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The pharmacophore hypotheses of I_{Kr} potassium channel blockers: novel class III antiarrhythmic agents

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Abstract—Predictive pharmacophore models were developed for a large series of I_{Kr} potassium channel blockers as class III antiarrhythmic agents using HypoGen in Catalyst software. The pharmacophore hypotheses were generated using a training set consisting of 34 compounds carefully selected from documents. Their biological data, expressed as IC_{50}, spanned from 1.5 nM to 2.8 mM with 7 orders difference. The most predictive hypothesis (Hypo1), consisting of four features (one positive ionizable feature, two aromatic rings and one hydrophobic group), had a best correlation coefficient of 0.825, a lowest rms deviation of 1.612, and a highest cost difference (null cost − total cost) of 77.552, which represents a true correlation and a good predictivity. The hypothesis Hypo1 was then validated by a test set consisting of 21 compounds and by a cross-validation of 95% confidence level with randomizing the data using CatScramble program. Accordingly, our model has strong predictivity to identify structural diverse I_{Kr} potassium channel blockers with desired biological activity by virtual screening.

1. Introduction

Since it has been known that sotalol increases the action potential duration (APD) by blocking the rapidly activating delayed rectifier potassium channel (I_{Kr}) in the heart, I_{Kr} has been the major target channel for the design of new compounds as class III antiarrhythmic drugs.[1] I_{Kr} is one of the voltage dependent potassium channels, composed of the tetrameric interaction of six transmembrane segments, presenting the cardiac cells. The elegant studies by Sanguinetti and his co-workers revealed that biophysically I_{Kr}-like channel, which is, however, not blocked by the methanesulfonamide class III antiarrhythmic drugs, was expressed in the *Xenopus oocytes* when the *human ether-a-go-go-related gene* (HERG) was injected.[2] It was also reported that HERG produces the I_{Kr}-like currents that are sensitively blocked by the I_{Kr} blockers when was expressed in the human cell line. IKr blockers can prolong atrial and ventricular APD (QT prolongation) and refractoriness in the absence of significant changes in conduction velocity (AH, HV and PR intervals). In various animal models, I_{Kr} blockers suppress ventricular tachycardia induced by programmed electrical stimulation or a new ischemic insult in dogs with prior infarct. The antiarrhythmic efficacy of I_{Kr} blockers has also been proven in clincial studies.[3]

It has been recently reported that the co-assembly between HERG and Mink-related protein 1 (MiRP1) encodes potassium channels that behave like native I_{Kr} more than HERG itself.[4] However, whether or not HERG indeed requires a subunit protein such as minK or MiRP1 to form native I_{Kr} potassium channel is still controversial.[5]

There is only one attempt to construct a pharmacophore hypothesis for I_{Kr} blockers, using DISCO module in SYBYL software package, by Matyus and his colleagues.[6] The results of their pharmacophore modeling study point out a five-point pharmacophore, including a hydrogen-bond donor, a hydrogen-bond acceptor, two aromatic rings and an aliphatic chain (as a hydrophobic group), identified for the most active compounds, whereas a four-point pharmacophore a hydrogen-bond donor, a hydrogen-bond acceptor, an aromatic ring and an aliphatic chain, forming a subset of the former one, developed by the less potent agents.

Keywords: Pharmacophore; Hypothesis; I_{Kr} potassium channel; Blockers.
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The approach we using here is to develop a pharmacophore model for I_{Kr} potassium channel blockers using HypoGen module implemented in Catalyst software package, a leading software products for the automated generation of pharmacophore models and 3D database searching, as a large number of successful application in medicinal chemistry clearly demonstrated.[7,8] We here have included the biological data of 34 compounds, which covers 7 orders of magnitude, for the training set, and of 21 compounds, which covers 6 orders, for the test set. These molecules were selected to span the range of activity from the most active compound available to almost inactive molecules that are publicly.

