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Pharmacophore identification of α_{1A}-adrenoceptor antagonists

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Abstract—A chemical feature based pharmacophore model was developed for α_{1A}-adrenoceptor antagonists by HypoGen module implemented in catalyst software package. The best scoring pharmacophore hypothesis, Hypo1, consisted of four important chemical features (one positive ion, one hydrogen-bond donor, one aromatic ring, and one hydrophobic group). The results of our study provide a valuable tool in designing new leads with desired biological activity by virtual screening.

1. Introduction

As a urological disorder prevalent in the aging male population, benign prostatic hyperplasia (BPH) is a manifestation of noncancerous proliferation of glandular and fibromuscular tissue in the transition and periurethral zones of the prostate gland. The enlarged prostate radially compresses the urethra thereby impairing urine flow. In addition to this static component, the adrenergic tone of the prostate is elevated in BPH patients, which results in further tightening of urethra as dynamic component. The typical symptoms of prostatism are obstructive (poor urine stream, dribbling, and large residual urine volume) and irritative (hesitancy, increased frequency of urination, and nocturia) in nature, and can significantly compromise the quality of life of patients.[1] While surgical procedures of the use of 5α-reductase inhibitors such as finasteride and dutasteride are used to reduce the prostatic mass, α_1-adrenoceptor antagonists such as alfuzosin and tamsulosin are administered to treat the dynamic component of the sympathetic nervous system.[2] However, some adverse effects, which including orthostatic hypotension, tachycardia, dizziness, impotence, and fatigue, have been reported with these α_1-adrenoceptor antagonists in some patients, and a dose titration is usually required. These cardiovascular side effects are attributed to a nonselective blockade of α_1-adrenoceptors present in vascular smooth muscle in addition to the required blockade of α_1-adrenoceptors in prostate.[3]

Pharmacological evidence and recent molecular cloning studies have demonstrated that α_1-adrenoceptors are not a homogeneous population and three distinct α_1-adrenoceptor subtypes, called α_{1A}, α_{1B}, and α_{1D}, have been characterized by functional, radio-ligand binding, and molecular biology studies.[4,5] Recent studies have demonstrated that α_{1A}-subtype is the predominantly expressed α_1-adrenoceptor in human prostate. In addition, the binding affinities of many antagonists for the recombinant α_{1A}-adrenoceptor were found to correlate well with the potencies of the same antagonists to block agonist-induced contraction of prostatic smooth muscles.[6] Collectively, these observations suggest a possibility that a powerful blocker of the α_{1A}-adrenoceptor could alleviate the symptoms associated with BPH with minimal cardiovascular side effects.

Accordingly, various compounds have been synthesized and tested against α_{1A}-adrenoceptor, and as there is no crystal for α_{1A}-adrenoceptor, the use of ligand-based drug design is the major route to antagonist development. In a ligand-based molecule design, identification of pharmacophore is one of the most important steps, especially when the structure and properties of the receptor remain unknown. A powerful approach in ligand-based pharmacophore design is the appearance of catalyst (available from Accelrys Inc.), one of the leading software products for the automated generation

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of pharmacophore models and 3D database searching,[7] as a large number of successful application in medicinal chemistry clearly demonstrated.[8–10]

There have also been attempts in the literature to develop a pharmacophore model for α_1-adrenoceptor antagonists,[11–13] but only Bremner et al. have made any attempt to study the specific structural requirements for antagonist affinity to the various three subtypes of α_1-adrenoceptor using catalyst software package.[12] They have described a minimal pharmacophore, consisting of three features including a positive charge in the middle of the system, a hydrogen-bond acceptor group and an aromatic ring system at opposite ends of the molecule, for the α_{1A}-subtype. Their pharmacophore hypothesis, however, has been generated by only four compounds which have strong bioactivity for the α_{1A}-subtype, and the number of compounds is not large enough to ensure the statistical significance of the resulting pharmacophore model.

The aim of this study was to construct a pharmacophore model based on common chemical features of compounds with six orders difference in antagonistic affinity for α_{1A}-adrenoceptor using the HypoGen module implemented in catalyst software package.[14] The obtained pharmacophore model can provide a rational hypothetical picture of the primary chemical features responsible for activity, and is expected to provide useful knowledge for developing new potentially active candidates targeting the α_{1A}-adrenoceptor.

