**Mass spectrometry data.**

**Supplementary file 3A**

**Disulfide library screened by mass spectrometry.**

|  |  |  |  |
| --- | --- | --- | --- |
| Compound | Properties | Commercial Codes | Structures |
| A1 | MW: 274.358Log P: 3.110N Heavy Atoms: 9+9 | NCI: NSC209ZINC: 1555356 |  |
| A2 | MW: 435.39Log P:4.964N Heavy Atoms: 13+13 | NCI: NSC659162ZINC: 1636936 |  |
| A3 | MW: 180.248Log P: -1.014N Heavy Atoms: 5+5 | NCI: NSC28727ZINC: 1646639 |  |
| A4 | MW: 154.251 Log P: 0.380N Heavy Atoms: 4+4 | NCI: NSC33920ZINC: 1665970 |  |
| A5 | MW: 312.325 Log P: 2.780N Heavy Atoms: 10+10 | NCI: NSC149336ZINC: 1734461 |  |
| A6 | MW: 240.300 Log P: -5.196N Heavy Atoms: 7+7 | NCI: NSC13203ZINC: 1529198 |  |
| A7 | MW: 268.354 Log P: -4.214N Heavy Atoms: 8+8 | NCI: NSC11337ZINC: 1529294 |  |
| A8 | MW: 332.394 Log P: 2.768N Heavy Atoms: 11+11 | NCI: NSC211ZINC: 1555358 |  |
| A9 a | MW: 296.474 Log P: 3.992N Heavy Atoms: 8+10 | NCI: NSC261199ZINC: 1558189 |  |
| A10 | MW: 534.694 Log P: 4.136N Heavy Atoms: 17+17 | NCI: NSC273905ZINC: 1561354 |  |
| A11 | MW: 240.300 Log P: -5.694N Heavy Atoms: 7+7 | NCI: NSC302851ZINC: 1567219 |  |
| A12 a | MW: 246.369 Log P: 1.442N Heavy Atoms: 4+11 | NCI: NSC3420025ZINC: 1579481 |  |
| A13 a | MW: 213.296 Log P: 3.333N Heavy Atoms: 3+10 | NCI: NSC342013ZINC: 1579486 |  |
| A14 a | MW: 200.344 Log P: 2.318N Heavy Atoms: 4+8 | NCI: NSC342020ZINC: 1579492 |  |
| A15 a | MW: 272.321 Log P: -0.643N Heavy Atoms: 7+10 | NCI: NSC342023ZINC: 1579494 |  |
| A16 | MW: 380.53 Log P: 4.388N Heavy Atoms: 13+13 | NCI: NSC3286ZINC: 1666690 |  |
| A17 | MW: 300.402 Log P: 4.188N Heavy Atoms: 10+10 | NCI: NSC35825ZINC: 1668414 |  |
| A18 | MW: 334.37 Log P: 2.584N Heavy Atoms: 11+11 | NCI: NSC38069ZINC: 1670426 |  |
| A19 | MW: 433.329 Log P: 3.812N Heavy Atoms: 13+13 | NCI: NSC39633ZINC: 1671480 |  |
| A20 a | MW: 373.424 Log P: -0.922N Heavy Atoms: 7+17 | NCI: NSC43135ZINC: 1675892 |  |
| A21 | MW: 424.458 Log P: 0.462N Heavy Atoms: 14+14 | NCI: NSC71895ZINC: 1697318 |  |
| A22 | MW: 416.467 Log P: 4.628N Heavy Atoms: 14+14 | NCI: NSC108934ZINC: 1701075 |  |
| A23 | MW: 508.617 Log P: 1.992N Heavy Atoms: 17+17 | NCI: NSC110311ZINC: 1702746 |  |
| A24 | MW: 304.341 Log P: 3.668N Heavy Atoms: 10+10 | NCI: NSC110311ZINC: 1704344 |  |
| A25 | MW: 353.438 Log P: 4.974N Heavy Atoms: 12+12 | NCI: NSC125034ZINC: 1714235 |  |
| A26 | MW: 420.592 Log P: 4.408N Heavy Atoms: 13+13 | NCI: NSC125828ZINC: 1714842 |  |
| A27 | MW: 352.427 Log P: 2.342N Heavy Atoms: 11+11 | NCI: NSC175829ZINC: 1716349 |  |
