**pKa measurements for the SAMPL6 prediction challenge for a set of kinase inhibitor-like fragments**

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SUPPLEMENTARY INFORMATION

**Table SI 1.** Procurement details of SAMPL6 pKa challenge compounds

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SAMPL6 Molecule ID** | **Group** | **Supplier** | **LOT** | **CAT** | **Supplier reported purity** | **CAS** | **eMolecules ID** | **canonical isomeric SMILES** | **Experimental Molecule ID** |
| SM01 | fragment-like | AchemBlock | 11549 | 10222 | 95% | 521937-07-05 | 6679830 | c1cc2c(cc1O)c3c(o2)C(=O)NCCC3 | M01 |
| SM02 | fragment-like | ChemDiv | CM02432403 | 3232-0333 |  |  | 1327907 | c1ccc2c(c1)c(ncn2)Nc3cccc(c3)C(F)(F)F | M02 |
| SM03 | fragment-like | ChemDiv |  | Z27474679 |  |  | 1228629 | c1ccc(cc1)Cc2nnc(s2)NC(=O)c3cccs3 | M03 |
| SM04 | fragment-like | ChemDiv |  | Z126957826 |  |  | 30719859 | c1ccc2c(c1)c(ncn2)NCc3ccc(cc3)Cl | M04 |
| SM05 | fragment-like | ChemDiv |  | Z119335440 |  |  | 18908671 | c1ccc(c(c1)NC(=O)c2ccc(o2)Cl)N3CCCCC3 | M05 |
| SM06 | fragment-like | ChemDiv |  | Z28487401 |  |  | 18893169 | c1cc2cccnc2c(c1)NC(=O)c3cc(cnc3)Br | M06 |
| SM07 | fragment-like | Enamine | 2017-0168841 | Z57161635 | 95% | 100818-54-0 | 1327878 | c1ccc(cc1)CNc2c3ccccc3ncn2 | M07 |
| SM08 | fragment-like | Enamine | 2017-0168838 | Z57157353 | 95% | 65418-08-8 | 1367649 | Cc1ccc2c(c1)c(c(c(=O)[nH]2)CC(=O)O)c3ccccc3 | M08 |
| SM09 | fragment-like | Enamine | 2017-0168839 | Z220564816 | 95% |  | 1865544 | COc1cccc(c1)Nc2c3ccccc3ncn2.Cl | M09 |
| SM10 | fragment-like | Enamine | 2017-0168843 | Z69130143 | 95% | 35056-22-5 | 23354217 | c1ccc(cc1)C(=O)NCC(=O)Nc2nc3ccccc3s2 | M10 |
| SM11 | fragment-like | Maybridge | 142989 | RJC00689SC | 90% | 5334-30-5 | 719540 | c1ccc(cc1)n2c3c(cn2)c(ncn3)N | M11 |
| SM12 | fragment-like | Maybridge | 265423 | DP00818SC |  |  | 1859493 | c1ccc2c(c1)c(ncn2)Nc3cccc(c3)Cl.Cl | M12 |
| SM13 | fragment-like | Maybridge | 248841 | GK03474SC |  |  | 5828805 | Cc1cccc(c1)Nc2c3cc(c(cc3ncn2)OC)OC | M13 |
| SM14 | fragment-like | Enamine |  | Z57290870 |  |  | 31653344 | c1ccc(cc1)n2cnc3c2ccc(c3)N | M15 |
| SM15 | fragment-like | Enamine |  | Z1318268952 |  |  | 37095168 | c1ccc2c(c1)ncn2c3ccc(cc3)O | M16 |
| SM16 | fragment-like | VitaScreen |  | STK098832 |  |  | 1284691 | c1cc(c(c(c1)Cl)C(=O)Nc2ccncc2)Cl | M18 |
| SM17 | fragment-like | VitaScreen |  | STK032731 |  |  | 1444229 | c1ccc(cc1)CSc2nnc(o2)c3ccncc3 | M19 |
| SM18 | drug-like | Enamine |  | Z278071350 |  |  | 18897105 | c1ccc2c(c1)c(=O)[nH]c(n2)CCC(=O)Nc3ncc(s3)Cc4ccc(c(c4)F)F | D01 |
| SM19 | drug-like | Enamine |  | Z30206127 |  |  | 3365457 | CCOc1ccc2c(c1)sc(n2)NC(=O)Cc3ccc(c(c3)Cl)Cl | D02 |
| SM20 | drug-like | VitaScreen |  | STL282831 |  |  | 46568819 | c1cc(cc(c1)OCc2ccc(cc2Cl)Cl)/C=C/3\C(=O)NC(=O)S3 | D05 |
| SM21 | drug-like | VitaScreen |  | STL368658 |  |  | 1574612 | c1cc(cc(c1)Br)Nc2c(cnc(n2)Nc3cccc(c3)Br)F | D06 |
| SM22 | drug-like | VitaScreen |  | STK070581 |  |  | 536848 | c1cc2c(cc(c(c2nc1)O)I)I | D07 |
| SM23 | drug-like | VitaScreen |  | STK097966 |  |  | 4375254 | CCOC(=O)c1ccc(cc1)Nc2cc(nc(n2)Nc3ccc(cc3)C(=O)OCC)C | D08 |
| SM24 | drug-like | VitaScreen |  | STK090644 |  |  | 1415746 | COc1ccc(cc1)c2c3c(ncnc3oc2c4ccc(cc4)OC)NCCO | D09 |

