

3rd Annual Predictive Toxicology
half day interactive pre-conference workshop

Tox IVIVE - inter-individual variability matters!

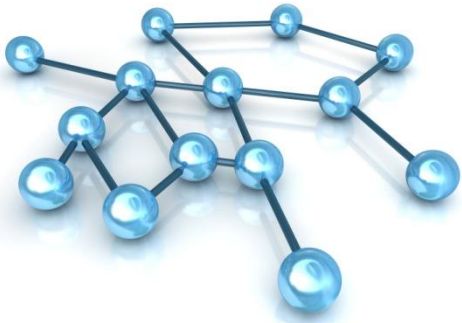
extended QSAR

Sebastian Polak



Unit of Pharmacoepidemiology and
Pharmacoeconomics
Jagiellonian University Medical College

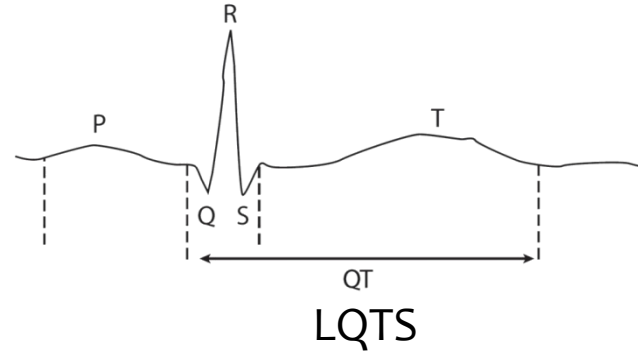




TdP – Torsades de Pointes

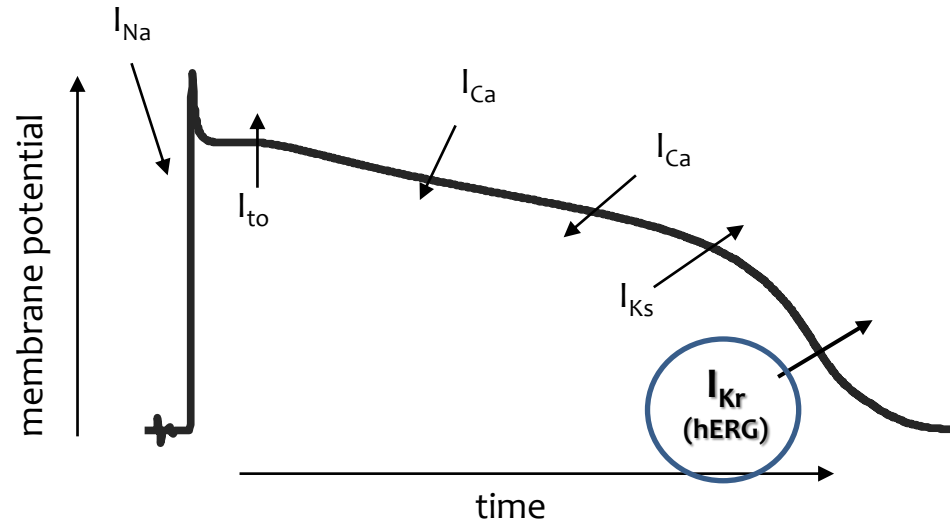


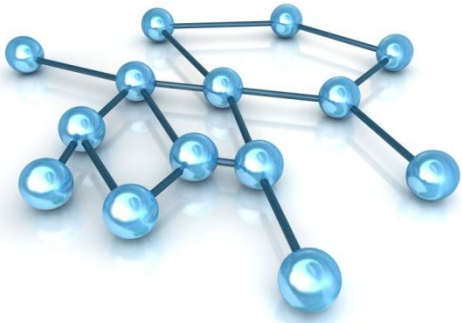
TdP



mechanism

- inhibition of the rapid delayed rectifier potassium current I_{Kr}
- inhibition of the channel encoded by the hERG gene





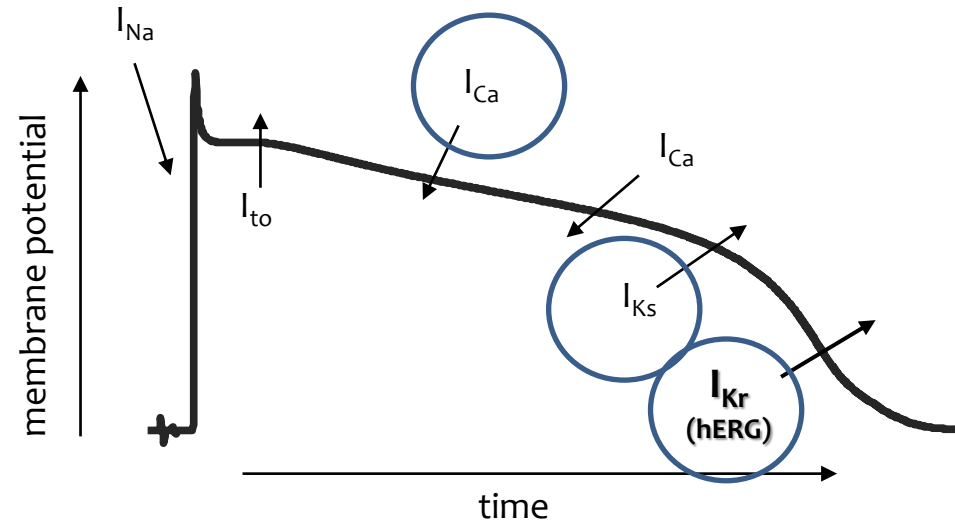
TdP – Torsades de Pointes

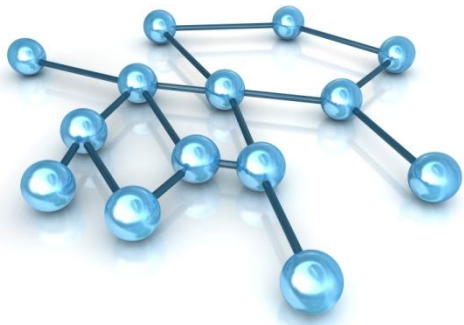
problems

- drugs inhibiting I_{Kr} potassium current in vitro – do not express same characteristic in vivo (verapamil)

potential reasons

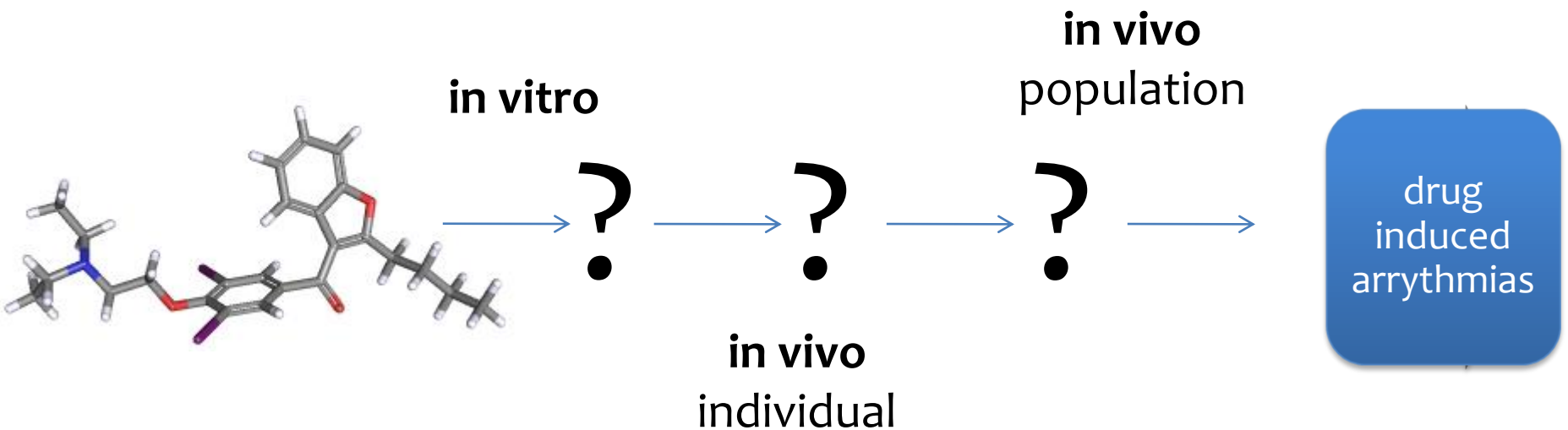
- interplay between various ionic channels inhibition

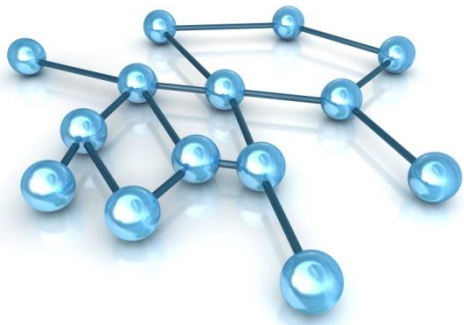




toxicity prediction

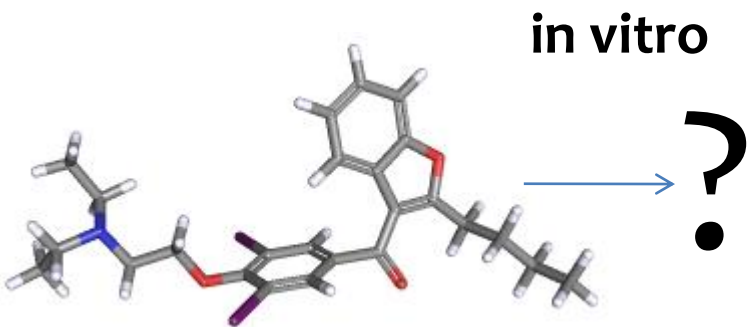
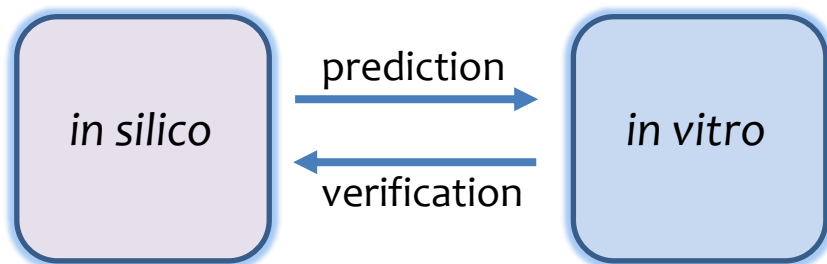
in silico – in vitro – in vivo extrapolation

