

Docking model of K_{Ca}**3.1 and K**_{Ca}**2.2 CaM-BD/CaM complexes with SKA-111.** In the K_{Ca}3.1 model the amino group of the benzothiazole ring system of SKA-111 forms hydrogen bonds with M51 and E54 in calmodulin. E54 is further stabilized by an extensive hydrogen bond network with R362, E295 and N300 in the K_{Ca}3.1 channel model, which we hypothesized to be responsible for the 100-fold K_{Ca}3.1 selectivity of SKA-111. In the K_{Ca}2.2 model, however, SKA-111 forms only hydrogen bonds with M51 and E54. Due to the shorter length of the N474 side chain in K_{Ca}2.2 E54 is not stabilized by additional hydrogen bonds. The CaM-BD is shown in light green; CaM is colored pink.

The eight 2-aminobenzothiazoles shown below, which all display some degree of selectivity for $K_{Ca}3.1$ over $K_{Ca}2$ channels, converged on similar binding poses as SKA-111 with an extensive hydrogen bond network centered around R362 in the $K_{Ca}3.1$ model and hydrogen bonds between the -NH₂ group and M51 and E54 in CaM in both the $K_{Ca}3.1$ and the $K_{Ca}2.2$ model.

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SKA-31	SKA-44	SKA-45	SKA-72	SKA-73	SKA-107	SKA-117	SKA-120

Chemical structures of the 168 virtually designed and docked compounds. Highlighted compounds were synthesized and tested.



