VEGFR2/Ang2 Compounds

The present invention relates to the field of medicine. More particularly, the present invention relates to compounds that bind to human vascular endothelial growth factor receptor-2 (VEGFR2) and human angiopoietin-2 (Ang2), and may be useful for treating cancer, especially solid tumors driven by VEGFR2 and Ang2, including gastric, hepatocellular carcinoma, ovarian, colorectal, non-small cell lung, biliary tract, and breast cancers.

A hallmark of cancer is persistent new blood vessel formation, called angiogenesis. The vascular endothelial growth factor (VEGF) pathway is an important signaling cascade in the regulation of angiogenesis; human VEGFR2 is a key receptor in the VEGF pathway. Ramucirumab (IMC-1121B) is an IgG1 antibody that targets human VEGFR2, and has been shown to have an antitumor effect in certain clinical studies (see, for example, Zhu et al., Clin Cancer Res (2013) 19:6614).

Angiopoietin-1 (Ang1) and Ang2 are members of another key pathway that regulate angiogenesis; Ang1 and Ang2 are secreted factors that bind to the endothelial cell–specific receptor tyrosine kinase Tie2. Ang1 is constitutively secreted by pericytes and stabilizes blood vessel integrity via the Tie2 receptor. Ang2 is released from endothelial cells only in response to stimulus (e.g. wound healing, tumor growth) and facilitates blood vessel sprouting and inhibits pericyte-endothelial cell interaction via Tie2 signaling. An antibody against human Ang2, when dosed in combination with the VEGF blocker aflibercept, has been shown to inhibit tumor growth and to decrease tumor vascularity in mouse xenograft tumor models (Daly et al., Cancer Res (2013) 73(1):108). Multiple investigational Ang2 antibodies are currently in clinical trials.

Inhibition of both the VEGF and Ang/Tie2 pathways of angiogenesis has been proposed for the potential to improve the outcome against cancer (see, for example, Daly et al., Cancer Res (2013) 73:108). Currently, co-administration of a VEGFR2 antibody and Ang-2 antibody would require either injections or infusions of two separate products or administration of a co-formulation of an antibody mixture. Separate administration would permit flexibility of dose amount and timing, but would be a potential issue for patient compliance and inconvenience due to increased infusion time. A co-formulation

might provide some flexibility of dosage amounts, but can be challenging to find formulation conditions that permit chemical and physical stability of both antibodies due to different molecular characteristics of the two different antibodies. Furthermore, co-administration or co-formulation involves the additive costs of two drug therapies.

WO2012/009705 disclosed complexes containing one or more modular recognition domains (MRDs) attached to scaffolds that include antibodies. Ang2 was listed as contemplated for the MRD portion of the complex, and a VEGFR2 antibody was specified as an antibody which MRDs could be attached. A MRD against Ang2 attached to a VEGFR2 antibody was not exemplified. Brown *et al.* (Mol Cancer Ther (2010) 9(1):145) disclosed a human monoclonal Ang2 antibody, 3.19.3. In a SW620 xenograft study, 3.19.3 was dosed in combination with DC101, a monoclonal antibody that binds murine VEGFR2.

When generating compounds (Compound A and B) that contain a VEGFR2 antibody portion containing the light chain variable region (LCVR) and heavy chain variable region (HCVR) of the IMC-1121B antibody in an IgG1 or IgG4 backbone, fused to a single chain Fv (scFv) portion binding human Ang2, expression and stability problems were observed by Applicant as part of the present invention. Specifically, chemical instability and unacceptable product quality in recombinantly expressed materials due to free light chain mis-pairing were surprisingly observed. As observed by Applicant as part of the present invention, compound engineering to improve chemical instability and product quality in the materials obtained from cell culture unexpectedly led to certain compounds with increased product heterogeneity due to Mab-diabody formation.

There remains a need to provide compounds that inhibit two angiogenesis pathways by binding and neutralizing both human VEGFR2 and human Ang2. In particular, there remains a need to provide compounds that inhibit two angiogenesis pathways by binding and neutralizing both human VEGFR2 and human Ang2, and without compromising significant Ang2 *in vitro* binding activity due to the use of an Ang2 scFv, and without compromising significant *in vitro* cell-based assay activity due to the combination of the VEGFR2 antibody and Ang2 scFv into one compound. There also remains a need to provide compounds that inhibit two angiogenesis pathways by binding

and neutralizing both human VEGFR2 and human Ang2, and avoid at least one of the above listed stability and product heterogeneity problems.

Accordingly, an embodiment of the present invention provides a compound, comprising an antibody fused by two linkers to two single chain fragment variable (scFv) polypeptides, wherein:

a) the antibody comprises two identical heavy chains (HCs) and two identical light chains (LCs), wherein each HC comprises a heavy chain variable region (HCVR) whose amino acid sequence is given in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, and wherein each LC comprises a light chain variable region (LVCR) whose amino acid sequence is given in SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15,

b) the two scFv polypeptides are identical and each comprise an HCVR operably linked to an LCVR, wherein each HCVR has the amino acid sequence given in SEQ ID NO: 21, or SEQ ID NO: 22, and wherein each LCVR has the amino acid sequence given in SEQ ID NO: 23, or SEQ ID NO: 24, and

c) the two linkers are identical glycine-rich linkers that each operably link the carboxy-terminus of one HC of the antibody to the amino-terminus of one of the scFv polypeptides.

In a further embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein the two scFv polypeptides each comprise the carboxy-terminus of the LCVR of one scFv polypeptide operably linked to the amino-terminus of the HCVR of one scFv polypeptide.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 1, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 13, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 2, each LCVR of the

antibody has the amino acid sequence given in SEQ ID NO: 14, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 3, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 15, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 4, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 13, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 22, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 24.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein the antibody comprises two heavy chains (HCs) and two light chains (LCs), wherein each HC has the amino acid sequence given in one of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, and each LC has the amino acid sequence given in one of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 5, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 16.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 6, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 17.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HC of the antibody

has the amino acid sequence given in SEQ ID NO: 7, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 18.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 8, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 16.

In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each scFv polypeptide has the identical amino acid sequence given in one of SEQ ID NO: 19 or SEQ ID NO: 20. In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each scFv polypeptide has the amino acid sequence given in SEQ ID NO: 19. In an embodiment, the present invention provides a compound comprising an antibody fused by two linkers to two scFv polypeptides, wherein each scFv polypeptide has the amino acid sequence given in SEQ ID NO: 20.

In an embodiment, the present invention provides a compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18. As shown in Table 1, the two first polypeptides comprise the HC of the antibody, the linker, and the scFv polypeptide; the two second polypeptides comprise the LC of the antibody.

In an embodiment, the present invention provides a compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 9, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16. In an embodiment, the present invention provides a compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 10, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 17. In an embodiment, the present invention provides a compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the

amino acid sequence of SEQ ID NO: 11, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 18. In an embodiment, the present invention provides a compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 12, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16.

In an embodiment, the present invention further provides a compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides forms an inter-chain disulfide bond with each of the second polypeptides, and the first polypeptide forms two inter-chain disulfide bonds with the other first polypeptide, and each of the first polypeptides forms an intra-chain disulfide bond.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein:

a) the antibody comprises two identical heavy chains (HCs) and two identical light chains (LCs), wherein each HC comprises a heavy chain variable region (HCVR) whose amino acid sequence is given in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, and wherein each LC comprises a light chain variable region (LVCR) whose amino acid sequence is given in SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15,

b) the two scFv polypeptides are identical and each comprise an HCVR operably linked to an LCVR, wherein each HCVR has the amino acid sequence given in SEQ ID NO: 21, or SEQ ID NO: 22, and wherein each LCVR has the amino acid sequence given in SEQ ID NO: 23, or SEQ ID NO: 24, and

c) the two linkers are identical glycine-rich linkers that each operably link the carboxy-terminus of one HC of the antibody to the amino-terminus of one of the scFv polypeptides.

In a further embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2

(SEQ ID NO: 33), wherein the carboxy-terminus of the LCVR of each scFv polypeptide is operably linked to the amino-terminus of the HCVR.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 1, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 13, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 2, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 14, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 3, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 15, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 4, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO:

13, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 22, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 24.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein the antibody comprises two heavy chains (HCs) and two light chains (LCs), wherein each HC has the amino acid sequence given in one of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, and each LC has the amino acid sequence given in one of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 5, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 16.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 6, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 17.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 7, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 18.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID

NO: 33), wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 8, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 16.

In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each scFv polypeptide has the identical amino acid sequence given in one of SEQ ID NO: 19 or SEQ ID NO: 20. In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each scFv polypeptide has the amino acid sequence given in SEQ ID NO: 19. In an embodiment, the present invention provides a compound that binds human VEGFR2 and human Ang2 comprising an antibody that binds human VEGFR2 (SEQ ID NO: 32) fused by two linkers to two scFv polypeptides that bind human Ang2 (SEQ ID NO: 33), wherein each scFv polypeptide has the amino acid sequence given in SEQ ID NO: 20.

In an embodiment, the present invention provides a compound that binds human VEGFR2 (SEQ ID NO: 32) and human Ang2 (SEQ ID NO: 33) comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18. In an embodiment, the present invention provides a compound that binds human VEGFR2 (SEQ ID NO: 32) and human Ang2 (SEQ ID NO: 33) comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 9, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16. In an embodiment, the present invention provides a compound that binds human VEGFR2 (SEQ ID NO: 32) and human Ang2 (SEQ ID NO: 33) comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 10, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 17. In an embodiment, the present invention provides a

compound that binds human VEGFR2 (SEQ ID NO: 32) and human Ang2 (SEQ ID NO: 33) comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 11, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 18. In an embodiment, the present invention provides a compound that binds human VEGFR2 (SEQ ID NO: 32) and human Ang2 (SEQ ID NO: 33) comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 12, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16.

In an embodiment, the present invention further provides a compound that binds human VEGFR2 (SEQ ID NO: 32) and human Ang2 (SEQ ID NO: 33) comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides forms an inter-chain disulfide bond with each of the second polypeptides, and the first polypeptide forms two inter-chain disulfide bonds with the other first polypeptide, and each of the first polypeptides forms an intra-chain disulfide bond.