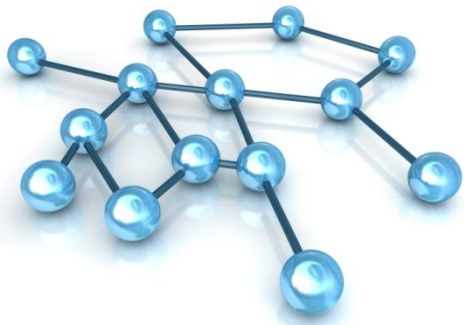




toxicity prediction

in silico prediction





proarrhythmic potency assessment

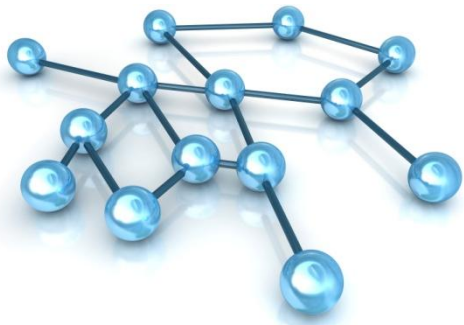
in vitro

in vitro



in vitro

- electrophysiological methods - **patch clamp**
 - various currents
 - HEK, CHO, XO cell lines
 - room/phys temperature
 - holding potential / depolarization level/ measurement potential
 - pulse duration
 - ionic concentration in bath



proarrhythmic potency assessment

in vitro hERG - database

in vitro

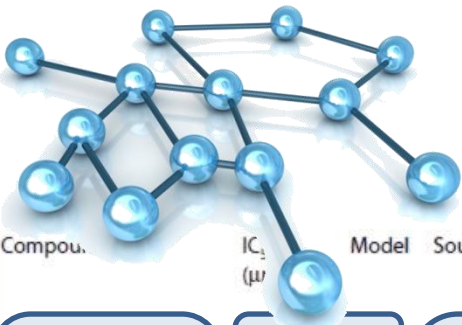


in vitro

- **hERG (I_{Kr})** - database

- Arizona CERT (Center for Education and Research on Therapeutics)
- Robert Fenichel database
- Olivier Roche database
- literature analysis

tox-portal.net



proarrhythmic potency assessment in vitro hERG - database

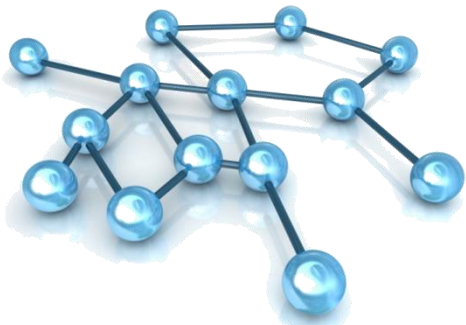
| Compo. | IC ₅₀ (μ) | Model | Source ^a | Transf. ^b | Temperature (°C) | Technique ^c | K rd (mm) | t ₁ pulse (s) | Hold ^e (mV) | Depol ^f (mV) | Measurement ^g (mV) | Protocol ^h |
|-------------------------------|-------------------------|-------|------------------------------------|----------------------|---------------------|---------------------------|-------------------------|--------------------------------|---------------------------|----------------------------|----------------------------------|-----------------------|
| 4,4'-Dimethyl-diphenhydramine | 18.5 | XO | Tagliatalata <i>et al.</i> (2000b) | | 23 | Voltage-clamp 2-electrode | 10 | 2 | -90 | 0 | -100 | Step |
| 4-Aminopyridine | 4400 | HEK | Ridley <i>et al.</i> (2003b) | Stable | 37 | | 4 | 1 | -40 | 30 | -40 | Step |
| 5-Hydroxypropafenone | 0.88 | CHO | Arias <i>et al.</i> (2003) | Stable | 21.5 | | 4 | 5 | -80 | -10 | -60 | Step |
| Ajmaline | 1.04 | HEK | Kiesecker <i>et al.</i> (2004) | Stable | 21 | | 5 | 0.4 | -80 | 40 | -120 | Step |
| Ajmaline | 42.3 | XO | Kiesecker <i>et al.</i> (2004) | | 21 | | 5 | 0.4 | -80 | 40 | -60 | Step |
| Ambasilide | 16.1 | CHO | Walker <i>et al.</i> (2000) | Stable | 22 | | 4.8 | 3.9 | -80 | -30 | -60 | Step |
| Ambasilide | 3.6 | CHO | Walker <i>et al.</i> (2000) | Stable | 22 | whole cell PC | 4.8 | 3.9 | -80 | 30 | -60 | Step |
| Amiodarone | 15 | XO | Niwa <i>et al.</i> (2003) | | 23 | Voltage-clamp 2-electrode | ? | 2 | -90 | 0 | -80 | Step |
| Amiodarone | 37.9 | XO | Kamiya <i>et al.</i> (2001b) | | 23 | | 5.4 | 2 | -90 | 40 | -70 | Step |
| Amiodarone | 9.8 | XO | Kiehn <i>et al.</i> (1999) | | 20 | | 5 | 0.4 | -80 | 30 | -60 | Step |
| Amiodarone | 2.347 | CHO | Guo and Guthrie (2005) | Stable | 23 | | 4 | 1 | -80 | 20 | -40 | Step |
| Amiodarone | 0.421 | CHO | Guo and Guthrie (2005) | Stable | 23 | | 4 | 1 | -80 | 20 | -40 | Step |
| Amiodarone | 0.048 | CHO | Guo and Guthrie (2005) | Stable | 35 | | 4 | 1 | -80 | 20 | -40 | Step |
| Amiodarone | 1.157 | CHO | Guo and Guthrie (2005) | Stable | 23 | | 4 | 4 | -80 | 20 | -40 | Step |
| Amiodarone | 0.244 | CHO | Guo and Guthrie (2005) | Stable | 23 | PatchXpress 7000A | 4 | 4 | -80 | 20 | -40 | Step |
| Amiodarone | 37 | XO | Lin <i>et al.</i> (2007a) | | 21.5 | | 5 | 1.2 | -80 | 40 | -40 | Step |
| Amiodarone | 24.7 | XO | Lin <i>et al.</i> (2007a) | | 21.5 | | 10 | 1.2 | -80 | 40 | -40 | Step |
| Vardenafil | 12.8 | HEK | Dustan Saraza <i>et al.</i> (2004) | Stable | 37 | | 4 | 0.5 | -75 | 10 | -40 | Step |
| Verapamil | 114 | XO | Duan <i>et al.</i> (2007) | | 21.5 | | 30 | 2 | -90 | 40 | -90 | Step |
| Verapamil | 0.136 | HEK | Kirsch <i>et al.</i> (2004) | Stable | 35 | | ? | 1 | -80 | 20 | -80 | Step ramp |
| Verapamil | 5.1 | XO | Duan <i>et al.</i> (2007) | | 21.5 | Voltage-clamp 2-electrode | 2 | 2 | -90 | 0 | -60 | Step |
| Verapamil | 0.444 | HEK | Kirsch <i>et al.</i> (2004) | Stable | 22 | | ? | 2 | -80 | 20 | -50 | Step |
| Verapamil | 0.37 | HEK | Kirsch <i>et al.</i> (2004) | Stable | 35 | | ? | 2 | -80 | 20 | -50 | Step |
| Verapamil | 0.41 | HEK | Martin <i>et al.</i> (2004) | Stable | 37 | | 5 | 3 | -80 | 0 | -50 | Step |
| Verapamil | 0.143 | HEK | Zhang <i>et al.</i> (1999) | Stable | 23 | | 4 | 4 | -80 | 20 | -50 | Step |
| Verapamil | 1.055 | CHO | Guo and Guthrie (2005) | Stable | 23 | | 4 | 1 | -80 | 20 | -40 | Step |
| Verapamil | 0.239 | CHO | Guo and Guthrie (2005) | Stable | 23 | | 4 | 1 | -80 | 20 | -40 | Step |
| Verapamil | 0.192 | CHO | Guo and Guthrie (2005) | Stable | 35 | whole cell PC | 4 | 1 | -80 | 20 | -40 | Step |
| Verapamil | 0.094 | HEK | Fossa <i>et al.</i> (2004) | Stable | 35 | Whole cell PC | 4 | 1 | -80 | 20 | -40 | Step ramp |
| Vesnarinone | 17.7 | XO | Kamiya <i>et al.</i> (2001a) | | 23 | Voltage-clamp 2-electrode | 2 | 2 | -90 | 0 | -80 | Step |
| Vesnarinone | 1.1 | HEK | Katayama <i>et al.</i> (2000) | ? | 23.5 | Whole cell PC | 5.4 | 4 | -80 | 20 | -60 | Step |
| Ziprasidone | 2.8 | XO | Su <i>et al.</i> (2006) | | 23 | Voltage-clamp 2-electrode | 2 | 5 | -90 | 0 | -90 | Step |
| Ziprasidone | 0.12 | HEK | Su <i>et al.</i> (2006) | Stable | 37 | Whole cell PC | 5 | 3 | -80 | 0 | -50 | Step |
| Ziprasidone | 0.24 | HEK | Ducroq <i>et al.</i> (2005) | ? | 20 | Whole cell PC | 4 | 1 | -80 | 30 | -40 | Step |
| Ziprasidone | 0.169 | CHO | Kongsamut <i>et al.</i> (2002) | ? | 23 | Whole cell PC | 5 | 2 | -80 | 20 | -40 | Step |