SKA-182	SKA-183	SKA-184	SKA-185	SKA-186	SKA-187	SKA-188	SKA-189
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SKA-198	SKA-199 ^{H₂N}	SKA-200	SKA-201 ^{H₂N}	SKA-202	SKA-203	SKA-204 \	SKA-205 I
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			SKA-265	SKA-266	SKA-267	SKA-268	SKA-269
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							SKA-269 \downarrow^{CF_3} \downarrow^{N} \downarrow^{N}
	SKA-203	SKA-272	SKA-273	SKA-266	SKA-267	SKA-276 SKA-276	SKA-269
	SKA-263	$\frac{C}{CF_{3}}$	SKA-265	SKA-266	SKA-267	SKA-268 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{-S} \downarrow^{N} \downarrow^{-S} SKA-276 \downarrow^{CF_3}	
$\begin{array}{c} SKA-202 \\ CI \\ CI \\ Fi \\ SKA-270 \\ SKA-270 \\ CF_3 \\ Fi \\ Fi \\ Fi \end{array}$	SKA-263	$\frac{SKA-264}{C}$	SKA-265 N HN SKA-273 CF_3 HN SKA-273	SKA-266 \downarrow^{N} \downarrow	$SKA-267$ $N \rightarrow KA-267$ CF_3 $SKA-275$ CF_3 $FN \rightarrow S$	SKA-268 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} SKA-276 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S}	SKA-269
	SKA-263	SKA-264	SKA-265	SKA-266 N HN N N HN N N N N N N N	$SKA-267$ $N \rightarrow CF_3$ $SKA-275$ $SKA-275$ CF_3 $HN \rightarrow CF_3$	SKA-268 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} SKA-276 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{CF_3} \downarrow^{CF_3}	SKA-269 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} SKA-277 H_2N \downarrow^{S}
$\frac{SKA-202}{CI}$	SKA-263	$\frac{SKA-264}{CF_{3}}$	SKA-265 N HN SKA-273 CF3 HN SKA-273	$\frac{SKA-266}{N}$	$\frac{SKA-267}{N}$	SKA-268 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} SKA-276 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} \downarrow^{S} \downarrow^{CF_3}	SKA-269
$\frac{SKA-202}{CI}$	SKA-263	$\frac{SKA-264}{CF_{3}}$	SKA-265 $N \rightarrow N$ $HN \rightarrow S$ $N \rightarrow CF_3$ $HN \rightarrow S$ $N \rightarrow CF_3$ $N \rightarrow CF_3$	$SKA-266$ $\xrightarrow{N}_{HN} \xrightarrow{S}_{N} \xrightarrow{S}_{V}$ $SKA-274$ $\xrightarrow{KA-274}_{V} \xrightarrow{CF_3}_{N} \xrightarrow{S}_{V} \xrightarrow{C}_{N}$	$\begin{array}{c} SKA-267 \\ & \overset{N}{\underset{HN}{\overset{S}{\underset{N}{\overset{N}{\underset{N}{\overset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\underset{N}{\overset{S}{\underset{N}{\atop\atopN}{\underset{N}{\underset{N}{\atopN}{\underset{N}{\underset{N}{\atopN}}}}}}}}}}}}}}}}}}}}$	SKA-268 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} \downarrow^{S} \downarrow^{CF_3} \downarrow^{S} \downarrow^{CF_3} \downarrow^{S} \downarrow^{S} \downarrow^{CF_3} \downarrow^{S}	SKA-269 \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow
SKA-202 \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow	SKA-263 \downarrow CI \downarrow CI \downarrow CI SKA-271 \downarrow CF ₃ \downarrow CF ₃ \downarrow CI \downarrow C	SKA-2704 HN $SKA-272KA-272FHN$ $FFFF$	SKA-273 SKA-273 \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow	SKA-266 $N \rightarrow N$ $HN \rightarrow SKA-274$ SKA-274 $V \rightarrow CF_{3}$ $HN \rightarrow SKA-282$	SKA-267 N HN CF_3 SKA-275 CF_3 HN N CF_3 CF_3 CF_3 HN N CF_3 C	SKA-268 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{O} SKA-276 \downarrow^{O}	SKA-269 \downarrow^{CF_3} \downarrow^{HN} \downarrow^{S} SKA-277 H_2N \downarrow^{H_2N} \downarrow^{S
$\begin{array}{c} SKA-202 \\ CI \\ CF_3 \\ $	SKA-263 \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow	SKA-264	SKA-265 N HN SKA-273 \downarrow CF ₃ HN \downarrow SKA-281 H ₂ N	SKA-266 N N N N N N N N	SKA-267 N HN CF_3 SKA-275 CF_3 HN SKA-275 CF_3 HN SKA-283 H_2N CF_3	SKA-268 CF3 SKA-276 SKA-276 SKA-284 SKA-284	SKA-269
$\begin{array}{c} SKA-270\\ CI\\ CI\\ F_{N}\\ SKA-270\\ CF_{3}\\ SKA-278\\ F_{N}\\ SKA-278\\ F_{N}\\$	SKA-263	$SKA-264$ $\downarrow \downarrow $	SKA-265 N HN SKA-273 G G G G G G G G	SKA-266 $SKA-274$ $SKA-274$ $SKA-274$ $SKA-282$ $SKA-282$ $SKA-282$	$\begin{array}{c} SKA-267 \\ & \searrow \\ & & & \\ N \\ & & & \\ N \\ & & & \\ N \\ & & \\ CF_3 \end{array}$ $\begin{array}{c} SKA-275 \\ & & & \\ SKA-275 \\ & & & \\ N \\ & & & \\ N \\ & \\ \\ N \\ & \\ \\ N \\ & \\ \\ N \\ & \\$	$\frac{SKA-268}{CF_{3}}$	SKA-269
$\begin{array}{c} SKA-202 \\ CI \\ CI \\ CI \\ SKA-270 \\ SKA-270 \\ CF_3 \\ SKA-278 \\ F_2 \\ N \\ SKA-278 \\ SKA-278$	SKA-263	SKA-264	SKA-273 SKA-273 \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow \downarrow	SKA-266 \downarrow_{HN} $\downarrow_{SKA-274}$ SKA-274 \downarrow_{HN} \downarrow_{CF_3} \downarrow_{HN} \downarrow_{CN} SKA-282 \downarrow_{HN} \downarrow_{SH_2} \downarrow_{HN} \downarrow_{SH_2}	SKA-267 $N \rightarrow CF_3$ SKA-275 SKA-275 $N \rightarrow CF_3$ $N \rightarrow CF_3$	SKA-268 $\downarrow_{H_{n}}^{CF_{3}}$ SKA-276 $\downarrow_{H_{n}}^{CF_{3}}$ $\downarrow_{H_{n}}^{O}$ SKA-284 $\downarrow_{H_{n}}^{S}$ SKA-284	SKA-269 SKA-277 SKA-277 H ₂ N SKA-285 F ₃ C SKA-285
$\begin{array}{c} SKA-270\\ KA-270\\ KA-270\\ KA-270\\ KA-270\\ KA-278\\ K$	SKA-263	$\begin{array}{c} SKA-264 \\ & \swarrow \\ H_{N} \downarrow \\ H$	SKA-265 N HN SKA-273 CF_3 HN SKA-281 H_2N SKA-281	SKA-266 \xrightarrow{N}_{HN} \xrightarrow{S}_{N} \xrightarrow{V}_{CN} SKA-274 \xrightarrow{V}_{N} \xrightarrow{V}_{CN} \xrightarrow{V}_{CN} \xrightarrow{V}_{N} \xrightarrow{V}_{CN} \xrightarrow{V}_{N} \xrightarrow{V}_{N	SKA-267 \downarrow_{HN} \downarrow_{S} \downarrow_{CF_3} SKA-275 \downarrow_{CF_3} \downarrow_{NO_2} SKA-283 \downarrow_{HN} \downarrow_{S} \downarrow_{NO_2}	$\frac{SKA-268}{CF_3}$	SKA-269 CF3 CF3 SKA-277 H2N SKA-277 H2N SKA-285 F3C SKA-285