| A28 | MW: 406.431 Log P: 2.400N Heavy Atoms: 13+13 | NCI: NSC175830ZINC: 1716350 |  |
| A29 a | MW: 181.276 Log P: -1.22N Heavy Atoms: 3+7 | NCI: NSC135126ZINC: 1721731 |  |
| A30 | MW: 304.341 Log P: 3.64N Heavy Atoms: 10+10 | NCI: NSC210281ZINC: 1745667 |  |
| A31 a | MW: 383.425 Log P: 4.847N Heavy Atoms: 8+16 | NCI: NSC213718ZINC: 1752388 |  |
| A32 | MW: 268.486 Log P: 0.502N Heavy Atoms: 7+7 | NCI: NSC18421ZINC: 1769219 |  |
| A33 a | MW: 244.354 Log P: 1.979N Heavy Atoms: 4+11 | NCI: NSC342027ZINC: 1579497 |  |
| A34 | MW: 346.426 Log P: -0.122N Heavy Atoms: 11+11 | NCI: NSC350993ZINC: 1581670 |  |
| A35 | MW: 460.654 Log P: 4.418N Heavy Atoms: 14+14 | NCI: NSC363956ZINC: 1585472 |  |
| A36 a | MW: 248.342 Log P: 2.330N Heavy Atoms: 4+11 | NCI: NSC369423ZINC: 1587145 |  |
| A37 | MW: 354.400 Log P: 3.734N Heavy Atoms: 12+12 | NCI: NSC370926ZINC: 1587470 |  |
| A38 | MW: 364.393 Log P: 4.058N Heavy Atoms: 12+12 | NCI: NSC372683ZINC: 1588809 |  |
| A39 | MW: 354.535 Log P: -0.49N Heavy Atoms: 10+10 | NCI: NSC403854ZINC: 1596138 |  |
| A40 | MW: 462.587 Log P: 3.728N Heavy Atoms: 16+16 | NCI: NSC611506ZINC: 1611876 |  |
| A41 a | MW: 197.299 Log P: -3.390N Heavy Atoms: 4+7 | NCI: NSC161601ZINC: 1623380 |  |
| A42 | MW: 210.271 Log P: 1.608N Heavy Atoms: 6+6 | NCI: NSC638710ZINC: 1625711 |  |
| A43 a | MW: 178.272 Log P: 2.648N Heavy Atoms: 4+6 | NCI: NSC96660ZINC: 1626922 |  |
| A44 a | MW: 303.292 Log P: 2.194N Heavy Atoms: 6+13 | NCI: NSC96669ZINC: 1626930 |  |
| A45 a | MW: 362.443 Log P: 4.087N Heavy Atoms: 10+14 | NCI: NSC96696ZINC: 1626956 |  |
| A46 | MW: 404.548 Log P: 5.792N Heavy Atoms: 12+12 | NCI: NSC97416ZINC: 1632630 |  |
| A47 | MW: 289.203 Log P: 4.406N Heavy Atoms: 8+8 | NCI: NSC98831ZINC: 1648618 |  |
| A48 | MW: 238.324 Log P: 1.828N Heavy Atoms: 7+7 | NCI: NSC308782ZINC: 1568750 |  |
| A49 | MW: 460.654 Log P: 4.346N Heavy Atoms: 14+14 | NCI: NSC342021ZINC: 1579493 |  |
| A50 a | MW: 270.412 Log P: -2.552N Heavy Atoms: 7+8 | NCI: NSC342029ZINC: 1579499 |  |
| A51 | MW: 442.551 Log P: 2.012N Heavy Atoms: 15+15 | NCI: NSC405812ZINC: 1598776 |  |
| A52 | MW: 272.432 Log P: -0.834N Heavy Atoms: 7+7 | NCI: NSC14558ZINC: 1653104 |  |
| A53 | MW: 254.289 Log P: -1.58N Heavy Atoms: 8+8 | NCI: NSC106685ZINC: 1697982 |  |
| A54 | MW: 340.415 Log P: -2.662N Heavy Atoms: 8+8 | NCI: NSC126672ZINC: 1715307 |  |
| A55 | MW: 262.346Log P: 4.702N Heavy Atoms: 8+8 | NCI: NSC15869ZINC: 1733696 |  |
| TCP | MW: 654.884Log P: 4.088N Heavy Atoms: 21+21 |  |  |