Due to such a fact there is no recent report on developing pharmacophore models using newly published I_{Kr} blockers, the present paper provides a hypothetical image of the primary pharmacophore features responsible for activity, and it is expected to provide useful knowledge for discovering novel potential blockers targeted to I_{Kr} potassium channel.

2. Pharmacophore generation

The training set consists of 34 compounds and was selected by considering structural diversity and wide coverage of activity range presented in Figure 1. Activities are reported as IC_{50} values spanning from 1.5 nM to 2.8 mM with 7 orders.[9–32] Each compound of the training set should provide new structural information to achieve a good pharmacophoric model in terms of

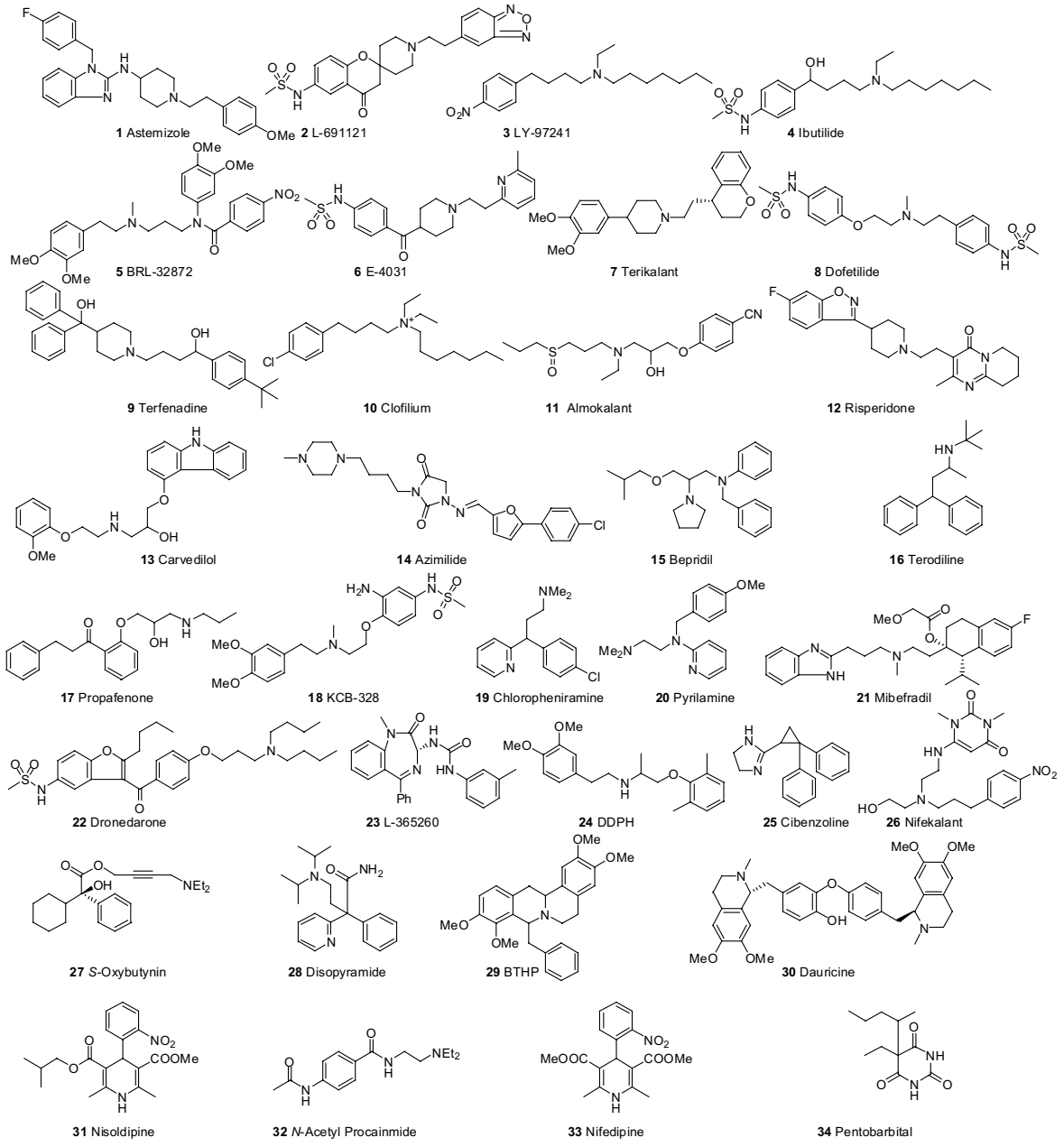

Figure 1. Chemical structures of 34 training set molecules used to form HypoGen pharmacophore hypotheses.

predictive power and statistical significance. In this case where the chirality of a stereogenic centre was not specified, all structures were built and minimized to the closest local minimum using the CHARMM-like force field within the Catalyst 4.9 software package installed on a SGI Origin 3800 workstation equipped with 48×400 MHz MIPS R12000 processors, and conformational analysis for each molecule was performed using the Poling algorithm, which the setting number of conformer was limited to a maximum value of 250 using 'best conformer generation' method with 20 kcal/mol energy cutoff. All other parameters used were default.

