Generation of Predictive Pharmacophore Models for CCR5 Antagonists: Study with Piperidine- and Piperazine-Based Compounds as a New Class of HIV-1 Entry Inhibitors

Asim Kumar Debnath[†]

Laboratory of Molecular Modeling & Drug Design, Lindsley F. Kimball Research Institute of The New York Blood Center, 310 E. 67th Street, New York, New York 10021

Received May 30, 2003

Predictive pharmacophore models were developed for a large series of piperidine- and piperazine-based CCR5 antagonists as anti-HIV-1 agents reported by Schering-Plough Research Institute in recent years. The pharmacophore models were generated using a training set consisting of 25 carefully selected antagonists based on well documented criteria. The activity spread, expressed in K_i, of training set molecules was from 0.1 to 1300 nM. The most predictive pharmacophore model (hypothesis 1), consisting of five features, namely, two hydrogen bond acceptors and three hydrophobic, had a correlation (r) of 0.920 and a root mean square of 0.879, and the cost difference between null cost and fixed cost was 44.46 bits. The model was cross-validated by randomizing the data using the CatScramble technique. The results confirmed that the pharmacophore models generated from the test set were not due to chance correlation. The best model (hypothesis 1) was validated using test set molecules (total of 78) and performed well in classifying active and inactive molecules correctly. The model was further validated by mapping onto it a diverse set of six CCR5 antagonists identified by five different pharmaceutical companies. The best model correctly predicted these compounds as being highly active. These multiple validation approaches provide confidence in the utility of the predictive pharmacophore model developed in this study as a 3D query tool in virtual screening to retrieve new chemical entities as potent CCR5 antagonists. The model can also be used in predicting biological activities of compounds prior to undertaking their costly synthesis.

Introduction

According to an UNAIDS report, about 42 million people are living with HIV/AIDS at the end of 2002.[1] There were 5 million new infections just in the past year, and more than 3 million people died from this deadly disease in 2002. These data show the enormity of the AIDS epidemic in the world, especially affecting sub-Saharan Africa and southeast Asia. The current trend in infection rate suggests that by the end of 2010 another 45 million people will be infected with HIV in the absence of effective global prevention measures. The currently available drugs approved by the U.S. FDA are only, until recently, reverse transcriptase and protease inhibitors and their combinations. Although these drugs are helping to reduce the morbidity and mortality of HIV infection, their very high cost is prohibitive for most people infected in sub-Saharan Africa and third-world countries. Besides, these drugs have well documented side effects,[2−6] and development of resistance has been reported.[7−9] Therefore, discovery of new classes of potent and less-toxic anti-HIV-1 drugs with a different mechanism of action is urgently needed. The recent approval of a new class of drug, known as entry/fusion inhibitor T-20 (Fuzeone)[10−13] by the U.S. FDA, has generated hope that it might help infected people who are resistant to other available retroviral therapies. This new drug has also showed promise and validated the notion that early stages of HIV infection, i.e., fusion/entry can be effectively inhibited.[14−16]

The early step of HIV-1 infections starts when the virus enters the target cells by attaching to the CD4 receptor and subsequently interacts with chemokine receptors such as CXCR4 and CCR5.[17−20] CCR5 belongs to the family of G-protein-coupled receptors (GPCR) and was discovered as the major chemokine receptor for the macrophage tropic strains (M-tropic) of HIV-1 to enter into monocytes, macrophages, and T-cells based on the observation that the β-chemokines, MIP-1α, MIP-1β, and RANTES inhibit infection of $CD4^+$ cells by nonsyncytium-inducing (NSI) strains of HIV-1.[21] The idea of using CCR5 as a possible target for therapeutic intervention emerged from the findings that some individuals who were homozygous for a defective CCR5 allele with internal 32 base pair deletion (CCR5-Δ32) were protected from HIV-1 infection and appeared to be healthy.[22−24] Since then, a great deal of activity in identifying small-molecule drugs against this target has been reported (recently elegantly reviewed by Kazmierski et al.[25]). The first discovery of a CCR5 inhibitor, TAK-779, was reported by Takeda Chemical Industries in Japan.[26] Recently, Schering-Plough Research Institute in the U.S. has reported the systematic discovery of highly potent CCR5 antagonists as anti-HIV-1 agents in a series of publications.[16,27−33] In the absence of any three-dimensional (3D) structure of CCR5, rational design of inhibitors against this receptor using a structure-based approach is not feasible. Therefore, it is

[†] Phone: (212) 570-3373. Fax: (212) 570-3299. E-mail: adebnath@nybloodcenter.org.

10.1021/jm030265z CCC: $25.00
Published on Web 09/11/2003

prudent to identify possible pharmacophores from a series of CCR5 antagonists with binding (or inhibitory) activities to understand the structural requirements for potent and selective drugs against this target. A pharmacophore represents the 3D arrangements of structural or chemical features of a drug (small organic compounds, peptides, peptidomimetics, etc.) that may be essential for interacting with the receptor for optimum binding. These pharmacophores can be used in different ways in drug design programs: (1) as a 3D query tool in virtual screening to identify potential new compounds from 3D databases of "drug-like"[34,35] molecules with patentable structures different from those already discovered; (2) to predict the activities of a set of new compounds yet to be synthesized; (3) to understand the possible mechanism of action. The concepts of pharmacophore, their development techniques, and applications have been elegantly compiled in a recently published book.[36] The pharmacophore generation approach is quite powerful and finds many applications in drug discovery research.[37−56] According to a recent report, it costs about $600 million to $800 million and 12−15 years to bring a compound from the identification stage to the market.[57] Pharmaceutical companies are hard-pressed to take a multitude of rational design approaches to shorten the time and reduce the cost of identifying new chemical entities (NCEs). Hypothesis generation by the Catalyst software is one of such approaches that has been successfully used in drug discovery and toxicology research (for comprehensive reference lists, see http://www.accelrys.com/references/rdd_pub.html#catalys). We have initiated a systematic study toward developing pharmacophore models from a large data set of CCR5 antagonists as part of our drug discovery program. In this report, we present the development of pharmacophore models of CCR5 antagonists as anti-HIV-1 agents using the Catalyst/HypoGen module and validate the model not only on large test sets of compounds but also on six of the most potent and structurally diverse CCR5 antagonists identified by five major pharmaceutical companies.

Materials and Methods

Molecular Modeling. Molecular modeling was performed on a Silicon Graphics Octane R12000 dual processor computer (sgi, 1600 Amphitheater Parkway, Mountain View, CA 94043). Catalyst 4.7 software (Accelrys Inc., San Diego, CA) was used to generate pharmacophore models.

Biological Data. The sources of the biological activity data (data for inhibition of RANTES binding to CCR5), represented as K_i in nM, were from the literature published by Schering-Plough Research Institute, Kenilworth, NJ[27,28,31,58,59] (U.S. Patents 6,387,930 (2002) and 6,391,865 (2002)). The chemical structures of the antagonists are listed in Charts 1 and 2. The datasets were divided into a training set and a test set. For estimation (prediction) purposes, the activity values were classified as follows: K_i (nM) ≤100 nM means the compounds are highly active (represented as ++); 100 nM < K_i (nM) ≤ 500 nM means the compounds are moderately active (represented as +); K_i (nM) > 500 nM means the compounds are inactive (represented as −). This classification scheme was created on the basis of the fact that the initial compound screening program at Schering-Plough Research Institute to find CCR5 antagonists identified a lead compound with an activity (K_i) of 1000 nM, and subsequent optimization of this molecule generated several potent CCR5 antagonists. The current literature data indicate that activity can reach subnanomolar levels. Therefore, we have chosen a much stricter cutoff value of less than 500 nM to consider any compound to be active. Because one of the major goals of pharmacophore generation is to utilize it in searching (virtual screening) 3D drug-like chemical databases to identify lead compounds, this classification scheme is more meaningful than actual prediction values and may help in identifying lead compounds with structures different from those reported in the literature.

Criteria of Selecting Training Set. The most critical aspect of pharmacophore hypothesis generation in the Catalyst software is the selection of the training set. Some basic strategies have been elegantly laid out by Li et al.[60] The basic guidelines are as follows. (1) A minimum of 16 diverse compounds should be selected to avoid any chance correlation. (2) The activity data should have a range of 4−5 orders of magnitude. (3) The compounds should be selected to provide clear and concise information and to avoid redundancy and bias in terms of both structural features and activity range. (4) The most active compounds should be included so that they provide information on most critical features required as a pharmacophore. (5) Inclusion of any compound known to be inactive because of steric hindrance must be avoided because current features in the Catalyst software cannot handle such cases.

On the basis of the above criteria, we have selected 25 compounds for the training set and 78 compounds for the test set.

Generation of Pharmacophores. All stereoisomeric centers in the molecules were appropriately assigned as indicated in the original data sources using the Catalyst software. Conformation models for all molecules (both training and test sets) were generated using the Catalyst/ConFirm module within the software, using the "best quality" conformational search option. A maximum of 250 conformations were generated using Charmm force field parameters[61] and a constraint of 10 kcal mol^{-1} energy thresholds above the global energy minimum. Catalyst selects conformers using the Poling algorithm,[62−64] which penalizes any newly generated conformer if it is too close to any already found conformers. This method ensures maximum coverage in conformation space. All other parameters were set to the default settings. An initial analysis of the "show function mapping" tools revealed that hydrogen bond acceptor (HA), hydrophobic (HY), ring aromatic (RA), and positive ionizable (PI) features could effectively map all critical chemical/structural features of all the training set molecules. During the initial phase of the hypothesis generation exercise, it was observed that only two features, i.e., HA and HY, out of those four mentioned above dominated in most of the useful hypotheses generated by the Catalyst software. Also, the maximum number of hydrogen bond acceptor features (HA) in those hypotheses was never more than two. Therefore, those two features were used to generate 10 pharmacophore hypotheses from the training set, using a default uncertainty value of 3. The minimum and maximum count of features for HY was 0 and 5, respectively, whereas for HA the values were 0 and 3, respectively. The Catalyst/HypoGen module can only generate a maximum of five features for a hypothesis.

