

Supporting Information

Multi-fold Computational Analysis to Discover Novel Putative Inhibitors of isethionate sulfite-lyase (Isla) from *Bilophila wadsworthia*: Combating colorectal cancer and inflammatory bowel diseases

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Table S1. Physiochemical properties of compounds **1-9** calculated by SwissADME server.

| Compounds | ZINC ID | Physiochemical properties | | | | | | Water Solubility | Lipophilicity | Drug likeness | | |
|---|---|---------------------------|------------------------|-----|-------|-----|-----|------------------|---------------|---------------|-------|--------|
| | | MWT (g/mol) | TPSA (Å ²) | NRB | MR | HBA | HBD | Class | Log Po/w | Lipinski | Veber | Muegge |
| CP1 | <chem>Fc1cnc(c(n1)C(=O)N)O</chem> | 157.1 | 89.1 | 1 | 32.11 | 5 | 2 | Very soluble | 0.7 | 0 | 0 | 1 |
| CP2 | <chem>Nn1c(=S)[nH]nc1c1ccncc1</chem> | 193.23 | 104.61 | 1 | 50.79 | 2 | 2 | Very soluble | 0.88 | 0 | 0 | 1 |
| CP3 | <chem>OC(=O)CCc1ccc(cc1)O</chem> | 166.17 | 57.53 | 3 | 44.82 | 3 | 2 | Very soluble | 1.19 | 0 | 0 | 1 |
| CP4 | <chem>Nn1c(=S)[nH]nc1c1ccncc1</chem> | 193.23 | 104.61 | 1 | 50.79 | 2 | 2 | Very soluble | 0.97 | 0 | 0 | 1 |
| CP5 | <chem>Sc1nnc(o1)c1cc2c([nH]1)ccccc2</chem> | 217.25 | 93.51 | 1 | 58.84 | 3 | 1 | Soluble | 2.11 | 0 | 0 | 0 |
| CP6 | <chem>Clc1ccc(c(c1)F)c1nnc(o1)S</chem> | 230.65 | 77.72 | 1 | 51.95 | 4 | 0 | Soluble | 2.32 | 0 | 0 | 0 |
| CP7 | <chem>Sc1nnc(o1)Cc1ccccc1F</chem> | 210.23 | 77.72 | 2 | 50.96 | 4 | 0 | Soluble | 2.2 | 0 | 0 | 0 |
| CP8 | <chem>NC(=S)N/N=C/c1ccc(cc1)[N+](=O)[O-]</chem> | 224.24 | 128.32 | 4 | 62.02 | 3 | 2 | Soluble | 1.36 | 0 | 0 | 0 |
| CP9 | <chem>CCNC(=S)N/N=C(/c1cc(sc1C)C)\C</chem> | 255.4 | 96.75 | 5 | 75.52 | 1 | 2 | Soluble | 3.1 | 0 | 0 | 0 |
| MWT = Molecular Weight, TPSA = Topological Polar Surface Area, NRB = Number of Rotatable bond, MR = Molar Refractivity, HBA = Hydrogen bond acceptor, HBD = Hydrogen bond donor, Log Po/w = Partition Coefficient octanol/water | | | | | | | | | | | | |

MWT = Molecular weight , TPSA = topological polar surface area, NRB = Number of rotatable bonds, MR = Molar refractivity, HBA = Hydrogen bond acceptor, HBD = hydrogen bond donor, Log Po/w = Partition coefficient octanol/water.

Table S2. Pharmacokinetics properties of compounds **1-9** calculated by SwissADME server.