2. Materials and methods

The training set consists of 30 compounds (No. **1**–**30**) and is selected to generate HypoGen hypotheses by considering structural diversity and wide coverage of activity range.[12,15–25] Affinity for α_{1A}-adrenoceptor in the training set are reported in Table 2 as K_i values spanning from 0.036 nM to 3.3 μM with six orders of difference. Structures are reported below in Figure 1. All structures in the training set were built and minimized to the closest local minimum based on a modified CHARMM force field[26] within Confirm module in Catalyst 4.9,[14] on a SGI Origin 3800 workstation equipped with 48×400 MHz MIPS R12000 processors. As a quasi-exhaustive search module, Confirm uses conformational models that are not limited to a specific reference conformer. Rather, every training set member is comprised of a collection of low energy conformers that covers the conformational space accessible to the molecule within a given energy range. In Confirm, conformational analysis for each molecule was performed using the Poling algorithm to improve the broad coverage of the available conformational space.[27,28] Poling explicitly promotes the conformational variation that forces similar conformers away from each other.[29] All conformers are treated equally; each is considered as a possible configuration of functional groups or features. In this case the setting in conformer generation was 250 as the maximum number of conformers for each molecule by using 'best conformer generation' option with 20 kcal/mol energy cutoff. All other parameters used were default.

As mentioned before, four chemical feature types such as hydrogen bond donor, hydrophobic, ring aromatic, and positive ionizable are of crucial relevance for the binding of α_{1A}-adrenoceptor antagonists to their receptor.[11–13] Hence, in this hypothesis generation process, four pharmacophore features, positive ionizable, hydrogen-bond donor, aromatic ring, and hydrophobic group were carefully selected to form the essential information. The uncertain factor for each compound represents the ratio range of uncertainty in the activity value based on the expected statistical straggling of biological data collection. Here this factor was defined as the default value of 3. Pharmacophores were then computed using HypoGen module implemented in catalyst software package and the top 10 scoring hypotheses were exported, composed of these four pharmacophore features.

3. Results and discussion

Catalyst produces 10 hypotheses and Hypo1 is the best significant pharmacophore hypothesis in this study, characterized by the highest cost difference, lowest error cost, closest weight cost to 2, and lowest root-mean-square divergence and has the best correlation coefficient. All 10 hypotheses have the same features: one positive ionizable, one hydrogen-bond donor, one aromatic ring, and one hydrophobic group.

The top scoring hypothesis is depicted in Figure 2 (with compound **1** (Silodosin; $K_i = 0.036$ nM) superposed as a representative example). As the best active compound in the training set, compound **1** shows a good fit with all features of the pharmacophore hypothesis Hypo1. In this case, the hydrogen-bond donor seems to be mapped by the hydroxy group, the hydrophobic feature is fitted by the condensed pyrrolidine ring of the terminal heterocyclic portion of the molecule, the positive ionizable sphere is mapped by an aliphatic nitrogen atom, and the aromatic feature is fitted by a phenyl plane. The distances and angles between pharmacophore features from this work and Bremner et al.'s are shown in Table 1.

In this study the null cost value of the top 10 hypotheses is 215.459, and the fixed cost value is 116.066. Configuration cost value is 14.033. All 10 hypotheses have a total cost close to the cost of the fixed hypothesis. The difference between the fixed cost and the null cost is 99.393 bits. Therefore, the probability that the cost difference of any hypothesis with the null hypothesis will be higher than 60 is small. The cost range between these hypotheses and the null hypothesis varies between 87.501 and 82.143 bits with a low cost range, 5.358 bits, between the first and the tenth hypothesis. Therefore, we can expect that for all these hypotheses, there is at least a 75–90% chance of representing a true correlation in the data. Table 2 shows these parameters of statistical significance and predicted power.