In a further embodiment, the present invention provides a compound of the present invention that has a dissociation equilibrium constant, K_D, of about 300 pM to about 1400 pM for human VEGFR2 and a K_D of about 300 pM to about 800 pM for human Ang2. The compound of the present invention is further characterized by a k_{on} rate to human VEGFR2 of about 0.1 x 10^5 $M^{-1}sec^{-1}$ to about 0.5 x 10^5 $M^{-1}sec^{-1}$ and a k_{on} rate to human Ang2 of about 1 x 10^5 $M^{-1}sec^{-1}$ to about 3 x 10^5 $M^{-1}sec^{-1}$. The compound of the present invention is further characterized by a k_{off} rate to human VEGFR2 of about 0.1 x 10^{-4} sec^{-1} to about 0.5 x 10^{-4} sec^{-1} and a k_{off} rate to human Ang2 of about 0.8 x 10^{-4} sec^{-1} to about 1.2 x 10^{-4} sec^{-1}. The K_D, k_{on}, and k_{off} values are established by binding kinetics at 25°C as described in "Binding kinetics, affinity, and selectivity" in the Assays section.

The compound of the present invention binds to human VEGFR2 and human Ang2 with high affinity. For the purposes of the present disclosure, the term "high affinity" refers to a K_D of less than about 1500 pM for human VEGFR2 and of less than about 1000 pM for human Ang2. The K_D values are established by binding kinetics at 25°C as described in "Binding kinetics, affinity, and selectivity" in the Assays section.

In an embodiment, the present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence a encoding a polypeptide selected from the group consisting of a polypeptide consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 and a polynucleotide sequence encoding a polypeptide selected from the group consisting of a polypeptide consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, wherein the cell is capable of expressing a compound comprising a first polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 and a second polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.

In an embodiment, the present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 9 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 16, wherein the cell is capable of expressing a compound comprising a first polypeptide having an amino acid sequence of SEQ ID NO: 9 and a second polypeptide having an amino acid sequence of SEQ ID NO: 16.

In an embodiment, the present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 10 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 17, wherein the cell is capable of expressing a compound comprising a first polypeptide having an amino acid sequence of SEQ ID NO: 10 and a second polypeptide having an amino acid sequence of SEQ ID NO: 17.

In an embodiment, the present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 11 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 18, wherein the cell is capable of expressing a compound comprising a first polypeptide having an amino acid sequence of SEQ ID NO: 11 and a second polypeptide having an amino acid sequence of SEQ ID NO: 18.

In an embodiment, the present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 12 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 16, wherein the cell is capable of expressing a compound comprising a first polypeptide having an amino acid sequence of SEQ ID NO: 12 and a second polypeptide having an amino acid sequence of SEQ ID NO: 16.

In an embodiment, the present invention provides a process for producing a compound comprising two first polypeptides selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 and two second polypeptides selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, comprising cultivating the mammalian cell of the present invention under conditions such that the compound is expressed, and recovering the expressed compound.

In an embodiment, the present invention provides a process for producing a compound comprising two first polypeptides of SEQ ID NO: 9 and two second polypeptides of SEQ ID NO: 16, comprising cultivating the mammalian cell of the present invention under conditions such that the compound is expressed, and recovering the expressed compound.

In an embodiment, the present invention provides a process for producing a compound comprising two first polypeptides of SEQ ID NO: 10 and two second polypeptides of SEQ ID NO: 17, comprising cultivating the mammalian cell of the present invention under conditions such that the compound is expressed, and recovering the expressed compound.

In an embodiment, the present invention provides a process for producing a compound comprising two first polypeptides of SEQ ID NO: 11 and two second polypeptides of SEQ ID NO: 18, comprising cultivating the mammalian cell of the present invention under conditions such that the compound is expressed, and recovering the expressed compound.

In an embodiment, the present invention provides a process for producing a compound comprising two first polypeptides of SEQ ID NO: 12 and two second

polypeptides of SEQ ID NO: 16, comprising cultivating the mammalian cell of the present invention under conditions such that the compound is expressed, and recovering the expressed compound.

In an embodiment of the above-described processes, the two polynucleotide sequences in the mammalian cell of the present invention are part of the same nucleic acid molecule.

In an embodiment, the present invention provides a compound obtainable by one of the aforementioned processes.

In an embodiment, the present invention provides a pharmaceutical composition, comprising a compound of the present invention, and an acceptable carrier, diluent, or excipient.

In an embodiment, the present invention provides a method of treating cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention. In a further embodiment, the present invention provides a method of treating cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, or hepatocellular carcinoma. In a further embodiment, the present invention provides a method of treating cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, biliary tract cancer, or hepatocellular carcinoma. In a further embodiment, these methods comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, ramucirumab, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), and cetuximab.

In a further embodiment, these methods comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or

sequential combination with one or more immuno-oncology agents selected from the group consisting of nivolumab, ipilimumab, pidilizumab, pembrolizumab, and durvalumab.

In a further embodiment, the present invention provides a method of treating cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention in simultaneous, separate, or sequential combination with one or more immuno-oncology agents, wherein the cancer is bladder cancer, kidney cancer, prostate cancer, or testicular cancer, and wherein the immuno-oncology agents are selected from the group consisting of nivolumab, ipilimumab, pidilizumab, pembrolizumab, and durvalumab.

In an embodiment, the present invention provides a method of treating breast cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention. In a further embodiment, these methods of treating breast cancer comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel protein-bound particles for injectable suspension, ixabepilone, and capecitabine.

In an embodiment, the present invention provides a method of treating ovarian cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention. In a further embodiment, these methods of treating ovarian cancer comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, and liposomal doxorubicin.

In an embodiment, the present invention provides a method of treating gastric cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention. In a further embodiment, these methods of treating gastric cancer comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with ramucirumab.

In an embodiment, the present invention provides a method of treating hepatocellular carcinoma, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention. In a further embodiment, these methods of treating hepatocellular carcinoma comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with ramucirumab.

In an embodiment, the present invention provides a method of treating colorectal cancer, comprising administering to a patient in need thereof, an effective amount of a compound of the present invention. In a further embodiment, these methods of treating colorectal cancer comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), and cetuximab.

In an embodiment, the present invention provides a compound of the present invention, for use in therapy. In an embodiment, the present invention provides a compound of the present invention, for use in the treatment of cancer. In a further embodiment, the present invention provides a compound of the present invention, for use in the treatment of cancer, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, or hepatocellular carcinoma. In a further embodiment, the present invention provides a compound of the present invention, for use in the treatment of cancer, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, biliary tract cancer, or hepatocellular carcinoma. In a further embodiment, for use in the treatment of cancer, the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, ramucirumab, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), and cetuximab.

In a further embodiment, for use in the treatment of cancer, the compound of the present invention in simultaneous, separate, or sequential combination with one or more immuno-oncology agents selected from the group consisting of nivolumab, ipilimumab, pidilizumab, pembrolizumab, and durvalumab.

In a further embodiment, for use in the treatment of cancer, the compound of the present invention in simultaneous, separate, or sequential combination with one or more immuno-oncology agents, wherein the cancer is bladder cancer, kidney cancer, prostate cancer, or testicular cancer, and wherein the immuno-oncology agents are selected from the group consisting of nivolumab, ipilimumab, pidilizumab, pembrolizumab, and durvalumab.

In an embodiment, the present invention provides a compound of the present invention, for use in the treatment of breast cancer. In a further embodiment, for use in the treatment of breast cancer, the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel protein-bound particles for injectable suspension, ixabepilone, and capecitabine.

In an embodiment, the present invention provides a compound of the present invention, for use in the treatment of ovarian cancer. In a further embodiment, for use in the treatment of ovarian cancer, the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, and liposomal doxorubicin.

In an embodiment, the present invention provides a compound of the present invention, for use in the treatment of gastric cancer. In a further embodiment, for use in the treatment of gastric cancer, the compound of the present invention in simultaneous, separate, or sequential combination with ramucirumab.

In an embodiment, the present invention provides a compound of the present invention, for use in the treatment of hepatocellular carcinoma. In a further embodiment, for use in the treatment of hepatocellular carcinoma, the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group ramucirumab.

In an embodiment, the present invention provides a compound of the present invention, for use in the treatment of colorectal cancer. In a further embodiment, for use in the treatment of colorectal cancer, the compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), and cetuximab.

In an embodiment, the present invention provides the use of a compound of the present invention for the manufacture of a medicament for the treatment of cancer. In a further embodiment, the present invention provides the use of a compound of the present invention for the manufacture of a medicament for the treatment of cancer, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, or hepatocellular carcinoma. In a further embodiment, the present invention provides the use of a compound of the present invention for the manufacture of a medicament for the treatment of cancer, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, biliary tract cancer, or hepatocellular carcinoma.

In a further embodiment , the present invention provides the use of a compound of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, ramucirumab, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), and cetuximab for the manufacture of a medicament for the treatment of cancer.

A compound of the present invention is an engineered, non-naturally occurring polypeptide complex. A DNA molecule of the present invention is a non-naturally occurring DNA molecule that comprises a polynucleotide sequence encoding a polypeptide having the amino acid sequence of one of the polypeptides in a compound of the present invention.

The antibody portion of the compound of the present invention is designed to have engineered CDRs and have some portions of the antibody (all or parts of the frameworks, hinge regions, and constant regions) to be of human origin that are identical with or

substantially identical (substantially human) with frameworks and constant regions derived from human genomic sequences. Fully human frameworks, hinge regions, and constant regions are those human germline sequences as well as sequences with naturally-occurring somatic mutations and those with engineered mutations. The antibody portion of the compound of the present invention may comprise framework, hinge, or constant regions derived from a fully human framework, hinge, or constant region containing one or more amino acid substitutions, deletions, or additions therein. Further, the antibody portion of the compound of the present invention is preferably substantially non-immunogenic in humans.

The antibody portion of the compound of the present invention is an IgG type antibody and has four amino acid chains (two "heavy" chains and two "light" chains) that are covalently stabilized via intra- and inter-chain disulfide bonds. Each heavy chain is comprised of an N-terminal HCVR and a heavy chain constant region ("HCCR"). Each light chain is comprised of a LCVR and a light chain constant region ("LCCR"). When expressed in certain biological systems, antibodies having native human Fc sequences are glycosylated in the Fc region. Typically, glycosylation occurs in the Fc region of the antibody at a highly conserved N-glycosylation site. N-glycans typically attach to asparagine. Antibodies may be glycosylated at other positions as well.

Optionally, the antibody portion of the compound of the present invention contains an Fc portion which is derived from human IgG_4 Fc region because of a reduced ability to engage Fc receptor-mediated inflammatory mechanisms or to activate complement resulting in reduced effector function.