> 350

> 700

> 250

15



proarrhythmic potency assessment

in vitro hERG – extrapolation factors

in vitro

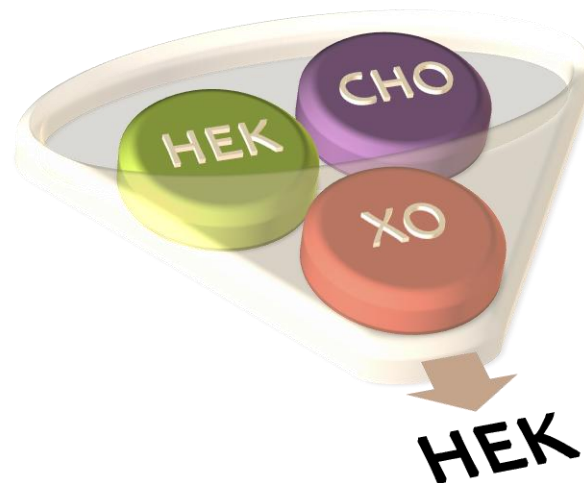
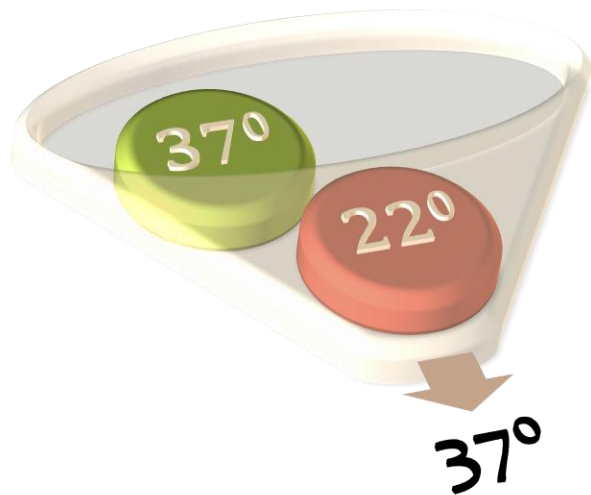


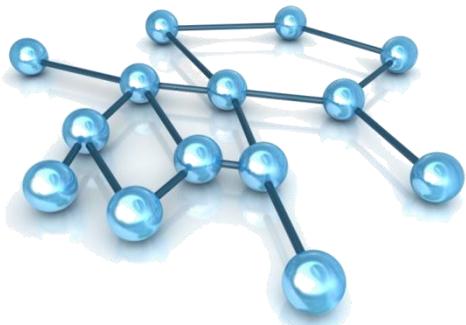
- **temperature extrapolation factor**

- HEK physiological temperature/HEK room temperature

- **temperature extrapolation factor**

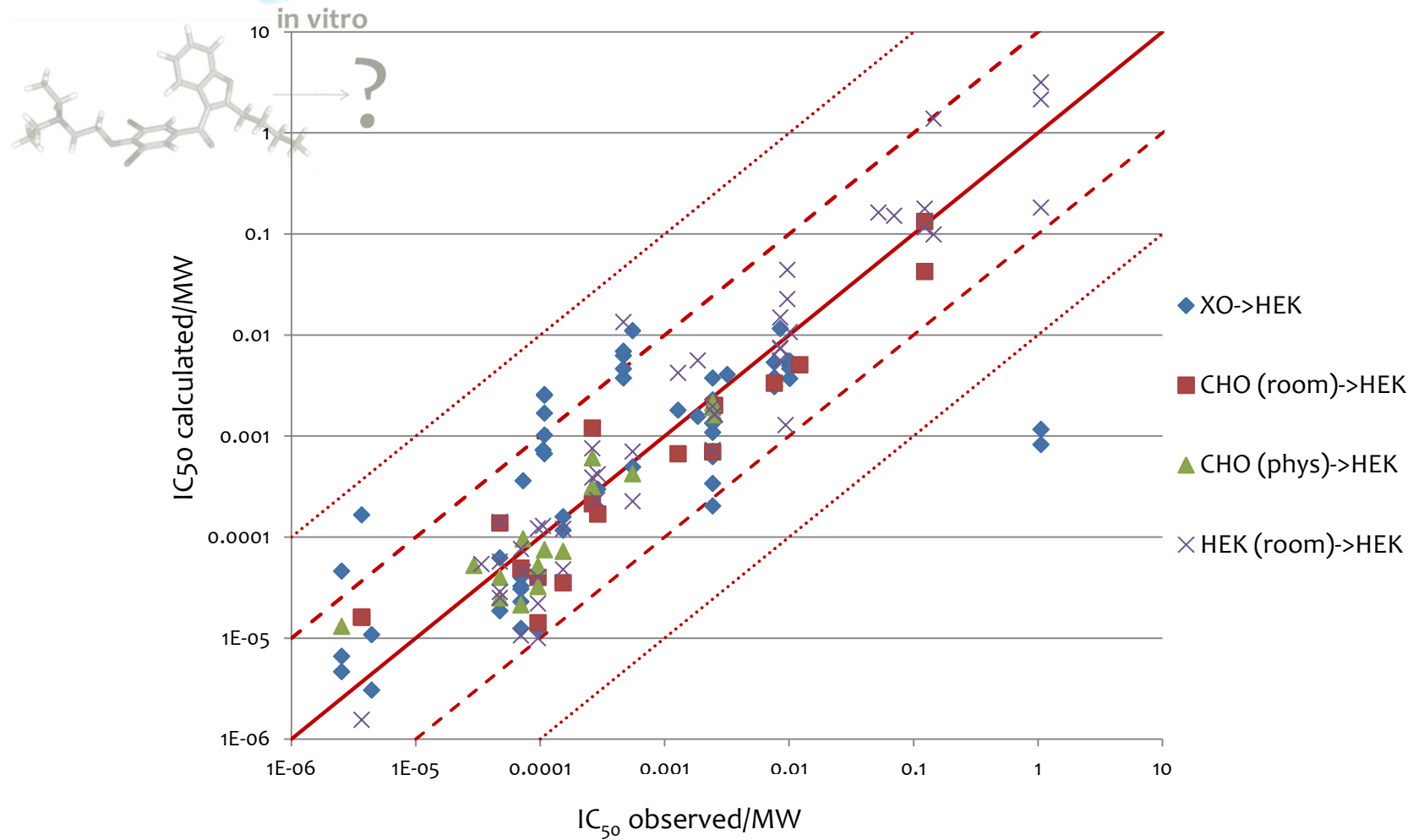
- inter-system ratios

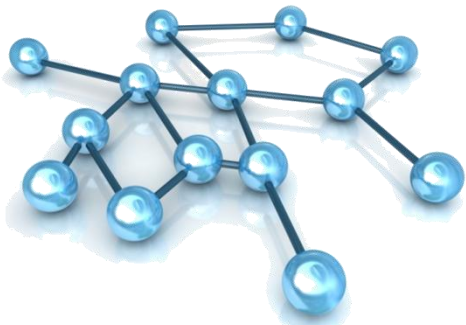




proarrhythmic potency assessment

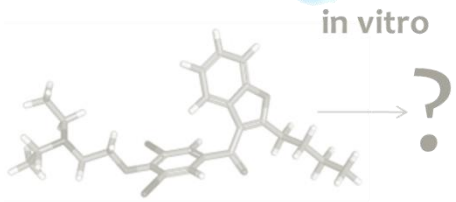
in vitro hERG – extrapolation factors



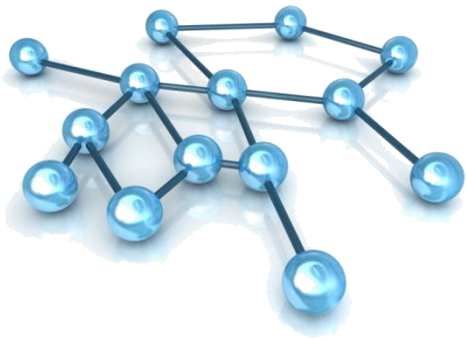


proarrhythmic potency assessment

in vitro hERG – extrapolation factors



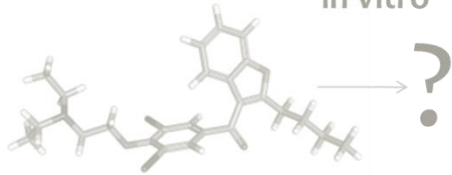
| | HEK_(room) HEK_(phys) | CHO_(room) HEK_(phys) | XO_(room) HEK_(phys) | CHO_(phys) HEK_(phys) |
|----------------|--|--|---|--|
| median | 1.42 | 2.38 | 22.85 | 1.02 |
| MAD | 0.97 | 1.68 | 17.31 | 0.41 |
| n (records) | 169 | 69 | 152 | 70 |
| n (substances) | 27 | 13 | 21 | 12 |
| zakres | 0.0312- 54.86 | 0.0767 - 31.25 | 0.0159 - 1740 | 0.049 – 16.67 |



proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods

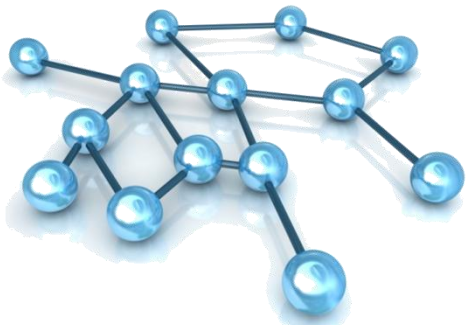
in vitro



- **INPUT:** in vitro experimental settings + phys-chem descriptors

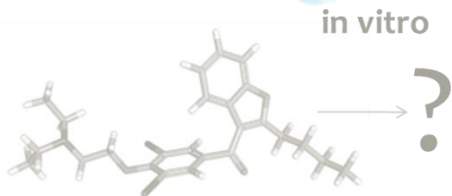
derived from the available literature

- cell model {XO / CHO / HEK}
- temperature [oC]
- K⁺ bath concentration [mM]
- t1 pulse [s]
- t2 pulse [s]
- holding potential [mV]
- depolarization level [mV]
- measurement potential [mV]



proarrhythmic potency assessment

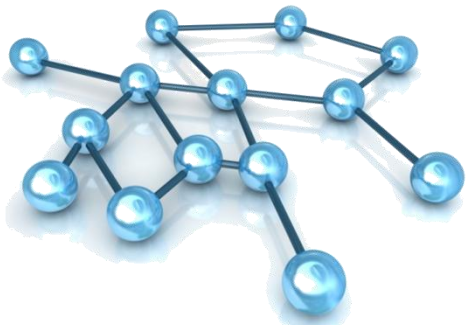
in vitro hERG - prediction algorithms & methods



- **INPUT:** in vitro experimental settings + phys-chem descriptors

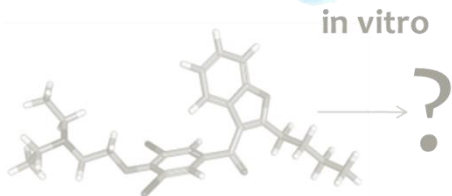
calculated in Marvin Beans package

- sdf files either derived from PubChem or drawn in MarvinSketch
- 41 plugins
- 107 numeric inputs natively
- 30 parameters after the sensitivity analysis



proarrhythmic potency assessment

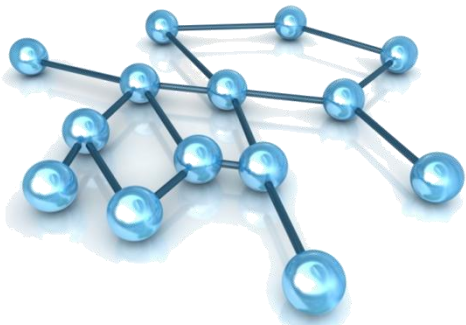
in vitro hERG - prediction algorithms & methods



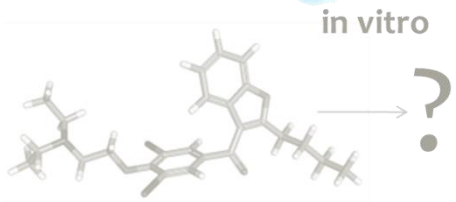
- **INPUT:** in vitro experimental settings + phys-chem descriptors

calculated in Marvin Beans package

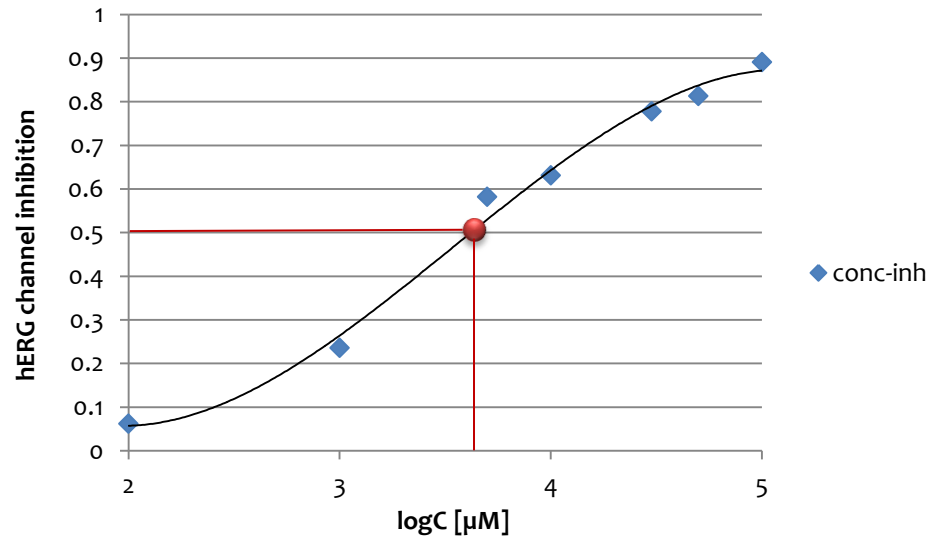
- aromaticAtomCount
- rotatableBondCount
- heteroaromaticRingCount
- ...
- dreidingEnergy
- ...
- logP
- bpKa1
- bpKa2

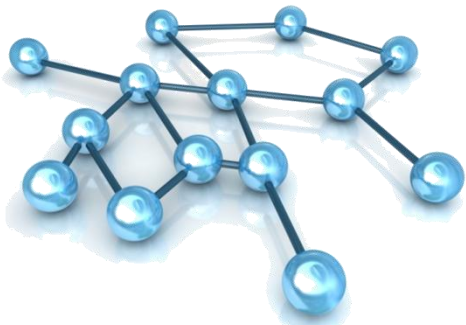


proarrhythmic potency assessment in vitro hERG - prediction algorithms & methods



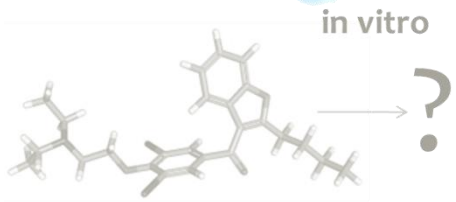
- **OUTPUT: IC_{50}**



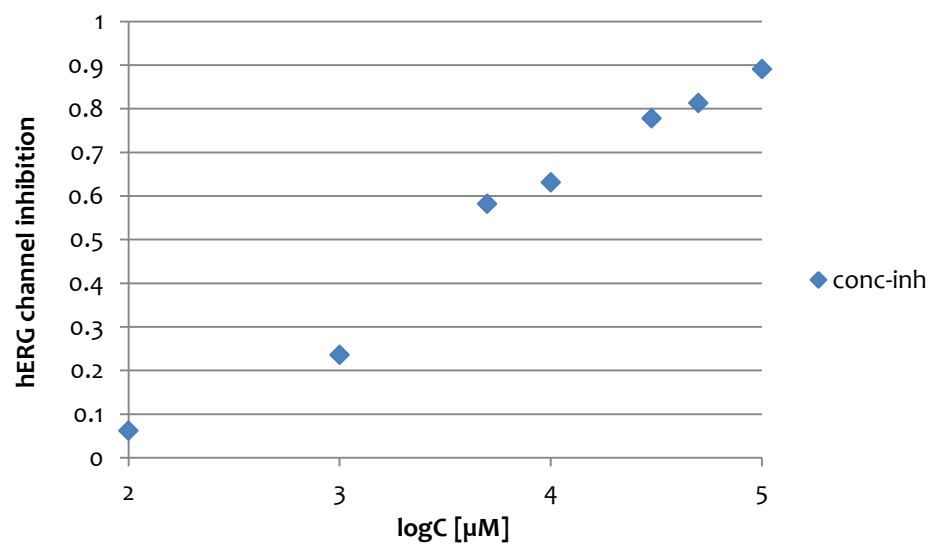


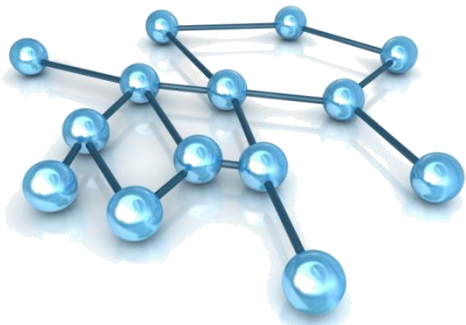
proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods



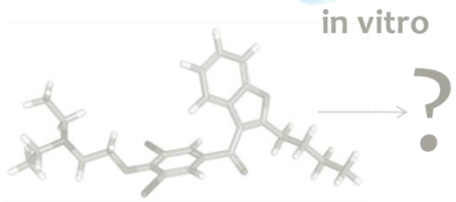
- **OUTPUT:** concentration – inhibition relation