**Table SI 2.** Calculated properties and descriptors of compounds of 28 compounds selected and procured for pKa challenge. pKa measurement experiments were successful 24 molecules labeled SM01-SM24.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SAMPL6 Molecule ID** | **Experimental Molecule ID** | **group** | **Epik pKas in [3,11] range** | **OpenEye XlogP** | **Molecular Weight (g/mol)** | **eMolecules reported availability (mg)** | **Number of rotatable bonds** | **Number of UV-chr. units** | **eMolecules ID** | **canonical isomeric SMILES** |
| SM01 | M01 | fragment-like | [9.119] | 3.27 | 289.26 | 184 | 0 | 27 | 6679830 | c1cc2c(cc1O)c3c(o2)C(=O)NCCC3 |
| SM02 | M02 | fragment-like | [4.05] | 3.61 | 301.39 | 101 | 3 | 36 | 1327907 | c1ccc2c(c1)c(ncn2)Nc3cccc(c3)C(F)(F)F |
| SM03 | M03 | fragment-like | [7.12] | 3.52 | 269.73 | 379 | 5 | 14 | 1228629 | c1ccc(cc1)Cc2nnc(s2)NC(=O)c3cccs3 |
| SM04 | M04 | fragment-like | [5.564] | 3.79 | 304.77 | 415.5 | 3 | 36 | 30719859 | c1ccc2c(c1)c(ncn2)NCc3ccc(cc3)Cl |
| SM05 | M05 | fragment-like | [5.346] | 2.92 | 328.16 | 424.3 | 4 | 18 | 18908671 | c1ccc(c(c1)NC(=O)c2ccc(o2)Cl)N3CCCCC3 |
| SM06 | M06 | fragment-like | [4.001, 10.328] | 2.91 | 235.28 | 406 | 3 | 37 | 18893169 | c1cc2cccnc2c(c1)NC(=O)c3cc(cnc3)Br |
| SM07 | M07 | fragment-like | [5.564] | 2.80 | 293.32 | 208.7 | 3 | 36 | 1327878 | c1ccc(cc1)CNc2c3ccccc3ncn2 |
| SM08 | M08 | fragment-like | [4.109] | 3.14 | 287.74 | 232.1 | 3 | 59 | 1367649 | Cc1ccc2c(c1)c(c(c(=O)[nH]2)CC(=O)O)c3ccccc3 |
| SM09 | M09 | fragment-like | [4.05] | 2.72 | 311.36 | 119.7 | 3 | 36 | 1865544 | COc1cccc(c1)Nc2c3ccccc3ncn2.Cl |
| SM10 | M10 | fragment-like | [8.672] | 1.50 | 211.22 | 149.1 | 6 | 28 | 23354217 | c1ccc(cc1)C(=O)NCC(=O)Nc2nc3ccccc3s2 |
| SM11 | M11 | fragment-like | [3.869] | 3.90 | 292.16 | 3430 | 1 | 31 | 719540 | c1ccc(cc1)n2c3c(cn2)c(ncn3)N |
| SM12 | M12 | fragment-like | [4.05] | 2.60 | 295.34 | 7366 | 2 | 36 | 1859493 | c1ccc2c(c1)c(ncn2)Nc3cccc(c3)Cl.Cl |
| SM13 | M13 | fragment-like | [4.267] | 2.91 | 235.28 | 1864 | 4 | 36 | 5828805 | Cc1cccc(c1)Nc2c3cc(c(cc3ncn2)OC)OC |
| - | M14 | fragment-like | [5.564] | 2.33 | 209.25 | 208.7 | 3 | 36 | 1327878 | c1ccc(cc1)CNc2c3ccccc3ncn2 |
| SM14 | M15 | fragment-like | [6.348] | 2.22 | 210.23 | 50213 | 1 | 40 | 31653344 | c1ccc(cc1)n2cnc3c2ccc(c3)N |
| SM15 | M16 | fragment-like | [5.82, 8.709] | 4.23 | 263.33 | 21650.2 | 1 | 40 | 37095168 | c1ccc2c(c1)ncn2c3ccc(cc3)O |
| - | M17 | fragment-like | [3.158] | 2.93 | 267.11 | 283.7 | 2 | 24 | 45809595 | CC(C)c1ccc(cc1)/C=C\2/c3ccccc3NC2=O |
| SM16 | M18 | fragment-like | [4.714, 9.847] | 3.31 | 269.32 | 385 | 3 | 20 | 1284691 | c1cc(c(c(c1)Cl)C(=O)Nc2ccncc2)Cl |
| SM17 | M19 | fragment-like | [4.902] | 3.34 | 426.44 | 170 | 4 | 30 | 1444229 | c1ccc(cc1)CSc2nnc(o2)c3ccncc3 |
| SM18 | D01 | drug-like | [9.381, 10.773] | 5.17 | 381.28 | 247.7 | 7 | 37 | 18897105 | c1ccc2c(c1)c(=O)[nH]c(n2)CCC(=O)Nc3ncc(s3)Cc4ccc(c(c4)F)F |
| SM19 | D02 | drug-like | [9.167] | 5.78 | 403.31 | 489.9 | 6 | 28 | 3365457 | CCOc1ccc2c(c1)sc(n2)NC(=O)Cc3ccc(c(c3)Cl)Cl |
| - | D03 | drug-like | [4.113] | 4.72 | 401.48 | 324.5 | 6 | 35 | 10794751 | CC(C)(C)c1cc(n(n1)c2ccccc2)NC(=O)Nc3cccc(c3Cl)Cl |
| - | D04 | drug-like | [3.199] | 5.24 | 380.25 | 636.9 | 5 | 34 | 3064762 | c1ccc(cc1)C(=O)Nc2ccc(cc2)Oc3c4c5c(sc4ncn3)CCCC5 |
| SM20 | D05 | drug-like | [8.05] | 4.14 | 438.09 | 154 | 4 | 24 | 46568819 | c1cc(cc(c1)OCc2ccc(cc2Cl)Cl)/C=C/3\C(=O)NC(=O)S3 |
| SM21 | D06 | drug-like | [3.892] | 3.37 | 396.95 | 222 | 4 | 28 | 1574612 | c1cc(cc(c1)Br)Nc2c(cnc(n2)Nc3cccc(c3)Br)F |
| SM22 | D07 | drug-like | [3.511, 6.794] | 2.94 | 420.46 | 239 | 0 | 29 | 536848 | c1cc2c(cc(c(c2nc1)O)I)I |
| SM23 | D08 | drug-like | [6.336] | 2.79 | 391.42 | 319 | 10 | 28 | 4375254 | CCOC(=O)c1ccc(cc1)Nc2cc(nc(n2)Nc3ccc(cc3)C(=O)OCC)C |
| SM24 | D09 | drug-like | [4.829] | 3.27 | 289.26 | 398 | 7 | 71 | 1415746 | COc1ccc(cc1)c2c3c(ncnc3oc2c4ccc(cc4)OC)NCCO |

