pharmacophore Model. 1. Fisher's Cross-Validation Test. A pharmacophore generated by the CatScramble technique in Catalyst was assessed for quality by Fisher's randomization test method. The purpose of this technique is to randomize the activity data associated with the training set compounds, and the randomized training sets are used to generate pharmacophore hypotheses using the same features and parameters to develop the original pharmacophore hypothesis. If the randomized data result in the generation of a pharmacophore with similar or better cost values, rms's, or correlations, the original hypothesis is considered as having been generated by chance. The results of such a run are shown in Table 3, and the resultant data clearly shows that none of the generated hypotheses after randomization had a cost value better than that of hypothesis 1. Out of the 19 runs, only one had a correlation close to 0.80, but the rms deviations were high and total costs were close to the null cost, which is not desirable for a good hypothesis. According to the software documentation and

Table 2. Actual and Estimated Activities of Training Set Molecules Calculated on the Basis of Hypothesis 1

			K_i (nM)[a]		activity scale[b]	
number	comp number	fit	actual	estimated	actual	estimated
1	1 (YF 476)	8.522	0.1[c]	0.17	+++	+++
2	2	8.514	0.2	0.17	+++	+++
3	3	7.717	0.93	1.1	+++	+++
4	4	6.541	8.3	16	+++	+++
5	5 (L365, 260)	6.278	5.2	30	+++	+++
6	6	7.512	0.6	1.8	+++	+++
7	7	5.494	44	180	++	++
8	8	5.326	190	270	++	+++
9	9	4.939	1100[c]	650	+	+
10	10	5.516	2100[c]	170	+	++
11	11	7.304	11[c]	2.8	+++	+++
12	12	5.011	590[c]	550	+	+
13	13	7.061	1.5[c]	4.9	+++	+++
14	14	7.424	1.2[c]	2.1	+++	+++
15	15	5.335	730[c]	260	+	+
16	16	5.339	370[c]	260	+	+
17	17	6.506	26[c]	18	+++	+++
18	18	6.988	9.3[c]	5.8	+++	+++
19	19	6.633	58[c]	13	++	+++
20	20	5.908	97	69	++	++
21	21	5.447	590	200	+	+
22	22	5.719	46	110	++	++
23	23	7.231	1.1	3.3	+++	+++
24	24	7.717	13	3.8	+++	+++
25	25	6.705	340	11	+	+++
26	26	5.338	410	260	+	+
27	27	6.772	6.1	9.5	+++	+++
28	28	5.339	87	260	++	+
29	29	6.767	14	9.7	+++	+++
30	30 (PD135308)	6.917	1.7	6.9	+++	+++
31	31	5.333	310	260	+	+
32	32	7.592	2.9	1.4	+++	+++
33	33	6.704	18	11	+++	+++

[a] Data for activities of cholecystokinin-B/gastrin receptor antagonists are from references listed in the Materials and Methods section. [b] Activity scale: +++ (0−30nM, highly active), ++ (30−200 nM, moderately active), + (>200 nM, poorly active). [c] Activities expressed as IC_{50} in nM.

Table 3. Results from Cross-Validation Run Using CatScramble[a]

hypothesis number	total cost	fixed cost	error cost	rms	correlation (*r*)	configuration cost
			Results for Unscrambled			
	152.78	128.40	132.92	1.152	0.8843	16.28
			Results for Scrambled			
trial 1	190.77	127.13	173.84	1.951	0.6063	15.01
trial 2	165.55	128.03	146.27	1.462	0.8049	15.91
trial 3	186.4	125.22	172.16	1.925	0.6186	13.09
trial 4	176.22	130.66	155.04	1.633	0.7471	18.54
trial 5	170.79	128.12	151.83	1.573	0.7689	15.99
trial 6	184.7	130.87	164.72	1.804	0.6765	18.75
trial 7	189.92	128.69	170.19	1.894	0.6392	16.56
trial 8	182.30	123.88	168.88	1.873	0.6455	11.75
trial 9	184.86	129.02	166.65	1.836	0.6621	16.89
trial 10	178.96	125.84	164.04	1.792	0.6817	13.72
trial 11	185.83	129.84	166.10	1.827	0.6675	17.72
trial 12	175.20	128.85	157.28	1.674	0.7300	16.73
trial 13	175.52	127.11	159.35	1.711	0.7154	14.98
trial 14	191.88	128.70	173.85	1.951	0.6051	16.58
trial 15	176.03	127.06	159.45	1.713	0.7153	14.93
trial 16	173.41	124.93	158.00	1.687	0.7269	12.81
trial 17	194.90	128.83	177.01	2.000	0.5776	16.71
trial 18	179.54	123.21	165.82	1.822	0.6709	11.087
trial 19	182.357	125.22	166.38	1.832	0.6673	13.09

[a] Null cost = 209.99. All costs are in units of bits.