According to earlier pharmacophore study for I_{Kr} potassium channel blockers, the hydrogen-bond donor, hydrogen-bond acceptor, aromatic ring and hydrophobic features were considered to be important. Hence, in the hypothesis generation process, a default uncertainty factor of 3 for each compound was defined, and four features, including hydrogen-bond donor, hydrogen-bond acceptor, aromatic ring and hydrophobic group, were selected to form the pharmacophore hypothesis using HypoGen module in Catalyst. The HypoGen algorithm was forced to find pharmacophore models that contain at least one and at most two of every features.

3. Results

Catalyst produces 10 hypotheses, and Hypo1 is the best significant pharmacophore hypothesis in this study, characterized by the highest cost difference, lowest error cost, and lowest root-mean-square divergence and has the best correlation coefficient. All 10 hypotheses have the same features: one positive ionizable, two aromatic rings and one hydrophobic group. Figure 2 represents the top scoring hypothesis Hypo1 aligned with the highest active compound **1** (Astemizole, IC_{50} = 1.5 nM) in the training set and the distance constraints between pharmacophore features.

Compound **1** shows a good fit with all features of the pharmacophore hypothesis Hypo1. In this case, the hydrophobic group seems to be mapped by a phenyl ring, the positive ionizable sphere is mapped by a nitrogen atom in piperidine moiety, and the two aromatic features is fitted by a 1*H*-benzo[*d*]imidazole plane and by a phenyl ring, respectively.

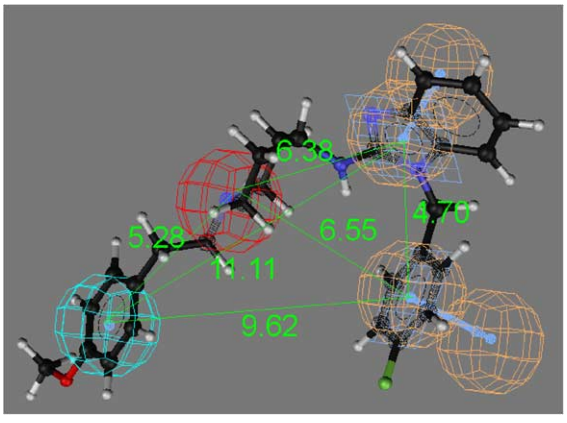

Figure 2. Top scoring HypoGen pharmacophore, Hypo1, is mapped to the most active compound **1** (Astemizole, IC_{50} = 1.5 nM) in the training set. Pharmacophore features are color-coded with: orange, aromatic rings; light-blue, hydrophobic groups; red, positive ionizable. Distances between pharmacophore features are reported in angstroms.