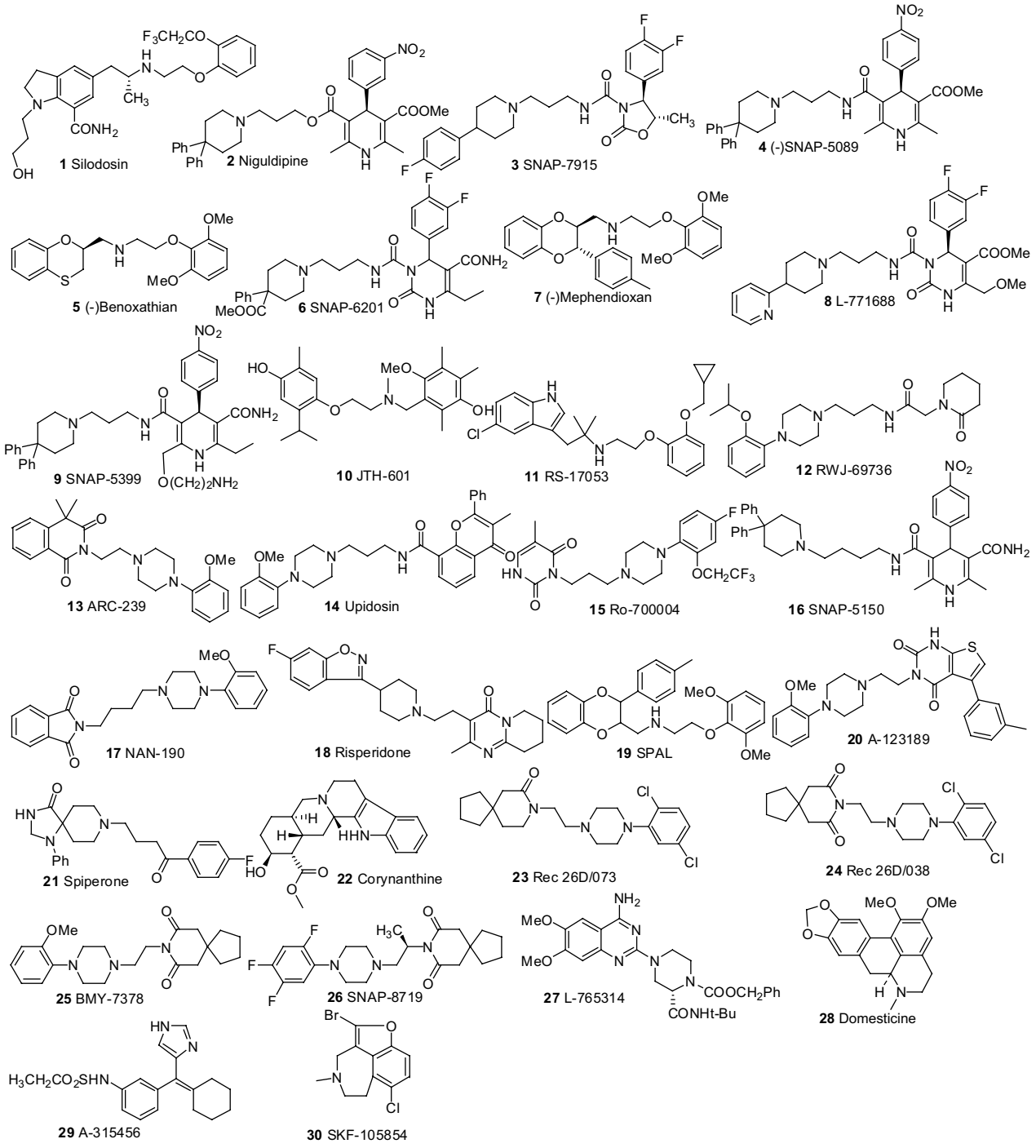

Figure 1. Structures of α_{1A}-adrenoceptor antagonists in the training set.

The rms factor indicates the quality of 'prediction' for the training set. In this case the rms deviation value of the best hypothesis Hypo1, 0.810, represents a good predictivity for Hypo1. The correlation coefficient for the top scoring hypothesis, 0.957, shows a good correlation by linear regression of the geometric fit index. Another validation method to characterize the quality of hypothesis is represented by its capacity for correct activity prediction. The difference between estimated activity values and experimental activity values are represented as error (ratio between the estimated and experimental activity), with a negative sign if the actual activity is higher than the estimated. In this study, the error values of all compounds were found to be less than 10, that means a not-more-than one order difference between estimated and actual activity.

All compounds both in the training set and in the test set were classified into three activity scales, highly active (<1 nM, +++), moderately active (1–100 nM, ++), and inactive (>100 nM, +) by their activity. Table 3 shows the actual and estimated affinity, and references of 30