Table 4. Actual and Estimated Activities of Test Set Molecules Calculated on the Basis of Hypothesis 1

			K_i (nM)[a]		activity scale[b]	
number	comp number	fit	actual	estimated	actual	estimated
1	34	7.619	1.2	1.4	+++	+++
2	35	5.566	52	150	++	++
3	36	7.948	0.21	0.63	+++	+++
4	37	7.944	1.8	0.64	+++	+++
5	38	7.554	0.676	1.6	+++	+++
6	39	7.542	13	1.6	+++	+++
7	40	6.858	1.73	7.8	+++	+++
8	41	4.621	1244[c]	1300	+	+
9	42	5.573	50[c]	150	++	++
10	43	7.806	1.9[c]	0.88	+++	+++
11	44	7.939	5.8[c]	0.65	+++	+++
12	45	5.339	5000[c]	260	+	+
13	46	5.322	262[c]	270	+	+
14	47	6.79	9[c]	14	+++	+++
15	48	5.34	890	260	+	+
16	49	5.339	4890	260	+	+
17	50	5.339	102	260	++	+
18	51	6.428	1.58	21	+++	+++
19	52	6.416	13.5	22	+++	+++
20	53	5.527	2880	170	+	++
21	54	6.348	12.3	25	+++	+++
22	55	6.293	36	29	++	+++
23	56	6.443	0.15	20	+++	+++
24	57	5.339	1766	260	+	+
25	58	7.7933	6.3	0.9	+++	+++
26	59	7.954	2.6	0.6	+++	+++
27	60	7.914	16	0.69	+++	+++

[a] Data for activities of cholecystokinin-B/gastrin receptor antagonists are from references listed in the Materials and Methods section. [b] Activity scale: +++ (0−30nM, highly active), ++ (30−200 nM, moderately active), + (>200 nM, poorly active). [c] Activities expressed as IC_{50} in nM.

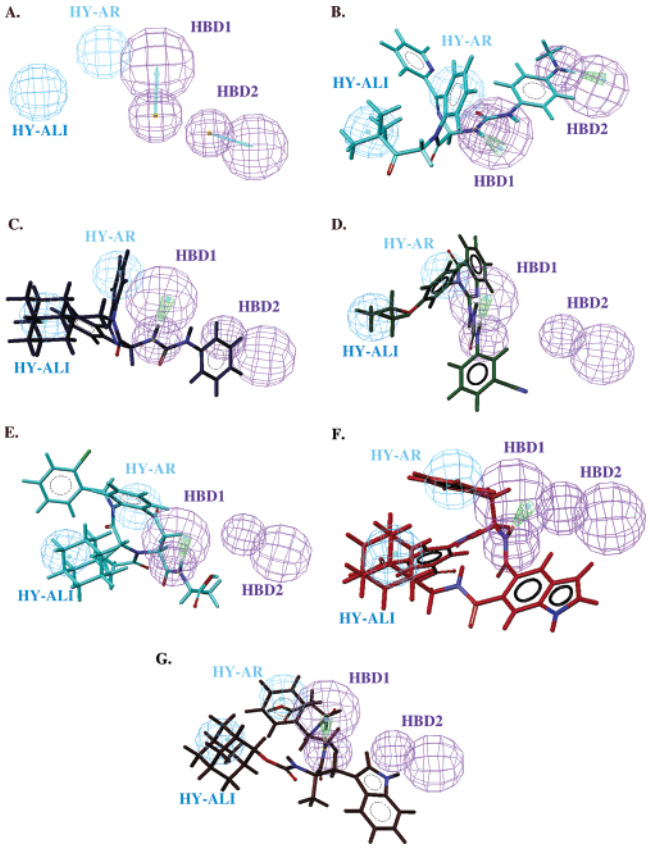


Figure 2. Mapping of the five chemotypes of CCK-BR antagonists from the training set onto the selected pharmacophore (hypothesis 1). (A) Pharmacophore (hypothesis 1); (B) benzodiazepine derivative, compound 1 (YF 476); (C) benzodiazepine derivative, compound 3; (D) quinazolinone derivative, compound 14; (E) benzodiazonine derivative, compound 22; (F) compound 23; (G) "dipeptoid" derivative (peptidomimetic), compound 30. The blue contour represents the HY-ALI features, the cyan contour represents HY-AR features, and the dark pink contour represents the HBD features.

the literature available, this result indicates that there is a 95% chance for hypothesis 1 to represent a true correlation in the training set

2. Using the Test Set. The purpose of the pharmacophore hypothesis generation is not just to predict the activity of the training set compounds accurately but also to verify whether the pharmacophore models are capable of predicting the activities of compounds of test series and classifying them correctly as active or inactive. We have constructed a set of test set compounds (27), and conformational studies were done as described earlier. The estimated activities were

