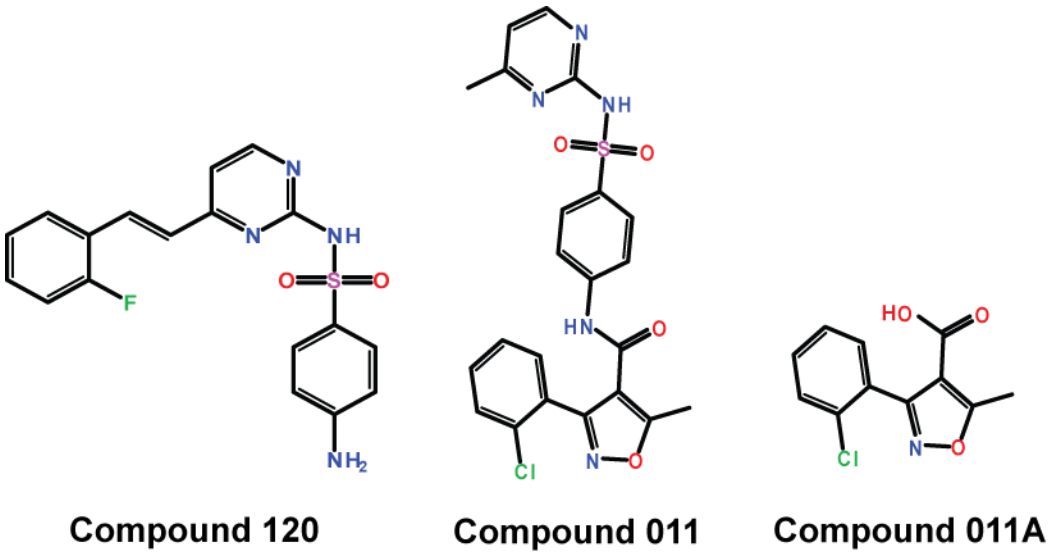
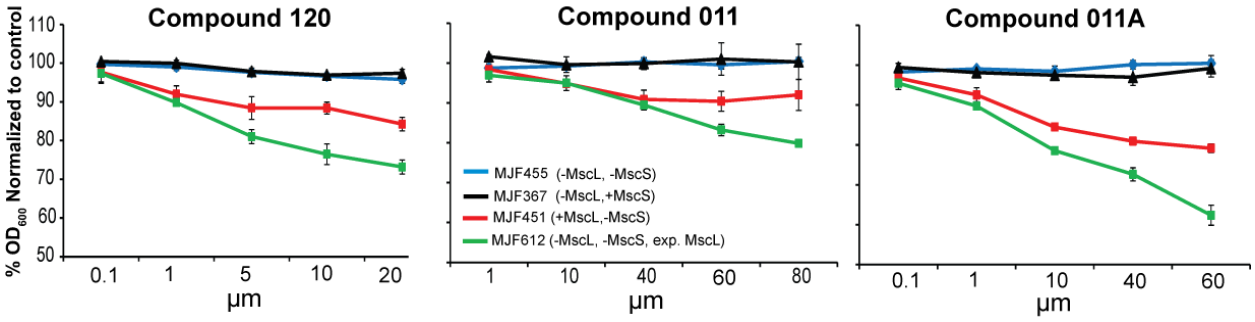


S1. Chemical structures of the three compounds used in this paper.



S2. All three compounds in this study affect cultures with endogenous levels of expression of Eco MscL. Shown are concentration curves for cultures treated with compounds 120, 011 and 011A. Cell lines used are MJF455 (ΔMscL, ΔMscS) in blue, MJF367 (ΔMscL, +MscS) in black, MJF451 (+MscL, ΔMscS) in red, with endogenous levels of expression of MscL, and MJF612 (ΔMscL, ΔMscS) expressing Eco-MscL in trans in green. Values represent the percentage of decreased growth (OD_{600}) in the presence of compounds, 120, 011 or 011A, relative to the non-treated n = 3 to 4.

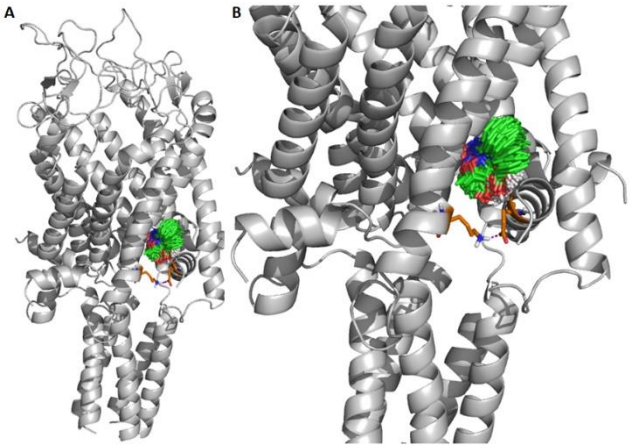


Fig. S3. The dynamics of 011A at the binding site. (A) All the 011A conformations mapped to the representative MD conformation of Eco-MscL/011A. (B) a focused look of Eco-MscL/011A binding. Glu6 and Lys369/97 forming a stable salt bridge are shown as brownish sticks. 011A, which has a large translational/rotation flexibility during MD simulations has occupied a large part of binding pocket. These data may help to explain why some cysteines in/near the pocket are not very sensitive to the MTS-PEG5000 experiments. We calculated the volume of individual 011A conformation and the whole conformational ensemble. The volume of the individual conformations, V_i, is 190.3 ± 1.3 Å^3, and the volume of ensemble, 011A is V_e is 949.4 Å^3. Because V_e is much larger than V_i,, 011A is able to avoid direct competition with the MTS-PEG5000 modified cysteine. However, with Glu6 and K396/97 the E6C and K97C mutations destroyed the salt bridge, which may normally play a critical role of maintaining the integrity of the binding pocket.