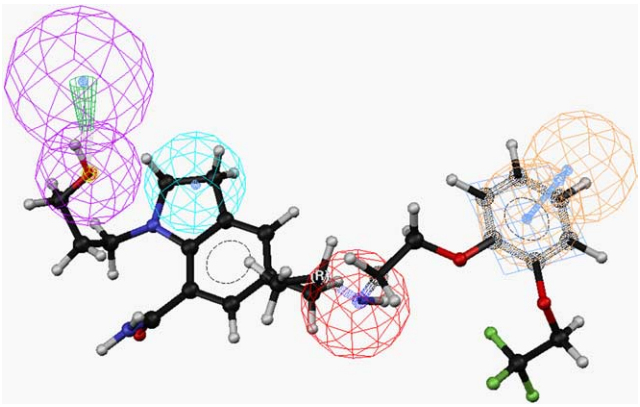

Figure 2. Top scoring HypoGen pharmacophore. Hypo1 is aligned to the most active compound **1** (Silodosin; $K_i = 0.036$ nM) in the training set. Pharmacophore features are color-coded (red, positive ionizable; orange, aromatic ring; blue, hydrophobic; violet, hydrogen-bond donor).

compounds in the training set. Out of 30 compounds in the training set, one inactive compound was predicted to be moderately active, one moderately active compound was predicted to be highly active, meanwhile one moderately active compound was predicted to be inactive and two highly active compounds were predicted to be moderately active.

The main purpose of a quantitative model is to identify active structures and to forecast their actual activity accurately. To verify if the hypothesis can also predict the activity of compounds that are structurally distinct from those included in the training set, we applied a test set of 30 compounds (No. **31**–**45**) selected from literature by different activity classes and of different structural information which was then analyzed.[12,15,17,19,20,30–33] Activities for α_{1A}-adrenoceptor in the test set are reported as K_i values spanning from 0.2 nM to 2.5 μM over a range of six orders of magnitude. All molecules in the test set were built and minimized as well as used in conformational analysis like all molecules in the training set. The structural data for the test set are shown in Figure 3. With the top scoring hypothesis generated using the test set, the correlation coefficient between actual and estimated activity, 0.841, shows a best correlation among all 10 hypotheses (Table 2).

In this test set analysis, out of 15 compounds, 13 compounds had the error values of less than 10, representing a not more than one order difference between estimated and actual activity. All inactive compounds are correctly predicted, meanwhile one moderately active compound is overestimated as highly active, and one highly active compound is underestimated as moderately active. Altogether, all fifteen compounds bar compound **33** in the test set are predicted correctly or better than their actual activity. The results are represented in Table 4.

The mapping of top scoring Hypo1 onto a highly active compound in the test set, compound **31** (Tamsulosin; $K_i = 0.2$ nM), is represented in Figure 4. Hypo1 features are all matched by the chemical groups of compound **31**. Thus, the methoxy substituent maps hydrophobic feature; the nitrogen atom of the aliphatic chain corresponds to the positive ionizable group; the sulfonamide group plays as a role of the hydrogen-bond donor; and the phenyl ring fits aromatic feature. This model also accurately estimates the K_i value for **31** (0.47 nM vs an experimental value of 0.2 nM).

To further evaluate the statistical quality of HypoGen model, the cross-validation based on Fischer's randomization test was applied using the CatScramble program.

Table 1. Distances and angles of the α_{1A}-adrenoceptor antagonists pharmacophore from this work and the pharmacophore of Bremner et al.

Pharmacophore	Distance ranges (Å) and angles (°) between features[a]
In this paper	P–R 5.82; P–D 9.08; P–H 7.53; R–P–D 125; D–R 13.27; D–H 3.95; H–R 10.87
Bremner et al.	P–R 5.5; P–A 7.1; A–P–A 100

[a] Abbreviations used for features: A, hydrogen-bond acceptor; D, hydrogen bond donor; H, hydrophobic group; P, positive charge; R, aromatic ring center.

Table 2. Information of statistical significance and predictive power presented in cost values for top 10 hypotheses[a]

Hypothesis no.	Features	Training set				Test set
		Total cost	ΔCost	rms Deviation	Correlation (r)	Correlation (r)
1	DHPR	127.958	87.501	0.810	0.957	0.841
2	DHPR	129.744	85.715	0.873	0.950	0.748
3	DHPR	129.817	85.642	0.818	0.957	0.794
4	DHPR	131.249	84.210	0.941	0.941	0.788
5	DHPR	131.285	84.174	0.844	0.956	0.783
6	DHPR	131.340	84.119	0.924	0.944	0.737
7	DHPR	131.491	83.968	0.951	0.940	0.745
8	DHPR	131.547	83.912	0.898	0.948	0.714
9	DHPR	132.416	83.043	0.975	0.937	0.812
10	DHPR	133.316	82.143	0.942	0.942	0.647

[a] Null cost of top-ten score hypotheses is 215.459 bits. Fixed cost is 116.066 bits. Configuration cost is 14.033 bits. Abbreviation used for features: D, hydrogen-bond donor; H, hydrophobic; P, positive ionizable; R, aromatic ring.