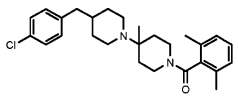
Assessment of the Quality of Pharmacophore Hypotheses. (a) Cost Function Analysis. The quality of the generated pharmacophore hypotheses was evaluated by considering the cost functions (represented in bits unit) calculated by the Catalyst/HypoGen module during hypothesis generation. Details of the cost function were reported by Sutter et al.[65] In brief, the cost (total cost) of a hypothesis is calculated by the following equation:

$$\text{cost} = eE + wW + cC$$

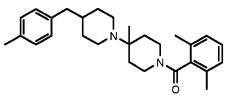
where e, w, and c are the coefficients associated with the error (E), weight (W), and configuration (C) components, respectively.

The other two important cost calculations are the "fixed cost" and the "null cost". The "fixed cost" represents the simplest

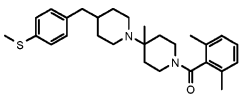
Chart 1. Chemical Structures of 25 Training Set Compounds[a]



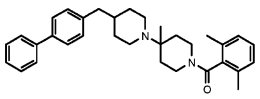
2



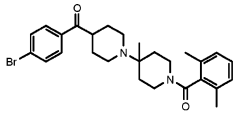
6



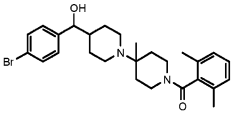
8



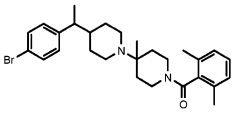
11



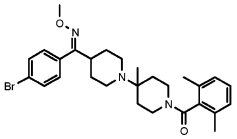
12



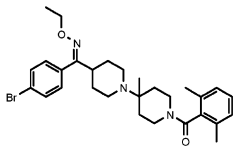
13



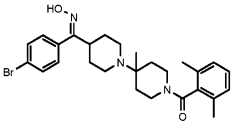
14



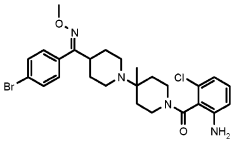
18



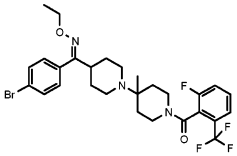
20



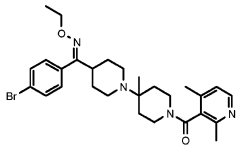
21



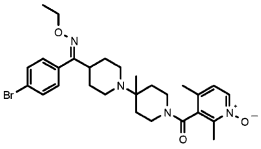
27



30

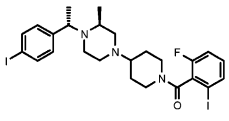


32

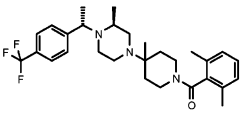


38

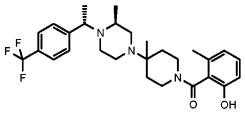
Sch-351125 (SCH-C)



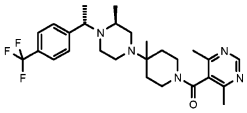
52



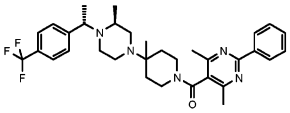
61



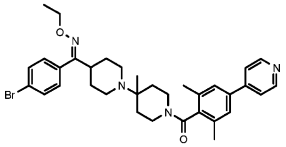
63



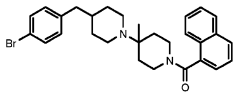
71



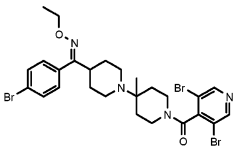
74



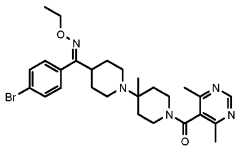
77



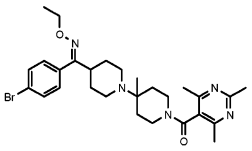
78



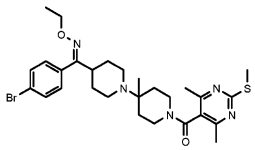
79



86



92



100

[a] All structures were drawn using ISIS Draw 2.5 (MDL Information Systems, Inc., San Leandro, CA). Numbers represent the compound numbers.

Chart 2. Chemical Structures of 78 Test Set Compounds[a]

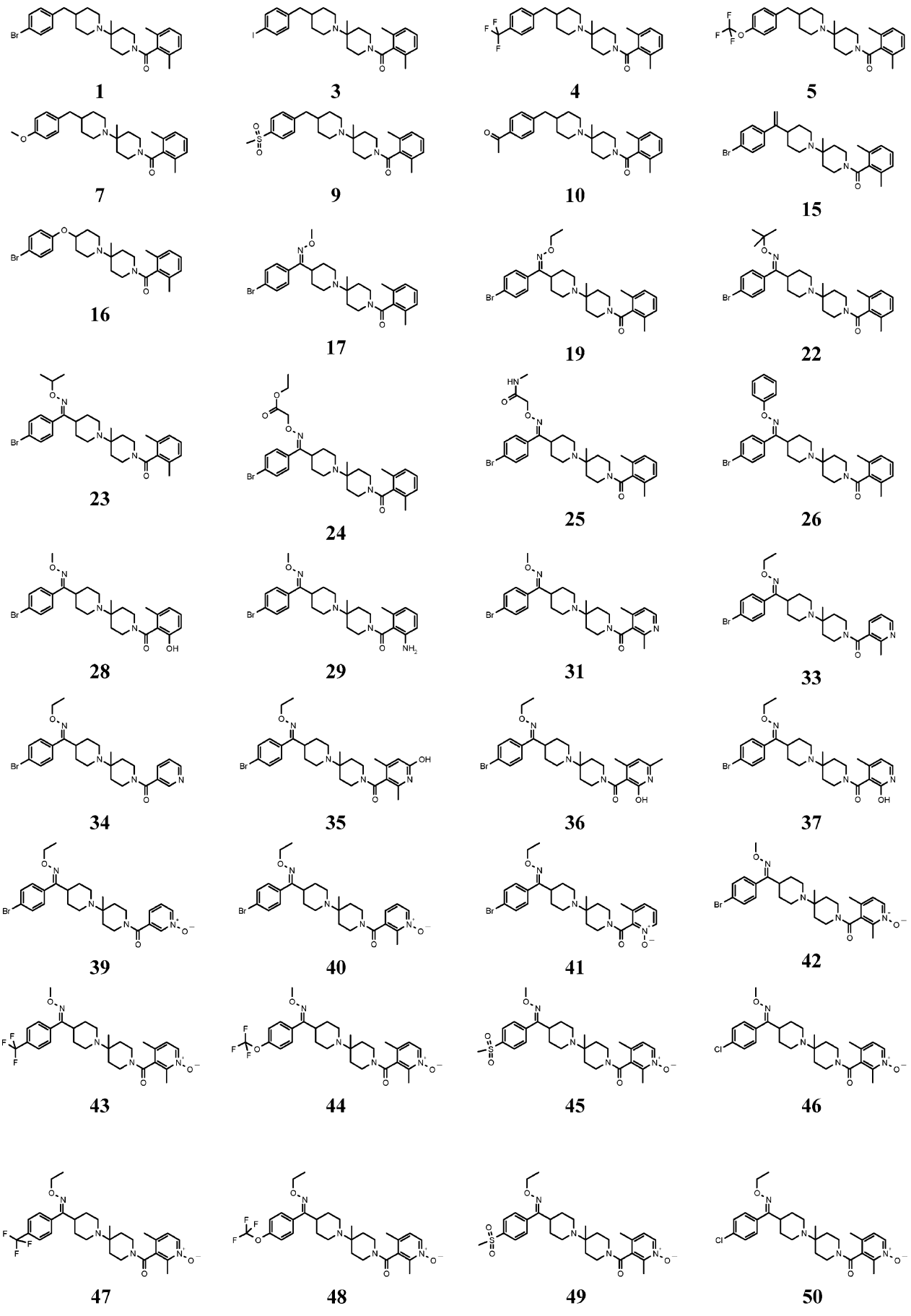
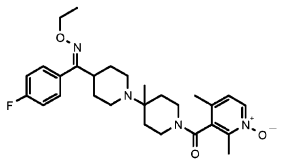
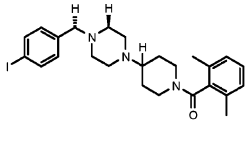


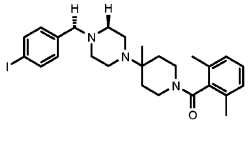
Chart 2 (Continued)