| Compo unds | SMILE | | Pharmacokinetics | | | | | | | | Log Kp |
|---------------|--|--|------------------|----------|---------------|-----------------------------|----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------|
| | | | GIA | BBB P | P- gp S | CYP 1A2 inhi bitor | CYP 2C1 9 inhi bitor | CYP 2C9 inhi bitor | CYP 2D6 inhi bitor | CYP 3A4 inhi bitor | |
| CP1 | <chem>Fc1cnc(c(n1)C(=O)N)O</chem> | | High | No | No | No | No | No | No | No | -7.29 |
| CP2 | <chem>Nn1c(=S)[nH]nc1c1ccncc1</chem> | | High | No | No | No | No | No | No | No | -7.61 |
| CP3 | <chem>OC(=O)CCc1ccc(cc1)O</chem> | | High | Yes | No | No | No | No | No | No | -6.49 |
| CP4 | <chem>Nn1c(=S)[nH]nc1c1cccnc1</chem> | | High | No | No | No | No | No | No | No | -7.61 |
| CP5 | <chem>Sc1nnc(o1)c1cc2c([nH]1)cccc2</chem> | | High | No | No | Yes | No | No | No | Yes | -6.16 |
| CP6 | <chem>Clc1ccc(c(c1)F)c1nnc(o1)S</chem> | | High | Yes | No | Yes | No | No | No | No | -5.85 |
| CP7 | <chem>Sc1nnc(o1)Cc1cccc1F</chem> | | High | Yes | No | Yes | No | No | No | No | -5.96 |
| CP8 | <chem>NC(=S)N/N=C/c1ccc(c1)[N+](=O)[O-]</chem> | | High | No | No | Yes | No | No | No | No | -6.69 |
| CP9 | <chem>CCNC(=S)N/N=C(/c1cc(sc1C)C)C</chem> | | High | No | No | Yes | Yes | Yes | No | No | -5.96 |

GIA = gastrointestinal absorption, BBBP = Blood brain barrier permeability, P-gp S = P-glycoprotein substrate, Log Kp = skin permeation.

Table S3. Drug likeness and medicinal chemistry properties of compounds **1-9** estimated by SwissADME server.

| Compounds | SMILE | Drug likeness | Medicinal chemistry | | |
|-----------|---|------------------------|---------------------|---------------|-------------------------|
| | | Bio Availability Score | PAINS Alert | Lead likeness | Synthetic accessibility |
| CP1 | <chem>Fc1cnc(c(n1)C(=O)N)O</chem> | 0.55 | 0 | 1 | 2.03 |
| CP2 | <chem>Nn1c(=S)[nH]nc1c1ccncc1</chem> | 0.55 | 0 | 1 | 2.21 |
| CP3 | <chem>OC(=O)CCc1ccc(cc1)O</chem> | 0.85 | 0 | 1 | 1 |
| CP4 | <chem>Nn1c(=S)[nH]nc1c1ccncc1</chem> | 0.55 | 0 | 1 | 2.34 |
| CP5 | <chem>Sc1nnc(o1)c1cc2c([nH]1)cccc2</chem> | 0.55 | 0 | 1 | 2.13 |
| CP6 | <chem>C1c1ccc(c(c1)F)c1nnc(o1)S</chem> | 0.55 | 0 | 1 | 2.12 |
| CP7 | <chem>Sc1nnc(o1)Cc1cccc1F</chem> | 0.55 | 0 | 1 | 2.02 |
| CP8 | <chem>NC(=S)N/N=C/c1ccc(cc1)[N+](=O)[O-]</chem> | 0.55 | 0 | 1 | 2.28 |
| CP9 | <chem>CCNC(=S)N/N=C(/c1cc(sc1C)C)\C</chem> | 0.55 | 0 | 0 | 3.09 |

Table S4. Hydrogen bond interactions of Isla with the selected ligands and reference (7KQ3) versus simulation time.