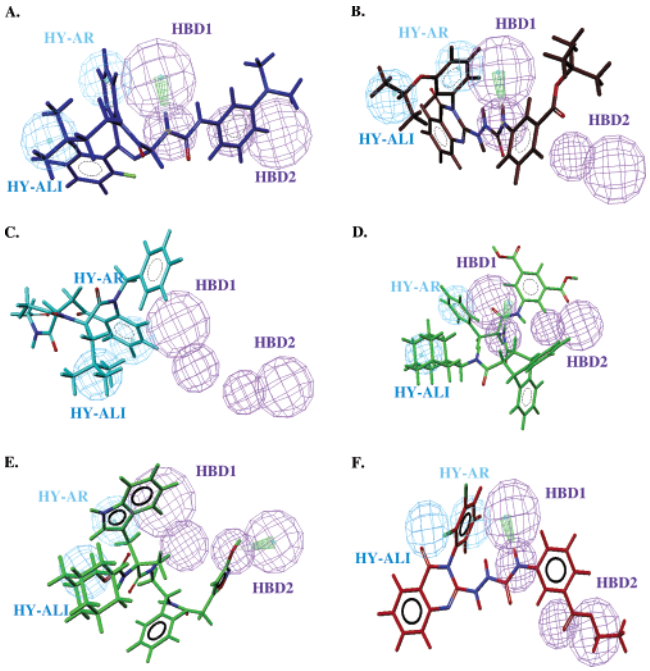
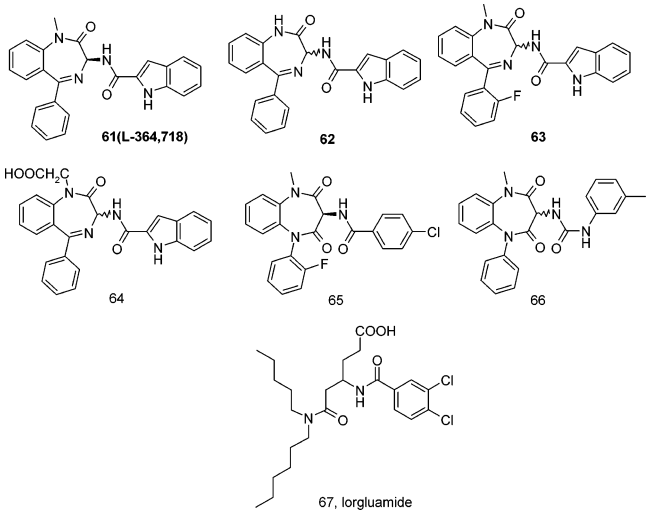


Figure 3. Mapping of the five chemotypes of CCK-BR antagonists from the test set onto the selected pharmacophore (hypothesis 1). (A) Benzodiazepine derivative, compound 36, (B) quinazolinone derivative, compound 43; (C) benzodiazonine derivative, compound 49; (D) compound 51; (E) "dipeptoid" derivative (peptidomimetic), compound 59; (F) one of the moderately active compounds, compound 42. The blue contour represents the HY-ALI features, the cyan contour represents HY-AR features, and the dark pink contour represents the HBD features.

scored using hypothesis 1 as the pharmacophore (Table 4). All highly active compounds (<30 nM) were accurately predicted as highly active, and two of the moderately active compounds (30−200 nM) were incorrectly predicted, one as highly active and other as poorly active. One poorly active compound was predicted to be moderately active.