**Table SI 3**. pKa results of replicate UV-metric pKa measurements. "UV-metric pKa" assay indicates spectrophotometric pKa measurements done with Sirius T3 in ISA water. "UV-metric pKa with cosolvent" assay refers to pKa determination by Yasuda-Shedlovsky extrapolation from psKa measurements in various ratios of ISA methanol:water mixtures. Triplicate measurements were performed at 25 ± 0.5°C and in the presence of approximately 150 mM KCl to adjust ionic strength.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Molecule ID | pKa1 | pKa2 | pKa3 | Assay Type | Experiment ID | Experiment Report |
| SM01 | 9.54 |  |  | UV-metric pKa | 17I-15024 | SM01\_17I-15024\_M01\_UV-metric pKa\_report.pdf |
| SM01 | 9.53 |  |  | UV-metric pKa | 17I-15025 | SM01\_17I-15025\_M01\_UV-metric pKa\_report.pdf |
| SM01 | 9.53 |  |  | UV-metric pKa | 17I-16001 | SM01\_17I-16001\_M01\_UV-metric pKa\_report.pdf |
| SM02 | 5.04 |  |  | UV-metric pKa with cosolvent | 17I-22022 | SM02\_17I-22022\_M02\_UV-metric psKa\_report.pdf |
| SM02 | 5.04 |  |  | UV-metric pKa with cosolvent | 17I-22023 | SM02\_17I-22023\_M02\_UV-metric psKa\_report.pdf |
| SM02 | 5.02 |  |  | UV-metric pKa with cosolvent | 17I-22024 | SM02\_17I-22024\_M02\_UV-metric psKa\_report.pdf |
| SM03 | 7.01 |  |  | UV-metric pKa with cosolvent | 17I-19004 | SM03\_17I-19004\_M03\_UV-metric psKa\_report.pdf |
| SM03 | 7.01 |  |  | UV-metric pKa with cosolvent | 17I-19005 | SM03\_17I-19005\_M03\_UV-metric psKa\_report.pdf |
| SM03 | 7.03 |  |  | UV-metric pKa with cosolvent | 17I-19006 | SM03\_17I-19006\_M03\_UV-metric psKa\_report.pdf |
| SM04 | 6.03 |  |  | UV-metric pKa | 17I-18018 | SM04\_17I-18018\_M04\_UV-metric pKa\_report.pdf |
| SM04 | 6.02 |  |  | UV-metric pKa | 17I-18019 | SM04\_17I-18019\_M04\_UV-metric pKa\_report.pdf |
| SM04 | 6.02 |  |  | UV-metric pKa | 17I-18020 | SM04\_17I-18020\_M04\_UV-metric pKa\_report.pdf |
| SM05 | 4.57 |  |  | UV-metric pKa with cosolvent | 17I-19007 | SM05\_17I-19007\_M05\_UV-metric psKa\_report.pdf |
| SM05 | 4.6 |  |  | UV-metric pKa with cosolvent | 17I-19008 | SM05\_17I-19008\_M05\_UV-metric psKa\_report.pdf |
| SM05 | 4.6 |  |  | UV-metric pKa with cosolvent | 17I-19009 | SM05\_17I-19009\_M05\_UV-metric psKa\_report.pdf |
| SM06 | 2.96 | 11.76 |  | UV-metric pKa | 17I-18021 | SM06\_17I-18021\_M06\_UV-metric pKa\_report.pdf |