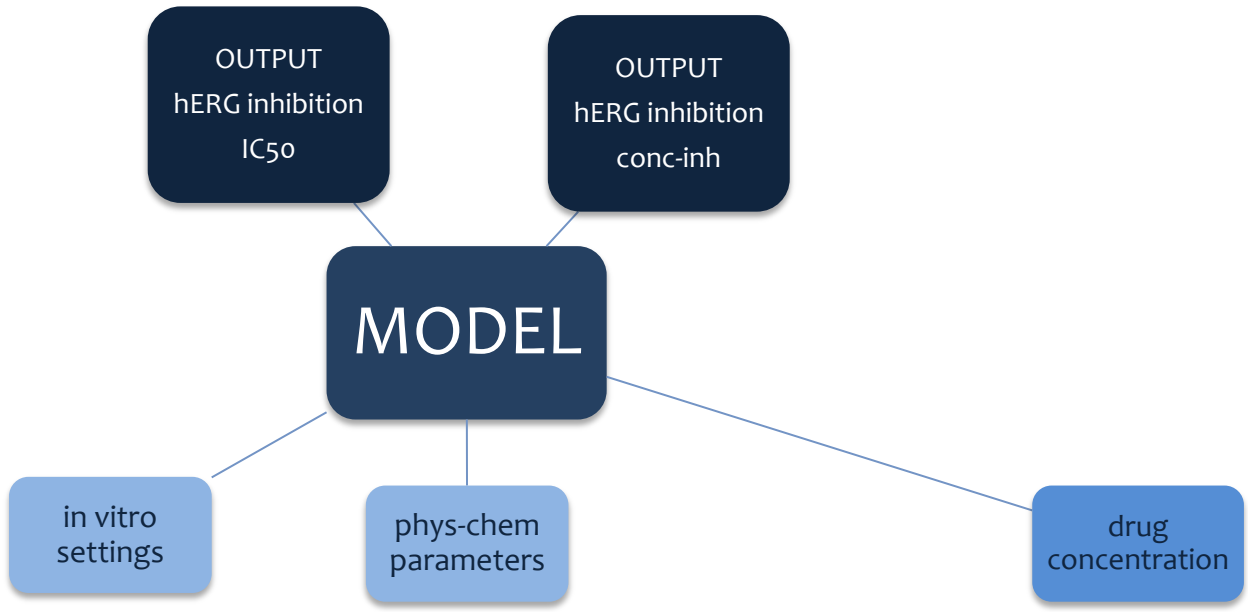


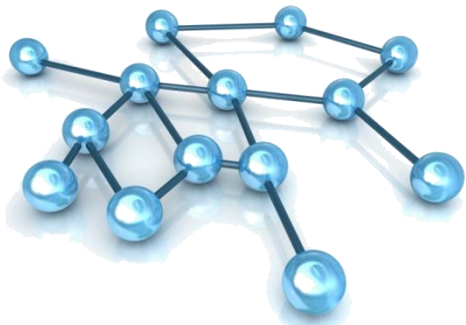
proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods



- **MODEL**

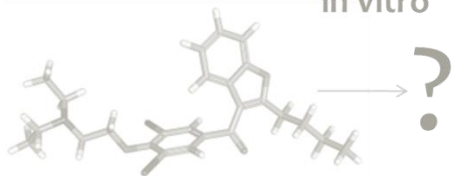




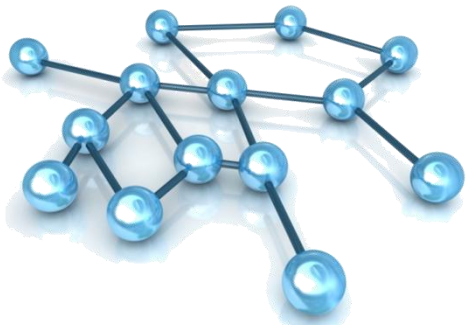
proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods

in vitro

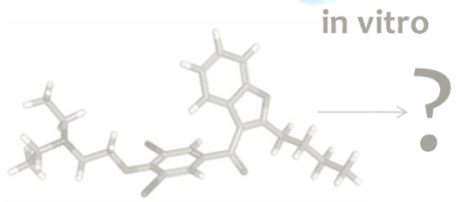


- **ALGORITHMS:** SVM; ANN; Random Forests (+ hybrid systems)
- **VALIDATION:** internal (10-fold CV) & external (separate dataset)
- **FLEXIBILITY:** various cell systems and settings



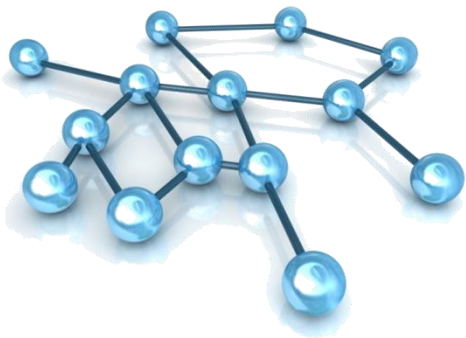
proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods



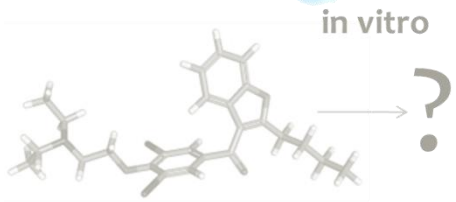
- **RESULTS conc-inh**

| model | RandomForest 100 trees | ANN 15_7_5 logistic | Expert committee (RF10+RF100+ANN) |
|-----------------|---------------------------|------------------------|--------------------------------------|
| validation RMSE | 0.21 | 0.20 | 0.20 |
| results | | | |



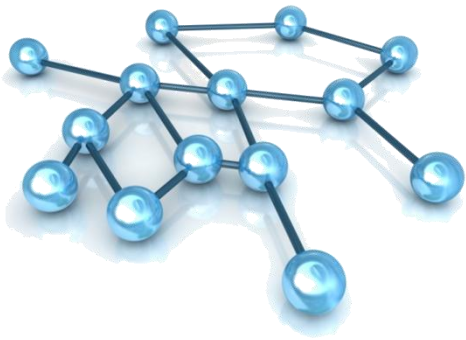
proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods



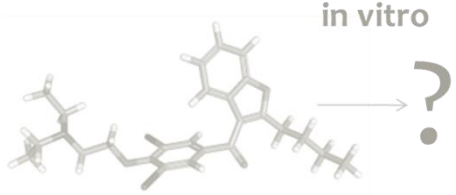
- **RESULTS conc-inh – expert committee**

| model | previously present structures | new structures |
|---------------|-------------------------------|----------------|
| within 2-fold | 89.41% | 76.85% |
| results | | |



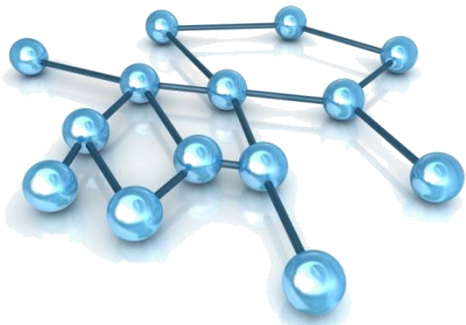
proarrhythmic potency assessment

in vitro hERG - prediction algorithms & methods



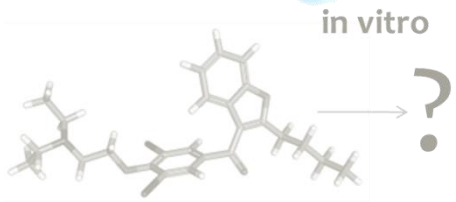
- **RESULTS conc-inh – expert committee**

| model | previously present structures | new structures |
|---------------|-------------------------------|--------------------|
| within 2-fold | 89.41% | 76.85% |
| results | <p>Methadone</p> | <p>Atomoxetine</p> |



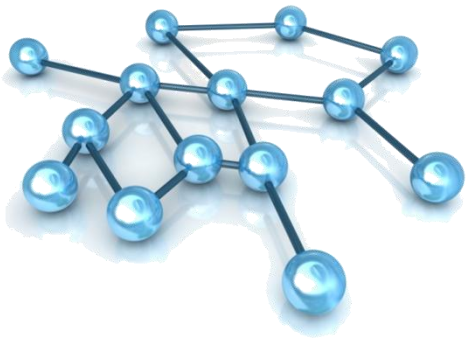
proarrythmic potency assessment

in vitro hERG - prediction algorithms & methods



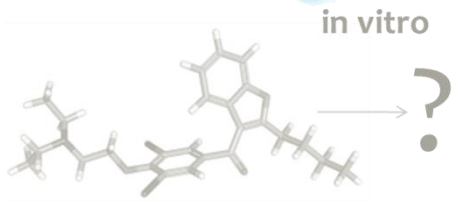
- **RESULTS conc-inh – expert committee**

| model | previously present structures | new structures |
|---------------|-------------------------------|--------------------|
| within 2-fold | 89.41% | 76.85% |
| results | <p>Sparfloxacin</p> | <p>Doxorubicin</p> |



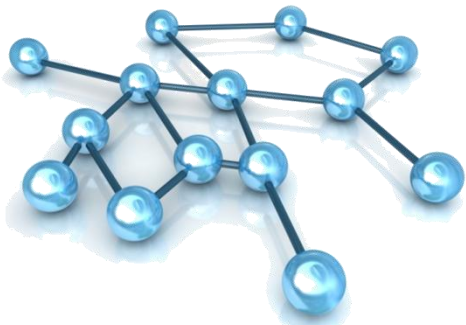
proarrhythmic potency assessment

hERG only?