| Compound | H-Acceptor | H-Donor | Bond life (%) | Avg-Distance (Å) | Avg-Angle° |
|----------|------------|------------|---------------|------------------|------------|
| 7KQ3 | GLU470-OE2 | 8X3831-O6 | 52.43% | 2.71 | 163.76 |
| | 8X3831-O4 | ARG678-NH1 | 42.34% | 2.81 | 160.92 |
| | 8X3831-O5 | ARG189-NH2 | 40.67% | 2.82 | 153.83 |
| | 8X3831-O7 | TYR485-OH | 36.45% | 2.73 | 161.65 |
| | 8X3831-O5 | ARG189-NH1 | 34.26% | 2.81 | 153.67 |
| | 8X3831-O7 | ARG189-NH1 | 28.00% | 2.81 | 156.12 |
| | 8X3831-O5 | ARG678-NH1 | 24.50% | 2.81 | 159.18 |
| | 8X3831-O5 | TYR485-OH | 23.98% | 2.74 | 161.77 |
| | 8X3831-O4 | TYR485-OH | 22.53% | 2.74 | 161.51 |
| | 8X3831-O4 | ARG189-NH1 | 22.47% | 2.78 | 155.23 |
| | 8X3831-O4 | TYR587-OH | 22.26% | 2.71 | 161.09 |
| | 8X3831-O7 | TYR587-OH | 22.08% | 2.69 | 161.89 |
| | 8X3831-O5 | TYR587-OH | 21.79% | 2.71 | 160.30 |
| | 8X3831-O7 | ARG189-NH2 | 20.22% | 2.83 | 152.06 |
| | 8X3831-O7 | ARG678-NH1 | 20.16% | 2.81 | 160.04 |
| | 8X3831-O7 | ARG678-NH2 | 19.09% | 2.86 | 160.81 |
| | 8X3831-O4 | ARG189-NH2 | 19.05% | 2.83 | 150.74 |
| | 8X3831-O6 | GLN193-NE2 | 16.70% | 2.90 | 159.33 |
| | 8X3831-O6 | CYS468-N | 15.89% | 2.88 | 158.70 |
| | 8X3831-O4 | ARG678-NH2 | 8.40% | 2.86 | 157.03 |
| | 8X3831-O5 | ARG678-NH2 | 6.80% | 2.85 | 153.83 |
| | 8X3831-O7 | THR312-OG1 | 6.44% | 2.77 | 157.18 |
| | THR312-OG1 | 8X3831-O6 | 6.24% | 2.84 | 159.78 |
| | GLU470-OE1 | 8X3831-O6 | 1.24% | 2.79 | 162.82 |
| | 8X3831-O7 | GLN193-NE2 | 0.54% | 2.85 | 166.16 |
| | 8X3831-O6 | THR312-OG1 | 0.45% | 2.83 | 164.88 |
| | VAL680-O | 8X3831-O6 | 0.17% | 2.82 | 156.84 |
| | 8X3831-O5 | THR312-OG1 | 0.09% | 2.72 | 158.50 |
| | TRP374-NE1 | 8X3831-O6 | 0.07% | 2.93 | 146.89 |
| | 8X3831-O6 | TYR587-OH | 0.02% | 2.88 | 152.78 |
| | 8X3831-O5 | TRP374-NE1 | 0.01% | 2.86 | 139.43 |
| | 8X3831-O6 | GLN193-N | 0.01% | 2.91 | 140.64 |
| | 8X3831-O6 | ARG678-NH1 | 0.01% | 2.92 | 142.68 |
| | 8X3831-O4 | GLY483-N | 0.01% | 2.93 | 163.93 |
| | 8X3831-O7 | TRP374-NE1 | 0.01% | 2.90 | 149.77 |
| | 8X3831-O6 | TRP374-NE1 | 0.01% | 2.96 | 144.22 |
| CPI | CP1831-O7 | ARG189-NH1 | 60.24% | 2.82 | 153.43 |
| | THR312-OG1 | CP1831-N6 | 52.20% | 2.87 | 158.11 |
| | CP1831-O10 | TYR587-OH | 44.53% | 2.78 | 160.36 |
| | CP1831-O7 | GLN193-NE2 | 34.99% | 2.85 | 157.99 |
| | CP1831-O10 | ARG189-NH2 | 32.87% | 2.86 | 155.73 |
| | GLU589-OE1 | CP1831-N9 | 22.41% | 2.83 | 153.98 |
| | GLU589-OE2 | CP1831-N9 | 22.03% | 2.82 | 158.68 |
| | CP1831-O10 | ARG189-NH1 | 13.54% | 2.87 | 150.30 |
| | GLY311-O | CP1831-N6 | 5.58% | 2.89 | 157.98 |
| | TYR485-OH | CP1831-N9 | 5.22% | 2.90 | 144.39 |
| | CP1831-O7 | GLN193-N | 3.93% | 2.92 | 153.09 |
| | CP1831-F11 | PHE682-N | 1.38% | 2.89 | 140.97 |
| | VAL680-O | CP1831-N9 | 1.33% | 2.85 | 143.80 |
| | TYR485-OH | CP1831-N9 | 0.55% | 2.85 | 151.43 |
| | CP1831-N9 | TYR485-OH | 0.48% | 2.89 | 144.91 |
| | CP1831-O7 | ARG189-NH2 | 0.31% | 2.91 | 145.44 |
| | CP1831-O10 | ARG678-NH2 | 0.20% | 2.88 | 148.13 |
| | GLU589-OE2 | CP1831-N9 | 0.19% | 2.87 | 145.00 |
| | GLU589-OE1 | CP1831-N9 | 0.17% | 2.84 | 145.44 |
| | CP1831-N9 | ARG678-NH2 | 0.10% | 2.90 | 141.91 |
| | CP1831-N3 | ARG678-NH2 | 0.09% | 2.89 | 141.50 |
| | CP1831-O10 | GLN193-NE2 | 0.08% | 2.93 | 139.33 |
| | ARG678-NH2 | CP1831-N9 | 0.05% | 2.90 | 137.91 |
| | CP1831-N9 | ARG678-NH2 | 0.03% | 2.96 | 139.20 |
| CP2 | TYR479-OH | CP2831-N7 | 78.58% | 2.80 | 152.16 |