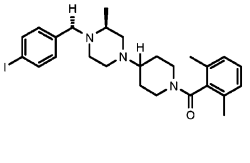
51



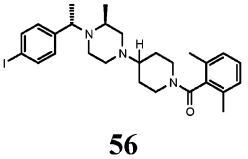
53



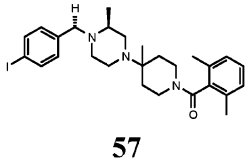
54



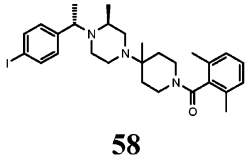
55



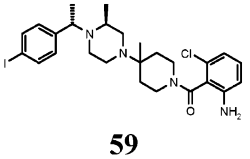
56



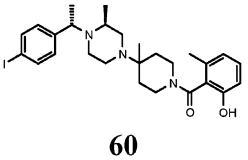
57



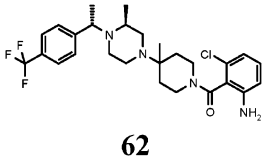
58



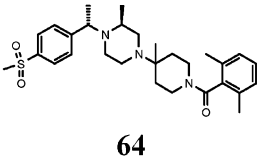
59



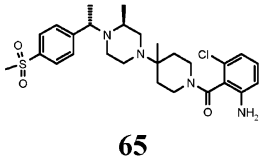
60



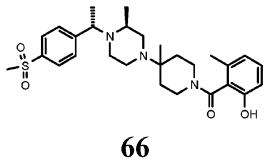
62



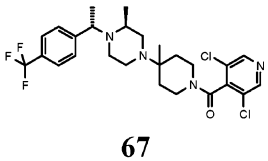
64



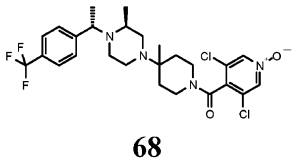
65



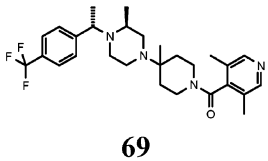
66



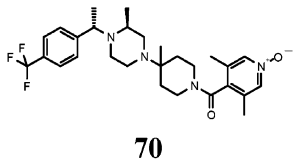
67



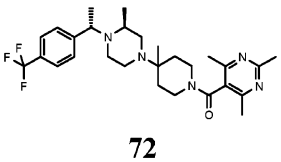
68



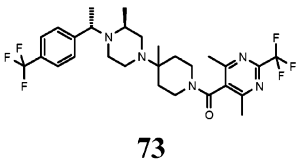
69



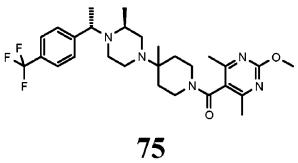
70



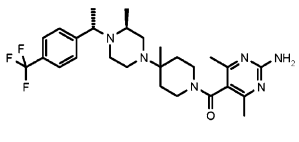
72



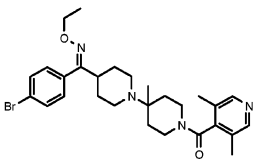
73



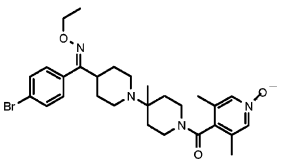
75



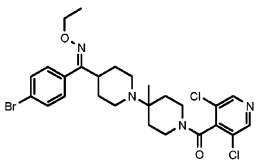
76



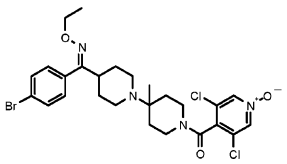
80



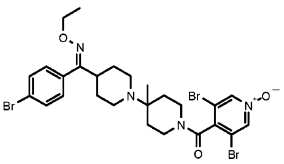
81



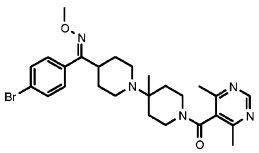
82



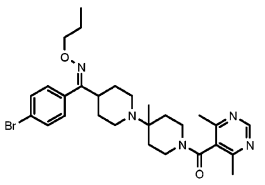
83



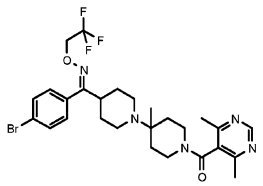
84



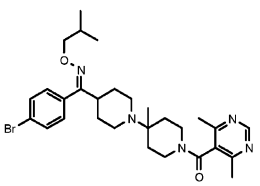
85



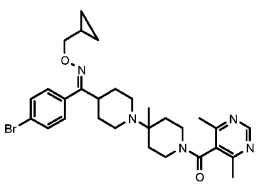
87



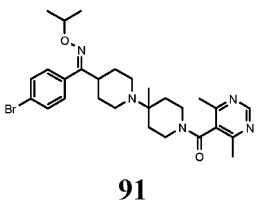
88



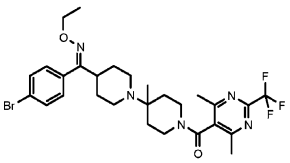
89



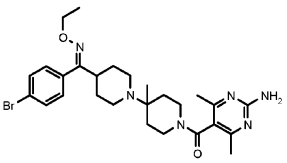
90



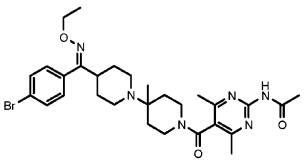
91



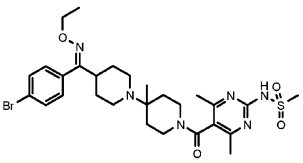
93



94

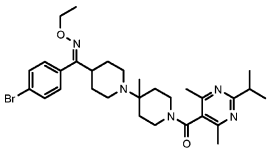


95

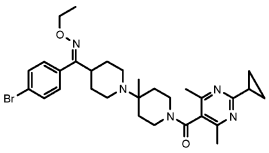


96

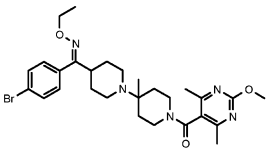
Chart 2 (Continued)



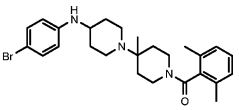
97



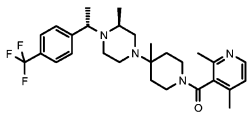
98



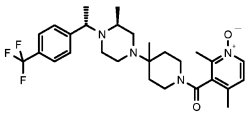
99



101



102



103
Sch-350634

[a] All structures were drawn using ISIS Draw 2.5 (MDL Information Systems, Inc., San Leandro, CA). Numbers represent the compound numbers.

model that perfectly fits the data and is calculated by the following equation:

$$\text{fixed cost} = eE(x=0) + wW(x=0) + cC$$

where x is the deviation from the expected values of weight and error.

The null cost is the cost of a pharmacophore when the activity data of every molecule in the training set is the average value of all activities in the set and the pharmacophore has no features. Therefore, the contribution from the weight or configuration component does not apply. The null cost is calculated from the following equation:

$$\text{null cost} = eE(\chi_{\text{est}}=\bar{\chi})$$

where χ_{est} is the averaged scaled activity of the training set molecules.

It has been suggested in the Catalyst software that the differences between cost of the generated hypothesis and the null hypothesis cost should be as large as possible; a value of 40−60 bits difference may indicate that most probably it has a 75−90% chance of representing a true correlation in the data set used. The total cost of any hypothesis should be toward the value of fixed cost to represent any meaningful model. Two other very important output parameters are the configuration cost (also known as entropy cost) and the error cost. The former depends on the complexity of the pharmacophore hypothesis space. Any value higher than 17 may indicate that the correlation from any generated pharmacophore is most likely due to chance, and some attention has to be given to selecting the training set molecules. The entropy cost value can be reduced by limiting the minimum and maximum features. The error cost increases as the value of the root mean square (rms) increases. The rms deviations represent the quality of the correlation between the estimated and the actual activity data.

(b) Cross-Validation Test. A validation technique, termed CatScramble, available in the Catalyst/HypoGen module was used to further assess the statistical significance of the pharmacophore hypotheses generated from the training set molecules. This validation technique is based on Fischer's randomization test. The purpose of this test is to validate the strong correlation between chemical structures and biological activity. The activity values of the training set molecules are reassigned by randomization using the CatScramble technique, and new spreadsheets are created. The number of spreadsheets depends on what level of statistical significance one wants to achieve. For a 95% confidence level, 19 spreadsheets are created. For 98% and 99% confidence levels, 49 and 99 spreadsheets, respectively, are created. In our validation test, we selected the 95% confidence level, and 19 spreadsheets were created by the CatScramble command. These spreadsheets were used to generate hypotheses using exactly the same features and parameters as used in generating the original pharmacophore hypotheses.

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

hypothesis no.	total cost	error cost	rms	correlation (r)	features[b]
1	112.50	93.77	0.879	0.920	HA, HA, HY, HY, HY
2	113.21	94.18	0.898	0.917	HA, HY, HY, HY, HY
3	113.81	93.60	0.872	0.925	HA, HA, HY, HY, HY
4	114.24	95.29	0.946	0.907	HA, HY, HY, HY, HY
5	114.97	96.79	1.007	0.893	HA, HY, HY, HY
6	115.02	96.86	1.011	0.892	HY, HY, HY, HY, HY
7	115.68	94.46	0.910	0.920	HY, HY, HY, HY
8	115.89	97.55	1.037	0.886	HA, HY, HY, HY, HY
9	116.73	98.11	1.058	0.881	HA, HY, HY, HY, HY
10	117.32	98.95	1.090	0.873	HA, HA, HY, HY

[a] Null cost = 146.71. Fixed cost = 102.25. Configuration = 17.0. All costs are in units of bits. [b] HA, hydrogen bond acceptor. HY, hydrophobic.

Results and Discussions

Pharmacophore Generation. A set of 10 pharmacophore hypotheses were generated using 25 training set compounds listed in Chart 1. The results of the hypotheses, which include different cost values calculated during hypotheses generation along with rms deviations, correlation (r), and pharmacophore features, have been listed in Table 1. The value of total cost of each hypothesis was close to the fixed cost values, which is expected for good hypotheses. The entropy (configuration cost) values of the hypotheses was also within the allowed range. The difference between null hypothesis and the fixed cost and the total cost of the best hypothesis (hypothesis 1) were 44.46 and 34.21 bits, respectively. These values were somewhat lower than recommended in the Catalyst software (see Catalyst Tutorial at www.accelrys.com). Despite the recommendation, lower values in cost differences have been reported by the developer of the Catalyst software in one of their case study reports published on their Web site (http://www.accelrys.com/cases/D1Agonists_full.html) as well as by others.[66] The possible explanations for the lower values provided in those reports were that (1) the molecules in the training set were fairly rigid and (2) the training set molecules were structurally homologous. In our case, the most likely cause for the lower

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

			K_i (nM)[a]		activity scale	
no.	compd no.	fit	actual	estimated	actual	estimated
1	**2**	7.49	68	78	++	++
2	**6**	6.89	110	310	+	+
3	**8**	6.83	86	350	++	+
4	**11**	6.71	360	460	+	+
5	**12**	8.10	54	19	++	++
6	**13**	8.00	190	24	+	++
7	**14**	8.34	62	11	++	++
8	**18**	8.86	2	3.3	++	++
9	**20**	9.05	2	2.1	++	++
10	**21**	8.18	78	16	++	++
11	**27**	9.06	3.4	2.1	++	++
12	**30**	9.04	3	2.2	++	++
13	**32**	8.98	1.1	2.5	++	++
14	**38** (Sch-351125)	9.05	2.1	2.1	++	++
15	**52**	6.97	1300	260	−	+
16	**61**	8.84	1	3.4	++	++
17	**63**	8.49	5	7.7	++	++
18	**71**	8.36	3	10	++	++
19	**74**	8.03	12	22	++	++
20	**77**	10.42	0.1	0.09	++	++
21	**78**	6.83	590	360	−	+
22	**79**	9.56	0.3	0.65	++	++
23	**86**	9.11	2.2	1.8	++	++
24	**92**	8.92	2.1	2.9	++	++
25	**100**	8.88	1.3	3.1	++	++

[a] Data for inhibition of RANTES binding to CCR5 are from references listed in Materials and Methods.

values was probably due to structural homology in the training set molecules. In both cases, the pharmacophore models were further cross-validated using a data randomization technique incorporated in the Catalyst software, and the models were shown to be valid and useful. We also followed the same approach to validate our model, which will be discussed in the next section.