Table 1. Information of statistical significance and predictive power presented in cost values measured in bits for top-ten hypotheses[a]

Hypothesis no.	Total cost	Δcost	Rms deviation	Correlation
1	175.368	77.552	1.612	0.825
2	175.609	77.311	1.616	0.824
3	175.814	77.106	1.618	0.824
4	175.856	77.064	1.621	0.823
5	176.397	76.523	1.631	0.821
6	176.693	76.227	1.636	0.820
7	176.697	76.223	1.636	0.819
8	176.765	76.155	1.637	0.819
9	176.950	75.970	1.641	0.818
10	177.086	75.834	1.643	0.818

[a] Null cost of top-ten scoring hypotheses is 252.920. Fixed cost value is 131.171. Configuration cost is 15.683. Δcost = null cost − total cost.

Table 1 lists the results of statistical significance and predictive ability. In this case the null cost value of the best 10 ranking hypothesis is 252.920, and the fixed cost value is 131.171. Configuration cost, a constant value less than 17, describing the complexity of the hypotheses space to explore, is 15.683. The first hypothesis Hypo1, as the best pharmacophore hypothesis, is characterized by the highest cost difference (77.552), the lowest rms deviation value (1.612) and the best correlation coefficient value (0.825), which represents a true correlation and a good predictivity.

Hypo1, identified as the best hypothesis, was then used to estimate the activity of the training set molecules. In this study all compounds were classified by their activity as highly active (<1 μM, +++), moderately active (1–100 μM, ++) and inactive (>100 μM, +). Table 2 represents the actual and estimated I_{Kr} potassium channel blocking activity of the 26 training set molecules, based on the best hypothesis Hypo1. Out of all 34 compounds in the training set, five moderately active compounds was predicted to be highly active, and two highly active compounds were predicted to be moderately active in the training set by Hypo1. Consequently, for 27 of 34 compounds, the predicted IC_{50} (μM) values were to be within the same activity scale as the experimental values in the training set.

4. Validation of pharmacophore hypothesis

In order to validate our pharmacophore hypothesis, we used a test set consisted of 21 molecules with I_{Kr} blocking activity by different activity classes and of different structural information.[9,10,12,21,24,33–39] Activities are reported as IC_{50} values spanning from 40 nM to 4.4 mM with 6 orders. 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.

Table 2. Actual biological data and estimated IC_{50} of training set molecules based on pharmacophore model Hypo1

No.	Compound	Actual IC_{50} (μM)	Estimated IC_{50} (μM)	Error	Activity scale[a]	Estimated activity scale[a]	Reference
1	Astemizole	0.0015	0.0014	−1.1	+++	+++	9
2	L-691121	0.008	0.13	16	+++	+++	10
3	LY-97241	0.019	0.16	8.6	+++	+++	11
4	Ibutilide	0.02	0.24	12	+++	+++	12
5	BRL-32872	0.028	0.14	4.9	+++	+++	12
6	E-4031	0.029	0.1	3.5	+++	+++	13
7	Terikalant	0.031	0.14	4.4	+++	+++	14
8	Dofetilide	0.032	0.18	5.8	+++	+++	12
9	Terfenadine	0.05	0.15	3	+++	+++	9
10	Clofilium	0.15	0.24	1.6	+++	+++	11
11	Almokalant	0.25	0.11	−2.2	+++	+++	15
12	Risperidone	0.26	0.83	3.2	+++	+++	16
13	Carvedilol	0.35	0.15	−2.4	+++	+++	17
14	Azimilide	0.4	5.5	14	+++	++	18
15	Bepridil	0.43	0.21	−2	+++	+++	19
16	Terodiline	0.50	8.3	17	+++	++	20
17	Propafenone	0.80	0.66	−1.2	+++	+++	21
18	KCB-328	1	0.17	−5.8	+++	+++	12
19	Chloropheniramine	1.1	6.5	5.9	++	++	9
20	Pyrilamine	1.1	0.8	−1.4	++	+++	9
21	Mibefradil	1.4	0.28	−5.1	++	++	22
22	Dronedarone	3	0.15	−20	++	+++	23
23	L-365260	5	5.8	1.2	++	++	24
24	DDPH	6.1	0.29	−21	++	+++	25
25	Cibenzoline	8.8	8.5	−1	++	++	26
26	Nifekalant	10	0.52	−19	++	+++	12
27	*S*-Oxybutynin	12	8	−1.5	++	++	20
28	Disopyramide	12	8	−1.5	++	++	26
29	BTHP	14	4.1	−3.3	++	++	27
30	Dauricine	16	0.48	−34	++	+++	28
31	Nisoldipine	23	31	1.3	++	++	29
32	*N*-Acetyl procainmide	100	8.1	−12	++	++	30
33	Nifedipine	280	730	2.7	+	+	31
34	Pentobarbital	2800	730	−3.7	+	+	32