Further, the antibody portion of certain compounds of the present invention contains an IgG_4-PAA Fc portion. The IgG_4-PAA Fc portion has a serine to proline mutation at position 224, a phenylalanine to alanine mutation at position 230, and a leucine to alanine mutation at position 231. The S224P mutation is a hinge mutation that prevents half-antibody formation (phenomenon of dynamic exchange of half-molecules in IgG_4 antibodies). The F230A and L231A mutations further reduce effector function of the already low human IgG_4 isotype.

An isolated DNA molecule encoding a HCVR region can be converted to a full-length heavy chain gene by operably linking the HCVR-encoding DNA to another DNA

molecule encoding heavy chain constant regions. The sequences of human, as well as other mammalian, heavy chain constant region genes are known in the art. DNA fragments encompassing these regions can be obtained *e.g.,* by standard PCR amplification.

An isolated DNA encoding a LCVR region may be converted to a full-length light chain gene by operably linking the LCVR-encoding DNA to another DNA molecule encoding a light chain constant region. The sequences of human, as well as other mammalian, light chain constant region genes are known in the art. DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region.

A "single chain fragment variable" or "scFv" or "scFv polypeptide" refers to an engineered, non-naturally occurring single folded polypeptide comprising the LCVR and the HCVR of an antibody linked through a scFv linker molecule. The scFv polypeptide portion of the compound of the present invention is an engineered, non-naturally occurring scFv that has been designed to have engineered CDRs and have some portions of the scFv (all or parts of the frameworks) to be of human origin that are identical with or substantially identical (substantially human) with frameworks derived from human genomic sequences. Fully human frameworks are those human germline sequences as well as sequences with naturally-occurring somatic mutations and those with engineered mutations. The scFv polypeptide portion of the compound of the present invention may comprise framework derived from a fully human framework containing one or more amino acid substitutions, deletions, or additions therein. Further, the scFv polypeptide portion of the compound of the present invention is preferably substantially non-immunogenic in humans. Optionally, the scFv polypeptide portion of the compound can have 44-100 disulfides from cysteine 44 in HCVR and cysteine 100 in LCVR (Cys44 and Cys100 numbering corresponds with a numbering that starts with the first amino acid of the scFv polypeptide). In such a scFv polypeptide, the HCVR and LCVR domains can be either in the HCVR – scFv linker - LCVR or LCVR – scFv linker - HCVR order. The scFv linker can be a flexible glycine-rich peptide linker which enables the HCVR and LCVR chains to be folded as a functional monomeric unit for recognizing an antigen.

Optionally, the scFv linker is a glycine-rich linker such as a 2x G4S linker, a 3x G4S linker, a 4x G4S linker, or a 5x G4S linker.

Fusion of a scFv to an antibody can allow for multiple structures to form during expression and secretion. Firstly, scFv elements fused to an antibody can fold independently via an intramolecular interaction by which individual HCVR and LCVR elements located within the same polypeptide fold to form two separate and autonomous units, referred here to as Mab-scFv. Secondly, via an alternative folding pathway, scFv elements fused to an antibody can fold via intermolecular interactions by which a HCVR present in one polypeptide will fold with a LCVR present in the adjacent polypeptide to form a single co-folded species, here referred to as Mab-Diabody.

The term "linker" and "scFv linker" both refer to glycine-rich peptide linkers. The "linkers" are utilized in certain embodiments of the invention to link the antibody to the scFv, and the "scFv linkers" are utilized in certain embodiments of the invention to link the LCVR of the scFv to the HCVR of the scFv. Preferably, the peptide linkers are glycine-rich peptides with at least 5 amino acids, preferably of at least 10 amino acids, more preferably between 10 and 50 amino acids. In some embodiments of the present invention, said glycine-rich peptide linker is $(G_xS)_n$ with G=glycine, S=serine, (x=3 and n=3, 4, 5 or 6) or (x=4 and n=2, 3, 4 or 5). For example, in some embodiments of the present invention, said glycine-rich peptide linker is $(G_xS)_n$ with G=glycine, S=serine, x=4 and n=2, 3, 4 or 5 (i.e., GGGGSGGGGS (SEQ ID NO: 34), GGGGSGGGGSGGGGS (SEQ ID NO: 35), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 36), or GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 37), respectively.

The polynucleotides of the present invention will be expressed in a host cell after the sequences have been operably linked to an expression control sequence. The expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline, neomycin, and dihydrofolate reductase, to permit detection of those cells transformed with the desired DNA sequences.

The compound of the present invention may readily be produced in mammalian cells such as CHO, NS0, HEK293 or COS cells. The host cells are cultured using techniques well known in the art.

The vectors containing the polynucleotide sequences of interest (e.g., the polynucleotides encoding the polypeptides of the compound and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host.

Various methods of protein purification may be employed and such methods are known in the art and described, for example, in Deutscher, *Methods in Enzymology* 182: 83-89 (1990) and Scopes, *Protein Purification: Principles and Practice*, 3rd Edition, Springer, NY (1994).

In another embodiment of the present invention, the compound, or the nucleic acids encoding the same, is provided in isolated form. As used herein, the term "isolated" refers to a protein, peptide, or nucleic acid which is free or substantially free from any other macromolecular species found in a cellular environment. "Substantially free" as used herein means the protein, peptide, or nucleic acid of interest comprises more than 80% (on a molar basis) of the macromolecular species present, preferably more than 90%, and more preferably more than 95%.

The compound of the present invention, or pharmaceutical compositions comprising the same, may be administered by parenteral routes (e.g., subcutaneous and intravenous). A compound of the present invention may be administered to a patient alone with pharmaceutically acceptable carriers, diluents, or excipients in single or multiple doses. Pharmaceutical compositions of the present invention can be prepared by methods well known in the art (e.g., *Remington: The Science and Practice of Pharmacy*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co.) and comprise a compound, as disclosed herein, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

The term "treating" (or "treat" or "treatment") refers to slowing, interrupting, arresting, alleviating, stopping, reducing, or reversing the progression or severity of an existing symptom, disorder, condition, or disease. A patient refers to a mammal,

preferably a human with a disease, disorder, or condition that would benefit from inhibition of VEGFR2 and/or Ang2.

"Binds" as used herein in reference to the affinity of a compound, antibody, or scFv polypeptide for human VEGFR2 or human Ang2 is intended to mean, unless indicated otherwise, a K_D of less than about 1 x 10^{-8} M, preferably, less than about 1 x 10^{-9} M as determined by common methods known in the art, including by use of a surface plasmon resonance (SPR) biosensor at 25°C or 37°C essentially as described herein. The term "selective" or "selectivity" used herein in reference to a compound of the present invention refers to a compound that binds a target, such as human Ang2, with a K_D about 1000-, 500-, 200-, 100-, 50-, or about 10-fold lower than the compound binds other proteins, including member of the target family such as human Ang1, as measured by surface plasmon resonance at 25°C or 37°C. Additionally, or alternatively, an Ang2 selective compound of the present invention binds human Ang2 but does not bind or only minimally binds human Ang1 when assayed by the methods described in the Example herein below.

"Effective amount" means the amount of a compound of the present invention or pharmaceutical composition comprising a compound of the present invention that will elicit the biological or medical response of or desired therapeutic effect on a tissue, system, animal, mammal or human that is being sought by the researcher, medical doctor, or other clinician. An effective amount of the compound may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effect of the compound is outweighed by the therapeutically beneficial effects.

This invention is further illustrated by the following non-limiting examples.

Example 1: Compound expression and purification

The polypeptides of the antibody portions, the scFv portions, and the antibody-linker-scFv of Compound C, Compound D, Compound E, and Compound F, and the nucleotide sequences encoding the same, are listed below in the section entitled "Amino Acid and Nucleotide Sequences." In addition, the SEQ ID NOs for the antibody portions,

the scFv portions, and the antibody-linker-scFv of Compound C, Compound D, Compound E, and Compound F are shown in Table 1.

The compounds of the present invention, including, but not limited to Compound C, Compound D, Compound E, and Compound F, can be made and purified essentially as follows. An appropriate host cell, such as HEK 293 or CHO, can be either transiently or stably transfected with an expression system for secreting compounds using an optimal predetermined HC-linker-scFv:LC vector ratio (such as 1:3 or 1:2) or a single vector system encoding both HC-linker-scFv and LC. Clarified media, into which the compound has been secreted, may be purified using any of many commonly-used techniques. For example, the medium may be conveniently applied to a MabSelect column (GE Healthcare), or KappaSelect column (GE Healthcare) for Fab fragment, that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column may be washed to remove nonspecific binding components. The bound compound may be eluted, for example, by pH gradient (such as 20 mM Tris buffer pH 7 to 10 mM sodium citrate buffer pH 3.0, or phosphate buffered saline pH 7.4 to 100 mM glycine buffer pH 3.0). Compound fractions may be detected, such as by SDS-PAGE, and then may be pooled. Further purification is optional, depending on the intended use. The compound may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, multimodal, or hydroxyapatite chromatography. The purity of the compound after these chromatography steps is greater than 95%. The product may be immediately frozen at -70°C or may be lyophilized.

Table 1: SEQ ID NOs

	Compound C	**Compound D**	**Compound E**	**Compound F**
HCVR of antibody	1	2	3	4
HC of antibody	5	6	7	8
HC of antibody + linker + scFv	9	10	11	12

polypeptide				
LCVR of antibody	13	14	15	13
LC of antibody	16	17	18	16
scFv polypeptide	19	19	19	20
HCVR of scFv polypeptide	21	21	21	22
LCVR of scFv polypeptide	23	23	23	24

Assays

Binding kinetics, affinity, and selectivity

The binding kinetics, affinity, and selectivity to human Ang2 and to human VEGFR2, for compounds of the present invention, may be determined by use of a surface plasmon resonance (SPR) biosensor such as a Biacore® 2000, Biacore® 3000, or a Biacore® T100 (GE HealthCare) according to methods known in the art.