Out of 10 hypotheses, seven had five feature hypotheses whereas three had four feature hypotheses. Out of seven five-feature hypotheses, two had two hydrogen bond acceptors and three hydrophobic features, four had one hydrogen bond acceptor and four hydrophobic features, and one had five hydrophobic features. Among three four-feature hypotheses, one had one hydrogen bond acceptor and three hydrophobic, one had two hydrogen bond acceptors and two hydrophobic, and the last one had all hydrophobic features. Hypotheses 1 and 3 had the best values in terms of total cost, error cost, rms differences, and the highest correlation and utilized identical features, namely, two hydrogen bond acceptors and three hydrophobic. Hypothesis 3 estimated the activities almost similarly (data not shown) to hypothesis 1. We have selected hypothesis 1 as the best pharmacophore. Table 2 shows the actual and estimated K_i values of training set compounds calculated on the basis of hypothesis 1. On the basis of the activity scale assigned and described in the Materials and Methods, only one highly active (++) compound was estimated to have moderate activity (+), one moderately active compound was estimated to be highly active, and two inactive (−) compounds were estimated to have moderate activity.

Pharmacophore Assessment. Cross-Validation Study. The quality of the pharmacophore was assessed using the CatScramble technique in the Catalyst. The purpose of using this technique is to randomize the activity data among the training set compounds and to generate pharmacophore hypotheses using the same features and parameters used to develop the original pharmacophore hypothesis. If the randomized sets generate pharmacophores with similar or better cost values, rms, and correlation, then the original pharmacophore can be considered as generated by chance. The results of the CatScramble runs are listed in Table 3, and the data clearly indicate that all values generated after randomization produced hypotheses with no predictive value. Besides, out of 19 runs, only 2 had a correlation close to 0.82, but the rms deviations were very high and the total cost values were close to the null cost, which is not desirable for a good hypothesis. This cross-validation technique provided confidence on the pharmacophore generated from the training set molecules.

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

validation	total cost	fixed cost	rms	correlation (r)	configuration cost
		Results for Unscrambled			
	112.50	102.25	0.88	0.920	17.00
		Results for Scrambled			
trial_1	139.57	101.34	1.73	0.636	16.12
trial_2	128.71	101.01	1.45	0.767	15.79
trial_3	124.61	98.68	1.34	0.812	13.47
trial_4	136.70	101.05	1.65	0.680	15.83
trial_5	133.56	102.29	1.57	0.711	17.07
trial_6	128.06	100.54	1.43	0.773	15.32
trial_7	132.78	98.56	1.60	0.706	13.34
trial_8	133.95	101.16	1.61	0.693	15.94
trial_9	126.25	101.29	1.31	0.822	16.07
trial_10	135.68	101.23	1.60	0.711	16.01
trial_11	134.00	101.52	1.59	0.706	16.30
trial_13	132.73	102.18	1.53	0.733	16.96
trial_14	127.83	100.83	1.42	0.779	15.76
trial_15	131.25	100.47	1.54	0.728	15.26
trial_16	135.75	99.42	1.63	0.703	14.20
trial_17	125.80	101.28	1.31	0.821	16.06
trial_18	136.58	100.45	1.65	0.684	15.23
trial_19	131.25	99.93	1.47	0.774	14.71

[a] Null cost = 146.71. All costs are in units of bits.

The selected pharmacophore was further validated by three techniques: (a) by assessing the predictive ability of the pharmacophore on a large set of test set molecules; (b) by verifying whether a series of potent and structurally unrelated CCR5 antagonists reported by five major pharmaceutical companies can effectively map onto the pharmacophore and predict and classify the activity of these antagonists correctly; (c) by incorporating an external set of negative controls consisting of five marketed drugs acting on central nervous systems (CNS), which target different G-protein-coupled receptors other than CCR5. This validation should confirm that the pharmacophore does not predict those drugs as "highly active" (represented as +) CCR5 antagonists.

Validation of Pharmacophores. 1. Validation of Pharmacophore Using Test Set Compounds. The validity of any pharmacophore model needs to be ascertained by applying that model to the test set to find out how correctly the model predicts the activity of the test set molecules and, most importantly, whether it can identify active and inactive molecules correctly. We have validated the selected pharmacophore with a large test set containing 78 piperidine- and piperazine-based CCR5 antagonists obtained from the same labo-

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

		K_i (nM)[a]		activity scale				K_i (nM)[a]		activity scale	
no.	compd no.	actual	estimated	actual	estimated	no.	compd no.	actual	estimated	actual	estimated
1	1	58	14	++	++	40	55	30	270	++	+
2	3	33	19	++	++	41	56	20	270	++	+
3	4	25	3.1	++	++	42	57	8	130	++	+
4	5	55	75	++	++	43	58	5	18	++	++
5	7	155	300	+	+	44	59	12	19	++	++
6	9	33	530	++	++	45	60	7	9.6	++	++
7	10	596	290	−	−	46	62	5	17	++	++
8	15	8	130	++	++	47	64	3	130	++	+
9	16	29	3.8	++	++	48	65	5	51	++	++
10	17	25	9.2	++	++	49	66	15	72	++	++
11	19	48	16	++	++	50	67	0.7	44	++	++
12	22	9	6	++	++	51	68	2.7	26	++	++
13	23	4	3.2	++	++	52	69	5.23	19	++	++
14	24	33	2.9	++	++	53	70	18	120	++	+
15	25	33	3.2	++	++	54	72	10	6.3	++	++
16	26	>30	2.7		++	55	73	10	11	++	++
17	28	18	3.6	++	++	56	75	11	29	++	++
18	29	4.5	6.7	++	++	57	76	38	7.5	++	++
19	31	1.1	3	++	++	58	80	2.3	2.5	++	++
20	33	26	4.6	++	++	59	81	8.8	2.4	++	++
21	34	>30	2.5		++	60	82	0.4	2	++	++
22	35	7	2.5	++	++	61	83	1.2	5.2	++	++
23	36	43	2.7	++	++	62	84	0.7	4.9	++	++
24	37	>30	2.7		++	63	85	4.7	4.1	++	++
25	39	>30	3.6		++	64	87	2	2.1	++	++
26	40	>30	1.9		++	65	88	14	2.6	++	++
27	41	16	2.4	++	++	66	89	8.1	4.1	++	++
28	42	19	2.4	++	++	67	90	11	4.4	++	++
29	43	5.3	7.8	++	++	68	91	3.3	2.4	++	++
30	44	7.6	5.2	++	++	69	93	3.2	3.3	++	++
31	45	30	430	++	+	70	94	5.7	3.1	++	++
32	46	24	3.6	++	++	71	95	60	0.07	++	++
33	47	3.8	2.6	++	++	72	96	315	0.38	+	++
34	48	4.4	26	++	++	73	97	43	3.2	++	++
35	49	20	380	++	+	74	98	25	4.1	++	++
36	50	5.6	2.2	++	++	75	99	5.3	3.3	++	++
37	51	30	590	++	−	76	101	7	110	++	+
38	53	440	380	+	+	77	102	2.3	150	++	+
39	54	62	50	++	++	78	103 (Sch-350634)	7	14	++	++

[a] Data for inhibition of RANTES binding to CCR5 are from references listed in Materials and Methods.

ratory as that of the training set compounds. This approach eliminates any interlaboratory variation in the data, which can introduce additional noises in the biological activity data. This validation gives additional confidence in the usability of the selected pharmacophore. The estimated activities were scored using hypothesis 1 and reported in Table 4. Out of 69 highly active compounds, 57 were accurately classified as highly active and 10 were classified as moderately active, whereas only two highly active compounds were classified as inactive. One moderately active compound was classified as highly active, and one inactive compound was classified as moderately active. The selected pharmacophore clearly showed minimal failure in classifying compounds correctly.

We have selected one of the most potent compounds reported and introduced as a clinical candidate by the Schering-Plough group,[25] Sch-351125 (also termed SCH-C; compound **38** in Chart 1 and Table 2) [Figure 1A], and one of the most inactive compounds reported by this group (compound **52** in Chart 1 and Table 2) from the training set [Figure 1B], and one potent "next generation" CCR5 antagonist known as Sch-350634 (compound **103** in Chart 2 and Table 4) from the test set [Figure 1C] to show how these molecules mapped onto the selected hypothesis. The "best fit" option was selected in all cases. Compound **38** mapped onto all five features very well, whereas in the case of compound **103**, it mapped to all but one hydrogen bond acceptor feature. The most inactive compound in the dataset (compound **52**) missed one hydrogen bond acceptor feature and one hydrophobic feature. Other inactive compounds also missed one or more features.