| | | | | | |
|-----|------------|------------|--------|------|--------|
| | ASN675-OD1 | CP2831-N13 | 75.73% | 2.72 | 145.32 |
| | CP2831-O11 | ALA484-N | 21.40% | 2.90 | 156.83 |
| | CP2831-N14 | TYR587-N | 0.14% | 2.96 | 144.17 |
| | GLN544-OE1 | CP2831-N13 | 0.13% | 2.70 | 144.49 |
| | TYR479-OH | CP2831-N13 | 0.01% | 2.82 | 139.66 |
| | GLY482-O | CP2831-N7 | 0.01% | 2.83 | 138.53 |
| CP4 | TYR587-O | CP4831-N14 | 61.47% | 2.85 | 159.09 |
| | SER481-O | CP4831-N11 | 15.32% | 2.91 | 152.38 |
| | TYR479-OH | CP4831-N11 | 4.11% | 2.90 | 151.32 |
| | GLN544-OE1 | CP4831-N14 | 3.00% | 2.87 | 147.99 |
| | CP4831-N11 | GLY483-N | 2.10% | 2.94 | 145.89 |
| | CP4831-N3 | GLY483-N | 1.06% | 2.95 | 155.68 |
| | CP4831-N14 | TYR587-N | 0.34% | 2.96 | 145.76 |
| | CP4831-N3 | THR185-OG1 | 0.01% | 2.97 | 162.45 |
| CP5 | GLU589-OE1 | CP5831-N4 | 96.38% | 2.75 | 162.09 |
| | GLU589-OE1 | CP5831-N7 | 37.20% | 2.87 | 147.74 |
| | CP5831-O15 | ARG189-NH1 | 16.20% | 2.88 | 152.27 |
| | CP5831-O14 | ARG189-NH1 | 14.16% | 2.88 | 152.54 |
| | CP5831-N3 | TYR485-OH | 14.11% | 2.87 | 162.63 |
| | TYR587-O | CP5831-N7 | 5.47% | 2.88 | 151.08 |
| | ALA484-O | CP5831-N7 | 4.24% | 2.89 | 143.61 |
| | CP5831-N4 | TYR485-OH | 1.01% | 2.93 | 145.22 |
| | CP5831-O15 | ARG678-NH2 | 0.95% | 2.90 | 147.57 |
| | CP5831-O14 | ARG678-NH2 | 0.88% | 2.91 | 149.07 |
| | CP5831-N3 | TYR587-OH | 0.86% | 2.89 | 157.04 |
| | GLY483-O | CP5831-N7 | 0.61% | 2.91 | 160.82 |
| | CP5831-N3 | ARG189-NH2 | 0.58% | 2.93 | 156.35 |
| | CP5831-O14 | ARG678-NH1 | 0.40% | 2.90 | 146.48 |
| | CP5831-O15 | ARG678-NH1 | 0.35% | 2.90 | 148.07 |
| | CP5831-O14 | ARG189-NH2 | 0.31% | 2.91 | 145.85 |
| | CP5831-O15 | ARG189-NH2 | 0.28% | 2.91 | 145.24 |
| | CP5831-O15 | CYS468-N | 0.24% | 2.90 | 147.41 |