[a] The error column shows the ratio of estimated activity to actual activity (or the ratio of actual/estimated, if that gives a number greater than 1, in which case the number is negative). Activity scale: highly active (<1 μM, +++), moderately active (1–100 μM, ++) and inactive (>100 μM, +).

The test set molecules were mapped onto the best pharmacophore hypothesis Hypo1 and the actual activity versus estimated activity are shown in Table 3. The correlation coefficient generated using the test set, 0.713, shows a good correlation between actual and estimated activity. In this case, all highly active compounds were predicted correctly, five moderately active compounds were overestimated as highly active, and one moderately active compounds were underestimated as inactive. Among inactive compounds, two were overestimated as false positive. In conclusion, all test set compounds, with exception of compound **46**, were predicted correctly or better than their actual activity.

The most potent compound **35** (Halofantrine, IC_{50} = 40 nM) in the test set, was selected to show the mapping of this molecule on Hypo1 represented in Figure 4. Compound **35** fits the hydrophobic group, the positive ionizable feature and the first aromatic ring, but does not fit the second aromatic ring.

Another approach to assess the quality of HypoGen hypothesis is to apply cross validation using the CatScramble program available in Catalyst. The validation procedure described here is based on Fischer's randomization test. The goal of this type of validation is to check whether there is a strong correlation between the chemical structures and the biological activity.

In this 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 hypothesis using exactly the same features as using in generating the initial hypothesis. The results of the CatScramble runs are listed in Table 4.

The data of cross validation clearly indicates that all values generated after randomization produced hypotheses with no predictive value. Besides, out of 19 runs, only one, trial 7, had a correlation value near 0.7, but the rms deviation and configuration cost were very high, which is not desirable for a good hypothesis. This cross validation also provided strong confidence on the initial pharmacophore Hypo1.

5. Conclusion

In the absence of detailed structural information on the I_{Kr} potassium channel binding site, we have employed a ligand-based computational approach to identify ligand requirements for blocking this ion channel. Using Hyp-