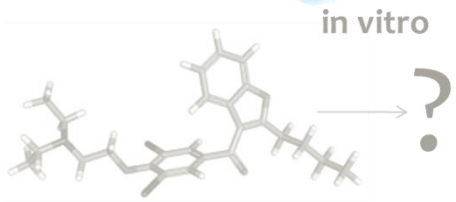
- multiple-channels inhibition

| Compound | IC [μM] | Model [XO/CHO/HEK] | temp [room/phys] | K+ [mM] | t1_puls [s] | t2_puls [s] | holding_pot [mV] | depol_pulse [mV] | measure_pot [mV] |
|-----------|-------------------------|-----------------------|---------------------|------------|----------------|----------------|---------------------|---------------------|---------------------|
| Verapamil | 114 | 1 | 1 | 30 | 2 | 2 | -90 | 40 | -90 |
| Verapamil | 5.1 | 1 | 1 | 2 | 2 | 2 | -90 | 0 | -60 |
| Verapamil | 0.094 | 3 | 2 | 4 | 1 | 120 | -80 | 20 | -40 |
| Verapamil | 0.192 | 2 | 2 | 4 | 1 | 0.5 | -80 | 20 | -40 |
| Verapamil | 0.136 | 3 | 2 | 4 | 1 | 200 | -80 | 20 | -80 |
| Verapamil | 0.37 | 3 | 2 | 4 | 2 | 2 | -80 | 20 | -50 |
| Verapamil | 0.444 | 3 | 1 | 4 | 2 | 2 | -80 | 20 | -50 |
| Verapamil | 0.41 | 3 | 2 | 5 | 3 | 4 | -80 | 0 | -50 |
| Verapamil | 0.215 | 3 | 2 | 4 | 2 | 4 | -80 | 20 | -40 |
| Verapamil | 0.94 | 2 | 1 | 4 | 0.4 | 0.4 | -80 | 20 | -40 |
| Verapamil | 0.143 | 3 | 1 | 4 | 4 | 6 | -80 | 20 | -50 |

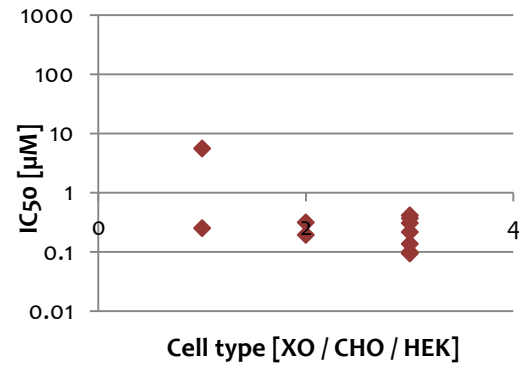
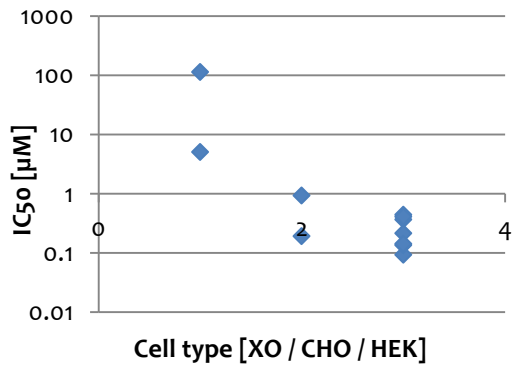


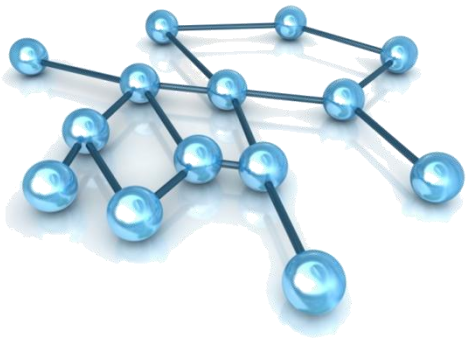
proarrhythmic potency assessment

hERG only?



- multiple-channels inhibition

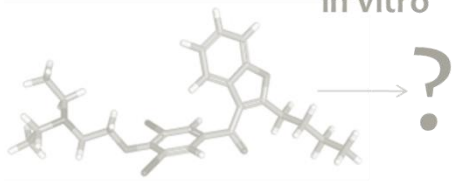




proarrhythmic potency assessment

hERG only?

in vitro



- multiple-channels inhibition

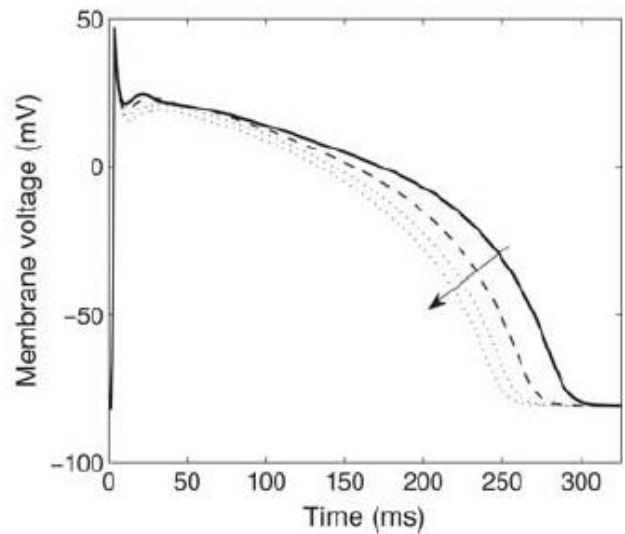
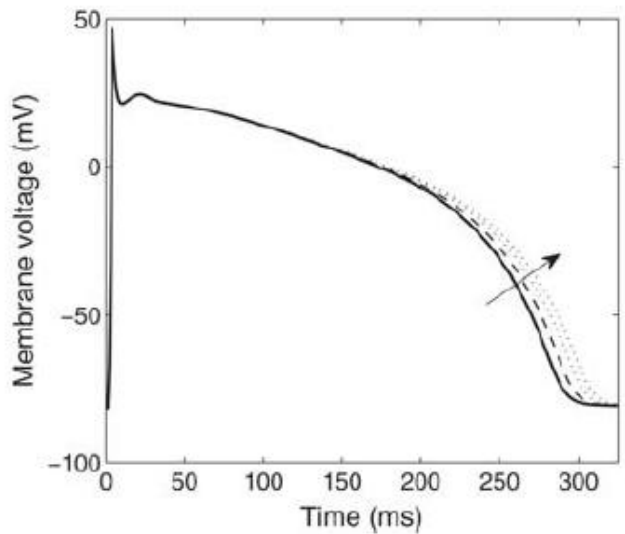
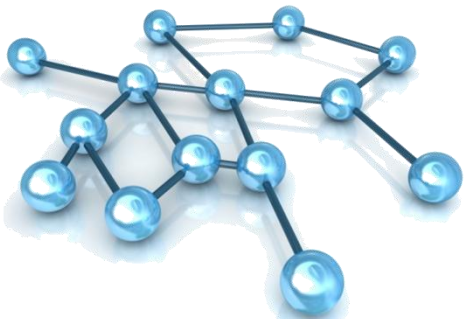


Figure 2 Simulation of steady-state 1 Hz pacing of the Grandi et al.²⁶ model under verapamil application when considering (left) a solely hERG block, and (right) a hERG, Na, and CaL block. Arrows indicate the effect on the AP of increasing drug concentration, displayed are: control (0 nM, solid line), low EFTPC (25 nM, dashed line), medium EFTPC (53 nM, dash-dotted line), and high EFTPC (81 nM, dotted line).

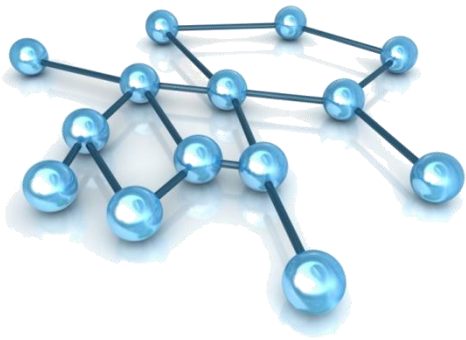


proarrhythmic potency assessment in vitro I_{K_S} (Kv7.1) – prediction algorithms & methods

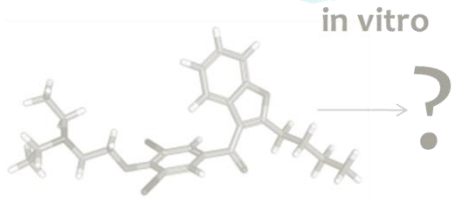
| Compound | IC ₅₀ | channel' | model' | source | temp' | K+ [mM] bath solution | t1 puls [s] | holding pot | depol puls to | mV pomiar |
|----------------|------------------|----------|--------|-----------------|-------|-----------------------|-------------|-------------|---------------|-----------|
| Amiodarone | 3.84 | 4 | 4 | Kamiya 2001a | 2 | 5.4 | 0.2 | -50 | 30 | -50 |
| Amiodarone | 1.2 | 4 | 4 | Zankov 2005 | 2 | | 2 | -50 | 30 | -50 |
| Amiodarone | 1.74 | 4 | 4 | Zankov 2005 | 2 | > 50 | 0.5 | -50 | 30 | -50 |
| AZD7009 | 193 | 1 | 2 | Persson 2005 | 1 | | 6 | -80 | 40 | 40 |
| Azimilide | 1.4 | 1 | 2 | Busch1998 | 1 | | 6 | -80 | 40 | 40 |
| Azimilide | 2.6 | 3 | 1 | Busch1998 | 1 | 2 | 15 | -80 | -10 | -10 |
| Azimilide | 3.1 | 3 | 1 | Persson 2005 | 1 | | 15 | -80 | -10 | -10 |
| Bepidil | 6.2 | 4 | 4 | Wang 1999a | 2 | 74 | 1 | -40 | 60 | -40 |
| Canrenoic_acid | 22.3 | 1 | 2 | Gomez 2005 | 1 | | 2 | -80 | 40 | -40 |
| Chromanol_293B | 26.9 | 2 | 1 | Loussouarn 1997 | 1 | | 3 | -80 | 40 | 40 |
| Chromanol_293B | 6.9 | 1 | 1 | Lerche 2007 | 1 | 2 | 12 | -80 | 40 | 40 |