| SM06 | 3.11 | 11.74 |  | UV-metric pKa | 17I-18022 | SM06\_17I-18022\_M06\_UV-metric pKa\_report.pdf |
| SM06 | 3.02 | 11.71 |  | UV-metric pKa | 17I-18023 | SM06\_17I-18023\_M06\_UV-metric pKa\_report.pdf |
| SM07 | 6.07 |  |  | UV-metric pKa | 17I-16002 | SM07\_17I-16002\_M07\_UV-metric pKa\_report.pdf |
| SM07 | 6.07 |  |  | UV-metric pKa | 17I-16004 | SM07\_17I-16004\_M07\_UV-metric pKa\_report.pdf |
| SM07 | 6.09 |  |  | UV-metric pKa | 17I-20001 | SM07\_17I-20001\_M07\_UV-metric pKa\_report.pdf |
| SM08 | 4.23 |  |  | UV-metric pKa | 17I-19001 | SM08\_17I-19001\_M08\_UV-metric pKa\_report.pdf |
| SM08 | 4.2 |  |  | UV-metric pKa | 17I-19002 | SM08\_17I-19002\_M08\_UV-metric pKa\_report.pdf |
| SM08 | 4.22 |  |  | UV-metric pKa | 17I-19003 | SM08\_17I-19003\_M08\_UV-metric pKa\_report.pdf |
| SM09 | 5.37 |  |  | UV-metric pKa with cosolvent | 17I-16014 | SM09\_17I-16014\_M09\_UV-metric psKa\_report.pdf |
| SM09 | 5.35 |  |  | UV-metric pKa with cosolvent | 17I-16015 | SM09\_17I-16015\_M09\_UV-metric psKa\_report.pdf |
| SM09 | 5.4 |  |  | UV-metric pKa with cosolvent | 17I-16016 | SM09\_17I-16016\_M09\_UV-metric psKa\_report.pdf |
| SM10 | 9.01 |  |  | UV-metric pKa with cosolvent | 17I-20020 | SM10\_17I-20020\_M10\_UV-metric psKa\_report.pdf |
| SM10 | 9.02 |  |  | UV-metric pKa with cosolvent | 17I-20021 | SM10\_17I-20021\_M10\_UV-metric psKa\_report.pdf |
| SM10 | 9.02 |  |  | UV-metric pKa with cosolvent | 17I-20022 | SM10\_17I-20022\_M10\_UV-metric psKa\_report.pdf |
| SM11 | 3.89 |  |  | UV-metric pKa | 17I-16005 | SM11\_17I-16005\_M11\_UV-metric pKa\_report.pdf |
| SM11 | 3.89 |  |  | UV-metric pKa | 17I-16006 | SM11\_17I-16006\_M11\_UV-metric pKa\_report.pdf |
| SM11 | 3.89 |  |  | UV-metric pKa | 17I-16007 | SM11\_17I-16007\_M11\_UV-metric pKa\_report.pdf |
| SM12 | 5.28 |  |  | UV-metric pKa | 17I-21002 | SM12\_17I-21002\_M12\_UV-metric pKa\_report.pdf |
| SM12 | 5.28 |  |  | UV-metric pKa | 17I-21003 | SM12\_17I-21003\_M12\_UV-metric pKa\_report.pdf |
| SM12 | 5.27 |  |  | UV-metric pKa | 17I-21004 | SM12\_17I-21004\_M12\_UV-metric pKa\_report.pdf |
| SM13 | 5.79 |  |  | UV-metric pKa | 17I-16011 | SM13\_17I-16011\_M13\_UV-metric pKa\_report.pdf |
| SM13 | 5.76 |  |  | UV-metric pKa | 17I-16012 | SM13\_17I-16012\_M13\_UV-metric pKa\_report.pdf |
| SM13 | 5.76 |  |  | UV-metric pKa | 17I-16013 | SM13\_17I-16013\_M13\_UV-metric pKa\_report.pdf |