Finally, the compounds were mapped onto the best hypothesis using the best fit and a conformational energy constraint of 10 kcal/mol^{-1}. One of the most active as well as most selective CCK-BR antagonists among all the compounds tested in all the series (compound 1) was selected from the training set to show the mapping of this compound on the selected pharmacophore (hypothesis 1, Figure 2A and B). This pharmacophore predicted the inhibitory activity of this compound against CCK-BR remarkably well (actual activity of 0.1 nM vs the estimated activity of 0.17 nM). Compound 1 mapped (Figure 2B) all four features of the hypothesis quite well with a fit value of 8.522 (maximum 10.68). Similarly, compound 2 also mapped all four features (fit value 8.514, figure not shown). None of the remaining training set compounds except these two mapped all four features. Out of 33 training set molecules, 21 compounds mapped one hydrogen bond door feature (HBD1, shown on left-hand side of Figure 2A) and 8 compounds mapped HBD2 (right side, Figure 2A). Two poorly active compounds did not map any of these HBD features. All compounds mapped the rest of the two features, HY-ALI and HY-AR. Compound 3, with a fit value of 7.717 (Figure 2C), and the other most active compounds having activity in the range 1−30 nM missed one of the two HBD features. This suggested that the three features—one HBD, one HY-ALI, and one HY-AR—are the minimum essential for the development of CCK-BR antagonists. The inclusion of a fourth feature as a HBD results in the improvement of potency from nanomolar (1−30 nM) to subnanomolar range. In other words, the three-feature hypothesis explains the most active compounds in the activity range 1−30 nM, but the four-feature hypothesis explains the improvement of activity of compound 1 (0.17 nM) over that of compound 3 (1.1 nM). To show that the other chemotypes also map well to hypothesis 1, one compound from each type—benzodiazepine derivatives, compounds 1 and 3 (Figure 2B and C, respectively); a quinazolinone derivative, compound 14 (Figure 2D); a benzodiazonine derivative, compound 22 (Figure 2E); compound 23 (Figure 2F); and a "peptoid" (peptidomimetics) derivative, compound 30 (Figure 2G)—were mapped to hypothesis 1. To give some indications of how well the structurally diverse compounds from the test set mapped this pharmacophore, one derivative from each subtype was selected and mapped on this pharmacophore and is shown in Figure 3. Among the test set compounds, 36 mapped the pharmacophore (fit value 7.7948) very well, and the estimated activity, calculated on the basis of this pharmacophore, was close to the actual activity (actual activity of 0.21 nM vs the estimated value of 0.63 nM) and is shown as +++ in Table 4. The classification of high (0−30 nM), moderate (30−200 nM), and weak activity (>200 nM) was done arbitrarily. Moderately active compounds were mapping to three features with partial overlaps, for example, compound 22 (fit value 5.719; Figure 2E) from the training set and compound 42 (fit value 5.573; Figure 3F) from the test set. The inactive compounds missed both HBD features (compound 49, Figure 3C).

Chart 3. Chemical Structures of 7 Test Set Compounds with Cholecystokinin-A Receptor Antagonist Activity[a]



[a] All structures were drawn using ISIS Draw 2.4 (MDL Information Systems, Inc., San Leandro, CA). Numbers represent the chemical numbers.

The validation study with five different classes of CCK-BR antagonists suggested that the pharmacophore was capable of mapping a structurally diverse group of compounds quite effectively and provided confidence that this pharmacophore could be used as a search query to identify new CCK-BR antagonists from drug-like chemical libraries.

Validation of the Pharmacophore Model Using Cholecystokinin-A-Receptor-Specific Antagonists as a Test Set (Test for Selectivity). A set of seven compounds[66,67] (Chart

Table 5. Actual and Estimated Activities of Test Set Molecules (CCK-AR Selective) Calculated on the Basis of Hypothesis 1

			K_i (nM)[a]		
number	comp number	fit	actual (CCK-A)	estimated (CCK−B)	predicted activity scale
1	61 (L364, 718)	5.306	0.71	280	+
2	62	5.327	4.7	260	+
3	63	5.324	1.4	270	+
4	64	5.337	1.4	260	+
5	65	5.433	2.9	210	+
6	66	6.636	7.1	13	+++
7	67 (lorgluamide)	6.607	49	14	+++

[a] Data for activities of cholecystokinin-A receptor antagonists are from references listed in the Materials and Methods section.

3) that target CCK-AR selectively were taken as negative controls to check the selectivity of the hypothesis for CCK-BR-targeted compounds versus CCK-AR. In fact, the most selective CCK-AR ligands were predicted as poorly active using pharmacophore mapping, thereby showing some kind of selectivity of the generated hypothesis. Hypothesis 1 predicted the activities of five out of the seven tested compounds to be inactive against CCK-BR, thereby showing the selectivity of the hypothesis to some extent. Compounds 66 and 67 were incorrectly predicted to be active against the CCK-BR. This suggests there may be similarities for feature requirements of the two kinds of receptors, and hypothesis 1 generated in this work , though predictive for the CCK receptor, may not be fully selective for CCK-BR. The results of the activity prediction of these compounds are shown in Table 5.

CONCLUSIONS

The work presented in this study shows how chemical features of a set of compounds along with their activities ranging over several orders of magnitudes can be used to generate pharmacophore hypotheses that can successfully predict activity. The models were not only predictive within the same series of compounds but six different classes of diverse compounds also effectively mapped onto most of the features important for activity. The pharmacophores generated from CCK-BR antagonists can be used (1) as a three-dimensional query in database searches to identify compounds with diverse structures that can potentially antagonize CCK-BR selectively and (2) to evaluate how well any newly designed compound maps on the pharmacophore before undertaking any further study including synthesis. Both these applications may help in identifying or designing compounds for further biological evaluation and optimization. The pharmacophore developed in this study using antagonists for CCK-BR showed distinct chemical features that may be responsible for the activity of the antagonists. We intend to utilize the information to undertake 3D searches on large databases of drug-like molecules to identify a new generation of cholecystokinin-B/gastrin receptors.