2. Validation of Pharmacophore Using Structurally Diverse and Potent CCR5 Antagonists in Either Clinical or Preclinical Development. One of the major goals of this study was to generate a predictive pharmacophore that can be utilized as a query tool[51,53,67−70] to search 3D databases of diverse drug-like compounds to identify new molecules with potent CCR5 inhibitory activities. This is of utmost importance to pharmaceutical companies for finding new chemical entities with potent activity against a target disease so that they can be patented and if clinically effective will add value to the company. We have initiated a validation study of the usefulness of the selected pharmacophore by using it to map on some potent diverse CCR5 antagonists, which have been either clinically introduced or under preclinical development. The rationale of this approach is that if the pharmacophore maps well onto those antagonists and predicts activities well, the pharmacophore is expected to be useful as a search tool to identify new CCR5 antagonists. The diversity of these compounds was

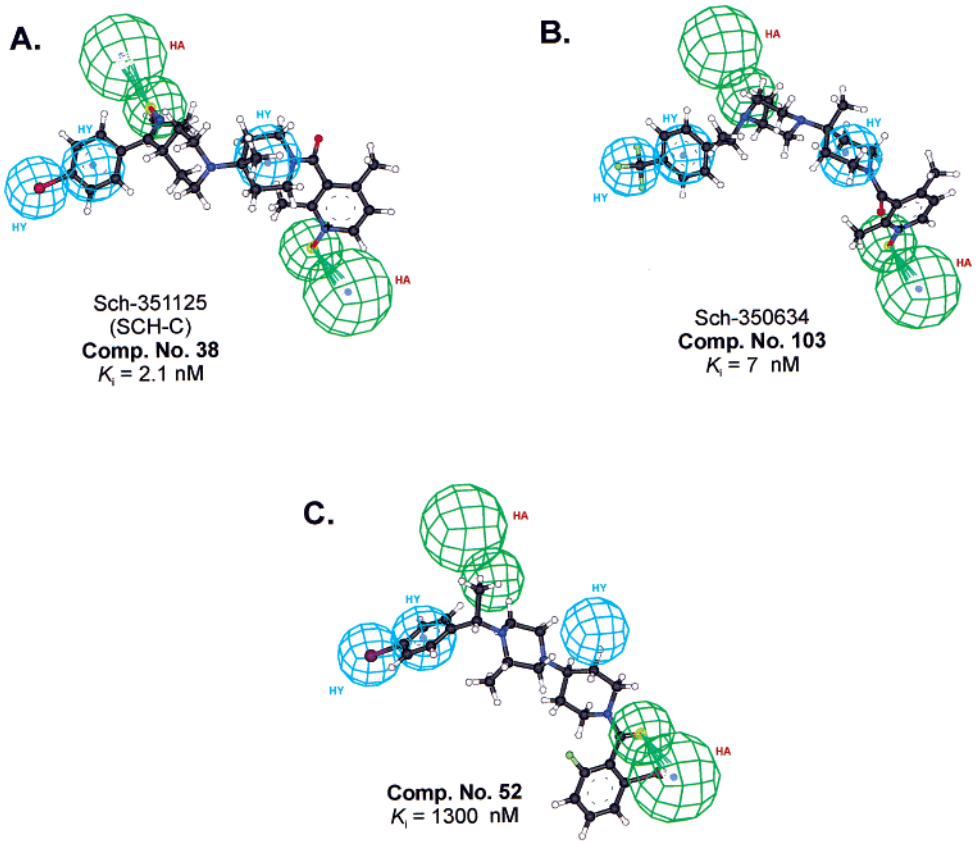


Figure 1. Mapping of two of the most active and one of the most inactive CCR5 antagonists onto the selected pharmacophore (hypothesis 1) (A) compound **38** (Sch-351125, also known as SCH-C) from the training set; (B) compound **103** (Sch-350634) from test set; (C) the most inactive compound in the entire set, compound **52**. The green and blue contours represent hydrogen bond acceptor (HA) and hydrophobic (HY) features, respectively.

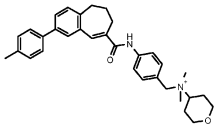
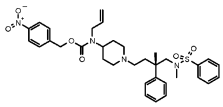
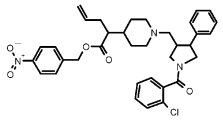
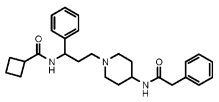
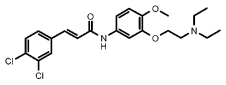
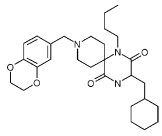
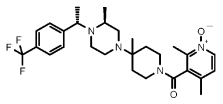
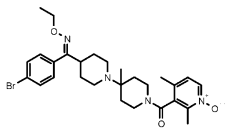
analyzed by calculating their Tanimoto coefficients. The QikSim option within QikProp software (Schrodinger, San Diego, CA) was used to calculate the Tanimoto coefficients. The mappings were done in Catalyst using the "best fit" option. Results of this study have been incorporated in Table 5 and Figure 2. No attempts were made to directly compare the estimated activities with actual activities of these six compounds because the data were collected from different laboratories and the activity data were also reported differently by different groups and in some cases no detailed activity data have been reported (especially in the patents). For example, TAK-779 was reported to have an IC_{50} of 1.4 nM in an assay measuring binding of [^{125}I]-RANTES to Chinese hamster ovary (CHO)/CCR5 cells, whereas the activities of the two compounds from Merck Research Laboratories have been reported as IC_{50} values in an assay assessing their ability to displace [^{125}I]-labeled MIP-1α from the CCR5 receptor expressed on CHO cell membranes. In the case of the Schering-Plough reported compounds, activity data used to generate the pharmacophores were based on an assay for binding of [^{125}I]-RANTES to the CCR5 receptor expressed on CHO cell membranes, similar to that reported by Takeda group for TAK-779[26] but expressed as K_i values.

The first CCR5 antagonist reported in the literature was TAK-779 (compound **104** in Table 5), which was discovered by Takeda Chemical Industries, Japan. The selected hypothesis mapped onto this molecule reasonably well but completely missed one of the two hydrogen bond acceptor features (Figure 2A). Although the estimated K_i value (72 nM) was somewhat higher than others in this validation, the mapping correctly classified this molecule as a highly active (++) CCR5 antagonist.

Merck Research Laboratories has recently published a series of papers on CCR5 antagonists.[71−83] One of the most potent analogues belonging to substituted phenylpiperidines (compound **105** in Table 5) mapped to four of the five features very well and missed one of the two HA features (Figure 2B). The estimated activity (K_i for RANTES binding to CCR5) was 2.6 nM and correctly classified the molecule as highly active. One of the other potent CCR5 antagonists reported by Merck, an analogue of 1,3,4-substituted pyrrolidine (compound **106** in Table 5), mapped to hypothesis features differently from the previous two compounds. One of the four hydrophobic features did not map on the molecule, but all hydrogen bond acceptor features mapped very well (Figure 2C). The mapping estimated the activity (K_i for RANTES binding to CCR5) to be 1.9 nM and classified the molecule as highly active.

Pfizer has recently reported piperidine-based CCR5 antagonists in their patent,[84,85] and although no specific activity data were available for these molecules, we have selected one compound (compound **107** in Table 5) for validation of the selected pharmacophore. This molecule also mapped very similarly to the Merck compound (compound **106**) where all but one feature (HY) did not map (Figure 2D). The estimated activity (K_i for RANTES binding to CCR5) was 2.0 nM and classified as highly active.

Table 5. Results of Validation with Structurally Unrelated Potent CCR5 Antagonists from Five Pharmaceutical Companies

Comp. No.	Structure (Code when available)	Company	CAS #	Tanimoto Coefficient[a]	IC_{50} (nM)		K_i (nM)		Activity Scale
					RANTES	MIP-1α	Actual	Estim.	
104	 **TAK-779**	Takeda	263765-56-6	0.442	1.4	-	-	72	++
105		Merck	502173-16-2	0.478	-	0.1	-	2.6	++
106		Merck	Not Registered	0.583	-	0.8	-	1.9	++
107		Pfizer	277744-99-7	0.455	-	-	-	2.0	++
108		GSK	Not Registered	0.568	-	-	-	2.0	++
109	 **E913**	Ono Pharmaceuticals	342394-93-8	0.576		2.0		6.1	++
103	 **Sch-350634**	Schering	306293-41-4	0.861			7	14	++
38	 **SCH-C** **(Sch-351125)**	Schering	305792-46-5	1.000			2.1	2.1	++

[a] Tanimoto coefficients were calculated using QikSim option in QikProp software (Schrodinger, San Diego, CA).

GlaxoSmithKline has also recently claimed heteroanilide derivatives as CCR5 antagonists in a patent.[86,87] No specific information was available on their binding activities. We have selected one such compound (com-

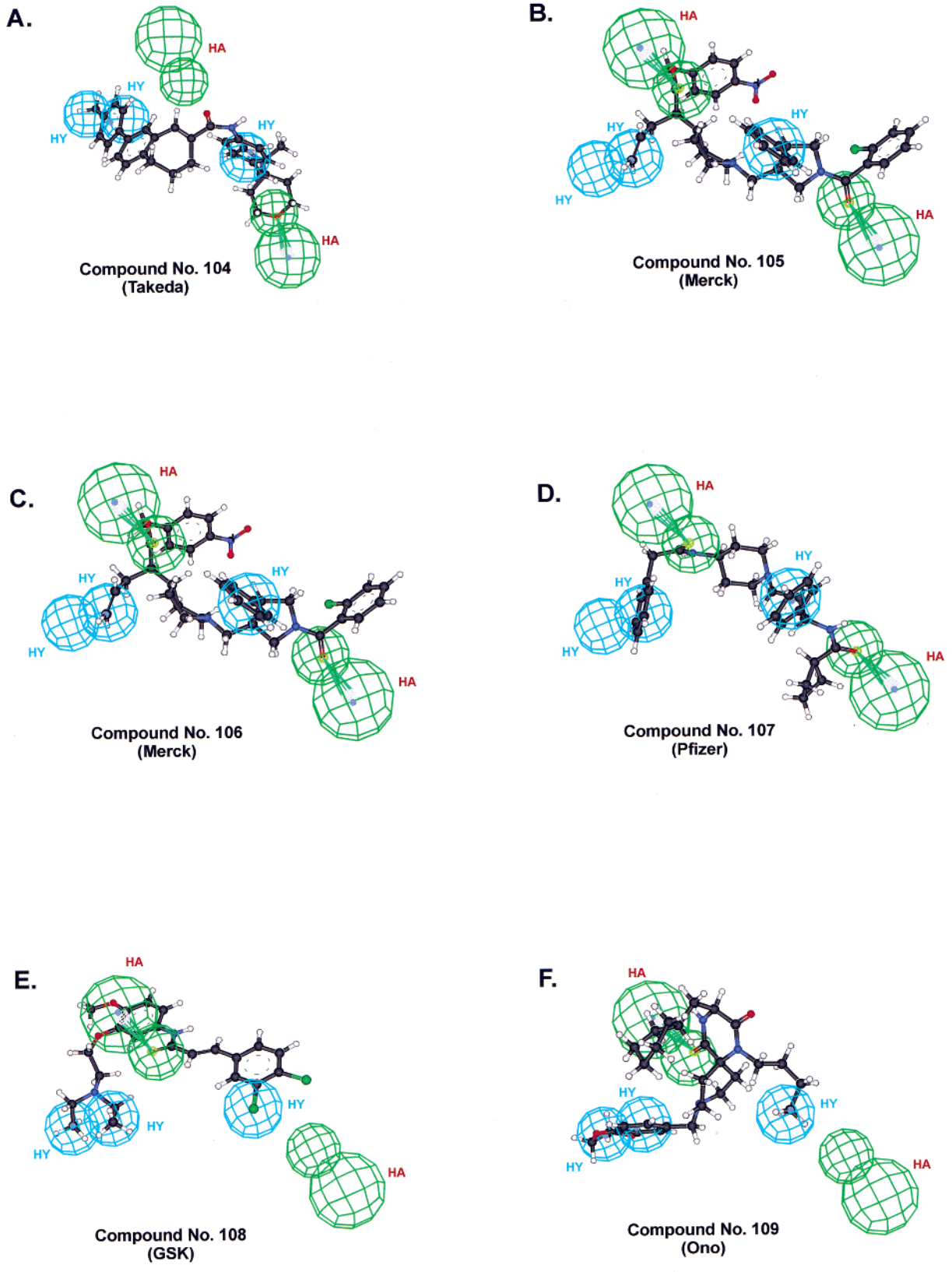
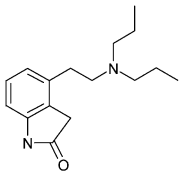
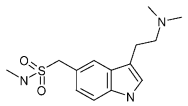
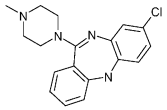
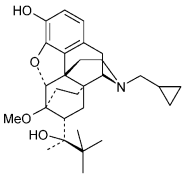
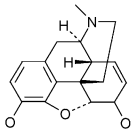