**Table SI 3**. Continued.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Molecule ID | pKa1 | pKa2 | pKa3 | Assay Type | Experiment ID | Experiment Report |
| SM14 | 2.59 | 5.31 |  | UV-metric pKa | 17I-29002 | SM14\_17I-29002\_M15\_UV-metric pKa\_report.pdf |
| SM14 | 2.57 | 5.29 |  | UV-metric pKa | 17I-29003 | SM14\_17I-29003\_M15\_UV-metric pKa\_report.pdf |
| SM14 | 2.57 | 5.29 |  | UV-metric pKa | 17I-29004 | SM14\_17I-29004\_M15\_UV-metric pKa\_report.pdf |
| SM15 | 4.71 | 8.96 |  | UV-metric pKa | 17K-10009 | SM15\_17K-10009\_M16\_UV-metric pKa\_report.pdf |
| SM15 | 4.7 | 8.94 |  | UV-metric pKa | 17K-10010 | SM15\_17K-10010\_M16\_UV-metric pKa\_report.pdf |
| SM15 | 4.69 | 8.92 |  | UV-metric pKa | 17K-10011 | SM15\_17K-10011\_M16\_UV-metric pKa\_report.pdf |
| SM16 | 5.37 | 10.64 |  | UV-metric pKa | 17J-03025 | SM16\_17J-03025\_M18\_UV-metric pKa\_report.pdf |
| SM16 | 5.37 | 10.65 |  | UV-metric pKa | 17J-03026 | SM16\_17J-03026\_M18\_UV-metric pKa\_report.pdf |
| SM16 | 5.38 | 10.65 |  | UV-metric pKa | 17J-03027 | SM16\_17J-03027\_M18\_UV-metric pKa\_report.pdf |
| SM17 | 3.15 |  |  | UV-metric pKa | 17J-02024 | SM17\_17J-02024\_M19\_UV-metric pKa\_report.pdf |
| SM17 | 3.16 |  |  | UV-metric pKa | 17J-02025 | SM17\_17J-02025\_M19\_UV-metric pKa\_report.pdf |
| SM17 | 3.17 |  |  | UV-metric pKa | 17J-02026 | SM17\_17J-02026\_M19\_UV-metric pKa\_report.pdf |
| SM18 | 2.1 | 9.51 | 10.93 | UV-metric pKa with cosolvent | 17I-28001 | SM18\_17I-28001\_D01\_UV-metric psKa\_report.pdf |
| SM18 | 2.15 | 9.59 | 11.03 | UV-metric pKa with cosolvent | 17I-28002 | SM18\_17I-28002\_D01\_UV-metric psKa\_report.pdf |
| SM18 | 2.19 | 9.63 | 11.11 | UV-metric pKa with cosolvent | 17I-28003 | SM18\_17I-28003\_D01\_UV-metric psKa\_report.pdf |
| SM19 | 9.59 |  |  | UV-metric pKa with cosolvent | 17I-28004 | SM19\_17I-28004\_D02\_UV-metric psKa\_report.pdf |
| SM19 | 9.58 |  |  | UV-metric pKa with cosolvent | 17I-28005 | SM19\_17I-28005\_D02\_UV-metric psKa\_report.pdf |
| SM19 | 9.52 |  |  | UV-metric pKa with cosolvent | 17I-28006 | SM19\_17I-28006\_D02\_UV-metric psKa\_report.pdf |
| SM20 | 5.72 |  |  | UV-metric pKa with cosolvent | 17J-12002 | SM20\_17J-12002\_D05\_UV-metric psKa\_report.pdf |
| SM20 | 5.75 |  |  | UV-metric pKa with cosolvent | 17J-12003 | SM20\_17J-12003\_D05\_UV-metric psKa\_report.pdf |
| SM20 | 5.64 |  |  | UV-metric pKa with cosolvent | 17K-16013 | SM20\_17K-16013\_D05\_UV-metric psKa\_report.pdf |
| SM21 | 4.11 |  |  | UV-metric pKa with cosolvent | 17J-06002 | SM21\_17J-06002\_D06\_UV-metric psKa\_report.pdf |
| SM21 | 4.09 |  |  | UV-metric pKa with cosolvent | 17J-06003 | SM21\_17J-06003\_D06\_UV-metric psKa\_report.pdf |
| SM21 | 4.09 |  |  | UV-metric pKa with cosolvent | 17J-06004 | SM21\_17J-06004\_D06\_UV-metric psKa\_report.pdf |
| SM22 | 2.45 | 7.42 |  | UV-metric pKa with cosolvent | 17J-07003 | SM22\_17J-07003\_D07\_UV-metric psKa\_report.pdf |
| SM22 | 2.37 | 7.45 |  | UV-metric pKa with cosolvent | 17J-07004 | SM22\_17J-07004\_D07\_UV-metric psKa\_report.pdf |
| SM22 | 2.37 | 7.41 |  | UV-metric pKa with cosolvent | 17J-07005 | SM22\_17J-07005\_D07\_UV-metric psKa\_report.pdf |
| SM23 | 5.45 |  |  | UV-metric pKa with cosolvent | 17J-07006 | SM23\_17J-07006\_D08\_UV-metric psKa\_report.pdf |
| SM23 | 5.44 |  |  | UV-metric pKa with cosolvent | 17J-07007 | SM23\_17J-07007\_D08\_UV-metric psKa\_report.pdf |
| SM23 | 5.45 |  |  | UV-metric pKa with cosolvent | 17J-07008 | SM23\_17J-07008\_D08\_UV-metric psKa\_report.pdf |
| SM24 | 2.61 |  |  | UV-metric pKa with cosolvent | 17J-06007 | SM24\_17J-06007\_D09\_UV-metric psKa\_report.pdf |
| SM24 | 2.58 |  |  | UV-metric pKa with cosolvent | 17J-06008 | SM24\_17J-06008\_D09\_UV-metric psKa\_report.pdf |
| SM24 | 2.6 |  |  | UV-metric pKa with cosolvent | 17J-06009 | SM24\_17J-06009\_D09\_UV-metric psKa\_report.pdf |