50

4



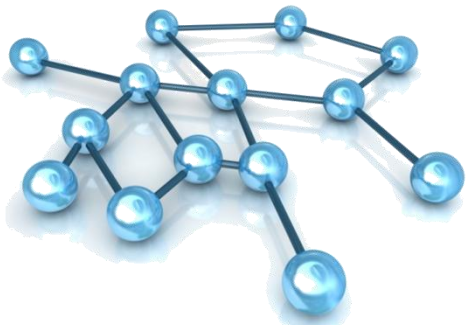
proarrhythmic potency assessment in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



- **INPUT:** in vitro experimental settings + phys-chem descriptors

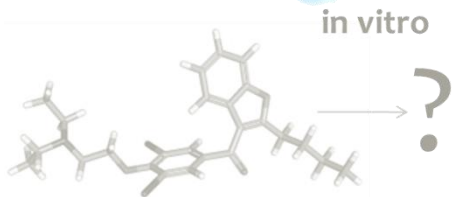
derived from the available literature

- cell model {XO / CHO / HEK / guinea pigs cm}
- channel type {LQT / minK}
- temperature [oC]
- t1 pulse [s]
- measurement potential [mV]



proarrhythmic potency assessment

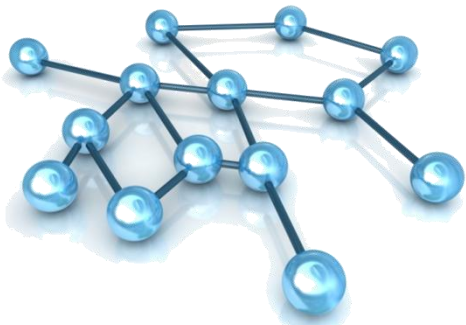
in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



- **INPUT:** in vitro experimental settings + phys-chem descriptors

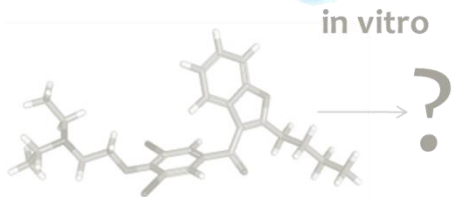
calculated in Marvin Beans package

- sdf files either derived from PubChem or drawn in MarvinSketch
- 41 plugins
- 107 numeric inputs natively
- 15 parameters after the sensitivity analysis



proarrhythmic potency assessment

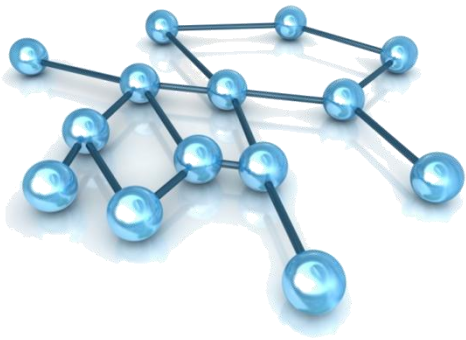
in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



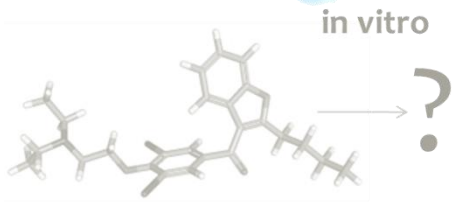
- **INPUT:** in vitro experimental settings + phys-chem descriptors

calculated in Marvin Beans package

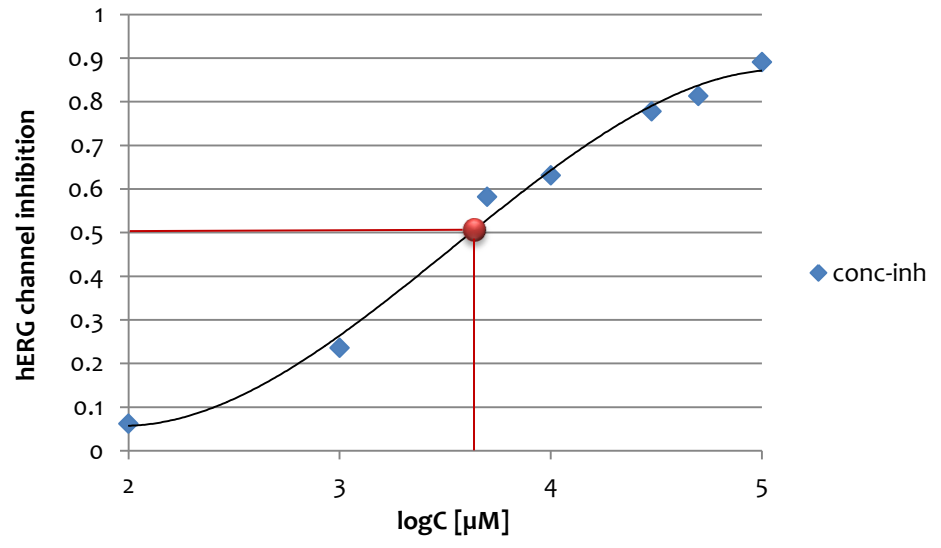
- Minimal_projection_area
- Largest_ring_size
- ...
- apKa2
- bpKa1
- ...
- Minimal_projection_radius
- Maximal_projection_radius

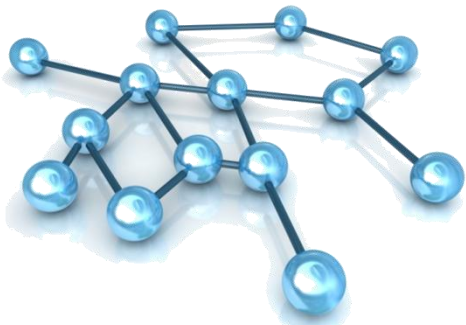


proarrhythmic potency assessment in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods

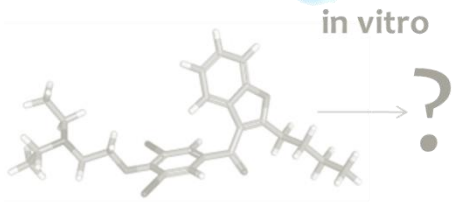


- **OUTPUT: IC_{50}**

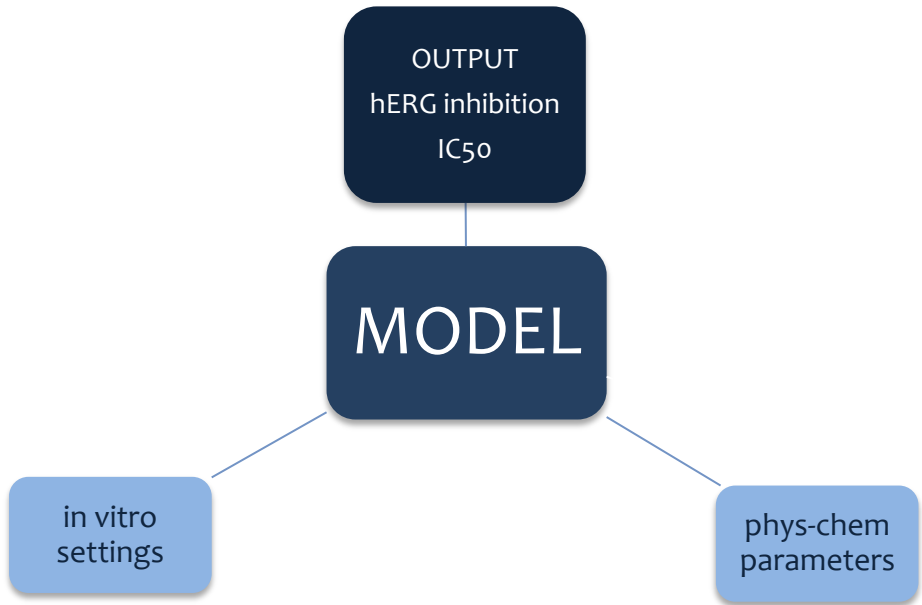


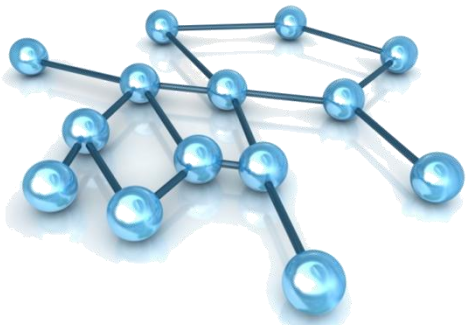


proarrhythmic potency assessment in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



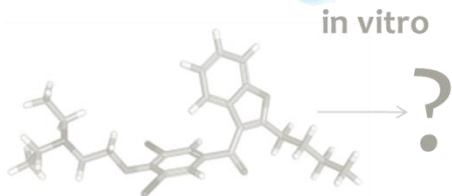
- **MODEL**



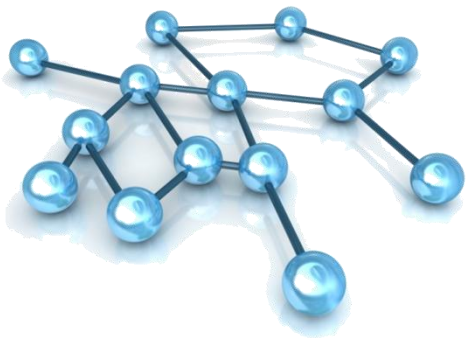


proarrhythmic potency assessment

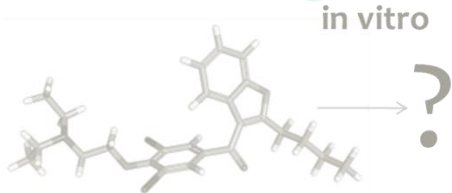
in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