Figure 2. Mapping of six structurally diverse CCR5 antagonists developed as clinical or preclinical candidates by five pharmaceutical companies onto the selected pharmacophore (hypothesis 1) (A) compound **104** (TAK-779) from Takeda Chemical Industries, Japan; (B) compound **105** from Merck Research Laboratories; (C) compound **106** from Merck Research Laboratories; (D) compound **107** from Pfizer; (E) compound **108** from GlaxoSmithKline; (F) compound **109** (E913) from Ono Pharmaceuticals, Japan. The contour notations are same as in Figure 1.

pound **108** in Table 5) and wanted to verify whether this molecule, like others mentioned above, maps onto the selected hypothesis. This molecule also mapped to all the features in the hypothesis except one HA feature

Table 6. Results of Validation with Five Marketed CNS Drugs (Negative Control) Known To Target GPCR Receptors Other than CCR5

Name of the Marketed Drug **(Company)**[a]	Target GPCR of the Drug	Structure of the Drug[b]	K_i (nM) Estimated[c]	Activity Scale
Ropinirole **(GlaxoSmithKline)**	Dopamine (D2/D3)		310	++
Sumatriptan **(GlaxoSmithKline)**	Serotonin (5-HT1D)		320	++
Clozapine **(Novartis)**	Serotonin (5-HT2A/2C)		7128	-
Buprenorphine **(Reckitt Benckiser)**	Opioid (K)		410	++
Morphine **(AstraZeneca, Purdue Frederick, Roxane, Elkins-Sinn, Faulding)**	Opioid (μ)		5700	-

[a] Obtained from Physician's Desk Reference (PDR), 2002. [b] Structures obtained from http://chembank.med.harvard.edu/bioactives/. [c] Estimated based on Hypothesis-1.

and the estimated activity (K_i for RANTES binding to CCR5) was 2.0 nM, showing that this molecule belongs to the highly active class.

Finally, we have selected for validation a spiroketopiperizine-based CCR5 inhibitor (code-named E913; compound **109** in Table 5) reported recently by Ono Pharmaceuticals in Japan. The IC_{50} value of this molecule in the [^{125}I]-labeled MIP-1α binding assay was reported as 2 nM. This molecule mapped very well to four features out of five. In this case, one of the hydrogen bond acceptor features was further away from the molecule. The estimated activity was 6.1 nM (K_i for RANTES binding to CCR5) and correctly classified the molecule as highly active. It is apparent from this mapping study that although the pharmacophore contains five features, three hydrophobic and two hydrogen bond acceptors, the most active compounds can be mapped with a combination of four features. In all the above cases, four features effectively classified all six very potent and diverse CCR5 antagonists as highly active in conformity with the reported results. The absence of one feature from most of the non-Schering compounds possibly indicates that these molecules may have alternative binding modes. Nevertheless, the pharmacophore was successful in mapping all these molecules and classified them accurately.

The above validation study with hypothesis 3 resulted in very similar results and accurately classified all the above molecules as highly active (data not shown).

The validation study with six different classes of CCR5 antagonists suggested that the selected pharmacophore was capable of mapping a diverse group of compounds quite effectively and provided confidence that this pharmacophore could be used as a search query to identify CCR5 antagonists from drug-like chemical libraries.

3. Validation of Pharmacophore Using Structurally Diverse Marketed CNS Drugs Known To Target GPCRs Other Than CCR5. We selected a set of five diverse marketed CNS drugs, which target a variety of GPCRs other than CCR5, as negative controls to validate that the CCR5-based pharmacophore did not predict these non-CCR5 targeted compounds as "highly active". The drug Ropinirole from GlaxoSmithKline, which targets dopamine receptor D2/D3, was predicted to have poor activity (300 nM). One of the two selected

serotonin receptor antagonists, Sumatriptan from GlaxoSmithKline that targets the 5-HT1D receptor, was also predicted to have similar poor activity. The other serotonin receptor antagonist, Clozapine from Novartis, which targets 5-HT2A/2C, was predicted to be inactive (7128 nM). Similarly, the selected pharmacophore predicted poor activity (410 nM) for one of the opioid receptor (*K*) antagonists, Buprenorphine, and no activity (5700 nM) for the other opioid receptor (μ) antagonist morphine. The results and the structures of these drugs were reported in Table 6.

This validation reconfirmed the usefulness of the derived pharmacophore model, which most likely will be effective in identifying compounds that target CCR5.

Conclusions

We initiated a systematic study to develop pharmacophores from a large series of piperidine- and piperazine-based CCR5 antagonists reported by the Schering-Plough Research Institute. The purpose of this study was twofold: (1) to generate pharmacophores as a powerful search tool to be used as a 3D query to identify new chemical entities from chemical databases as potential CCR5 antagonists and (2) to utilize the pharmacophore as a predictive tool for estimating biological activity of virtual compounds or compounds designed on the basis of structure−activity analyses. The study presented here clearly indicates that the selected pharmacophore can be used for the above two purposes. The pharmacophore successfully predicted biological activities of six very diverse classes of CCR5 antagonists from five different pharmaceutical companies and classified them accurately. It confirmed that correct mapping to four out of five features is sufficient to identify compounds successfully. The selected pharmacophore is expected to help in identifying new classes of CCR5 inhibitors as HIV-1 entry inhibitors.

Acknowledgment. I thank Dr. A. Robert Neurath for reading the manuscript thoroughly and for critical comments. I sincerely appreciate the comments of the reviewers, which helped to reshape the manuscript to the current form. This work was supported by NIH Grants AI54147 (for A.K.D. as Principal Investigator) and AI46221 (for A.K.D. as Co-Principal Investigator) and by the Campbell Foundation (for A.K.D. as Principal Investigator).