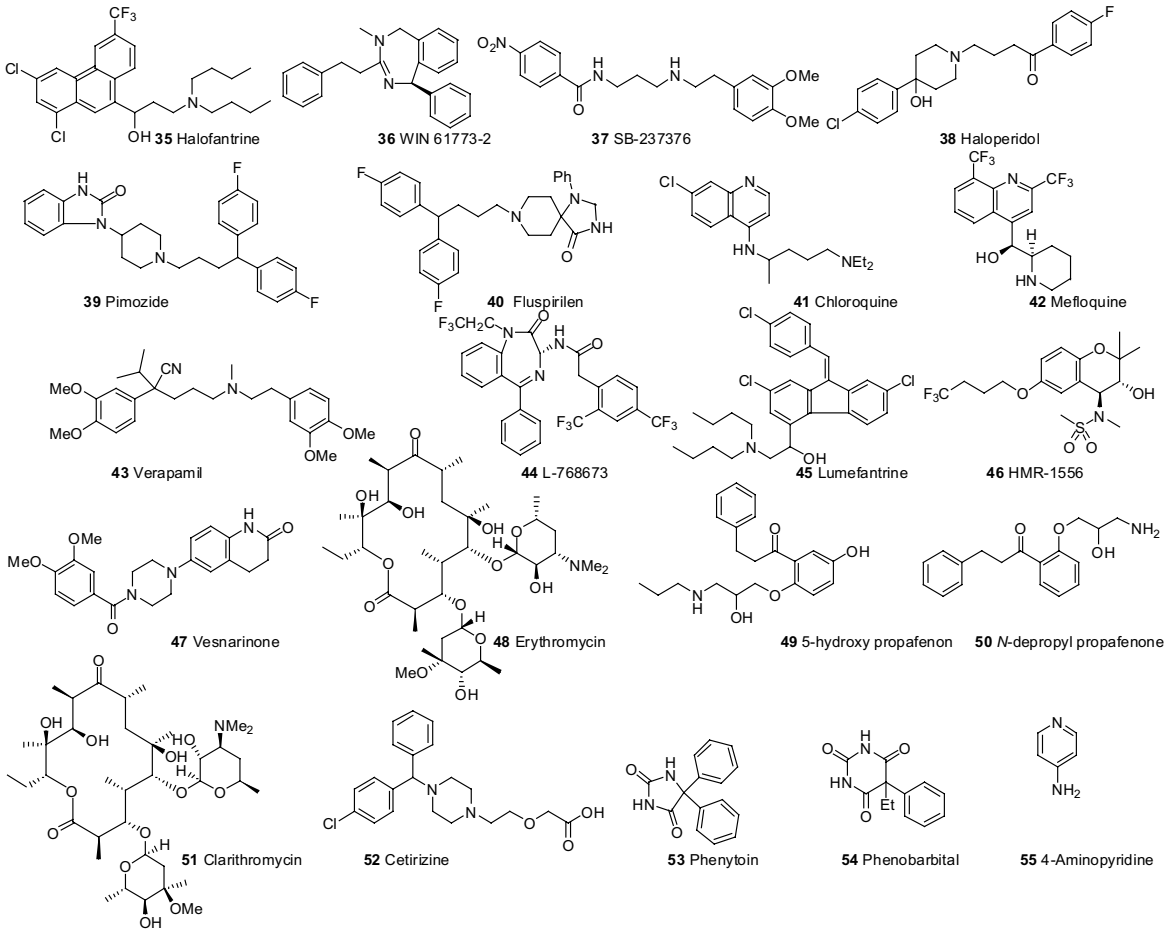

Figure 3. Chemical structures of 21 test set molecules.

Table 3. Actual biological data and estimated IC_{50} of test set molecules based on pharmacophore model Hypo1

No.	Compound	Actual IC_{50} (μM)	Estimated IC_{50} (μM)	Error	Activity scale[a]	Estimated activity scale[a]	Reference
35	Halofantrine	0.04	0.11	2.8	+++	+++	33
36	WIN 61773-2	0.092	0.75	8.2	+++	+++	10
37	SB-237376	0.42	0.22	−1.9	+++	+++	12
38	Haloperidol	1.4	0.18	−7.9	++	+++	34
39	Pimozide	1.7	0.28	−6.1	++	+++	34
40	Fluspirilen	2.3	0.3	−7.7	++	+++	34
41	Chloroquine	2.5	8.1	3.3	++	++	33
42	Mefloquine	2.6	8.1	3.1	++	++	33
43	Verapamil	3.1	0.12	−27	++	+++	35
44	L-768673	6	3.9	−1.5	++	++	24
45	Lumefantrine	8.1	0.1	−78	++	+++	33
46	HMR-1556	12.6	740	59	++	+	24
47	Vesnarinone	18	8.3	−2.2	++	++	36
48	Erythromycin	39	8.7	−4.5	++	++	37
49	5-Hydroxy propafenone	40	1.1	−36	++	++	21
50	*N*-Depropyl propafenone	44	5.6	−7.9	++	++	21
51	Clarithromycin	46	8.7	−5.3	++	++	37
52	Cetirizine	108	1.7	−64	+	++	9
53	Phenytoin	240	8.4	−29	+	++	38
54	Phenobarbital	3000	740	−4	+	+	38
55	4-Aminopyridine	4400	740	−5.9	+	+	39