Table 3. Actual and estimated activity K_i (nM) in the training set based on the best pharmacophore hypothesis Hypo1

No.	Name or code	Actual K_i (nM)	Estimated K_i (nM)	Error	Activity scale[a]	Estimated activity scale[a]	Reference
1	Silodosin	0.036	0.011	−3.3	+++	+++	12
2	Niguldipine	0.16	0.26	+1.6	+++	+++	15
3	(+)SNAP-7915	0.17	0.6	+3.5	+++	+++	15
4	(−)SNAP-5089	0.18	0.18	−1	+++	+++	15
5	(−)Benoxathian	0.2	0.42	+2.1	+++	+++	12
6	SNAP-6201	0.2	0.39	+1.9	+++	+++	15
7	(−)Mephendioxan	0.35	0.7	+2	+++	+++	16
8	L-771688	0.36	0.68	+1.9	+++	+++	15
9	(−)SNAP-5399	0.4	0.74	+1.9	+++	+++	15
10	JTH-601	0.4	1.8	+4.4	+++	++	12
11	RS-17053	0.6	0.68	+1.1	+++	++	12
12	RWJ-69736	0.65	1.8	+2.8	+++	++	17
13	ARC-239	1	2	+2	++	++	18
14	Upidosin	1	1.2	+1.2	++	++	12
15	Ro-700004	1.3	1	−1.3	++	++	19
16	SNAP-5150	1.9	1	−1.9	++	++	15
17	NAN-190	2	2.5	+1.3	++	++	12
18	Risperidone	2.8	4.3	+1.5	++	++	18
19	SPAL	4.1	0.7	−5.8	++	+++	18
20	A-123189	4.2	1.4	−3.1	++	++	20
21	Spiperone	7.9	5.7	−1.4	++	++	12
22	Corynanthine	29	150	+5.3	++	+	12
23	Rec 26D/073	42	33	−1.3	++	++	21
24	Rec 26D/038	130	28	−4.5	+	++	21
25	BMY-7378	250	130	−2	+	+	12
26	SNAP-8719	290	150	−1.9	+	+	12
27	L-765314	420	150	−2.8	+	+	22
28	Domesticine	430	170	−2.6	+	+	23
29	A-315456	590	210	−2.8	+	+	24
30	SKF-105854	3300	5500	+1.7	+	+	25

[a] Activity scale: highly active (<1 nM, +++), moderately active (1-100 nM, ++), and inactive (>100 nM, +). Detailed information of synthesis, separation, and biological data is reported in literature.