Supporting Information Available: Listing of sdf files for structures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *AIDS Epidemic Update−2002*. UNAIDS/WHO: Geneva, 2003.
(2) Weitzel, T.; Plettenberg, A.; Albrecht, D.; Lorenzen, T.; Stoehr, A. Severe anemia as a newly recognized side-effect caused by lamivudine. *AIDS* **1999**, *13*, 2309−2311.
(3) Fortuny, C.; Vicente, M. A.; Medina, M. M.; Gonzalez-Ensenat, A. Rash as side-effect of nelfinavir in children. *AIDS* **2000**, *14*, 335−336.
(4) Das, S. J.; Olsen, I. Keratinocyte growth factor is upregulated by the hyperplasia-inducing drug nifedipine. *Cytokine* **2000**, *12*, 1566−1569.
(5) Savini, C. J.; James, C. W.; Miller, J. L.; Wilson, S. A.; Cohen, D. M.; Perlada, D. E. Evidence of hypertriglyceridemia in managing HIV patients on efavirenz. *J. Assoc. Nurses AIDS Care* **2001**, *12*, 95−97.
(6) Foli, A.; Benvenuto, F.; Piccinini, G.; Bareggi, A.; Cossarizza, A.; Lisziewicz, J.; Lori, F. Direct analysis of mitochondrial toxicity of antiretroviral drugs. *AIDS* **2001**, *15*, 1687−1694.
(7) Ahlsen, G.; Hulten, J.; Shuman, C. F.; Poliakov, A.; Lindgren, M. T.; Alterman, M.; Samuelsson, B.; Hallberg, A.; Danielson, U. H. Resistance profiles of cyclic and linear inhibitors of HIV-1 protease. *Antiviral Chem. Chemother.* **2002**, *13*, 27−37.
(8) Blaise, P.; Clevenbergh, P.; Vaira, D.; Moutschen, M.; Dellamonica, P. HIV resistance to antiretroviral drugs: mechanisms, genotypic and phenotypic resistance testing in clinical practice. *Acta Clin. Belg.* **2002**, *57*, 191−201.
(9) Kuritzkes, D. R. Resistance to protease inhibitors. *J. HIV Ther.* **2002**, *7*, 87−91.
(10) Fusion inhibitors: T-20 and T-1249. *Treatmentupdate* **2001**, *12*, 2−3.
(11) James, J. S. T-20: most expensive AIDS drug ever at $25,000 per year? *AIDS Treat. News* **2003**, 6−7.
(12) Hanna, L. T-20: first of a new class of anti-HIV drugs. *BETA* **1999**, *12*, 7−8.
(13) Sharp, M.; Camp, R. FUZEON (enfuvirtide, T-20). Breaking barriers or breaking the bank? *GMHC Treat. Issues* **2003**, *17*, 7−8.
(14) Cooley, L. A.; Lewin, S. R. HIV-1 cell entry and advances in viral entry inhibitor therapy. *J. Clin. Virol.* **2003**, *26*, 121−132.
(15) Ketas, T. J.; Klasse, P. J.; Spenlehauer, C.; Nesin, M.; Frank, I.; Pope, M.; Strizki, J. M.; Reyes, G. R.; Baroudy, B. M.; Moore, J. P. Entry Inhibitors SCH-C, RANTES, and T-20 Block HIV Type 1 Replication in Multiple Cell Types. *AIDS Res. Hum. Retroviruses* **2003**, *19*, 177−186.
(16) Trkola, A.; Kuhmann, S. E.; Strizki, J. M.; Maxwell, E.; Ketas, T.; Morgan, T.; Pugach, P.; Xu, S.; Wojcik, L.; Tagat, J.; Palani, A.; Shapiro, S.; Clader, J. W.; McCombie, S.; Reyes, G. R.; Baroudy, B. M.; Moore, J. P. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 395−400.
(17) Brelot, A.; Alizon, M. HIV-1 entry and how to block it. *AIDS* **2001**, *15* (Suppl. 5), S3−S11.
(18) Moore, J. P.; Trkola, A.; Dragic, T. Co-receptors for HIV-1 entry. *Curr Opin. Immunol.* **1997**, *9*, 551−562.
(19) Sodroski, J. G. HIV-1 entry inhibitors in the side pocket. *Cell* **1999**, *99*, 243−246.
(20) Stein, B. S.; Engleman, E. G. Mechanism of HIV-1 entry into CD4+ T cells. *Adv. Exp. Med. Biol.* **1991**, *300*, 71−86.
(21) Cocchi, F.; DeVico, A. L.; Garzino-Demo, A.; Arya, S. K.; Gallo, R. C.; Lusso, P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* **1995**, *270*, 1811−1815.
(22) Liu, R.; Paxton, W. A.; Choe, S.; Ceradini, D.; Martin, S. R.; Horuk, R.; MacDonald, M. E.; Stuhlmann, H.; Koup, R. A.; Landau, N. R. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **1996**, *86*, 367−377.
(23) Marmor, M.; Sheppard, H. W.; Donnell, D.; Bozeman, S.; Celum, C.; Buchbinder, S.; Koblin, B.; Seage, G. R., III. Homozygous and heterozygous CCR5-Delta32 genotypes are associated with resistance to HIV infection. *J. Acquired Immune Defic. Syndr.* **2001**, *27*, 472−481.
(24) Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C. M.; Saragosti, S.; Lapoumeroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. G.; Doms, R. W.; Vassart, G.; Parmentier, M. Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **1996**, *382*, 722−725.
(25) Kazmierski, W.; Bifulco, N.; Yang, H.; Boone, L.; DeAnda, F.; Watson, C.; Kenakin, T. Recent Progress in Discovery of Small-Molecule CCR5 Chemokine Receptor Ligands as HIV-1 Inhibitors. *Bioorg. Med. Chem.* **2003**, *11*, 2663−2667.
(26) Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5698−5703.
(27) Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J.; Endres, M.; Baroudy, B. M. Discovery of 4-[(*Z*)-(4-bromophenyl)-(ethoxyimino)methyl]-1′-[(2,4-dimethyl-3-pyridinyl)carbonyl]-4′-methyl-1,4′-bipiperidine *N*-oxide (SCH 351125): an orally bioavailable human CCR5 antagonist for the treatment of HIV infection. *J. Med. Chem.* **2001**, *44*, 3339−3342.
(28) Palani, A.; Shapiro, S.; Josien, H.; Bara, T.; Clader, J. W.; Greenlee, W. J.; Cox, K.; Strizki, J. M.; Baroudy, B. M. Synthesis, SAR, and biological evaluation of oximino-piperidino-piperidine amides. 1. Orally bioavailable CCR5 receptor antagonists with potent anti-HIV activity. *J. Med. Chem.* **2002**, *45*, 3143−3160.
(29) Strizki, J. M.; Xu, S.; Wagner, N. E.; Wojcik, L.; Liu, J.; Hou, Y.; Endres, M.; Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Tagat, J. R.; McCombie, S.; Cox, K.; Fawzi, A. B.; Chou, C. C.; Pugliese-Sivo, C.; Davies, L.; Moreno, M. E.; Ho, D. D.; Trkola, A.; Stoddart, C. A.; Moore, J. P.; Reyes, G. R.; Baroudy,

B. M. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12718−12723.

(30) Tsamis, F.; Gavrilov, S.; Kajumo, F.; Seibert, C.; Kuhmann, S.; Ketas, T.; Trkola, A.; Palani, A.; Clader, J. W.; Tagat, J. R.; McCombie, S.; Baroudy, B.; Moore, J. P.; Sakmar, T. P.; Dragic, T. Analysis of the mechanism by which the small-molecule CCR5 antagonists SCH-351125 and SCH-350581 inhibit human immunodeficiency virus type 1 entry. *J. Virol.* **2003**, *77*, 5201−5208.

(31) Tagat, J. R.; Steensma, R. W.; McCombie, S. W.; Nazareno, D. V.; Lin, S. I.; Neustadt, B. R.; Cox, K.; Xu, S.; Wojcik, L.; Murray, M. G.; Vantuno, N.; Baroudy, B. M.; Strizki, J. M. Piperazine-based CCR5 antagonists as HIV-1 inhibitors. II. Discovery of 1-[(2,4-dimethyl-3-pyridinyl)carbonyl]-4-methyl-4-[3(*S*)-methyl-4-[1(*S*)-[4-(trifluoromethyl)phenyl]ethyl]-1-piperazinyl]-piperidine N1-oxide (Sch-350634), an orally bioavailable, potent CCR5 antagonist *J. Med. Chem.* **2001**, *44*, 3343−3346.

(32) Tagat, J. R.; McCombie, S. W.; Steensma, R. W.; Lin, S.; Nazareno, D. V.; Baroudy, B.; Vantuno, N.; Xu, S.; Liu, J. Piperazine-based CCR5 antagonists as HIV-1 inhibitors. I: 2(*S*)-Methyl piperazine as a key pharmacophore element. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2143-2146.

(33) McCombie, S. W.; Tagat, J. R.; Vice, S. F.; Lin, S. I.; Steensma, R.; Palani, A.; Neustadt, B. R.; Baroudy, B. M.; Strizki, J. M.; Endres, M. Piperazine-based CCR5 antagonists as HIV-1 inhibitors. III: Synthesis, antiviral and pharmacokinetic profiles of symmetrical heteroaryl carboxamides. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 567−571.

(34) Lipinski, C. A. Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235−249.

(35) 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.

(36) Güner, O. F. *Pharmacophore Perception, Development, and Use in Drug Design*; International University Line: La Jolla, CA, 1999.

(37) Barreca, M. L.; Gitto, R.; Quartarone, S.; De Luca, L.; De Sarro, G.; Chimirri, A. Pharmacophore modeling as an efficient tool in the discovery of novel noncompetitive AMPA receptor antagonists. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 651−655.

(38) Beno, B. R.; Mason, J. S. The design of combinatorial libraries using properties and 3D pharmacophore fingerprints. *Drug Discovery Today* **2001**, *6*, 251−258.

(39) Bostrom, J.; Gundertofte, K.; Liljeforsa, T. A pharmacophore model for dopamine D4 receptor antagonists. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 769−786.

(40) Bremner, J. B.; Coban, B.; Griffith, R. Pharmacophore development for antagonists at alpha 1 adrenergic receptor subtypes. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 545−557.

(41) Carlson, H. A.; Masukawa, K. M.; Rubins, K.; Bushman, F. D.; Jorgensen, W. L.; Lins, R. D.; Briggs, J. M.; McCammon, J. A. Developing a dynamic pharmacophore model for HIV-1 integrase. *J. Med. Chem.* **2000**, *43*, 2100−2114.

(42) Chen, X.; Rusinko, A., III; Tropsha, A.; Young, S. S. Automated pharmacophore identification for large chemical data sets. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 887−896.

(43) Debnath, A. K. Pharmacophore mapping of a series of 2,4-diamino-5-deazapteridine inhibitors of Mycobacterium avium complex dihydrofolate reductase. *J. Med. Chem.* **2002**, *45*, 41−53.

(44) 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.

(45) Ekins, S.; Stresser, D. M.; Andrew Williams, J. In vitro and pharmacophore insights into CYP3A enzymes. *Trends Pharmacol. Sci.* **2003**, *24*, 161−166.

(46) Enyedy, I. J.; Sakamuri, S.; Zaman, W. A.; Johnson, K. M.; Wang, S. Pharmacophore-based discovery of substituted pyridines as novel dopamine transporter inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 513−517.

(47) Giannakakou, P.; Gussio, R.; Nogales, E.; Downing, K. H.; Zaharevitz, D.; Bollbuck, B.; Poy, G.; Sackett, D.; Nicolaou, K. C.; Fojo, T. A common pharmacophore for epothilone and taxanes: molecular basis for drug resistance conferred by tubulin mutations in human cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904−2909.

(48) Guner, O. F. History and evolution of the pharmacophore concept in computer-aided drug design. *Curr. Top. Med. Chem.* **2002**, *2*, 1321−1332.

(49) Hirashima, A.; Eiraku, T.; Shigeta, Y.; Kuwano, E. Three-dimensional pharmacophore hypotheses of octopamine/tyramine agonists which inhibit [1-14C]acetate incorporation in Plodia interpunctella. *Bioorg. Med. Chem.* **2003**, *11*, 95−103.

(50) Joseph-McCarthy, D.; Thomas, B. E.; Belmarsh, M.; Moustakas, D.; Alvarez, J. C. Pharmacophore-based molecular docking to account for ligand flexibility. *Proteins* **2003**, *51*, 172−188.

(51) Koide, Y.; Tatsui, A.; Hasegawa, T.; Murakami, A.; Satoh, S.; Yamada, H.; Kazayama, S.; Takahashi, A. Identification of a stable chymase inhibitor using a pharmacophore-based database search. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 25−29.