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REFERENCES AND NOTES

(1) Walsh, J. H. Gastrin. In *Gut Peptides*; Walsh, J. H., Dockray, G. J., Eds.; Raven Press: New York, 1994; pp 75−121.
(2) Johnson, L. R. Trophic Effects of Gut Peptides. In *Handbook of Physiology;* Makhlouf, G. M., Ed.; American Physiological Society: Bethesda, MD, 1989; Section 6, pp 291−310.
(3) Johnson, L. R. The Trophic Action of Gastrointestinal Hormones. *Gastroenterology* **1976**, *70*, 278−288.
(4) Hakanson, R.; Sundler, F. Tropic Effects of Gastrin. *Scand. J. Gastroenterology* **1991**, *70* (Suppl. 180), 130−136.
(5) Kopin, A. S.; Lee, Y.; McBride, E. W.; Miller, L. J.; Lu, M.; Lin, H. Y.; Kolakowski, L. F.; Beinborn, M. Expression, Cloning and Characterization of the Canine Parietal Cell Gastrin Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3605−3609.
(6) Wank, S. A.; Pisegna, J. R.; de Weerth, A. Brain and Gastrointestinal Cholecystokinin Receptor Family: Structure And Functional Expression. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8691−8695.
(7) Mantyh, C. R.; Pappas, T. N.; Vigna, S. R. Localization of the Cholecystokinin A and Cholecystokinin B/Gastrin Receptors in the Canine Upper Gastrointestinal Tract. *Gastroenterology* **1994**, *107*, 1019−1030.
(8) Nakamura, M.; Oda, M.; Kaneko, K.; Akaiwa, Y.; Tsukada, N.; Komatsu, H.; Tsuchiya, M. Autoradiographic Demonstration of Gastrin-Releasing Peptide-Binding Sites in the Rat Gastric Mucosa. *Gastroenerology* **1988**, *94*, 968−976.
(9) Schjoldager, B.; Molero, X.; Miller, L. J. Functional and Biochemical Characterization of the Human Gall Bladder Muscularis Cholecystokinin Receptor. *Gastoenterology* **1989**, *96*, 1119−1125.
(10) Sandvik, A. K.; Waldum, H. L. CCK-B (Gastrin) Receptor Regulates Gastric Histamine Release and Acid Secretion. *Am. J. Physiol.* **1991**, *260*, G925−G928.
(11) Walsh, J. H. Role of Gastrin as a Trophic Hormone. *Digestion* **1990**, *47*, 11−16.
(12) Reubi, J. C. Neuropeptide Receptors in Health and Disease: The Molecular Basis for In Vivo Imaging. *J. Nucl. Med.* **1995**, *3*, 1825−35.
(13) Krenning, E. P.; Kwekkeboon, D. J.; Pauwels, S. Somatostatin Receptor Scintigraphy. *Nucl. Med. Ann.* **1995**, 1−50.
(14) Otte, A.; Mullar- Brand, J.; Dellas, S. Yttrium-90-Labeled Somatostatin- Analogue for Cancer Treatment. *Lancet* **1998**, *351*, 417−18.
(15) Reubi, J. C.; Waser, B. Cholecystokinin (CCK-A) and CCK-B/Gastrin Receptors in Human Tumors. *Cancer Res.* **1997**, *57*, 1377−86.
(16) Behr, T. M.; Jenner, N.; Behe, M.; Angerstein, C.; Gratz, S.; Raue, F.; Becker, W. Radiolabeled Peptide for Targeting Cholecystokinin-B/Gastrin Receptor—Expressing Tumors. *J. Nucl. Med.* **1999**, *40*, 1029−1044.
(17) Trivedi, B. K. Ligands for Cholecystokinin Receptors: Recent Developments. *Curr. Opin. Ther. Pat.* **1994**, *4* (1), 31−44.
(18) Trivedi, B. K. Cholecystokinin Receptor Antagonists: Current Status. *Curr. Med. Chem.* **1994**, *1*, 313−327.
(19) Blommaert, A. G. S.; Weng, J.-H.; Dorville, A.; McCort, I.; Ducos, B.; Durieux, C.; Roques, B. P. Cholecystokinin Peptidomimetics as Selective CCK-B Antagonists: Design, Synthesis, and in Vitro and in Vivo Biochemical Properties. *J. Med. Chem.* **1993**, *36*, 2868−2877.
(20) Trivedi, B. K.; Padia, J. K.; Holmes, A.; Rose, S.; Wright, D. S.; Hinton, J. P.; Pritchard, M. C.; Eden, J. M.; Kneen, C.; Webdale, L.; Suman-Chauhan, N.; Boden, P.; Singh, L.; Field, M. J.; Hill, D. Second Generation "Peptoid" CCK-B Receptor Antagonists: Identification and Development of *N*-(Adamantyloxycarbonyl)-α-methyl-(*R*)-tryptophan Derivative (CI-1015) with an Improved Pharmacokinetic Profile *J. Med. Chem.* **1998**, *41*, 38−45.
(21) Lipinski, C. A. Drug-like Properties and the Causes of Poor Solubility and Poor Permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235−249.
(22) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.
(23) *Pharmacophore Perception, Development, and Use in Drug Design*; Güner, O. F., Ed.; International University Line: La Jolla, CA, 2000.
(24) Martin, Y. C. Pharmacophore mapping. In *Designing Bioactive Molecules: Three-Dimensional Techniques and Application*; Martin, Y. C., Willett, P., Eds.; American Chemical Society: Washington, DC, 1998; pp 121−148.
(25) Clark, D. E.; Westhead, D. R.; Sykes, R. A.; Murray, C. W. Activesite-Directed 3D Database Searching: Pharmacophore Extraction and Validation of hits. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 397−416.
(26) Doweyko, A. M. Three-dimensional Pharmacophores from Binding Data. *J. Med. Chem.* **1994**, *37*, 1769−1778.
(27) Mason, J. S.; Good, A. C.; Martin, E. J. 3-D Pharmacophores in Drug Discovery. *Curr. Pharm. Des.* **2001**, *7*, 567−597.
(28) Milne, G. W.; Nicklaus, M. C.; Wang, S. Pharmacophores in Drug Design and Discovery. *SAR QSAR Environ. Res.* **1998**, *9*, 23−38.
(29) PhRMA: New Medicines in Development Series, 2001 (http://www.phrma.org/publications/documents/factsheets/2001-03-01.210.phtml).