[a] The error column shows the ratio of estimated activity to actual activity (or the ratio of actual/estimated, if that gives a number greater than 1, in which case the number is negative). Activity scale: highly active (<1 μM, +++), moderately active (1–100 μM, ++), and inactive (>100 μM, +).

oGen module within the Catalyst software package, several pharmacophore hypotheses were obtained. The best pharmacophore model in terms of predictive values consisted of a four-feature HypoGen model (one positive

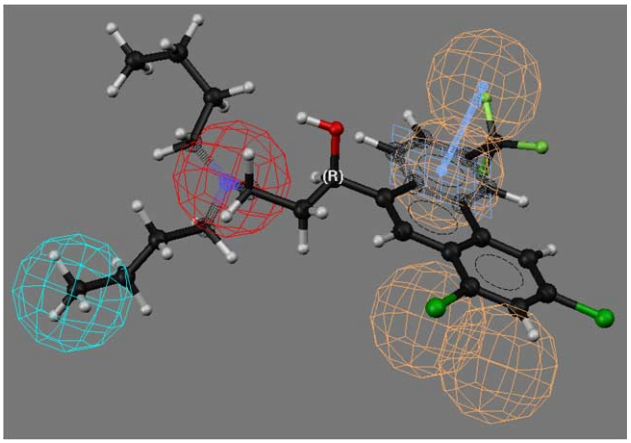

Figure 4. Top scoring HypoGen pharmacophore, Hypo1, is mapped to the most active compound **35** (Halofantrine, $IC_{50}=40$ nM) in the test set. Pharmacophore features are color-coded with: orange, aromatic rings; light-blue, hydrophobic groups; red, positive ionizable.

Table 4. Results from cross-validation using CatScramble in catalyst[a]

Trial no.	Total cost	Fixed cost	Rms deviation	Correlation (r)	Configuration cost
		Results for unscrambled			
	175.368	158.551	1.612	0.825	15.683
		Results for scrambled			
1	215.661	131.379	2.196	0.642	15.891
2	218.735	130.615	2.275	0.604	15.128
3	244.718	125.440	2.636	0.385	9.953
4	216.850	135.826	2.173	0.649	20.338
5	228.403	125.086	2.465	0.504	9.598
6	240.589	133.319	2.504	0.481	17.831
7	207.366	134.551	2.049	0.697	19.064
8	225.494	122.726	2.456	0.510	7.238
9	224.523	131.886	2.328	0.579	16.398
10	256.436	128.956	2.733	0.291	13.469
11	243.324	128.932	2.568	0.438	13.444
12	225.563	130.877	2.360	0.563	15.390
13	220.610	130.288	2.304	0.591	14.800
14	223.343	131.005	2.330	0.578	15.518
15	224.125	132.032	2.327	0.579	16.545
16	208.903	124.050	2.226	0.626	8.562
17	252.571	127.030	2.716	0.309	11.542
18	224.826	130.819	2.352	0.567	15.332
19	253.284	127.318	2.713	0.313	11.830

[a] Null cost = 252.92.

ionizable feature, two aromatic rings and one hydrophobic group). This study can open the way for elucidating the mechanisms of I_{Kr} potassium channel receptor-ligand interaction and for identifying new classes of I_{Kr} potassium channel blockers as class III antiarrhythmic drugs.

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