*Electronic Supplementary Information*

First-in-class small molecule inhibitors of ICOS/ICOSL interaction as a novel class of immunomodulators

*Somaya A. Abdel-Rahman,a,b* [*Katarzyna Świderek*](https://chemistry-europe.onlinelibrary.wiley.com/action/doSearch?ContribAuthorRaw=%C5%9Awiderek%2C+Katarzyna)*,c and Moustafa T. Gabra\**

*a* *Department of Radiology, Molecular Imaging Innovations Institute (MI3), Weill Cornell Medicine, New York, NY 10065, USA. Email:* [*mog4005@med.cornell.edu*](mailto:mog4005@med.cornell.edu)

*b* *Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.*

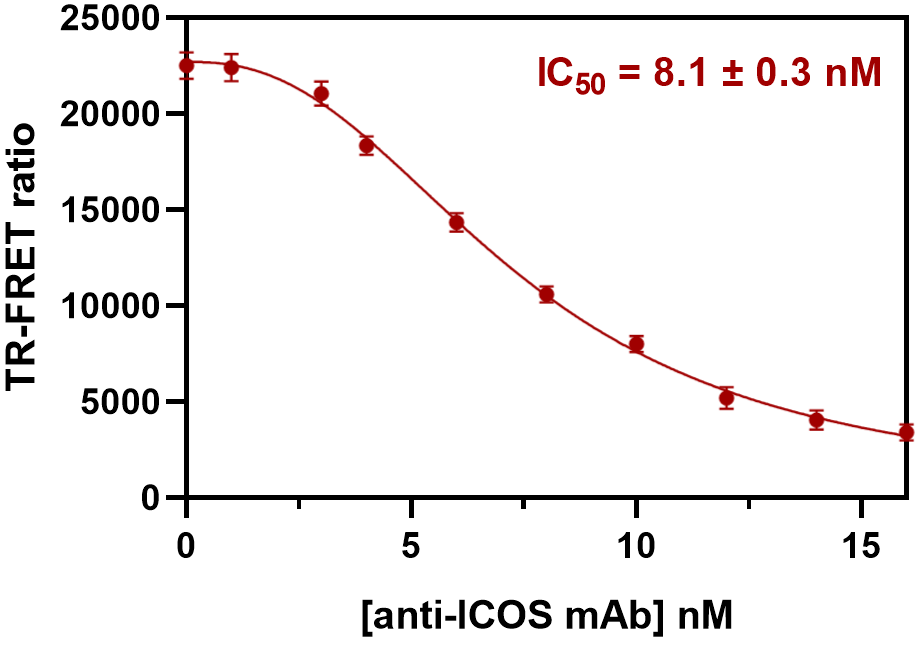
*c BioComp Group, Institute of Advanced Materials (INAM), Universitat Jaume I, 12071 Castellon, Spain.*

|  |  |
| --- | --- |
| **Contents** |  |
|  |  |
| Changes in TR-FRET ratio upon variation of the acceptor to donor ratio in the developed TR-FRET assay for ICOS/ICOSL interaction | S2 |
| Dose-response curve of anti-ICOS mAb binding in ICOS TR-FRET assay | S2 |
| Dose-response curve of the inhibitory profile of **AG-120** in ICOS/ICOSL ELISA. Error bars represent standard deviation (n = 3). | S3 |
| Overlay of ICOS structures from 6x4g and 7joo crystal structures with indicated missing residues in the former. | S3 |
| Schematic representation of interactions established between **AG-120** and its binding site in the proximity of ICOS/ICOSL interface from blind docking studies. | S4 |
| Atom types and atom point charges assigned to **AG-120** atoms using GAFF and geometry optimization at the AM1 level. | S5 |
| *In vitro* pharmacokinetic profiling of **AG-120-X** | S7 |

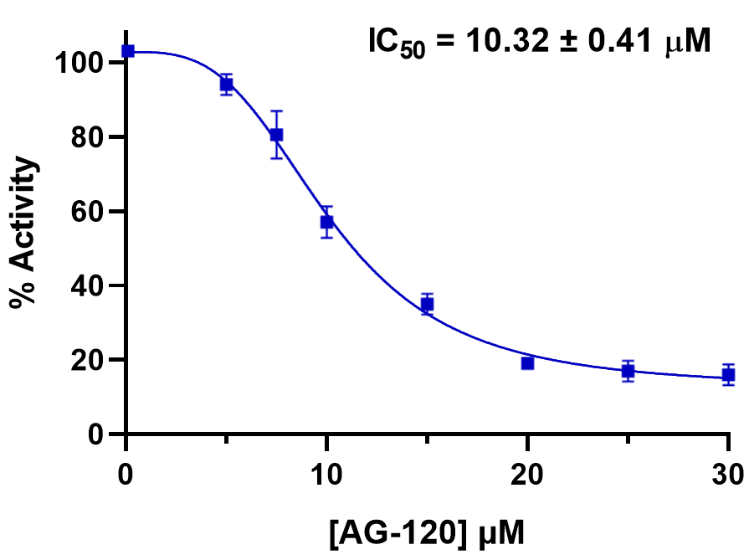
**Chart, scatter chart

Description automatically generated**

**Figure S1**. Changes in TR-FRET ratio upon variation of the acceptor to donor ratio in the developed TR-FRET assay for ICOS/ICOSL interaction. Error bars represent standard deviation (n = 3).