(30) *Catalyst*, version 4.7; Accelrys, Inc. (previously known as Molecular Simulations, Inc.): San Diego, CA, 2000.
(31) Greenidge, P. A.; Weiser, J. A Comparison of Methods for Pharmacophore Generation with the Catalyst Software and their use for 3D-QSAR: Application to a set of 4-Aminopyridine Thrombin Inhibitors. *Mini-Rev. Med. Chem.* **2001**, *1*, 79−87.
(32) Palomer, A.; Pascual, J.; Cabre, F.; Garcia, M. L.; Mauleon, D. Derivation of Pharmacophore and CoMFA Models for Leukotriene D(4) Receptor antagonists of the quinolinyl(bridged)aryl series. *J. Med. Chem.* **2000**, *43*, 392−400.
(33) Duffy, J. C.; Deardon, J. C.; Green, D. S. V. Use of Catalyst in the Design of Novel Non-Steroidal Anti-inflammatory Analgesic Drugs. In *QSAR and Molecular Modelling: Concepts, Computational Tools and Biological Applications*; Sanz, F., Giraldo, J., Manaut, F., Eds.; Prous Science Publishers: Barcelona, Spain, 1995; pp 289−291.
(34) Hoffman, R. D.; Bourguignon, J. J. Building a Hypothesis for CCK-B Antagonists Using Catalyst Program. In *QSAR and Molecular Modelling: Concepts, Computational Tools and Biological Applications*; Sanz, F., Giraldo, J., Manaut, F., Eds.; Prous Science Publishers: Barcelona, Spain, 1995; pp 298−300.
(35) Kaminski, J. J.; Rane, D. F.; Snow, M. E.; Weber, L.; Rothofsky, M. L.; Anderson, S. D.; Lin, S. L. Identification of Novel Farnesyl Protein Transferase Inhibitors Using Three-Dimensional Database Searching Methods. *J. Med. Chem.* **1997**, *40*, 4103−4112.
(36) Sprague, P. W. Automated Chemical Hypothesis Generation and Database Searching with Catalyst. In *Perspectives in Drug Discovery and Design*; Müller, K., Eds.; ESCOM Science Publishers B. V.: Leiden, The Netherlands, 1995; pp 1−21.
(37) Quintana, J.; Contijoch, M.; Cuberes, R.; Frigola, J. Structure Reactivity Relationships and Molecular Modeling Studies of a Series of H1 Antihistamines. In *QSAR and Molecular Modelling: Concepts, Computational Tools and Biological Applications*; Sanz, F., Giraldo, J., Manaut, F., Eds.; Prous Science Publishers: Barcelona, Spain, 1995; pp 282−288.
(38) Barbaro, R.; Betti, L.; Botta, M.; Corelli, F.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Corsano, S. Synthesis, Biological Evaluation, and Pharmacophore Generation of New Pyridazinone Derivatives with Affinity toward R1- and R2-Adrenoceptors. *J. Med. Chem.* **2001**, *44*, 2118−2132.
(39) Baringhaus, K. H.; Matter, H.; Stengelin, S.; Kramer, W. Substrate Specificity of the Ileal and the Hepatic Na(+)/Bile Acid Cotransporters of the Rabbit. II. A Reliable 3D QSAR Pharmacophore Model for the Ileal Na(+)/Bile Acid Cotransporter. *J. Lipid Res.* **1999**, *40*, 2158−2168.
(40) Ekins, S.; de Groot, M. J.; Jones, J. P. Pharmacophore and Three-Dimensional Quantitative Structure Activity Relationship Methods for Modeling Cytochrome P450 Active Sites. *Drug Metab. Dispos.* **2001**, *29*, 936−944.
(41) Hirashima, A.; Rafaeli, A.; Gileadi, C.; Kuwano, E. Three-Dimensional Pharmacophore Hypotheses of Octopamine Receptor Responsible for the Inhibition of Sex-Pheromone Production in Helicoverpa Armigera. *J. Mol. Graphics Model.* **1999**, *17*, 43−44.
(42) Karki, R. G.; Kulkarni, V. M. A Feature Based Pharmacophore for *Candida Albicans* MyristoylCoA: Protein *N*-Myristoyltransferase Inhibitors. *Eur. J. Med. Chem.* **2001**, *36*, 147−163.