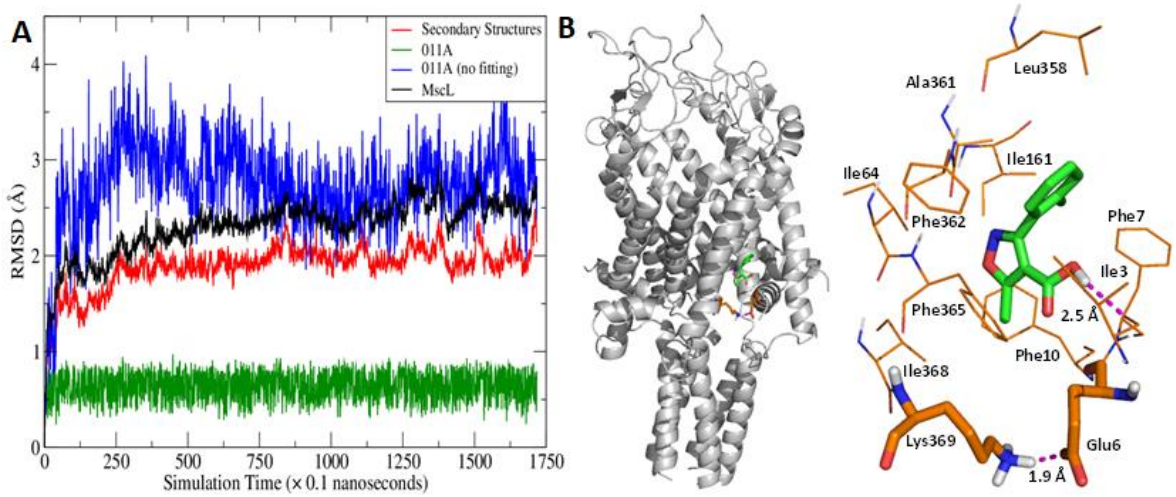
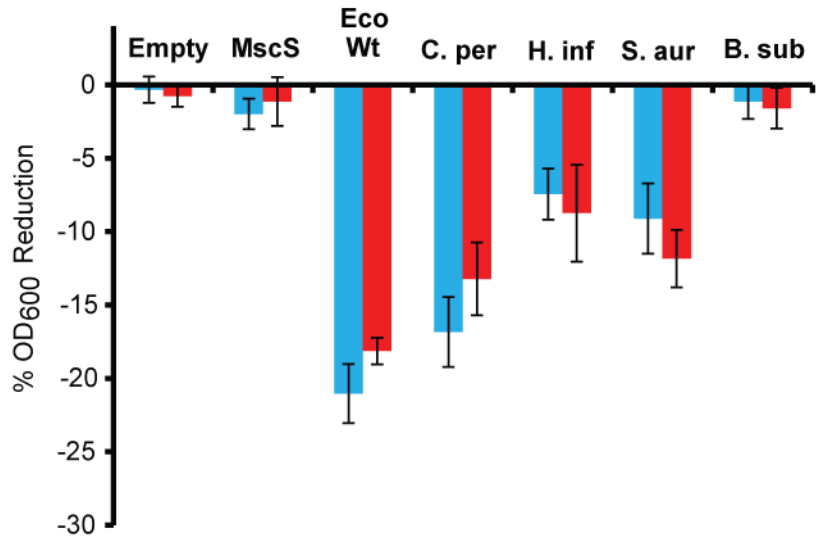


Fig. S4. MD simulation for Eco-MscL/011A. (A): the RMSDs ~ Simulation time plots. The system is stable after ~20 nanosecond equilibrium phase as measured by the root-mean-square deviation (RMSD) of the secondary structures of Eco-MscL (red curve). The conformation of 011A is very stable as its RMSD after fitting are around 0.5 Å (green curve), however, 011A has considerable translational/rotational mobility as its RMSD without fitting are much larger (blue curve). (B) The representative conformation, which has the smallest RMSD to the average MD structure. C) key residues for Eco-MscL/011A binding. 011A is shown as green sticks and Lys 360/97 and Glu6 which form a salt bridge are shown as brown sticks. A hydrogen bond is formed between the carboxyl hydrogen of 011A and the carbonyl oxygen of Ile3. It is pointed out that a strong salt bridge is formed between Glu6 and Lys 369/97. The salt bridge is very stable within the whole MD simulation period: its occupancy is 84.3% if the distance cutoff of 2.0 Å is applied; and the occupancy increases to 92.1% if a larger cutoff of 2.5 Å is applied. The distance is the shortest distance formed by two carboxyl oxygen atoms of Glu6 and the three-ammonium hydrogen atoms of Lys369.



S5. Bsub-MscL is resistant to compounds 011 and 011A. Reduction in bacterial growth (OD_{600}) for cultures treated with compounds 011 (red) and 011A (blue) relative to non-treated are shown. The effects of the compounds at a 40 µM concentration were studied in cultures of MJF455 carrying an empty plasmid (empty) or expressing Eco-MscS (MscS), Eco-MscL (Eco Wt), a slight GOF mutation for Eco-MscL K55T (Eco K55T), and the orthologues of MscL from *Clostridium perfringens (C. per), Haemophilus influenzae* (H. inf), *Staphylococcus aureus* (S. aur) and *Bacillus subtilis* (B. sub) n = 3 to 5.