- **ALGORITHMS:** SVM; ANN; Random Forests (+ hybrid systems)
- **VALIDATION:** internal (10-fold CV)
- **FLEXIBILITY:** various cell systems and settings

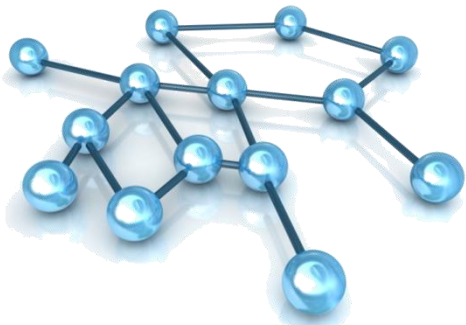


proarrhythmic potency assessment in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



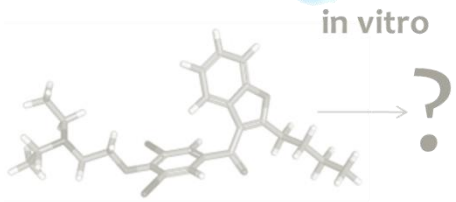
- RESULTS IC₅₀**

| model | SMO reg | ANN expert system | Expert committee (RF10+RF100+ANN) |
|----------------|---------|-------------------|-----------------------------------|
| val RMSE | 0.159 | 0.14 | 0.144 |
| R ² | 0.46 | 0.52 | 0.50 |
| results | | | |

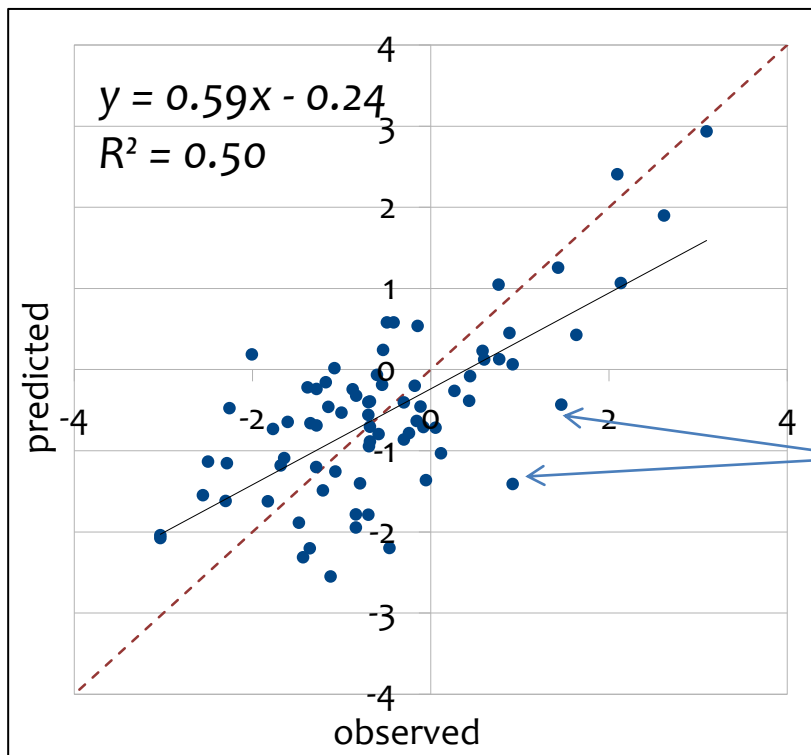


proarrhythmic potency assessment

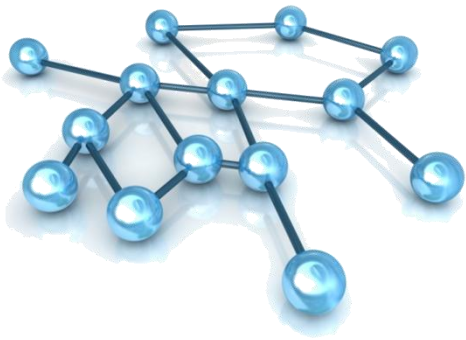
in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods



- **RESULTS IC₅₀ – expert system**

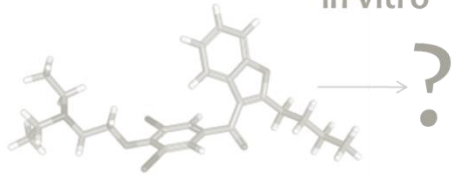


- tends to underpredict
- relatively small number of both tyoe of errors

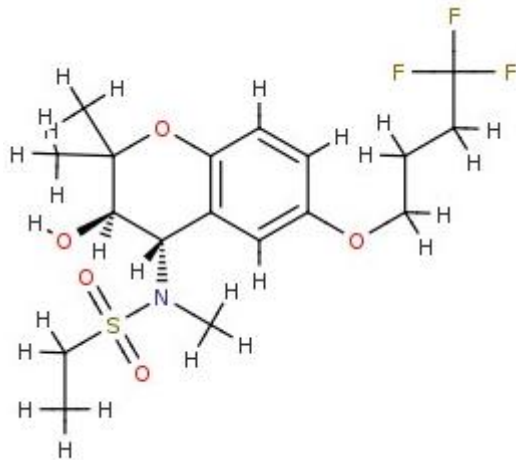


proarrhythmic potency assessment in vitro I_{Ks} (Kv7.1) – prediction algorithms & methods

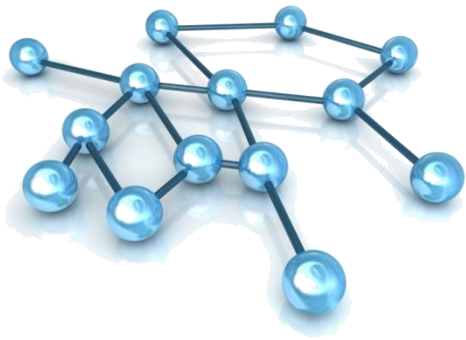
in vitro



- **RESULTS IC₅₀ – HMR1556**

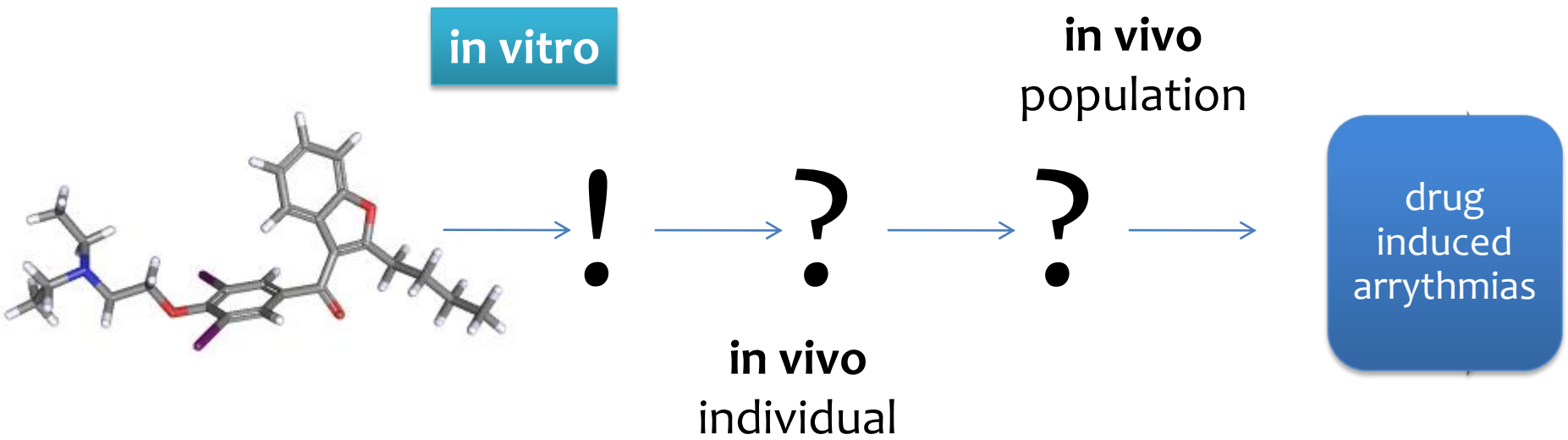


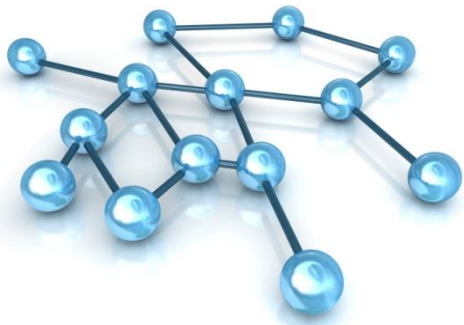
- structurally/physically similar to chromanols
- various in vitro settings tested



proarrhythmic potency assessment

in silico – in vitro – in vivo extrapolation





acknowledgements

team

Barbara Wiśniowska PhD

Aleksander Mendyk PhD

Miłosz Polak

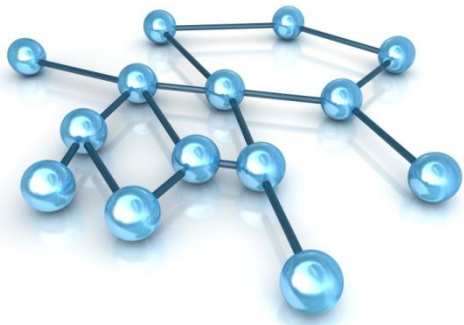
Kamil Fijorek

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THANK YOU



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Jagiellonian University Medical College