(43) Manetti, F.; Corelli, F.; Biava, M.; Fioravanti, R.; Porretta, G. C.; Botta, M. Building a Pharmacophore Model for a Novel Class of Antitubercular Compounds. *Farmaco* **2000**, *55*, 484−491.
(44) Ekins, S.; Bravi, G.; Ring, B. J.; Gillespie, T. A.; Gillespie, J. S.; Vandenbranden, M.; Wrighton, S. A.; Wikel, J. H. Three-Dimensional Quantitative Structure Activity Relationship Analyses of Substrates for CYP2B6. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 21−29.
(45) Ekins, S.; Bravi, G.; Binkley, S.; Gillespie, J. S.; Ring, B. J.; Wikel, J. H.; Wrighton, S. A. Three- and Four-Dimensional Quantitative Structure Activity Relationship Analyses of Cytochrome P-450 3A4 Inhibitors. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 429−438.
(46) Ekins, S.; Bravi, G.; Binkley, S.; Gillespie, J. S.; Ring, B. J.; Wikel, J. H.; Wrighton, S. A. Three and four dimensional Quantitative Structure Activity Relationship (3D/4D-QSAR) Analyses of CYP2D6 Inhibitors. *Pharmacogenetics* **1999**, *9*, 477−489.
(47) Grigorov, M.; Weber, J.; Tronchet, J. M.; Jefford, C. W.; Milhous, W. K.; Maric, D. A QSAR Study of the Antimalarial Activity of Some Synthetic 1,2,4-Trioxanes. *J. Chem. Inf. Comput. Sci.* **1997**, *37*, 124−130.
(48) Lopez-Rodriguez, M. L.; Porras, E.; Benhamu, B.; Ramos, J. A.; Morcillo, M. J.; Lavandera, J. L. First Pharmacophoric Hypothesis for 5-HT7 Antagonism. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1097−1100.
(49) Daveu, C.; Bureau, R.; Baglin, I.; Prunier, H.; Lancelot, J. C.; Rault, S. Definition of a Pharmacophore for Partial Agonists of Serotonin 5-HT3 Receptors. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 362−369.
(50) Norinder, U. Refinement of Catalyst Hypotheses Using Simplex Optimisation. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 545−557.
(51) Bureau, R.; Daveu, C.; Baglin, I.; Sopkova-De Oliveira, S. J.; Lancelot, J. C.; Rault, S. Association of two 3D QSAR Analyses. Application to the Study of Partial Agonist Serotonin-3 Ligands. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 815−823.
(52) Kurogi, Y.; Güner, O. F. Pharmacophore Modeling and Three-Dimensional Database Searching for Drug Design Using Catalyst. *Curr. Med. Chem.* **2001**, *8*, 1035−1055.
(53) Güner, O. F.; Waldman, M.; Hoffman, R.; Kim, J.-H. Strategies for Database Mining and Pharmacophore Development. In *Pharmacophore Perception, Development, and Use in Drug Design*; Güner, O. F., Eds.; International University Line: La Jolla, CA, 2000; pp 213−236.
(54) Langer, T.; Hoffman, R. D.; Bachmair, F.; Begle, S. Chemical Function Based Pharmacophore Models as Suitable Filters for 3D Database Searching. *THEOCHEM* **2000**, *500*, 59−72.
(55) Kurogi, Y.; Miyata, K.; Okamura, T.; Hashimoto, K.; Tsutsumi, K.; Nasu, M.; Moriyasu, M. Discovery of Novel Mesangial Cell Proliferation Inhibitors using a Three-Dimensional Database Searching Method. *J. Med. Chem.* **2001**, *44*, 2304−2307.
(56) Briens, F.; Bureau, R.; Rault, S. Applicability of CATALYST in Ecotoxicology, a New Promising Tool for 3D-QSAR: Study of Chlorophenols. *Ecotoxicol. Environ. Saf.* **1999**, *43*, 241−251.