The kinetics and equilibrium dissociation constant (K_D) for multiple species of soluble Ang2 (human, cyno, mouse, rabbit and dog) and VEGFR2 extracellular domain (ECD) (human, cyno, rabbit and dog) may be determined for compounds of the present invention at 25^{0}C or 37^{0}C using Biacore surface plasmon resonance methods. Human Ang2 and VEGFR2-ECD may be purchased from R&D Systems and Sino Biological, respectively. Protein A surface for capture of antibodies may be prepared using the following methods. Immobilization of soluble Protein A (Calbiochem, Cat: 539202) on a CM4 (Biacore # BR-1005-34) or CM5 (Biacore #BR-1000-99) may be prepared using EDC/NHS amine coupling method (Biacore # BR-1000-50). Briefly, the surfaces of all four flow cells may be activated by injecting a 1:1 mixture of EDC/NHS for seven minutes at 10 µL/min. After which, soluble protein A may be diluted to 50-100 µg/mL in 10 mM acetate buffer, pH 4.5, and immobilized for seven minutes onto flow cell (Fc) 2, 3 or 4 at a flow rate of 10 µL/min. Un-reacted sites still remaining on the chip surface may be blocked with a seven minute injection of ethanolamine at 10 µL/min. Running buffer may be HBS-EP+ (Biacore # BR-1006-69). Compound samples may be prepared at 1 µg/mL by dilution into running buffer. Discrete concentrations of Ang2 ligands ranging

from 50 nM to 1.56 nM may be prepared using a two-fold serial dilution into running buffer. Each analysis cycle may consist of a series of five separate steps: (1) capture of compound onto separate flow cells (Fc2, Fc3, and Fc4), (2) injection (using kinject) of 250 µL (300-second surface contact time) of discrete concentrations of Ang2 or VEGFR2-ECD over all Fc at 50 µL/min, (3) return to buffer flow for 20 minutes to monitor dissociation phase, (4) regeneration of chip surfaces with a 10 µL (30-second contact time) injection of 10 mM glycine, pH1.5, (5) equilibration of chip surface with a 15 µL (45-second contact time) injection of HBS-EP+ running buffer. Resultant data may be processed using standard double-referencing and fit to a 1:1 binding model using Biacore 2000 Evaluation software, version 4.1, to determine the association rate (k_{on}, $M^{-1}s^{-1}$ units), dissociation rate (k_{off}, s^{-1} units). Calculation of the equilibrium dissociation constant (K_D) may be calculated from the following relationship, $K_D = k_{off}/k_{on}$, and is presented in molar units.

In experiments performed essentially as described in this assay, Compound C, Compound D, Compound E, and Compound F bind human Ang2 at 25^{0}C with a K_D in the range of 407 pM to 673 pM (Table 2). Compound C, Compound D, Compound E, and Compound F bind human VEGFR2-ECD at 25^{0}C with a K_D in the range of 528 pM to 1110 pM (Table 3). These compounds of the present invention demonstrate high affinity for both human Ang2 and human VEGFR2.

In experiments performed essentially as described in this assay, Compound F exhibited comparable binding kinetics and affinities for human Ang2 relative an Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F (Tables 4 and 5). This demonstrates that the potent binding kinetics of the Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F is retained in Compound F.

In experiments performed essentially as described in this assay, Compound F binds human, cynomolgus monkey, mouse rabbit and dog Ang2 with comparable high affinities, and Compound F binds human, cynomolgus monkey, rabbit and dog VEGFR2 with comparable high affinities.

Table 2

Biacore SPR hAng2 at 25°C			
	k_{on} (10^5 1/Ms)	k_{off} (10^{-4} 1/s)	K_D (pM)
Compound A	1.83	<0.1	<100pM
Compound B	2.85	<0.1	<100pM
Compound C	2.26	1.05	462
Compound D	1.75	0.86	489
Compound E	2.39	0.97	407
Compound F	1.56	1.05	673

Table 3

Biacore SPR hVEGFR2-ECD at 25°C			
	k_{on} (10^5 1/Ms)	k_{off} (10^{-4} 1/s)	K_D (pM)
Compound A	0.68	0.48	711
Compound B	0.81	0.41	513
Compound C	0.38	0.20	528
Compound D	0.34	0.29	860
Compound E	0.32	0.35	1110
Compound F	0.25	0.18	721

Table 4

Ang2 antibody (same HCDRs/LCDRs as scFv polypeptide portion of Cmpd F) Biacore SPR Ang2 Binding at 37°C (n=3)			
	k_{on} (1/Ms)	k_{off} (1/s)	K_D (pM)
human Ang2	1.0 (± 0.4) x 10^6	0.7 (± 0.2) x 10^{-4}	80 ± 50
cyno Ang2	2.7 (± 0.2) x 10^6	2.9 (± 0.1) x 10^{-4}	107 ± 6
mouse Ang2	1.0 (± 0.2) x 10^6	1.1 (± 0.1) x 10^{-4}	109 ± 9
rabbit Ang2	7.3 (± 1.9) x 10^5	1.5 (± 0.1) x 10^{-4}	210 ± 40
dog Ang2	7.9 (± 3.2) x 10^5	1.1 (± 0.4) x 10^{-4}	140 ± 10

Table 5

Compound F Biacore SPR Ang2 Binding at 37°C (n=3)			
	k_{on} (1/Ms)	k_{off} (1/s)	K_D (pM)

human Ang2	8.2 (± 2.4) x 10^5	0.7 (± 0.3) x 10^{-4}	100 ± 70
cyno Ang2	9.6 (± 1.3) x 10^5	2.8 (± 0.1) x 10^{-4}	300 ± 30
mouse Ang2	3.6 (± 0.9) x 10^5	0.8 (± 0.1) x 10^{-4}	230 ± 20
rabbit Ang2	2.7 (± 0.7) x 10^5	1.1 (± 0.1) x 10^{-4}	420 ± 60
dog Ang2	3.6 (± 1.6) x 10^5	0.9 (± 0.3) x 10^{-4}	240 ± 30

Table 6

Compound F Biacore SPR VEGFR2-ECD Binding at 37°C (n=3)			
	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
human VEGFR2	2.5 (± 0.1) x 10^4	1.2 (± 0.2) x 10^{-4}	4.6 ± 0.7
cyno VEGFR2	3.4 (± 0.2) x 10^4	1.1 (± 0.1) x 10^{-4}	3.3 ± 0.4
rabbit VEGFR2	3.8 (± 0.5) x 10^4	2.3 (± 0.1) x 10^{-4}	6.1 ± 1.0
dog VEGFR2	4.1 (± 0.3) x 10^4	1.5 (± 0.7) x 10^{-3}	37 ± 1

Inhibition of human Ang2 to human Tie2 interaction

The blocking of human Ang2 binding to its receptor human Tie 2 by a compound of the present invention may be measured in a solid phase *in vitro* ELISA assay. The aforementioned *in vitro* cell-based assay may also be used to establish comparable blocking activity of a compound of the present invention to an Ang2 antibody with the same HCDRs and LCDRs sequences as the scFv polypeptide portion of the compound.

For the assay, high binding 96-well ELISA plates (Costar #2592) may be coated with 4 μg/ml (in 100 μl) recombinant human Tie2-Fc (R&D Systems #313-TI), overnight at room temperature. The plates may be washed 3X with TBST (Tris buffered saline containing 0.05% Tween 20) and then may be blocked with 300 μl per well of blocking buffer (0.5% BSA/D-PBS) (BSA: Jackson ImmunoResearch #001-000-162; IgG-free, protease-free) for 1-2 hours at room temperature on an orbital shaker. During the blocking step, in separate polypropylene mutiwell plates, 75 μl of 2X test antibodies (serially diluted 1:3 in blocking buffer) may be added with 75 μl of 2X biotinylated human Ang2 (R&D Systems #BT623) (also diluted in blocking buffer). The

antibody/biotinylated Ang2 mixtures may then be incubated for 1 hour at 37°C (final biotinylated Ang2 concentration was 100 ng/ml). The blocking solution may be removed from the Tie2-Fc coated ELISA plates, after which 50 µl per well of the antibody/biotinylated Ang2 mixtures may be added (in duplicate wells). The plates may then be incubated for 2 hours at room temperature, covered with plate sealers, on an orbital shaker. Plates may then be washed 3X, after which 100 µl per well of streptavidin-HRP (R&D Systems #DY998, may be diluted 1:200 in blocking buffer) may be added. Plates may then be incubated for 45 minutes at room temperature, covered with plate sealers, on an orbital shaker. Plates may then be washed again 3X.

Plates may then be developed by adding 100 µl per well of One Component TMB substrate (may be warmed to room temperature) (Surmodics/BioFX Labs #TMBW-1000-01). Development may be allowed to progress for 10 minutes at room temperature (plates may be covered with aluminum foil). Development may be stopped with 100 µl per well of acid stop solution (TMB stop solution, Surmodics/BioFX Labs #LSTP-1000-01). Plates may be mixed on an orbital shaker after which they may be read at 450 nm on an ELISA reader (Molecular Devices SpectraMax 190), using SOFTmax PRO 5.4.1 software (Molecular Devices Corp.). The A450 values reflect the amount of biotinylated Ang2 that remained bound to Tie-2-Fc. Reduction of A450 values reflected blocking of biotinylated Ang2 binding to Tie-2-Fc.

IC50 values for inhibition of Ang2 binding to Tie-2 may be calculated with GraphPad Prism 6, using Log-transformed X values. Nonlinear regression (curve fit) analysis (sigmoidal dose response, variable slope) may be performed on the log-transformed data to obtain IC50 values. If an experiment is performed more than once, the geometric mean IC50 value (and 95% confidence interval) between experiments may be calculated.

In experiments performed essentially as described in this assay, Compound F and an Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F result in geometric mean IC50 values (n=2) of 0.034 nM and 0.027 nM respectively. Compound F dose dependently blocks human Ang2 binding to human Tie-2 comparably to the Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F. This data indicates that the Ang2 scFv polypeptide

portion of the compound has maintained potency in this assay that is comparable to that of the Ang2 antibody.

Neutralization of Ang2 induced phosphorylation of Tie2, but not Ang1 mediated phosphorylation.

The *in vitro* cell-based inhibition of human Ang2 by a compound of the present invention may be measured in a cell-based assay where Ang1 and Ang2 bind to and induce human Tie2 phosphorylation in a dose-dependent manner. The *in vitro* cell-based assay may be used to evaluate the ability of compounds of the present invention to selectively neutralize Ang2 and not Ang1 mediated phosphorylation of the Tie-2 receptor in a dose-dependent manner. An Ang2 antibody, an Ang1 antibody, and a control human IgG4 PAA isotype antibody may be included as positive and negative controls respectively.