**Figure S2**. Dose-response curve of anti-ICOS mAb binding in ICOS TR-FRET assay. Error bars represent standard deviation (n = 3).

****

**Figure S3**. Dose-response curve of the inhibitory profile of **AG-120** in ICOS/ICOSL ELISA. Error bars represent standard deviation (n = 3).

A picture containing background pattern

Description automatically generated

**Figure S4.** Overlay of ICOS structures from 6x4g and 7joo crystal structures with indicated missing residues in the former.

A picture containing diagram

Description automatically generated

**Figure S5.** Schematic representation of interactions established between **AG-120** and its binding site in the proximity of ICOS/ICOSL interface from blind docking studies.

**Table S1.** Atom types and atom point charges assigned to **AG-120** atoms using GAFF and geometry optimization at the AM1 level.

|  |
| --- |
|  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Atom name** | **Atom type** | **Charge** | **Atom name** | **Atom type** | **Charge** |
| C35 | ca | 0.4252 | C4 | ca | -0.1375 |
| N38 | nb | -0.67 | C3 | ca | 0.3862 |
| C31 | ca | 0.4726 | H9 | h4 | 0.0301 |
| C39 | c3 | -0.1664 | N2 | nb | -0.643 |
| H42 | hc | 0.0667 | C1 | ca | 0.3632 |
| H40 | hc | 0.0667 | H7 | h4 | 0.0241 |
| H41 | hc | 0.0667 | C6 | ca | -0.1893 |
| H37 | h4 | 0.0281 | H10 | ha | 0.147 |
| C33 | ca | -0.2593 | C5 | cp | -0.052 |
| H36 | ha | 0.151 | C17 | cp | -0.069 |
| C32 | ca | -0.064 | C16 | ca | -0.069 |
| H34 | ha | 0.15 | H18 | ha | 0.158 |
| C30 | ca | -0.2759 | C15 | ca | -0.0146 |
| C28 | c | 0.6867 | Cl | cl | -0.0764 |
| O29 | o | -0.5971 | C14 | ca | 0.0871 |
| N22 | n | -0.4411 | O21 | os | -0.3439 |
| H23 | hn | 0.3205 | C24 | c3 | 0.1087 |
| C13 | ca | 0.0176 | H27 | h1 | 0.0467 |
| C12 | ca | 0.0028 | H25 | h1 | 0.0467 |
| N19 | na | -0.145300 | H26 | h1 | 0.0467 |
| H11 | hn | 0.315700 |  |  |  |
| **Parameters** | | |  |  |  |
| **Bond** | | | **Dihderals** | | |
| ca-nb 488.00 1.339  ca-h4 341.50 1.089  ca-ca 461.10 1.398  c3-ca 321.00 1.516  c3-hc 330.60 1.097  ca-ha 345.80 1.086  c -ca 345.90 1.491  c -o 637.70 1.218  c -n 427.60 1.379  hn-n 403.20 1.013  ca-n 384.20 1.412  ca-na 420.50 1.384  ca-cp 450.20 1.406  hn-na 408.40 1.010  cp-cp 351.40 1.485  ca-cl 305.60 1.750  ca-os 376.60 1.370  c3-os 308.60 1.432  c3-h1 330.60 1.097 | | | c3-ca-nb-ca 2 9.600 180.000 2.000  ca-ca-nb-ca 2 9.600 180.000 2.000  ca-ca-ca-ha 4 14.500 180.000 2.000  ca-ca-ca-ca 4 14.500 180.000 2.000  ha-ca-ca-nb 4 14.500 180.000 2.000  ca-ca-ca-nb 4 14.500 180.000 2.000  hc-c3-ca-nb 6 0.000 0.000 2.000  c -ca-ca-nb 4 14.500 180.000 2.000  o -c -ca-ca 4 4.000 180.000 2.000  n -c -ca-ca 4 4.000 180.000 2.000  ha-ca-cp-cp 4 14.500 180.000 2.000  cl-ca-ca-cp 4 14.500 180.000 2.000  ca-ca-ca-cp 4 14.500 180.000 2.000  cl-ca-ca-ha 4 14.500 180.000 2.000  ca-ca-ca-cl 4 14.500 180.000 2.000  cl-ca-ca-os 4 14.500 180.000 2.000  ca-ca-ca-na 4 14.500 180.000 2.000  h1-c3-os-ca 3 1.150 0.000 3.000  ca-ca-na-ca 4 1.200 180.000 2.000  ca-ca-cp-cp 4 14.500 180.000 2.000  ca-ca-cp-ca 4 14.500 180.000 2.000  ca-ca-os-c3 2 1.800 180.000 2.000  ca-ca-ca-os 4 14.500 180.000 2.000  cp-ca-na-ca 4 1.200 180.000 2.000  ha-ca-cp-ca 