| | CP5831-O15 | TRP374-NE1 | 0.15% | 2.90 | 143.00 |
| | CP5831-O14 | TRP374-NE1 | 0.14% | 2.91 | 141.38 |
| | CP5831-N13 | ARG189-NH1 | 0.13% | 2.96 | 148.60 |
| | CP5831-O15 | TYR485-OH | 0.07% | 2.85 | 147.35 |
| | CP5831-O14 | TYR485-OH | 0.04% | 2.89 | 143.26 |
| | CP5831-O14 | ARG189-NH1 | 0.02% | 2.85 | 143.57 |
| | ALA484-O | CP5831-N7 | 0.02% | 2.88 | 151.60 |
| | CP5831-O15 | THR312-OG1 | 0.01% | 2.76 | 140.87 |
| | CP5831-O15 | ARG189-NH1 | 0.01% | 2.84 | 144.17 |
| | CP5831-O14 | THR312-OG1 | 0.01% | 2.95 | 148.11 |
| CP8 | GLU589-OE1 | CP8831-N12 | 63.64% | 2.80 | 162.38 |
| | GLY483-O | CP8831-N10 | 39.57% | 2.79 | 150.80 |
| | CP8831-N2 | GLN193-NE2 | 24.07% | 2.92 | 166.19 |
| | GLU589-OE1 | CP8831-N12 | 23.22% | 2.82 | 158.58 |
| | TYR587-O | CP8831-N12 | 10.00% | 2.90 | 158.15 |
| | CP8831-N11 | ARG189-NH2 | 9.99% | 2.92 | 151.07 |
| | CP8831-N11 | THR185-OG1 | 2.36% | 2.88 | 150.28 |
| | TYR587-O | CP8831-N12 | 1.91% | 2.91 | 158.33 |
| | THR185-OG1 | CP8831-N10 | 0.53% | 2.84 | 148.48 |
| | CP8831-N2 | GLN193-N | 0.52% | 2.96 | 154.19 |
| | CP8831-N11 | ARG189-NE | 0.15% | 2.94 | 149.16 |
| | TYR479-OH | CP8831-N12 | 0.11% | 2.93 | 153.40 |
| | TYR485-OH | CP8831-N12 | 0.09% | 2.90 | 155.88 |
| | GLU470-OE2 | CP8831-N12 | 0.07% | 2.85 | 148.78 |
| | GLU470-OE2 | CP8831-N12 | 0.06% | 2.83 | 151.69 |
| | CP8831-N11 | TYR485-OH | 0.06% | 2.94 | 152.14 |
| | TYR485-OH | CP8831-N10 | 0.01% | 2.96 | 148.40 |
| | TYR479-OH | CP8831-N12 | 0.01% | 2.93 | 141.07 |
| CP9 | CP9831-N11 | THR185-OG1 | 60.24% | 2.80 | 162.23 |
| | GLY483-O | CP9831-N10 | 59.63% | 2.82 | 160.41 |
| | GLN193-OE1 | CP9831-N12 | 18.96% | 2.85 | 152.76 |
| | SER481-O | CP9831-N12 | 17.22% | 2.85 | 163.24 |
| | GLU470-OE2 | CP9831-N12 | 12.60% | 2.85 | 150.52 |
| | CP9831-N3 | ARG189-NH2 | 12.57% | 2.90 | 150.03 |