**Table SI 4**. UV-metric pKa measurments with and without cosolvent for 12 pKa challenge compounds with higher aqueous solubility and pyridoxine HCl. pKa values were measured with both methods to test if cosolvent method introduces bias.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Molecule ID** | **pKa1** | **pKa2** | **pKa3** | **Assay Type** | **Experiment ID** | **Experiment Report** |
| SM01 | 9.54 |  |  | UV-metric pKa | 17I-15024 | SM01\_17I-15024\_M01\_UV-metric pKa\_report.pdf |
| SM01 | 9.53 |  |  | UV-metric pKa | 17I-15025 | SM01\_17I-15025\_M01\_UV-metric pKa\_report.pdf |
| SM01 | 9.53 |  |  | UV-metric pKa | 17I-16001 | SM01\_17I-16001\_M01\_UV-metric pKa\_report.pdf |
| SM01\* | 9.71 |  |  | UV-metric pKa with cosolvent | 17J-06011 | SM01\_17J-06011\_M01\_UV-metric psKa\_report.pdf |
| SM04 | 6.03 |  |  | UV-metric pKa | 17I-18018 | SM04\_17I-18018\_M04\_UV-metric pKa\_report.pdf |
| SM04 | 6.02 |  |  | UV-metric pKa | 17I-18019 | SM04\_17I-18019\_M04\_UV-metric pKa\_report.pdf |
| SM04 | 6.02 |  |  | UV-metric pKa | 17I-18020 | SM04\_17I-18020\_M04\_UV-metric pKa\_report.pdf |
| SM04\* | 5.97 |  |  | UV-metric pKa with cosolvent | 17K-09020 | SM04\_17K-09020\_M04\_UV-metric psKa\_report.pdf |
| SM06 | 2.96 | 11.76 |  | UV-metric pKa | 17I-18021 | SM06\_17I-18021\_M06\_UV-metric pKa\_report.pdf |
| SM06 | 3.11 | 11.74 |  | UV-metric pKa | 17I-18022 | SM06\_17I-18022\_M06\_UV-metric pKa\_report.pdf |
| SM06 | 3.02 | 11.71 |  | UV-metric pKa | 17I-18023 | SM06\_17I-18023\_M06\_UV-metric pKa\_report.pdf |
| SM06\* | 3.46 | >12 |  | UV-metric pKa with cosolvent | 17K-09021 | SM06\_17K-09021\_M06\_UV-metric psKa\_report.pdf |
| SM06\* | 3.4 | >12 |  | UV-metric pKa with cosolvent | 17K-10008 | SM06\_17K-10008\_M06\_UV-metric psKa\_report.pdf |
| SM06\* | 3.45 | >12 |  | UV-metric pKa with cosolvent | 17K-16011 | SM06\_17K-16011\_M06\_UV-metric psKa\_report.pdf |
| SM07 | 6.07 |  |  | UV-metric pKa | 17I-16002 | SM07\_17I-16002\_M07\_UV-metric pKa\_report.pdf |
| SM07 | 6.07 |  |  | UV-metric pKa | 17I-16004 | SM07\_17I-16004\_M07\_UV-metric pKa\_report.pdf |
| SM07 | 6.09 |  |  | UV-metric pKa | 17I-20001 | SM07\_17I-20001\_M07\_UV-metric pKa\_report.pdf |
| SM07\* | 5.96 |  |  | UV-metric pKa with cosolvent | 17J-12006 | SM07\_17J-12006\_M07\_UV-metric psKa\_report.pdf |
| SM08 | 4.23 |  |  | UV-metric pKa | 17I-19001 | SM08\_17I-19001\_M08\_UV-metric pKa\_report.pdf |
| SM08 | 4.2 |  |  | UV-metric pKa | 17I-19002 | SM08\_17I-19002\_M08\_UV-metric pKa\_report.pdf |
| SM08 | 4.22 |  |  | UV-metric pKa | 17I-19003 | SM08\_17I-19003\_M08\_UV-metric pKa\_report.pdf |
| SM08\* | 4.47 |  |  | UV-metric pKa with cosolvent | 17J-12007 | SM08\_17J-12007\_M08\_UV-metric psKa\_report.pdf |
| SM11 | 3.89 |  |  | UV-metric pKa | 17I-16005 | SM11\_17I-16005\_M11\_UV-metric pKa\_report.pdf |
| SM11 | 3.89 |  |  | UV-metric pKa | 17I-16006 | SM11\_17I-16006\_M11\_UV-metric pKa\_report.pdf |
| SM11 | 3.89 |  |  | UV-metric pKa | 17I-16007 | SM11\_17I-16007\_M11\_UV-metric pKa\_report.pdf |
| SM11\* | 3.74 |  |  | UV-metric pKa with cosolvent | 17J-04003 | SM11\_17J-04003\_M11\_UV-metric psKa\_report.pdf |
| SM12 | 5.28 |  |  | UV-metric pKa | 17I-21002 | SM12\_17I-21002\_M12\_UV-metric pKa\_report.pdf |
| SM12 | 5.28 |  |  | UV-metric pKa | 17I-21003 | SM12\_17I-21003\_M12\_UV-metric pKa\_report.pdf |
| SM12 | 5.27 |  |  | UV-metric pKa | 17I-21004 | SM12\_17I-21004\_M12\_UV-metric pKa\_report.pdf |
| SM12\* | 5.16 |  |  | UV-metric pKa with cosolvent | 17J-11014 | SM12\_17J-11014\_M12\_UV-metric psKa\_report.pdf |
| SM13 | 5.79 |  |  | UV-metric pKa | 17I-16011 | SM13\_17I-16011\_M13\_UV-metric pKa\_report.pdf |
| SM13 | 5.76 |  |  | UV-metric pKa | 17I-16012 | SM13\_17I-16012\_M13\_UV-metric pKa\_report.pdf |
| SM13 | 5.76 |  |  | UV-metric pKa | 17I-16013 | SM13\_17I-16013\_M13\_UV-metric pKa\_report.pdf |
| SM13\* | 5.68 |  |  | UV-metric pKa with cosolvent | 17J-04005 | SM13\_17J-04005\_M13\_UV-metric psKa\_report.pdf |
| SM14 | 2.59 | 5.31 |  | UV-metric pKa | 17I-29002 | SM14\_17I-29002\_M15\_UV-metric pKa\_report.pdf |
| SM14 | 2.57 | 5.29 |  | UV-metric pKa | 17I-29003 | SM14\_17I-29003\_M15\_UV-metric pKa\_report.pdf |
| SM14 | 2.57 | 5.29 |  | UV-metric pKa | 17I-29004 | SM14\_17I-29004\_M15\_UV-metric pKa\_report.pdf |
| SM14\* | 2.56 | 5.29 |  | UV-metric pKa with cosolvent | 17J-12008 | SM14\_17J-12008\_M15\_UV-metric psKa\_report.pdf |