The CHO-Tie2 cell line may be generated by stable transfection of a full-length human Tie2 receptor (with a 3X FLAG tag at the C-terminus). CHO-Tie2 cells may be maintained in complete medium of Hams F-12 (CellGro/Mediatech #10-080-CV), 10% heat inactivated FBS (Life Technologies/Invitrogen #10082-147), 1X antibiotic-antimycotic (Life Technologies/Invitrogen #15240-062), 1.25 mg/ml G418 (Corning Cellgro #30-234-CI), 10 μg/ml puromycin (Calbiochem #540411), and 0.078% sodium bicarbonate (Thermo Hyclone #SH30033.01).

For the assay, CHO-Tie2 cells may be resuspended to 10,000 cells per well (in 100ul growth medium), into the inner 60 wells of poly-lysine coated 96-well plates (BD Biocoat #356640). 200 μl of D-PBS may be placed into the edge wells to reduce evaporation. Cells may be incubated overnight at 37°C, 95% RH, 5% CO_2. The next day, cells may be washed once and medium may be replaced with 100 μl serum-free growth medium containing 0.1% BSA (Sigma #A7979, low endotoxin). Cells may then be starved for 7.5 to 24 hours in serum-free medium at 37°C, 95% RH, 5% CO_2. During the starvation period, compounds (at 6X the final concentrations) may be serially diluted 1:2 in polypropylene plates in serum-free growth medium containing 0.1% BSA. Human Ang2 (R&D Systems #623-AN, reconstituted in D-PBS/0.1% BSA) and human Ang1 (R&D Systems #923-AN, reconstituted in D-PBS/0.1% BSA) may also be diluted to 6X

the final concentration in serum-free growth medium containing 0.1% BSA. Compounds and the Ang2 or Ang1 ligand may then be mixed at a 1:1 ratio in polypropylene plates and may be incubated for 1 hour at 37°C. The compound/ligand mixtures may then be added at 50 µl per well to the cells (in triplicate wells per treatment) and may be incubated for 13 minutes to 21 hours at 37°C, 95% RH, 5% CO_2. The final concentration range of compounds may be 0.0625 – 283 nM, and the final concentration of human Ang2 and Ang1 may be 0.3 µg/ml (approx. 6 nM) and 0.5 µg/ml (approx. 8.9 nM), respectively. After the incubation time, medium may be quickly and fully removed from the cells, and cells may be lysed in 60 µl per well of cold 1X Tris Lysis Buffer (Meso Scale Discovery #R60TX; 150 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100) which may contain freshly added protease and phosphatase inhibitors (1X protease inhibitor cocktail, Sigma #P8340; 1X phosphatase inhibitor cocktail 2, Sigma #P5726; 1X phosphatase inhibitor cocktail 3, Sigma #P0044; 1 mM final activated sodium orthovanadate (EMD Chemicals #567540)). Plates may then be placed on ice for 10 minutes, after which they may be placed on an orbital shaker at low speed for 25 minutes at 4°C. The plates may then be sealed and frozen at -80°C.

The day before analysis for phospho-Tie2 (with a human phospho-Tie2 DuoSet ELISA kit from R&D Systems, #DYC2720), high binding ELISA plates (Greiner BioOne, #655081) may be coated overnight at 4°C with 4 µg/ml mouse anti-human total Tie2 capture antibody in 1X ELISA coating buffer (Surmodics/BioFX Labs #COAT-1000-01).

The day of phospho-Tie2 measurement, plates containing lysates may be thawed on ice. The coated ELISA plates may be washed with wash buffer (1X TBST containing 0.05% Tween 20) and blocked with 300 µl per well of blocking buffer (1% BSA (Jackson ImmunoResearch #001-000-162; IgG-free, protease-free), 0.01% sodium azide) for a minimum of 1 hour at room temperature on an orbital shaker (while covered with plate sealers). During blocking, lysates may be diluted 1:5 or 1:10 in polypropylene plates in cold lysis buffer containing protease and phosphatase inhibitors. ELISA plates may be blocked and washed 4X, and 100 µl per well of diluted lysates (or phospho-Tie2 ELISA standards) may be added and incubated for 2 hours at room temperature, covered with sealers, on an orbital shaker. Plates may be washed 4X and 100 µl per well of HRP

conjugated mouse anti-phospho tyrosine (diluted as recommended on the vial, in TBST/0.1% BSA) may be added. Plates may then be covered with sealers, and incubated for 2 hours at room temperature on an orbital shaker. Plates may then be washed 6X and removal of liquid from the wells may be ensured. Plates may then be developed by adding 100 µl per well of One Component TMB substrate (Surmodics/BioFX Labs #TMBW-1000-01). Plates may be allowed to develop for 20 or 30 minutes at room temperature covered with aluminum foil. Development may be stopped with 100 µl per well of acid stop solution (TMB stop solution, Surmodics/BioFX Labs #LSTP-1000-01). Plates may then be mixed on an orbital shaker. The ELISA plates may be read at 450 nm on an ELISA reader (Molecular Devices SpectraMax 190), using SOFTmax PRO 5.4.1 software (Molecular Devices Corp.). Phospho-Tie2 values for the samples may be obtained from the standard curve (4-parameter logistic fit), and multiplied by the dilution factor of 5 or 10. Percent inhibition may be calculated by the following formula: (pTie2 value of treatment-mean pTie2 value of Ang2 alone treatment) / (mean medium alone pTie2 value- mean pTie2 value of Ang2 alone treatment)*100.

IC50 values for inhibition of Ang2 induced phospho-Tie2 may be calculated with GraphPad Prism 4, using Log-transformed X values. Nonlinear regression (curve fit) analysis (sigmoidal dose response, variable slope) may be performed on the log-transformed data to obtain IC_{50} values. If an experiment was performed more than once, the geometric mean IC_{50} value (and 95% confidence interval) between experiments may be calculated.

In experiments performed essentially as described in this assay, Compound F dose-dependently neutralizes human Ang2 induced phospho-Tie2 in CHO-Tie2 cells with an IC50 of 0.587 nM (n=3) while an Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F has an IC50 of 0.773 nM. The results indicate that Compound F neutralizes Ang2 induced phospho-Tie2, but does not neutralize human Ang1 induced phospho-Tie2 in CHO-Tie-2 cells when compared to the positive control Ang1 antibody. Moreover, this data indicates that the Ang2 scFv polypeptide portion of Compound F has maintained potency in this assay that is comparable to that of the Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F.

Neutralization of VEGF165 induced phosphorylation of VEGFR2

The *in vitro* cell-based inhibition of human VEGFR2 may be measured in a cell-based assay where binding of VEGF165 to the VEGFR2 on a VEGFR2 expressing cell line, induces VEGFR2 phosphorylation in a dose-dependent manner. The aforementioned assay may be used to evaluate the ability of a compound of the present invention to selectively neutralize VEGF165 mediated phosphorylation of the VEGFR2 receptor in a dose-dependent manner. A VEGFR2 antibody and an irrelevant antibody human IgG4 PAA isotype may be included as a positive and negative control, respectively.

For the assay, VEGFR2 expressing human ECFC (endothelial colony forming cells, derived from umbilical cord blood endothelial progenitors) (Endgenitor Technologies, Lot 100506-14-P4, passages 10-12) may be seeded at 14,000 cells per well (in 100 µl growth medium), into the inner 60 wells of collagen I coated 96-well plates (BD Biocoat #35-4407) in growth medium: EGM-2MV BulletKit (Lonza #CC-4147). Components of the included EGM-2MV Singlequot bag may be added to 500 ml of EBM-2 basal medium, adjusted to 10% final FBS concentration (Life Technologies/Invitrogen #10082-147, heat inactivated). 250 µl of growth medium may be placed into the edge wells to reduce evaporation. Cells may be incubated ON at 37°C, 95% RH, 5% CO_2. The next day, medium may be removed and replaced with 100 µl serum-free EBM-2 basal medium containing 0.1% BSA (Sigma #A7979, low endotoxin). Cells may be starved for 6 ½ hours at 37°C, 95% RH, 5% CO_2. During the starvation period, compounds (at 6X the final concentrations) may be serially diluted 1:4 in polypropylene plates in EBM-2/0.1% BSA. Human VEGF165 may be diluted to 6X the final concentration in EBM-2/ 0.1% BSA. Compounds (or EBM-2/0.1% BSA medium alone) may be added in triplicate at 25 µl to the cells, and cells may be incubated for 45 minutes at 37°C, 95% RH, 5% CO_2. Cells may be treated with 25 µl of 6X VEGF165 for 5 minutes at 37°C, 95% RH, 5% CO_2. (The final concentration range of compounds may be 0.018 – 300 nM, and the final concentration of human VEGF165 may be 0.16 nM). Medium may be removed from the cells, and cells may be lysed in 60 µl per well of cold 1X Tris Lysis Buffer (Meso Scale Discovery #R60TX; 150 mM NaCl, 20 mM Tris pH

7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100) containing freshly added 1X protease and phosphatase inhibitors (included with the phospho-VEGFR2 assay kit). Plates may be placed on ice for 10 minutes, then on an orbital shaker at low speed for 20 minutes at 4°C. Plates may then be sealed and frozen at -80°C.

The day of phospho-VEGFR2 measurement, plates containing lysates may be thawed on ice. Phospho-VEGFR2 levels may be measured using a phospho-VEGFR2 (Tyr1054) whole cell lysate kit, (Meso Scale Discovery #K151DJD). Meso Scale assay plates, pre-coated with an antibody against phospho-VEGFR2, may be blocked with 150 µl per well of blocking buffer (3% blocker A in TBST) for a minimum of 1 hour at room temperature on an orbital shaker (while covered with plate sealers). The plates may be washed 3X with 1X Meso Scale wash buffer, and 50 µl of lysates may be added per well (may be incubated for 1 hour at room temperature, covered with sealers, on an orbital shaker). The plates may be washed, and 3X, 25 µl per well of 1X MSD SULFO-TAG™ conjugated anti-total VEGFR2 (diluted in the manufacturer's recommended antibody diluent) may be added, and incubated for 1 hour at room temperature, covered with sealers, on an orbital shaker. Plates may be washed 3X and removal of liquid from the wells may be ensured. 150 ul per well of 1X Read Buffer T may be added to the plates, and they may be read immediately on a Meso Scale Discovery SECTOR Imager MA6000. Percent inhibition may be calculated by the following formula: (signal value of treatment-mean signal value of VEGF+huIgG treatment) / (mean starve medium alone signal value-mean signal value of VEGF+huIgG treatment)*100.