(57) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: A Program for Macromolecular Energy, Minimization, and Dynamics Calculations. *J. Comput. Chem.* **1983**, *4*, 187−217.
(58) Semple, G.; Ryder, H.; Rooker, D. P.; Batt, A. R.; Kendrick, D. A.; Szelke, M.; Ohta, M.; Satoh, M.; Nishida, A.; Akuzawa, S.; Miyata, K. (3*R*)-*N*-(1-(*tert*-Butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1*H*-1,4-benzodiazepin-3-yl)-*N*-(3-(methylamino)phenyl)urea (YF476): A Potent and Orally Active Gastrin/CCK-B Antagonist. *J. Med. Chem.* **1997**, *40*, 331−341.
(59) Kalindjian, S. B.; Buck, I. M.; Davies, J. M. R.; Dunstone, D. J.; Hudson, M. L.; Low, C. M. R.; McDonald, I. M.; Pether, M. J.; Steel, K. I. M.; Tozer, M. J.; Vinter, J. G.; Non-Peptide Cholecystokinin-B/Gastrin Receptor Antagonists Based on Bicyclic, Heteroaromatic Skeletons. *J. Med. Chem.* **1996**, *39*, 1806−1815.
(60) Blommaert, A. G. S.; Weng, J.-H.; Dorville, A.; McCort, I.; Ducos, B.; Durieux, C.; Roques, B. P. Cholecystokinin Peptidomimetics as Selective CCK-B Antagonists: Design, Synthesis, and in Vitro and in Vivo Biochemical Properties. *J Med. Chem*, **1993**, *36*, 2868−2877.
(61) Yu, M. J.; Thrasher, K. J.; McCowan, J. R.; Mason, N. R.; Mendelsohn, L. G. Quinazolinone Cholecystokinin-B Receptor Ligands. *J. Med. Chem.* **1991**, *34*, 1505−1508.
(62) Drysdale, M. J.; Pritchard, J. M. C.; Horwell, D. C. Rationally Designed "Dipeptoid" Analogues of CCK. Acid Mimics of the Potent and Selective Non-Peptide CCK-B Receptor Antagonist CI-988. *J. Med. Chem.* **1992**, *35*, 2573−2581.
(63) Padia, J. K.; Field, M.; Hinton, J.; Meecham, K.; Pablo, J.; Pinnock, R.; Roth, B. D.; Singh, L.; Suman-Chauhan, N.; Trivedi, B. K.; Webdale, L. Novel Nonpeptide CCK-B Antagonists: Design and Development of Quinazolinone Derivatives as Potent, Selective, and Orally Active CCK-B Antagonists. *J. Med. Chem.* **1998**, *41*, 1042−1049.
(64) McDonald, I. M.; Dunstone, D. J.; Kalindjian, S. B.; Linney, I. D.; Low, C. M. R.; Pether, M. J.; Steel, K. I. M.; Tozer, M. J.; Vinter, J. G. 2,7-Dioxo-2,3,4,5,6,7-hexahydro-1H-benzo[*h*][1,4]diazonine as a new template for the design of CCK_2 Receptor Antagonists. *J. Med. Chem.* **2000**, *43*, 3518−3529.
(65) Urisini, A.; Capelli, A. M.; Carr, R. A. E.; Cassara, P.; Corsi, M.; Curcuruto, O.; Curotto, G.; Cin, M. D.; Davali, S.; Donati, D.; Feriani, A. Finizia, G.; Gaviraghi, G.; Marien, M.; Pentassuglia, G.; Polinelli, S.; Ratti, E.; Reggiani, A.; Tarzia, G.; Tedesco, G.; Tranquillini, M. E.; Trist, D. G.; Van Amsterdam, F. T. M. Synthesis and SAR of New 5-phenyl-3-ureido-1,5-benzodiazepines as cholecystokinin-B Receptor Antagonists. *J. Med. Chem.* **2000**, *43*, 3596−3613.
(66) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. Benzodiazepine gastrin and brain cholecystokinin receptor ligands; L-365, 260. *J. Med. Chem.* **1989**, *32* (1), 13−16.
(67) Evans, B. E.; Bock, M. G.; Rittle, K. E.; DiPardo, R. M.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. Design of Potent, Orally Effective, Nonpeptidal Antagonists of the Peptide Hormone Cholecystokinin. *PNAS* **1986**, *83* (13), 4918−4922.
(68) Tromelin, A.; Guichard, E. Use of Catalyst in a 3D-QSAR Study of the Interactions between Flavor Compounds and β-Lactoglobulin. *J. Agric. Food Chem.* **2003**, *51*, 1977−1983.

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