| | | | | | |
|--|------------|------------|--------|------|--------|
| | GLN193-OE1 | CP9831-N12 | 12.14% | 2.85 | 152.90 |
| | GLU470-OE2 | CP9831-N12 | 9.02% | 2.84 | 152.17 |
| | ALA484-O | CP9831-N10 | 5.47% | 2.88 | 148.73 |
| | TYR587-O | CP9831-N10 | 4.03% | 2.80 | 141.43 |
| | SER481-O | CP9831-N12 | 2.13% | 2.87 | 163.31 |
| | CP9831-N12 | GLN193-NE2 | 1.07% | 2.92 | 147.04 |
| | CP9831-N10 | THR185-OG1 | 0.52% | 2.95 | 144.10 |
| | CP9831-N3 | ARG189-NE | 0.45% | 2.95 | 144.30 |
| | CP9831-N3 | TYR485-OH | 0.14% | 2.84 | 159.58 |
| | GLY483-N | CP9831-N10 | 0.09% | 2.95 | 140.27 |
| | CP9831-N12 | ARG678-NH1 | 0.09% | 2.94 | 149.87 |
| | CP9831-N11 | TYR485-OH | 0.08% | 2.86 | 161.44 |
| | TYR587-OH | CP9831-N12 | 0.07% | 2.94 | 152.90 |
| | ARG678-NH2 | CP9831-N12 | 0.06% | 2.93 | 153.20 |
| | CP9831-N3 | ARG189-NH2 | 0.05% | 2.88 | 145.30 |
| | TYR485-OH | CP9831-N12 | 0.05% | 2.95 | 155.91 |
| | ARG678-NH1 | CP9831-N12 | 0.04% | 2.89 | 148.34 |
| | CP9831-N3 | ARG189-NH1 | 0.04% | 2.90 | 152.50 |
| | CP9831-N11 | GLN193-NE2 | 0.04% | 2.90 | 165.08 |
| | GLY483-O | CP9831-N12 | 0.04% | 2.92 | 160.74 |
| | TYR485-OH | CP9831-N12 | 0.04% | 2.95 | 152.67 |
| | ARG678-NH1 | CP9831-N12 | 0.04% | 2.95 | 153.42 |
| | GLN544-OE1 | CP9831-N10 | 0.03% | 2.91 | 148.93 |
| | ARG678-NH2 | CP9831-N12 | 0.02% | 2.97 | 146.71 |
| | TYR479-OH | CP9831-N12 | 0.01% | 2.95 | 154.22 |
| | GLN193-NE2 | CP9831-N12 | 0.01% | 2.94 | 150.60 |