IC50 values for inhibition of VEGF165 induced phospho-VEGFR2 may be calculated with GraphPad Prism 6, using Log-transformed X values. Nonlinear regression (curve fit) analysis (sigmoidal dose response, variable slope) may be performed on the log-transformed data to obtain IC50 values. If an experiment was performed more than once, the geometric mean IC50 value (and 95% confidence interval) between experiments may be calculated.

In experiments performed essentially as described in this assay, Compound F dose dependently neutralizes human VEGF165-induced phospho-VEGFR2 in ECFC with an IC50 mean of 0.83 nM (n=3), while IMC-1121B has an IC50 mean of 0.52 nM (n=3).

This indicates that the VEGFR2 antibody portion of the compound has maintained potency in Compound F that is comparable to that of IMC-1121B in this cell based assay.

Neutralization of VEGF165 induced cell proliferation.

The *in vitro* cell-based inhibition of human VEGFR2 by a compound of the present invention may be measured in a cell-based assay where VEGF165 induces human VEGFR2 proliferation in a dose-dependent manner. The ability of a compound of the present invention to neutralize human VEGF165 induced proliferation via VEGFR2 may be measured in human HMVEC-d (dermal microvascular endothelial cells). VEGFR2, Ang2, and irrelevant human IgG4 PAA antibodies may be included as positive and negative controls.

Human dermal microvascular endothelial cells may be isolated from newborn male foreskin, and may be confirmed for CD31, VEGFR2, and acetylated LDL expression. HMVEC-d may be maintained in complete growth medium MCDB131 (Mediatech #15-100-CV), 2 mM L-glutamine (Thermo Scientific #SH30034.01), 1X penicillin-streptomycin (Life Technologies/Invitrogen #15140), MGVS supplement (Life Technologies/Invitrogen #S-005-25) as directed for 500 ml medium; which may be supplemented to contain 4.9% FBS, 1 µg/ml hydrocortisone, 3ng/ml human FGF, 10 µg/ml heparin, 1 ng/ml human EGF, and 0.08 mM dibutyryl cyclic AMP.

For the assay, HMVEC-d cells at passage 5 may be washed once in pre-warmed growth medium, and may be resuspended to 2,000 cells per well (in 100 µl growth medium), into the inner 60 wells of white-walled, clear bottom 96-well plates (BD #35-3377). 250 µl of supplement-free medium may be added to the edge wells to reduce evaporation. Compounds and human VEGF165 (Lot ALY-BE01241-033) at 4X the final concentrations may be serially diluted 1:4 in polypropylene plates in supplement-free medium. Compounds (or supplement-free medium alone) may be added to the cells in triplicates at 50 µl per well, which may be followed by 50 µl per well of the 4X VEGF165. The plates may then be incubated for 5 days at 37°C, 95% RH, 5% CO2. The final concentration range of compounds may be 0.012 – 800 nM, and the final concentration of human VEGF165 may be 0.5 nM.

After the incubation period, the plates and CellTiter Glo substrate (Promega #G7571) may be equilibrated to room temperature for 30 minutes. 100 µl per well of CellTiter Glo reagent may be added, and plates may be placed on an orbital shaker for 2 minutes at room temperature. Plates may be incubated for an additional 10 minutes, then luminescence may be recorded (1 second integration time) on a Perkin Elmer Wallac Victor 3 Model 1420 reader.

IC_{50} values for inhibition of VEGF165 induced proliferation may be calculated with GraphPad Prism 6, using Log-transformed X values. Medium alone values may be included as the highest point of curves; the X-value (concentration) for medium alone may be set to 100X higher than the highest X value. Also, the VEGF alone values may be included as the lowest point of the curves; the concentration for VEGF alone may be set to 100X lower than the lowest X value. Nonlinear regression (curve fit) analysis (sigmoidal dose response, variable slope) may be performed on the log-transformed data to obtain IC50 values.

In experiments performed essentially as described in this assay, Compound F dose dependently neutralizes human VEGF165-induced proliferation of HMVEC-d similarly to IMC-1121B with IC_{50} means of 19.24 nM and 31.36 nM, respectively (n=2). This indicates that the VEGFR2 antibody portion of the compound has maintained potency in Compound F that is comparable to IMC-1121B in this cell based assay.

Inhibition of VEGF165 Induced Cord Formation

The *in vitro* inhibition of VEGF induced cord formation may be measured in an *in vitro* co-culture system. The aforementioned assay may be used to measure inhibition of VEGF induced cord formation by a compound of the present invention. A VEGFR2 antibody may be included as a positive control.

For this assay, adipose derived stem cells (ADSC; Lonza # PT5006, lot#OF4505-01) may be cultured on Corning culture flasks (Corning #431082) in EGM-2MV medium (Lonza # CC3202). Endothelial colony forming cells (ECFC; Lonza, lot# EGT-ECFC100506r) may be cultured on Collagen I coated flasks (BD Biosciences #356486) in EGM-2MV medium supplemented with 5% heat inactivated FBS (Gibco # 10082-147). ADSC at passages 4-6 may be harvested from culture flasks which may be rinsed with

DPBS (Hyclone #SH30028.03) followed by TrypLE Express (Gibco #12605-010). ADSC cells may be suspended in Basal Medium (MCDC-131 (Gibco #10372-019) supplemented with 10 µg/ml insulin, 1 µM dexamethasone, 30 µg/ml ascorbic acid, 10 µg/ml human transferrin and 50 µg/ml tobramycin). Viable cell count may be determined and cells may be seeded onto black, clear bottomed 96-well plates (BD Falcon #353219) at $4x10^4$ cells per well in 100 µl Basal Medium. Cells may be incubated at 37°C in 5% CO_2 overnight to allow attachment. Next day, ECFC at passages 7-10 may be harvested in Basal Medium as above and viable cell count may be adjusted to $4x10^4$ cells per ml. Medium may be removed from ADSC cells and 100 µl ECFC cell suspension may be added to each well. Plates may be incubated at 37°C in 5% CO_2 for 2-3 hours to allow cells to settle on top of the ADSC monolayer. IMC-1121B and compounds of the present invention may be diluted to 80 µg/ml in Basal Medium, and then may be serially diluted 1:3 with Basal Medium to produce a nine point dose response series. 50 µl of each dilution of compound may be added to the co-culture. 50 µl of an 80 ng/ml solution of rhVEGF (R&D #293-VE/CF, 50 µg/ml in DPBS) prepared in Basal Medium may be added to the co-culture + compound combination. Final concentrations for compounds and rhVEGF may be 20 µg/ml and 20 ng/ml respectively. Positive control for the assay may include 20 ng/ml rhVEGF in the absence of compound. Negative control for the assay may include Basal Medium without rhVEGF. Plates may then be incubated at 37°C in 5% CO_2 for 3 days to allow cords to form.

At the end of the incubation period, medium may be aspirated from each well and 100ul room temperature 80% ethanol may be carefully added. Plates may be incubated at room temperature for 20 minutes. Ethanol solution may be aspirated and wells may be washed twice with 150ul DPBS. Anti-huCD31 (R&D #AF806 Affinity purified sheep IgG, 200ug/ml) and MAB Anti-Actin, alpha-Smooth Muscle-Cy3 (Sigma #C6198) may be each diluted 1:250 in 2.5% FBS/DPBS. 100 µl antibody mix may be added to wells and plates may be incubated at 37°C in 5% CO_2 for 2 hours. Plates may then be aspirated and wells may be washed twice with 150ul DPBS. Alexa Fluor 488 donkey anti-sheep IgG (H+L) (Life Technologies #A11015) may be diluted 1:400 and Hoescht 33342 (Life Technologies #H3570) may be diluted 1:1000 in 2.5% FBS/DPBS and 100 µl per well

may be added to plates. Plates may be incubated at room temperature protected from light for 30 minutes. Wells may then be washed twice with 150 µl DPBS. 150 µl DPBS may be added to each well and plates may be sealed with black adhesive seals (PerkinElmer #6050173).

Plates may be read on the ArrayScan VTI HCS Reader (Cellomics-Thermo Fisher) using the Tube Formation Bio-application. Total Tube Area data may be plotted against compound concentrations in nM in GraphPad Prism 6. Compound concentrations may be transformed into log data and IC_{50} values for inhibition may be calculated by nonlinear regression (sigmoidal dose response, variable slope). Each experiment may represent the mean of triplicates and triplicate experiments may be expressed as the geometric means and 95% confidence intervals may be calculated.

In experiments performed essentially as described in this assay, Compound F dose dependently inhibits human VEGF-induced cord formation in the ADSC/ECFC co-culture system, comparably to IMC-1121B with mean IC_{50} of 6.04 nM and 5.6 nM, respectively (n=3). This indicates that the VEGFR2 antibody portion of the compound has maintained potency in Compound F that is comparable to IMC-1121B in this cell based assay.

Repression of Ang2 Induced Blood Vessel Development.

The *in vivo* repression of physiological angiogenesis by an Ang2 antibody may be measured in a model of blood vessel development in the mouse retina. The aforementioned assay may be used to study the ability of compounds of the present invention to repress physiological angiogenesis in the mouse retina.

For this assay, the day of mouse pup delivery by the pregnant females may be marked P0 (postnatal day 0). Following delivery, at days two and four (P2 and P4) pups may be injected with vehicle control (PBS) or 10 mg/kg of Ang2 antibody or 13.5 mg/kg of the compound to maintain comparable molar amounts of the molecules. At P5 mice may be sacrificed and eyes may be harvested and may be fixed in formalin for 5 hours and may be washed with PBS.

Retinas may then be dissected, and may be stained with anti-CD31 diluted at 1:200 (BD Pharmingen; clone MEC 13.3; Catalog 553370), and anti-SMA-FTIC diluted

at 1:200 (Sigma; Clone1A4 Catalog F3777). For the anti-CD31 treated retinas an anti-Rat Alexa-647 diluted at 1:400 (Jackson Immuno Research; Catalog 712-606-153) may be used as a secondary antibody. Acquisition of the retinas may be done by using Nikon Ti, and quantifications of vascular progression, number of sprouting tip cells, and vascular density of remodeling plexus may be performed by using FIJI software. High magnification images may be acquired using a confocal Nikon A1.

In experiments performed essentially as described in this assay, Compound F and an Ang-2 Ab with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F comparably repress vascular progression, reduce both the number of endothelial tip cells and vascular density, as well as increase pericyte coverage (Table 7). These results from this *in vivo* model indicate that the Ang2 scFv polypeptide portion of Compound F has maintained function and potency that is comparable to an Ang2 antibody with the same HCDRs and LCDRs as the scFv polypeptide portion of Compound F.