(52) Krovat, E. M.; Langer, T. Non-peptide angiotensin II receptor antagonists: chemical feature based pharmacophore identification. *J. Med. Chem.* **2003**, *46*, 716−726.

(53) Kurogi, Y.; Guner, O. F. Pharmacophore modeling and three-dimensional database searching for drug design using catalyst. *Curr. Med. Chem.* **2001**, *8*, 1035−1055.

(54) Malawska, B.; Scatturin, A. Application of pharmacophore models for the design and synthesis of new anticonvulsant drugs. *Mini-Rev. Med. Chem.* **2003**, *3*, 341−348.

(55) Marriott, D. P.; Dougall, I. G.; Meghani, P.; Liu, Y. J.; Flower, D. R. Lead generation using pharmacophore mapping and three-dimensional database searching: application to muscarinic M(3) receptor antagonists. *J. Med. Chem.* **1999**, *42*, 3210−3216.

(56) Patel, Y.; Gillet, V. J.; Bravi, G.; Leach, A. R. A comparison of the pharmacophore identification programs: Catalyst, DISCO and GASP. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 653−681.

(57) DiMasi, J. A.; Hansen, R. W.; Grabowski, H. G. The price of innovation: new estimates of drug development costs. *J. Health Econ.* **2003**, *22*, 151−185.

(58) McCombie, S. W.; Tagat, J. R.; Vice, S. F.; Lin, S. I.; Steensma, R.; Palani, A.; Neustadt, B. R.; Baroudy, B. M.; Strizki, J. M.; Endres, M.; Cox, K.; Dan, N.; Chou, C. C. Piperazine-based CCR5 antagonists as HIV-1 inhibitors. III: Synthesis, antiviral and pharmacokinetic profiles of symmetrical heteroaryl carboxamides. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 567−571.

(59) Palani, A.; Shapiro, S.; Clader, J. W.; Greenlee, W. J.; Vice, S.; McCombie, S.; Cox, K.; Strizki, J.; Baroudy, B. M. Oximino-piperidino-piperidine-based CCR5 antagonists. Part 2: synthesis, SAR and biological evaluation of symmetrical heteroaryl carboxamides. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 709−712.

(60) Li, H.; Sutter, J.; Hoffman, R. HypoGen: An automated system for generating 3D predictive pharmacophore models. In *Pharmacophore Perception, Development, and Use in Drug Design*; Guner, O. F., Ed.; International University Line: La Jolla, CA, 1999; pp 173−189.

(61) 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.

(62) Smellie, A. Poling: Promoting Conformational Variation. *J. Comput. Chem.* **1995**, *16*, 171−187.

(63) Smellie, A.; Kahn, S. D.; Teig, S. L. Analysis of Conformational Coverage. 1. Validation and Estimation of Coverage. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 285−294.

(64) Smellie, A.; Kahn, S. D.; Teig, S. L. Analysis of Conformational Space. 2. Application of Conformational Models *J. Chem. Inf. Comput. Sci.* **1995**, *16*, 295−304.

(65) Sutter, J.; Güner, O. F.; Hoffman, R.; Li, H.; Waldman, M. Effect of Variable Weight and Tolerances on Predictive Model Generation. In *Pharmacophore Perception, Development, and Use in Drug Design*; Guner, O. F., Ed.; International University Line: La Jolla, CA, 1999; pp 501−511.

(66) Hirashima, A.; Shigeta, Y.; Eiraku, T.; Kuwano, E. Inhibitors of Calling Behavior of *Plodia interpunctella*. *J. Insect Sci.* **2003**, *3*, 4.

(67) Bremner, J. B.; Castle, K.; Griffith, R.; Keller, P. A.; Ridley, D. D. Mining the Chemical Abstracts database with pharmacophore-based queries. *J. Mol. Graphics Modell.* **2002**, *21*, 185−194.

(68) Clark, D. E.; Westhead, D. R.; Sykes, R. A.; Murray, C. W. Active-site-directed 3D database searching: pharmacophore extraction and validation of hits. *J. Comput.-Aided Mol. Des.* **1996**, *10*, 397−416.

(69) Nicklaus, M. C.; Neamati, N.; Hong, H.; Mazumder, A.; Sunder, S.; Chen, J.; Milne, G. W.; Pommier, Y. HIV-1 integrase pharmacophore: discovery of inhibitors through three-dimensional database searching. *J. Med. Chem.* **1997**, *40*, 920−929.

(70) Sprague, P. W. Automated chemical hypothesis generation and database searching with Catalyst. In *Perspectives in Drug Discovery and Design*; Müller, K., Ed.; ESCOM Science Publishers B.V.: Leiden, The Netherlands, 1995; pp 1−21.

(71) Hale, J. J.; Budhu, R. J.; Holson, E. B.; Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Siciliano, S.; Malkowitz, L.; Schleif, W. A.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Holmes, K.; Lineberger, J.; Carella, A.; Carver, G.; Emini, E. 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 2: lead optimization affording selective, orally bioavailable compounds with potent anti-HIV activity. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2741−2745.

(72) Hale, J. J.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Siciliano, S.; Gould, S. L.; DeMartino, J. A.; Springer, M. S. 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 1: discovery of the pyrrolidine scaffold and determination of its stereochemical requirements. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1437−1440.

(73) Hale, J. J.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Siciliano, S. J.; Malkowitz, L.; Schleif, W. A. 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 3: polar functionality and its effect on anti-HIV-1 activity. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2997−3000.

(74) Lynch, C. L.; Hale, J. J.; Budhu, R. J.; Gentry, A. L.; Mills, S. G.; Chapman, K. T.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L. 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. Part 4: Synthesis of N-1 acidic functionality affording analogues with enhanced antiviral activity against HIV. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3001−3004.

(75) Lynch, C. L.; Willoughby, C. A.; Hale, J. J.; Holson, E. J.; Budhu, R. J.; Gentry, A. L.; Rosauer, K. G.; Caldwell, C. G.; Chen, P.; Mills, S. G. 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists: modifications of the arylpropylpiperidine side chains. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 119−123.

(76) Lynch, C. L.; Gentry, A. L.; Hale, J. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J. CCR5 antagonists: bicyclic isoxazolidines as conformationally constrained N-1-substituted pyrrolidines. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 677−679.

(77) Castonguay, L. A.; Weng, Y.; Adolfsen, W.; Di Salvo, J.; Kilburn, R.; Caldwell, C. G.; Daugherty, B. L.; Finke, P. E.; Hale, J. J.; Lynch, C. L.; Mills, S. G.; MacCoss, M.; Springer, M. S.; DeMartino, J. A. Binding of 2-aryl-4-(piperidin-1-yl)butanamines and 1,3,4-trisubstituted pyrrolidines to human CCR5: a molecular modeling-guided mutagenesis study of the binding pocket. *Biochemistry* **2003**, *42*, 1544−1550.

(78) Dorn, C. P.; Finke, P. E.; Oates, B.; Budhu, R. J.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 1: discovery and initial structure−activity relationships for 1-amino-2-phenyl-4-(piperidin-1-yl)butanes. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 259−264.

(79) Finke, P. E.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 4: synthesis and structure−activity relationships for 1-[*N*-(methyl)-*N*-(phenylsulfonyl)amino]-2-(phenyl)-4-(4-(*N*-(alkyl)-*N*-(benzyloxycarbonyl)amino)piperidin-1-yl)butanes. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2475−2479.

(80) Finke, P. E.; Meurer, L. C.; Oates, B.; Shah, S. K.; Loebach, J. L.; Mills, S. G.; MacCoss, M.; Castonguay, L.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A. Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 3: a proposed pharmacophore model for 1-[*N*-(methyl)-*N*-(phenylsulfonyl)amino]-2-(phenyl)-4-[4-(substituted)piperidin-1-yl]butanes. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2469−2473.

(81) Finke, P. E.; Meurer, L. C.; Oates, B.; Mills, S. G.; MacCoss, M.; Malkowitz, L.; Springer, M. S.; Daugherty, B. L.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Carella, A.; Carver, G.; Holmes, K.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M.; Schleif, W. A.; Emini, E. A. Antagonists of the human CCR5 receptor as anti-HIV-1 agents. Part 2: structure−activity relationships for substituted 2-aryl-1-[*N*-(methyl)-*N*-(phenylsulfonyl)amino]-4-(piperidin-1-yl)butanes. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 265−270.

(82) Kim, D.; Wang, L.; Caldwell, C. G.; Chen, P.; Finke, P. E.; Oates, B.; MacCoss, M.; Mills, S. G.; Malkowitz, L.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Carver, G.; Carella, A.; Holmes, K.; Lineberger, J.; Schleif, W. A.; Emini, E. A. Design, synthesis, and SAR of heterocycle-containing antagonists of the human CCR5 receptor for the treatment of HIV-1 infection. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3103−3106.

(83) Kim, D.; Wang, L.; Caldwell, C. G.; Chen, P.; Finke, P. E.; Oates, B.; MacCoss, M.; Mills, S. G.; Malkowitz, L.; Gould, S. L.; DeMartino, J. A.; Springer, M. S.; Hazuda, D.; Miller, M.; Kessler, J.; Danzeisen, R.; Carver, G.; Carella, A.; Holmes, K.; Lineberger, J.; Schleif, W. A.; Emini, E. A. Discovery of human CCR5 antagonists containing hydantoins for the treatment of HIV-1 infection. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3099−3102.

(84) Armour, D. R.; Price, D. A.; Stammen, B. L. C.; Wood, A.; Perros, M.; Edwards, M. P. Piperidines as CCR5 Modulators. Patent WO 00/39125, 2000.

(85) Armour, D. R.; Price, D. A.; Stammen, B. L. C.; Wood, A.; Perros, M.; Edwards, M. P. Aminoazacycloalkanase as CCR5 modulators. Patent EP-1013276, 2000.

(86) Bondinell, W. E. Propenamides as CCR5 Modulators. Patent WO 00/06153, 2000.

(87) Ku, T. W.; Bondinell, W. E.; Neeb, M. J. Substituted Anilide Compounds and Methods. Patent WO 00/06146, 2000.

JM030265Z