\* These UV-metric pKa measurement with cosolvent were not used in the calculation of pKa mean and SEM in the experimental data reported for SAMPL6 pKa challenge because replicate water based experiments also existed for these molecules.

**Table SI 4**. Continued.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Molecule ID** | **pKa1** | **pKa2** | **pKa3** | **Assay Type** | **Experiment ID** | **Experiment Report** |
| SM15 | 4.71 | 8.96 |  | UV-metric pKa | 17K-10009 | SM15\_17K-10009\_M16\_UV-metric pKa\_report.pdf |
| SM15 | 4.7 | 8.94 |  | UV-metric pKa | 17K-10010 | SM15\_17K-10010\_M16\_UV-metric pKa\_report.pdf |
| SM15 | 4.69 | 8.92 |  | UV-metric pKa | 17K-10011 | SM15\_17K-10011\_M16\_UV-metric pKa\_report.pdf |
| SM15\* | 4.67 | 9.01 |  | UV-metric pKa with cosolvent | 17J-12009 | SM15\_17J-12009\_M16\_UV-metric psKa\_report.pdf |
| SM16 | 5.37 | 10.64 |  | UV-metric pKa | 17J-03025 | SM16\_17J-03025\_M18\_UV-metric pKa\_report.pdf |
| SM16 | 5.37 | 10.65 |  | UV-metric pKa | 17J-03026 | SM16\_17J-03026\_M18\_UV-metric pKa\_report.pdf |
| SM16 | 5.38 | 10.65 |  | UV-metric pKa | 17J-03027 | SM16\_17J-03027\_M18\_UV-metric pKa\_report.pdf |
| SM16\* | 5.35 | 11.01 |  | UV-metric pKa with cosolvent | 17J-12010 | SM16\_17J-12010\_M18\_UV-metric psKa\_report.pdf |
| SM17 | 3.15 |  |  | UV-metric pKa | 17J-02024 | SM17\_17J-02024\_M19\_UV-metric pKa\_report.pdf |
| SM17 | 3.16 |  |  | UV-metric pKa | 17J-02025 | SM17\_17J-02025\_M19\_UV-metric pKa\_report.pdf |
| SM17 | 3.17 |  |  | UV-metric pKa | 17J-02026 | SM17\_17J-02026\_M19\_UV-metric pKa\_report.pdf |
| SM17\* | 3.2 |  |  | UV-metric pKa with cosolvent | 17J-12011 | SM17\_17J-12011\_M19\_UV-metric psKa\_report.pdf |
| Pyridoxine HCl | 4.81 | 8.85 |  | UV-metric pKa | 18E-22003 | 18E-22003\_Pyridoxine HCl\_UV-metric pKa\_0417936-0002.pdf |
| Pyridoxine HCl | 4.83 | 8.87 |  | UV-metric pKa | 18E-22004 | 18E-22004\_Pyridoxine HCl\_UV-metric pKa\_0417936-0002.pdf |
| Pyridoxine HCl | 4.84 | 8.86 |  | UV-metric pKa | 18E-22010 | 18E-22010\_Pyridoxine HCl\_UV-metric pKa\_0417936-0002.pdf |
| Pyridoxine HCl | 4.85 | 8.79 |  | UV-metric pKa with cosolvent | 18E-22011 | 18E-22011\_Pyridoxine HCl\_UV-metric psKa\_0417936-0002.pdf |
| Pyridoxine HCl | 4.85 | 8.79 |  | UV-metric pKa with cosolvent | 18E-22012 | 18E-22012\_Pyridoxine HCl\_UV-metric psKa\_0417936-0002.pdf |
| Pyridoxine HCl | 4.86 | 8.77 |  | UV-metric pKa with cosolvent | 18E-22013 | 18E-22013\_Pyridoxine HCl\_UV-metric psKa\_0417936-0002.pdf |