Table 7

Parameters	Vehicle	Ang2 mAb	Compound F
Vascular progression			
Mean (%)	100	57.29	49.01
Std. Error of Mean	2.956	4.296	3.679
P value (Vehicle vs. Compounds) (Dunnett's test)		< 0.0001	< 0.0001
P value (Ang2 mAb vs. Compound F) (Dunnett's test)			0.2543
Number of tip cells			
Mean (%)	100	61.77	47.26
Std. Error of Mean	6.749	6.658	4.719
P value (Vehicle vs. Compounds) (Dunnett's test)		< 0.0001	< 0.0001
P value (Ang2 mAb vs. Compound F) (Dunnett's test)			0.1354
Vascular density			
Mean (%)	100	60.52	62.97
Std. Error of Mean	5.728	2.178	2.935

P value (Vehicle vs. Compounds) (Dunnett's test)		< 0.0001	< 0.0001
P value (Ang2 mAb vs. Compound F) (Dunnett's test)			0.9476

Physical stability, chemical stability, and product quality

Product qualities, including aggregate level, homogeneity during expression, physical stability, and chemical stability, are evaluated to identify any issues and ensure suitability for therapeutic uses.

Free light chain mis-pairing

During the purification and analysis of Compound A (two first polypeptides of SEQ ID NO: 38 and two second polypeptides of SEQ ID NO: 39), and Compound B (two first polypeptides of SEQ ID NO: 40 and two second polypeptides of SEQ ID NO: 39), the presence of non-covalent light chains from the antibody portion of the compound is detected. Purification to remove the mis-folded species results in poor final yield of the desired compound. The instability of the interface between VH and VL of the antibody portion of the compound, and the folding of the domains of the scFv polypeptide are both found to contribute to the problem. Mutations in the framework of the antibody portion of the compound are used to eliminate this light chain mis-pairing problem, however, the problem was only reduced and not resolved. A number of germline frameworks in the scFv polypeptide are tried, but the expression profiles are not improved. Engineering of the CDRs and frameworks of the antibody portion of the compound in combination with scFv polypeptide engineering is required to produce the desired result of acceptable levels of light chain mis-pairing.

Mass-spec analysis confirms the existence of free LC associated with Compound A, either as non-covalently linked association or as covalently linked species. To further quantify the percentage of LC associations, a HIC-HPLC (TSKgel butyl-NPR 4.6mm ID x 10cm, 2.5um; TOSOH cat# 42168) method is employed. In this analysis, a protein sample is injected onto a Butyl-NPR column and eluted according to their

hydrophobicity. Through weak hydrophobic interaction, monomer, aggregates and various states of LC associated species are resolved sequentially during elution. Method development such that test articles are prepared in 1 mg/ml solution in buffer 50 mM Potassium Phosphate, 1 M ammonium sulphate, pH 6.7 and 50 µg of sample is injected onto a TSKgel butyl-NPR column at flow rate of 1 ml/min on Agilent LC 1260 system. With a salt gradient from 1 M ammonium sulphate to zero in 50 mM Potassium Phosphate buffer, pH 6.7, three protein peaks are resolved and fractionated. Each fractionated peak is analyzed by LCMS and identified as monomer Mab-scFv, bi-cysteinylated Mab-scFv with one extra cysteinylated LC, and bi-cysteinylated Mab-scFv with two extra cysteinylated LC. With peak assignments confirmed by LCMS, the final chromatograph (A214 detection) is integrated to calculate the total percentage of LC association.

In experiments performed as described above, the compound with an M111L mutation of Compound B to fix oxidation was found to exhibit 55% LC association. Through engineering of the variable domain of VR2 and the frameworks, the final molecules Compound C, Compound D, Compound E, and Compound F were found to exhibit no detectable LC association as detected by HIC-HPLC analysis (Table 8).

Table 8

	LC%
Compound B with M111L	55
Compound C	0
Compound D	0
Compound E	0
Compound F	0

Mab-Diabody formation

To facilitate measurement of Mab-Diabody/Mab-scFv ratios in compounds of the present invention, the following treatments are performed. Typically 20 µg of the compound of the present invention is buffer exchanged into 50 mM sodium phosphate containing 150 mM sodium chloride, pH 6.6 using an Amicon Ultra-0.5mL centrifugal

filter device. Samples are concentrated to approximately 30 µl and then mixed with 1 µl of freshly prepared FABricator enzyme (Genovis, Cat: A0-FR1-020; 2000 U dissolved in 30 µl of ddH_2O) and incubated at 37°C overnight. Digested samples (1 µl) are subjected to LC/UV/MS analysis using the Waters Acquity UPLC coupled to a Waters Xevo G2-S mass spectrometer. The samples are loaded onto a PLRP-S 50x1.0mm, 1000 Å, 5um reverse phase column (Proxeon, Cat: PL1312-1502) at a flow rate of 0.3 ml/min and column temperature of 80°C. Samples are eluted from the column using a gradient of TFA in Acetonitrile. The eluate is analyzed first by UV at 214 nm with the flow then directed to the mass spectrometer for analysis using sensitivity mode, positive polarity with an acquisition range of 400-4000 m/z.

In experiments performed essentially as described in this assay, Compound B exhibits less than 1% Mab-Diabody. Compound C, Compound D, and Compound E, incorporating all the modifications made to Compound B to reduce oxidation and light chain mis-pairing problems, surprisingly had approximately 5-6% Mab-Diabody. The sequence changes incorporated into Compound F had a Mab-Diabody percentage of less than 1% Mab-Diabody which is comparable to Compound B.

Oxidation

Chemical stability of compounds of the present invention may be evaluated by producing varied formulation conditions which may be subjected to temperature hold stress. Changes in chemical stability may be monitored by established LCMS peptide mapping techniques. Briefly, test articles may be obtained and diluted to a final concentration of 1 mg/ml in the following formulation buffers: 10mM Citrate, pH 5.0, 10mM Citrate, pH 6.5, 1X PBS, pH 7.4 and 10mM Tris, pH 8.0 and then subjected to exhaustive dialysis at 4°C in each respective buffer to ensure complete buffer exchange. Buffer exchanged samples may then be subjected to incubation at either 4°C or 40°C for four weeks, after which samples may be analyzed by LCMS as follows. Stressed material may be buffer exchanged into 8M guanidine to denature prior to reduction with DTT followed by subsequent alkylation by iodoacetamide. Reduced and alkylated protein may then be buffer exchanged into Tris, pH 7.5 and digested with trypsin at a 20:1 molar ratio at 37°C for four hours. Digestion may be quenched with the addition of 1 µL glacial

acetic acid. Separation of digested peptide fragments may be achieved by capture onto a Zorbax 1.8μm C18 2.1mm x 50mm pre-equilibrated 0.2% Formic Acid in water and subsequent elution using a 0.2% Formic acid in acetonitrile gradient operated at 0.3 ml/min. Eluted peptides may be immediately analyzed using a Agilent ESI-QTOF set to scan in positive ion mode from 300m/z to 2000m/z at 1 scan/second. The ESI source may be set at 4000 V and the temperature at 350°C, nebulizer gas at 40 psi and cone gas at 12 psi. Agilent Mass Hunter Bio-confirm software may be used to align the tryptic peptide mass spectrum to the protein sequence.

In experiments performed essentially as described in this assay, Compound B exhibits 14.2% oxidation at position M111 on the heavy chain of the VEGFR2 antibody portion of the compound when subjected to temperature stress at 40°C at pH 8.0. To circumvent issues related to oxidation at this position, a single point mutation that changes methionine 111 to leucine in Compounds C, D, E, and F is made to eliminate the possibility of chemical modification at this position.

Plasma pharmacokinetics (PK) and pharmacodynamics (PD) following a single intravenous dose to male Cynomolgus monkeys

The PK and PD of compounds of the present invention may be measured in Cynomolgus Monkeys after a single intravenous dose.

Male Cynomolgus Monkeys (n=2/group) may be administered a single intravenous dose of a compound of the present invention. Blood may be sampled between 2-672 hours post-dose and plasma isolated for quantifying compound plasma levels using three ELISA methods.

The total human IgG method may utilize an ELISA format to measure the concentration of the compound of the present invention (total human IgG). Standards, controls, and test samples may be incubated with goat anti-human F(ab')2 which has been immobilized on a microtiter plate. After incubation, a mouse anti-human IgG_4-HRP (horseradish peroxidase) may be added to the wells. Once unbound enzyme is washed away, SureBlue® TMB (tetramethylbenzidine) substrate solution may be added to the wells. The color development may be stopped by the addition of an acidic solution and

the optical density measured at 450 nm with wavelength correction set to 630 nm. The assay range may be 30-700 ng/ml.

The VEGFR2 antigen capture method may utilize an ELISA format to measure the concentration of the compounds of the present invention (VEGFR2 antigen capture). Standards, controls and test samples may be incubated on a microtiter plate coated with human VEGFR2. After incubation, a mouse anti-human IgG_4-HRP (horseradish peroxidase) may be added to the wells. Once unbound enzyme is washed away, SureBlue® TMB (tetramethylbenzidine) substrate solution may be added to the wells. The color development may be stopped by the addition of an acidic solution and the optical density measured at 450 nm with wavelength correction set to 630 nm. The assay range may be 80-2000 ng/ml.

The Ang2 antigen capture method utilizes an ELISA format to measure the concentration of the compounds of the present invention (Ang2 antigen capture). Standards, controls and test samples may be incubated on a microtiter plate coated with Ang2. After incubation, a mouse anti-human IgG_4-HRP (horseradish peroxidase) is added to the wells. Once unbound enzyme is washed away, SureBlue® TMB (tetramethylbenzidine) substrate solution may be added to the wells. The color development may be stopped by the addition of an acidic solution and the optical density measured at 450 nm with wavelength correction set to 630 nm. The assay range may be 30-700 ng/ml.

Noncompartmental analysis may be performed using Phoenix WinNonlin 6.3. Plots may be generated using SigmaPlot v11, and data processing may be performed using Microsoft Excel 2010.

In experiments performed essentially as described in this assay, the PK was measured for Compound E and Compound F after one dose at 1, 10, and 25 mg/kg. For Compound E, terminal half-life, as measured in all three assays, was within the range of 8.96-37.9 h depending on the dose group (Table 9), while the terminal half-life for Compound F was within the range of 18.6-79.5 h depending on the dose group (Table 10). These results from three different binding assays, that each measure different parts of the compound, each demonstrate a higher terminal half-life for Compound F compared to Compound E.