a For asymmetric disulphide compounds the two possible binding fragments are indicated by “a” and “b”.

**Supplementary file 3B**

**Mass spectrometry screening results: compounds detected bound to hTS C195S-Y202C.**

|  |  |  |
| --- | --- | --- |
| Compound | MALDI | ESI-QToF |
| **A2** | detected | not detected |
| **A4** | detected | detected |
| **A5** | detected | detected |
| **A6** | detected | detected |
| **A7** | detected | not detected |
| **A9** | detected | not detected |
| **A10** | detected | detected |
| **A11** | detected | not detected |
| **A14** | detected (both fragments) | detected (both fragments) |
| **A15** | detected (both fragments) | detected (both fragments) |
| **A16** | not detected | detected |
| **A20** | detected (fragment b) | detected (fragment b) |
| **A21** | detected | not detected |
| **A22** | detected | not detected |
| **A25** | detected | not detected |
| **A26** | detected | not detected |
| **A27** | detected | not detected |
| **A28** | detected | not detected |
| **A29** | detected (both fragments) | detected (both fragments) |
| **A30** | detected | detected |
| **A36** | detected (both fragments) | detected (fragment b) |
| **A37** | detected | not detected |
| **A38** | detected | detected |
| **A41** | detected | not detected |
| **A42** | detected | not detected |
| **A43** | detected | detected (both fragments) |
| **A47** | detected | detected |
| **A49** | detected | not detected |
| **A55** | detected | not detected |
| **TCP** | detected | detected |

**Supplementary file 3C**

**Results of the mass spectrometry experiments on C195S-Y202C hTS protein-ligand complexes digested with trypsin and analyzed by MALDI (M) and ESI (E) MS**. If the ligand is not identified through E it is indicated as (E-).

|  |  |  |
| --- | --- | --- |
| Compound | CYS 180 | CYS 199, 202, 210  |
| (177)- (185)IIM**C**AWNPR(sequence A) | (176) –(185)RIIM**C**AWNPR(sequence B) | (186)- (215)DLPLMALPPSHAL**C**QF**C**VVNSELS**C**QLYQR(sequence C) |
| Cam (1x)1160.5708 Da | Lig (1x)a | Cam (1x)1316.6720 Da | Lig (1x)a | Cam (3x)3546.7006 Da | Lig (1x)Cam (2x)a |
| **A5** | M E | M E | NDb | NDb | M E | M |
| **A6** | M E | NDb | NDb | M E | M E- | NDb |
| **A10** | M E | M | E | NDb | M E | M |
| **A15 (both fragments)** | M E | E(a) | NDb | M(a)-E(ab) | M E- | NDb |
| **A20 (fragment b)** | M E | M(b) E(ab) | E | NDb | M E- | M(b) E(b) |
| **A38** | M E | NDb | NDb | M E | M E | NDb |
| **TCP** | M E | M E | NDb | NDb | M E | M |

aThe ligands bound to the Cys-containing peptides were identified by replacing the MW of the carbamidomethylic group (56 Da) with the MW of the thiol originated from the disulphides on the prototypic reporter ions 1160.5, 1316.7, 3546.7. For symmetric disulphides, the MW of the tethered-ligand corresponds to half of the MW of the molecule; for asymmetric disulphides, the MW of the tethered-ligand corresponds to the MW of either the **a** or **b** portions of the molecule; b ND= not detected