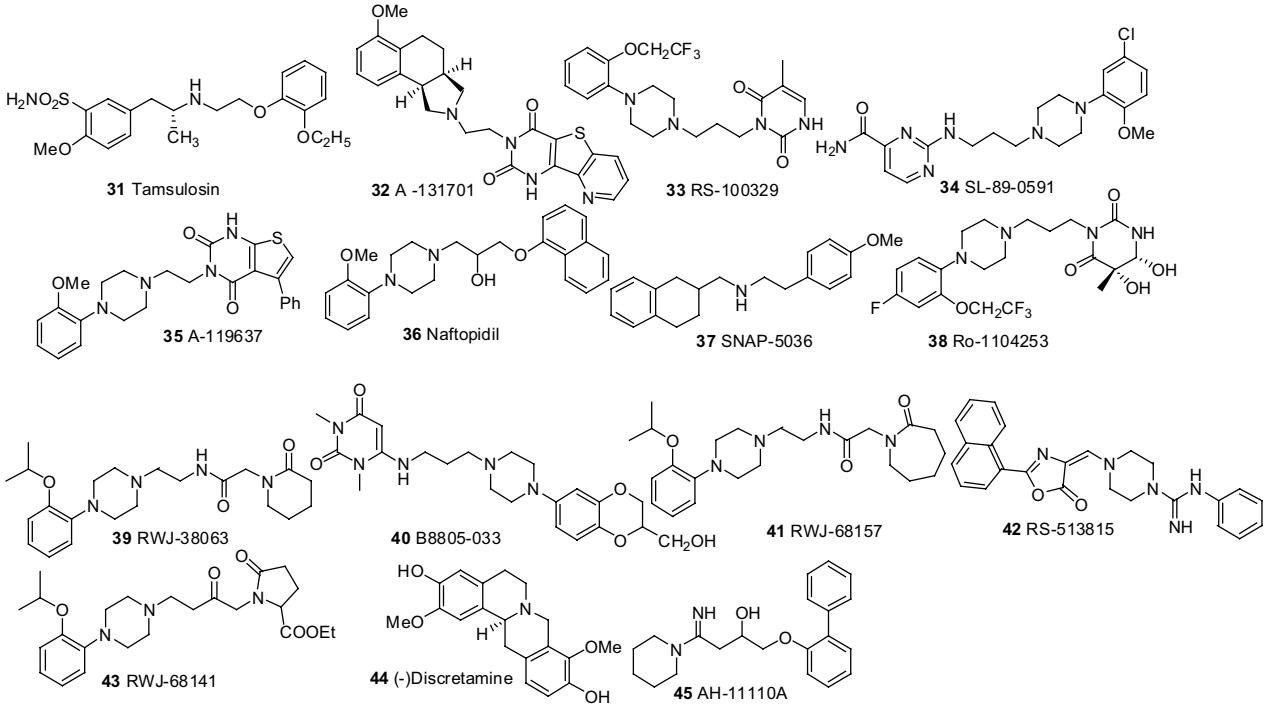

Figure 3. Structures of α_{1A}-adrenoceptor antagonists in the test set.

Table 4. Actual and estimated activity K_i (nM) in the test set based on the best pharmacophore hypothesis Hypo1

No.	Name or code	Actual K_i (nM)	Estimated K_i (nM)	Error	Activity scale[a]	Estimated activity scale[a]	Reference
31	Tamsulosin	0.2	0.47	+2.3	+++	+++	12
32	A-131701	0.22	0.49	+2.2	+++	+++	30
33	RS-100329	0.25	1.3	+5.3	+++	++	19
34	SL-89-0591	2	0.52	−3.9	++	+++	12
35	A-119637	2.6	1.5	−1.7	++	++	20
36	Naftopidil	3.7	2.4	−1.6	++	++	15
37	SNAP-5036	4.4	14	+3.3	++	++	12
38	Ro-1104253	5	4	−1.2	++	++	19
39	RWJ-38063	9.3	1.5	−6.3	++	++	17
40	B8805-033	20	1	−20	++	++	31
41	RWJ-68157	22	1.4	−15	++	++	17
42	RS-513815	41	16	−1.6	++	++	32
43	RWJ-68141	59	5.9	−9.9	++	++	17
44	(−)Discretamine	630	210	−3.3	+	+	12
45	AH-11110A	2500	150	−16	+	+	12

[a] Activity scale: highly active (<1 nM, +++), moderately active (1–100 nM, ++), and inactive (>100 nM, +). Detailed information of synthesis, separation, and biological data is reported in literature.

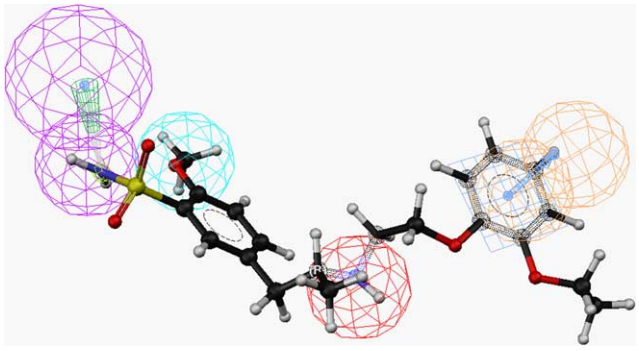

Figure 4. Top scoring HypoGen pharmacophore Hypo1 is aligned to the most active compound **31** (Tamsulosin; $K_i = 0.2$ nM) in the test set. Pharmacophore features are color-coded (red, positive ionizable; orange, aromatic ring; blue, hydrophobic; violet, hydrogen-bond donor).