4 14.500 180.000 2.000  na-ca-cp-cp 4 14.500 180.000 2.000  na-ca-cp-ca 4 14.500 180.000 2.000  h4-ca-ca-na 4 14.500 180.000 2.000  na-ca-ca-nb 4 14.500 180.000 2.000  cp-ca-na-hn 4 1.200 180.000 2.000  ca-cp-cp-ca 4 4.000 180.000 2.000  cp-ca-ca-nb 4 14.500 180.000 2.000  cp-ca-ca-h4 4 14.500 180.000 2.000  c3-ca-ca-ca 4 14.500 180.000 2.000  c -ca-ca-c3 4 14.500 180.000 2.000  h4-ca-nb-ca 2 9.600 180.000 2.000  h4-ca-ca-ha 4 14.500 180.000 2.000  ca-ca-ca-h4 4 14.500 180.000 2.000  c -ca-ca-ca 4 14.500 180.000 2.000  ha-ca-ca-ha 4 14.500 180.000 2.000  c -ca-ca-ha 4 14.500 180.000 2.000  hc-c3-ca-ca 6 0.000 0.000 2.000  ca-c -n -hn 4 10.000 180.000 2.000  ca-c -n -ca 4 10.000 180.000 2.000  ca-ca-n -c 4 1.800 180.000 2.000  o -c -n -hn 1 2.500 180.000 -2.000  o -c -n -hn 1 2.000 0.000 1.000  o -c -n -ca 4 10.000 180.000 2.000  n -ca-ca-na 4 14.500 180.000 2.000  cp-ca-ca-n 4 14.500 180.000 2.000  ca-ca-ca-n 4 14.500 180.000 2.000  n -ca-ca-os 4 14.500 180.000 2.000  ca-ca-n -hn 4 1.800 180.000 2.000  ca-ca-na-hn 4 1.200 180.000 2.000 | | |
| Angle | | |
| ca-nb-ca 68.300 117.220  ca-ca-ha 48.200 119.880  ca-ca-ca 66.600 120.020  h4-ca-nb 51.900 116.030  ca-ca-nb 68.800 122.940  c3-ca-nb 67.300 116.680  ca-c3-hc 46.800 110.470  c -ca-ca 64.300 120.330  c3-ca-ca 63.500 120.770  hc-c3-hc 39.400 107.580  ca-ca-h4 48.100 120.340  ca-c -o 68.700 122.600  ca-c -n 67.700 115.250  c -n -hn 48.300 117.550  c -n -ca 63.800 123.710  n -c -o 74.200 123.050  ca-ca-n 67.900 120.190  ca-n -hn 47.600 116.000  ca-ca-na 69.100 118.340  ca-ca-cp 66.300 120.690  ca-ca-os 69.600 119.200  ca-na-hn 46.600 125.540  ca-na-ca 65.300 120.050  ca-cp-cp 64.000 121.110  ca-cp-ca 66.700 118.380  cp-ca-na 71.900 108.790  cp-ca-ha 48.000 119.860  ca-ca-cl 57.900 119.390  c3-os-ca 62.500 117.960  h1-c3-os 50.800 109.780  h1-c3-h1 39.200 108.460 | | |
| **Improper** | | |
| ca-h4-ca-nb 1.1 180.0 2.0 Using the default value  c3-ca-ca-nb 1.1 180.0 2.0 Using the default value  ca-ca-ca-ha 1.1 180.0 2.0 Using general improper torsional angle X- X-ca-ha, penalty score= 6.0)  c -ca-ca-ca 1.1 180.0 2.0 Using the default value  ca-n -c -o 10.5 180.0 2.0 Using general improper torsional angle X- X- c- o, penalty score= 6.0)  c -ca-n -hn 1.1 180.0 2.0 Using general improper torsional angle X- X- n-hn, penalty score= 6.0)  ca-ca-ca-n 1.1 180.0 2.0 Using the default value  ca-cp-ca-na 1.1 180.0 2.0 Using the default value  ca-ca-na-hn 1.1 180.0 2.0 Using general improper torsional angle X- X-na-hn, penalty score= 6.0)  ca-cp-ca-ha 1.1 180.0 2.0 Using general improper torsional angle X- X-ca-ha, penalty score= 6.0)  ca-ca-cp-cp 1.1 180.0 2.0 Using the default value  ca-ca-ca-cl 1.1 180.0 2.0  ca-ca-ca-os 1.1 180.0 2.0 Using the default value | | |

**Table S2.** *In vitro* pharmacokinetic profiling of **AG-120-X**.

|  |  |
| --- | --- |
| **Test** | **AG-120-X** |
| Stability in simulated fluids (t1/2, min)  Gastric (pH 1.6)  Intestinal (pH 6.5)  Stability in human plasma (% remaining at 120 min)  CACO cell permeability  *Papp*, A  B (10-6 cm/s)  *Papp*, B  A (10-6 cm/s)  Stability in rat liver microsomes (t1/2, min)  Cytotoxicity in HEL 299 cells (IC50, µM) | 385  592  98.3  14.3  32.6  405  >50 |