QPatch screening of activator compounds on $K_{Ca}3.1$ and $K_{Ca}2.2$ channels with 250 nM of free Ca^{2+} in the internal solution.



Raw current traces (top) and plot of current slope (measured between - 85 and - 65 mV) versus time for K_{Ca} activation (bottom). SKA-31 was used as the positive control. Saline or drugs were perfused during the first 15 sec of each so-called liquid period.



Raw current traces (top) and plot of current slope (measured between - 85 and - 65 mV) versus time for K_{Ca} activation (bottom). SKA-31 was used as the positive control.

Note: $K_{Ca}2.2$ currents are smaller than $K_{Ca}3.1$ currents and show some rectification at positive potentials.



VDW interactions

Hydrogen bonds

SKA-111 in Kca3.1 open state 2 (pdb id: 6cno)

The channel is shown in gray and the CaM N-lobe in yellow. Hydrogen bonds are shown in black, van der Waals interactions are visualized in purple. SKA-111 forms one hydrogen bond with M51 (right) and makes 12 van der Waals contacts with residues in the S₄₅A helix and the CaM N-lobe (left).



Overlay of the Rosetta docking models of SKA111 in open state 1 (yellow and light grey) and open state 2 (orange and sky blue). SKA-111 is shown in dark grey in open state 1 and in light blue in open state 2.



SKA-31 (left) and SKA-121 (right) in KCa3.1 open state 1 (pdb id: 6cnn)

The channel is shown in gray and the CaM N-lobe in yellow. Hydrogen bonds are shown in black, van der Waals interactions are visualized in purple. For clarity, not all side chains of CaM residues within contact range of ligand are explicitly shown.



Rosetta ligand docking models of the top 50 lowest energy-binding poses of SKA-111 in the interface between the CaM N-lobe (yellow: WT, green: S372R) and the S₄₅A helix (light gray) of wild type KCa3.1 and the S372R K_{Ca}3.1 mutant. Molecular docking shows that SKA-111 converged well in wild type K_{Ca}3.1 but not in the S372R KCa3.1 mutant channel.