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. To achieve a 95% confidence level 19 random spreadsheets have to be generated ($y = 20$) and every generated spreadsheet is submitted to HypoGen using the same experimental conditions (functions and parameters) as the initial run. 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 reasoning behind this procedure is that if the randomized data sets produced a

Table 5. Results from cross-validation using CatScramble implemented in catalyst software package[a]

Validation no.	Total cost	Cost	Fixed cost	rms Deviation	Correlation (r)	Configuration cost
Results for unscrambled						
Hypo 1	127.958	87.501	116.066	0.810	0.957	14.033
Results for scrambled						
Trial 1	199.037	16.422	105.618	2.493	0.432	3.585
Trial 2	201.852	13.607	109.733	2.474	0.446	7.7
Trial 3	215.459	0	100.908	2.763	0	0
Trial 4	205.81	9.649	112.651	2.478	0.444	10.618
Trial 5	216.217	0	100.908	2.763	0	0
Trial 6	207.182	8.277	114.961	2.467	0.452	12.928
Trial 7	179.488	35.971	113.993	2.075	0.661	11.96
Trial 8	192.897	22.562	111.525	2.328	0.539	9.492
Trial 9	212.828	2.631	109.121	2.618	0.323	7.087
Trial 10	202.651	12.808	111.673	2.459	0.457	9.64
Trial 11	190.369	25.09	110.888	2.295	0.558	0
Trial 12	215.459	0	100.908	2.763	0	6.229
Trial 13	179.025	36.434	112.502	2.085	0.658	10.469
Trial 14	186.055	29.404	113.727	2.195	0.608	11.694
Trial 15	215.459	0	100.908	2.763	0	0
Trial 16	202.479	12.98	110.814	2.468	0.45	8.781
Trial 17	203.044	12.415	110.758	2.462	0.455	8.724
Trial 18	213.706	1.753	115.161	2.528	0.407	13.128
Trial 19	215.459	0	100.908	2.763	0	0

[a] Null cost = 215.459.

hypothesis with a high correlation value, then the methodology of the pharmacophore generation is flawed.

In this cross-validation test, we select the 95% confidence level, and the 19 spreadsheets were generated by the CatScramble command. These random spreadsheets were used to generate hypotheses using exactly the same features as used in generating the original pharmacophore hypothesis. The results of the CatScramble runs are listed in Table 5.

The results of CatScramble clearly indicate that all values generated after randomization produced hypotheses with no predictive value. Besides, out of 19 runs, only three, trial 7, trial 13, and trial 14 had a correlation value more than 0.6, but the rms deviation was very high and the total cost value was almost equal to the null cost value, which is not desirable for a good hypothesis. The cross-validation strongly supports that the Hypo1 hypothesis is not generated by chance since its statistics are far more superior to those of the 19 random hypotheses generated.

4. Conclusion

The pharmacophore models generated using a training set of thirty compounds in this study highlight the pattern with significance for α_{1A}-adrenoceptor antagonists. This pharmacophore hypothesis in terms of predictive ability, which consisted of one positive ionizable, one hydrogen-bond donor, one aromatic ring, and one hydrophobic group, is further validated by using an external test set of fifteen compounds. The most active compound **1** (Silodosin; $K_i = 0.036$ nM) in the training set fits very well with this top scoring pharmacophore hypothesis, so does compound **31** as the most active compound in the test set. Consequently, the knowledge of this four-feature pharmacophore hypothesis for α_{1A}-adrenoceptor antagonists can be used for virtual screening to discover more novel potential molecules for the treatment of benign prostatic hyperplasia.