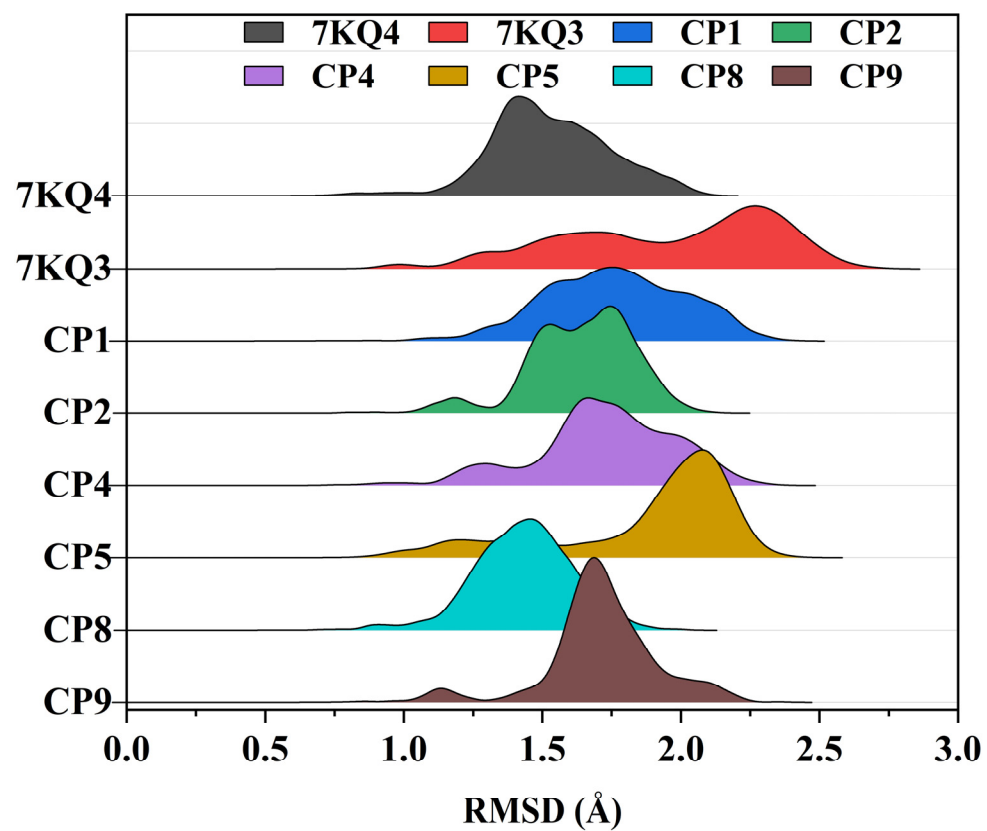


Figure S1 Root mean square histogram analysis of Isla in free state (7KQ4), reference substrate attached (7KQ3) and in complex with selected putative inhibitors.

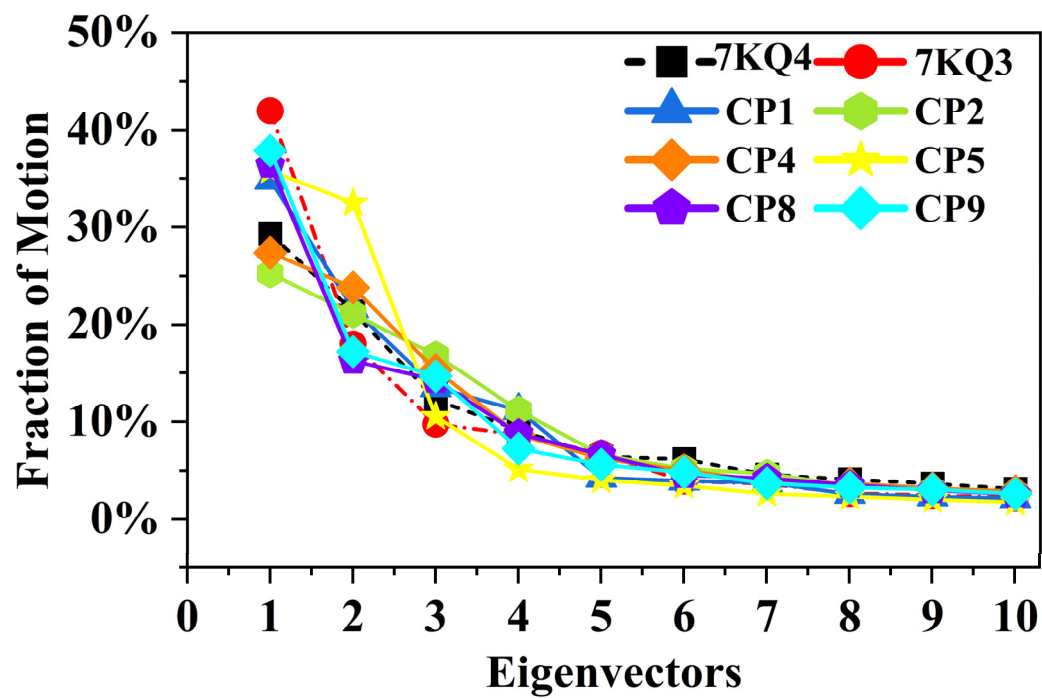


Figure S2. The covariance matrix plotted against the corresponding eigenvector from each system to obtain the first ten eigenvectors in fraction. The eigenvectors contribution for each system is reported in percentage.