\* These UV-metric pKa measurement with cosolvent were not used in the calculation of pKa mean and SEM in the experimental data reported for SAMPL6 pKa challenge because replicate water based experiments also existed for these molecules.

**Table SI 5.** pKa mean and SEM results of with (cosolvent) and without cosolvent (water) replicate experiment. pKa values without SEM reported were measured with 1 replicate. pKa values reported with SEM were measured in triplicates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Molecule ID** | **pKa ID** | **pKa mean water** | **pKa SEM water** | **pKa mean cosolvent** | **pKa SEM cosolvent** |
| SM01 | SM01\_pKa1 | 9.53 | 0.01 | 9.71 |  |
| SM04 | SM04\_pKa1 | 6.02 | 0.01 | 5.97 |  |
| SM06 | SM06\_pKa1 | 3.03 | 0.04 | 3.44 | 0.02 |
| SM07 | SM07\_pKa1 | 6.08 | 0.01 | 5.96 |  |
| SM08 | SM08\_pKa1 | 4.22 | 0.01 | 4.47 |  |
| SM11 | SM11\_pKa1 | 3.89 | 0.01 | 3.74 |  |
| SM12 | SM12\_pKa1 | 5.28 | 0.01 | 5.16 |  |
| SM13 | SM13\_pKa1 | 5.77 | 0.01 | 5.68 |  |
| SM14 | SM14\_pKa1 | 2.58 | 0.01 | 2.56 |  |
| SM14 | SM14\_pKa2 | 5.3 | 0.01 | 5.29 |  |
| SM15 | SM15\_pKa1 | 4.7 | 0.01 | 4.67 |  |
| SM15 | SM15\_pKa2 | 8.94 | 0.01 | 9.01 |  |
| SM16 | SM16\_pKa1 | 5.37 | 0.01 | 5.35 |  |
| SM16 | SM16\_pKa2 | 10.65 | 0.01 | 11.01 |  |
| SM17 | SM17\_pKa1 | 3.16 | 0.01 | 3.2 |  |
| Pyridoxine HCl | Pyridoxine HCl\_pKa1 | 4.83 | 0.01 | 4.85 | 0.01 |
| Pyridoxine HCl | Pyridoxine HCl\_pKa2 | 8.86 | 0.01 | 8.78 | 0.01 |

- TABLE SI 6: Summary of LC-MS purity results

% purity\_of\_SAMPL6\_pKa\_compounds\_determined\_by\_LCMS.csv

- LC-MS Figures

- NMR spectra of SM07 microstate characterization