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References and notes

1. Thorpe, A.; Neal, D. *Lancet* **2003**, *361*, 1359.
2. Chess-Williams, R. *Expert Opin. Pharmacother.* **2002**, *3*, 167.
3. Pool, J. L.; Kirby, R. S. *Int. Urol. Nephrol.* **2001**, *33*, 407.
4. Bylund, D. B.; Eikenburg, D. C.; Heible, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R.; Trendelenburg, U. *Pharmacol. Rev.* **1994**, *46*, 121.
5. Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. *Pharmacol. Rev.* **1995**, *47*, 267.
6. Price, D. T.; Schwinn, D. A.; Lomasney, J. W.; Allen, L. F.; Caron, M. G.; Lefkowitz, R. J. *J. Urol.* **1993**, *150*, 546.
7. Kurogi, Y.; Güner, O. F. *Curr. Med. Chem.* **2001**, *8*, 1035.
8. Debnath, A. K. *J. Med. Chem.* **2002**, *45*, 41.
9. Du, L. P.; Tsai, K. C.; Li, M. Y.; You, Q. D.; Xia, L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4771.
10. Li, H.; Sutter, J.; Hoffmann, R. In *Pharmacophore Perception, Development and Use in Drug Design*; Güner, O. F., Ed.; International University Line: La Jolla, CA, 2000; p 171.
11. Bremner, J. B.; Coban, B.; Griffith, R. *J. Comput. Aided Mol. Des.* **1996**, *10*, 545.
12. Bremner, J. B.; Coban, B.; Griffith, R.; Groenewoud, K. M.; Yates, B. F. *Bioorg. Med. Chem.* **2000**, *8*, 201.
13. Fang, H.; Lu, J. F.; Xia, L. *J. Chin. Pharm. Sci.* **2003**, *12*, 188.
14. CATALYST 4.9 User Guide, Accelrys Inc., San Diego, CA, USA, **2003**.
15. Lagu, B. *Drugs Future* **2001**, *26*, 757.
16. Quaglia, W.; Pigini, M.; Tayebati, S. K.; Piergentili, A.; Giannella, M.; Leonardi, A.; Taddei, C.; Melchiorre, C. *J. Med. Chem.* **1996**, *39*, 2253.
17. Pulito, V. L.; Li, X.; Varga, S. S.; Mulcahy, L. S.; Clark, K. S.; Halbert, S. A.; Reitz, A. B.; Murray, W. V.; Jolliffe, L. K. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 224.
18. Menziani, M. C.; Montorsi, M.; De Benedetti, P. G.; Karelson, M. *Bioorg. Med. Chem.* **1999**, *7*, 2437.
19. Lopez, F. J.; Arias, L.; Chan, R.; Clarke, D. E.; Elworthy, T. R.; Ford, A. P. D. W.; Guzman, A.; Jaime-Figueroa, S.; Jasper, J. R.; Morgans, D. J.; Padilla, F.; Perez-Medrano, A.; Quintero, C.; Romero, M.; Sandoval, L.; Smith, S. A.; Williams, T. J.; Blue, D. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1873.
20. Carroll, W. A.; Sippy, K. B.; Esbenshade, T. A.; Buckner, S. A.; Hancock, A. A.; Meyer, M. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1119.
21. Leonardi, A.; Barlocco, D.; Montesano, F.; Cignarella, G.; Motta, G.; Testa, R.; Poggesi, E.; Seeber, M.; De Benedetti, P. G.; Fanelli, F. *J. Med. Chem.* **2004**, *47*, 1900.
22. Patane, M. A.; Scott, A. L.; Broten, T. P.; Chang, R. S. L.; Ransom, R. W.; DiSalvo, J.; Forray, C.; Bock, M. G. *J. Med. Chem.* **1998**, *41*, 1205.
23. Indra, B.; Matsunaga, K.; Hoshino, O.; Suzuki, M.; Ogasawara, H.; Muramatsu, I.; Taniguchi, T.; Ohizumi, Y. *Eur. J. Pharmacol.* **2002**, *445*, 21.
24. Buckner, S. A.; Milicic, I.; Daza, A.; Lynch, J. J.; Kolasa, T.; Nakane, M.; Sullivan, J. P.; Brioni, J. D. *Eur. J. Pharmacol.* **2001**, *433*, 123.
25. Low, A. M.; Lu-Chao, H.; Wang, Y. F.; Brown, R. D.; Kwan, C. Y.; Daniel, E. E. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 894.
26. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
27. Smellie, A.; Kahn, S. D.; Teig, S. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 285.
28. Smellie, A.; Kahn, S. D.; Teig, S. *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 295.
29. Smellie, A.; Teig, S.; Towbin, P. *J. Comput. Chem.* **1995**, *16*, 171.
30. Meyer, M. D.; Altenbach, R. J.; Basha, F. Z.; Carroll, W. A.; Drizin, I.; Elmore, S. W.; Ehrlich, P. P.; Lebold, S. A.; Tietje, K.; Sippy, K. B.; Wendt, M. D.; Plata, D. J.;

Plagge, F.; Buckner, S. A.; Brune, M. E.; Hancock, A. A.; Kerwin, J. F., Jr. *J. Med. Chem.* **1997**, *40*, 3141.
31. Eltze, M.; Boer, R.; Michel, M. C.; Hein, P.; Testa, R.; Ulrich, W. R.; Kolassa, N.; Sanders, K. H. *Naunyn Schmiedebergs Arch. Pharmacol.* **2001**, *363*, 649.
32. Conley, R. K.; Williams, T. J.; Ford, A. P.; Ramage, A. G. *Br. J. Pharmacol.* **2001**, *133*, 61.
33. Fischer, R. *The Principle of Experimentation, Illustrated by a Psycho-Physical Experiment. The Design of Experiments*, 8th ed.; Hafner: New York, 1966; Chapter II.