Table 9

	Assay	Dose	Mean $t_{1/2}$ (n=2)
		(mg/kg)	(h)
Compound E	Ang2 Ag Capt	1	8.96
		10	24.4
		25	35.9
	Total Human IgG	1	35.8
		10	30.2
		25	36.7
	VEGFR2 Ag Capture	1	15.6
		10	23.2
		25	37.9

Table 10

	Assay	Dose	Mean $t_{1/2}$ (n=2)
		(mg/kg)	(h)
Compound F	Ang2 Ag Capt	1	20.1
		10	54.3
		25	75.2
	Total Human IgG	1	18.6
		10	52.6
		25	79.5
	VEGFR2 Ag Capture	1	27.5
		10	54.3
		25	71.2

Amino Acid and Nucleotide Sequences

SEQ ID NO: 1 (HCVR of antibody – Compound C)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSAISSS
SSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTEAFDIWGQ
GTLVTVSS

SEQ ID NO: 2 (HCVR of antibody – Compound D)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMLWVRQAPGKGLEWVSAISSSS
SYTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQ
GTLVTVSS

SEQ ID NO: 3 (HCVR of antibody – Compound E)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSAISSS
SSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQ
GTLVTVSS

SEQ ID NO: 4 (HCVR of antibody – Compound F)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMLWVRQAPGKGLEWVSAISSSS
SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQG
TLVTVSS

SEQ ID NO: 5 (HC of antibody – Compound C)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSAISSS
SSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTEAFDIWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP

ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLG

SEQ ID NO: 6 (HC of antibody – Compound D)

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMLWVRQAPGKGLEWVSAISSSS
SYTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLG

SEQ ID NO: 7 (HC of antibody – Compound E)

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSAISSS
SSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLG

SEQ ID NO: 8 (HC of antibody – Compound F)

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMLWVRQAPGKGLEWVSAISSSS
SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGP
PCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV

DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLG

SEQ ID NO: 9 (HC of antibody/linker/scFv polypeptide – Compound C)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSAISSS
SSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTEAFDIWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYSFTDYNM
VWVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTMTTDTSTSTAYMELRSLR
SDDTAVYYCARTRDRYDVWYFDVWGQGTLVTVSSGGGGSGGGGSGGGGSGGG
GSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQDVYIAVAWYQQKPGKAPKLLI
YWASTRDTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGCGTKV
EIK

SEQ ID NO: 10 (HC of antibody/linker/scFv polypeptide – Compound D)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMLWVRQAPGKGLEWVSAISSSS
SYTYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYSFTDYNM

VWVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTMTTDTSTSTAYMELRSLR
SDDTAVYYCARTRDRYDVWYFDVWGQGTLVTVSSGGGGSGGGGSGGGGSGGG
GSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQDVYIAVAWYQQKPGKAPKLLI
YWASTRDTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGCGTKV
EIK

SEQ ID NO: 11 (HC of antibody/linker/scFv polypeptide – Compound E)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSAISSS
SSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQ
GTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYSFTDYNM
VWVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTMTTDTSTSTAYMELRSLR
SDDTAVYYCARTRDRYDVWYFDVWGQGTLVTVSSGGGGSGGGGSGGGGSGGG
GSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQDVYIAVAWYQQKPGKAPKLLI
YWASTRDTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGCGTKV
EIK

SEQ ID NO: 12 (HC of antibody/linker/scFv polypeptide – Compound F)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMLWVRQAPGKGLEWVSAISSSS
SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGP
PCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYV
DGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIE
KTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS

LSLGGGGSGGGGSGGGGSDIVMTQSPDSLAVSLGERATINCKASQDVYIAVAWY
QQKPGQPPKLLIYWASTRDTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQY
SSYPPTFGCGTKVEIKGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSV
KVSCKASGYSFTDYNMVWVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTIT
ADESTSTAYMELSSLRSEDTAVYYCARTRDRYDVWYFDVWGQGTLVTVSS

SEQ ID NO: 13 (LCVR of antibody – Compound C and Compound F)
DIQMTQSPSSVSASVGDRVTITCRASRGIDNWLTWYQQKPGKAPKLLIYEASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKAFPPTFGGGTKVDIK

SEQ ID NO: 14 (LCVR of antibody – Compound D)
DIQMTQSPSSVSASVGDRVTITCRASQGIDNWLTWYQQKPGKAPKLLIVEASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKRFPPTFGGGTKVDIK

SEQ ID NO: 15 (LCVR of antibody – Compound E)
DIQMTQSPSSVSASVGDRVTITCRASQGIDNWLTWYQQKPGKAPKLLIVEASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKAFPPTFGGGTKVDIK

SEQ ID NO: 16 (LC of antibody – Compound C and Compound F)
DIQMTQSPSSVSASVGDRVTITCRASRGIDNWLTWYQQKPGKAPKLLIYEASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKAFPPTFGGGTKVDIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 17 (LC of antibody – Compound D)
DIQMTQSPSSVSASVGDRVTITCRASQGIDNWLTWYQQKPGKAPKLLIVEASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKRFPPTFGGGTKVDIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 18 (LC of antibody – Compound E)

DIQMTQSPSSVSASVGDRVTITCRASQGIDNWLTWYQQKPGKAPKLLIVEASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKAFPPTFGGGTKVDIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 19 (scFv polypeptide – Compound C, Compound D, Compound E)
QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYNMVWVRQAPGQCLEWMGYID
PYNGGTGYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARTRDRYDV
WYFDVWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVS
ASVGDRVTITCKASQDVYIAVAWYQQKPGKAPKLLIYWASTRDTGVPSRFSGSG
SGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGCGTKVEIK

SEQ ID NO: 20 (scFv polypeptide – Compound F)
DIVMTQSPDSLAVSLGERATINCKASQDVYIAVAWYQQKPGQPPKLLIYWASTR
DTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYSSYPPTFGCGTKVEIKGG
GGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYNMV
WVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTITADESTSTAYMELSSLRSE
DTAVYYCARTRDRYDVWYFDVWGQGTLVTVSS

SEQ ID NO: 21 (HCVR of scFv polypeptide – Compound C, Compound D, Compound E)
QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYNMVWVRQAPGQCLEWMGYID
PYNGGTGYNQKFEGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARTRDRYDV
WYFDVWGQGTLVTVSS

SEQ ID NO: 22 (HCVR of scFv polypeptide – Compound F)
QVQLVQSGAEVKKPGSSVKVSCKASGYSFTDYNMVWVRQAPGQCLEWMGYID
PYNGGTGYNQKFEGRVTITADESTSTAYMELSSLRSEDTAVYYCARTRDRYDVW
YFDVWGQGTLVTVSS

SEQ ID NO: 23 (LCVR of scFv polypeptide – Compound C, Compound D, Compound E)

DIQMTQSPSSVSASVGDRVTITCKASQDVYIAVAWYQQKPGKAPKLLIYWASTR
DTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGCGTKVEIK

SEQ ID NO: 24 (LCVR of scFv polypeptide – Compound F)

DIVMTQSPDSLAVSLGERATINCKASQDVYIAVAWYQQKPGQPPKLLIYWASTR
DTGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCHQYSSYPPTFGCGTKVEIK

SEQ ID NO: 25 (DNA of HC of antibody/linker/scFv polypeptide – Compound C)

GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATAGCATGAAC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCCATTAGTA
GTAGTAGTAGTTACATATACTACGCAGACTCAGTGAAGGGCCGATTCACCAT
CTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGA
GCCGAGGACACGGCTGTGTATTACTGTGCGAGAGTCACAGAGGCTTTTGATA
TCTGGGGCCAAGGGACACTGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCC
ATCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCC
GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTG
GAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGT
CCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTG
GGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGG
TGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGC
ACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAG
GACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGT
GAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACC
GTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGA
GTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCC
CATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAA

AGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGGCAGCCG
GAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT
CCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTC
TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGA
GCCTCTCCCTGTCTCTGGGTGGCGGAGGCTCCGGGGGAGGGGGTAGCGGAGG
AGGGGGATCCCAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCT
GGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACTCATTCACTGACTA
CAACATGGTGTGGGTGCGACAGGCCCCTGGACAATGCCTTGAGTGGATGGGA
TATATTGATCCTTACAATGGTGGTACTGGCTACAACCAGAAGTTCGAGGGCA
GAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGAG
GAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAACGAGGGAT
AGATACGACGTCTGGTACTTCGATGTCTGGGGCCAGGGAACCCTGGTCACCG
TCTCCTCAGGAGGCGGAGGTTCCGGGGGAGGGGGCAGCGGAGGAGGCGGAT
CGGGCGGAGGAGGAAGTGGAGGCGGAGGCAGCGACATCCAGATGACCCAGT
CTCCATCTTCCGTGTCTGCATCTGTTGGCGACAGAGTCACCATCACTTGTAAG
GCCAGTCAGGATGTGTATATTGCTGTAGCCTGGTATCAGCAGAAACCAGGGA
AAGCCCCTAAGCTCCTGATCTATTGGGCATCCACCCGGGACACTGGGGTCCCA
TCAAGGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCA
GCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCACCAATATAGCAGCTAT
CCTCCTACGTTCGGCTGCGGGACCAAGGTGGAGATCAAA

SEQ ID NO: 26 (DNA of LC of antibody– Compound C and Compound F)

GACATCCAGATGACCCAGTCTCCATCTTCTGTGTCTGCATCTGTAGGAGACAG
AGTCACCATCACTTGTCGGGCGAGTCGTGGTATTGACAACTGGTTAACGTGGT
ATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATTTACGAAGCATCCAG
TTTGCAATCAGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAT
TTCACTCTCACTATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTG
TCAACAAGCTAAGGCTTTTCCTCCCACTTTCGGCGGAGGGACCAAGGTGGAC
ATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA
GCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATC
CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA

CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
AGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA
ACAGGGGAGAGTGC

SEQ ID NO: 27 (DNA of HC of antibody/linker/scFv polypeptide – Compound D)

GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATAGCATGCTT
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCCATTAGTA
GTAGTAGTAGTTACACCTACTACGCAGACTCAGTGAAGGGCCGATTCACCAT
CTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGA
GCCGAGGACACGGCTGTGTATTACTGTGCGAGAGTCACAGATGCTTTTGATAT
CTGGGGCCAAGGGACACTGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCCA
TCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTC
CTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGT
GGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCA
CCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG
ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTG
AGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCG
TGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAG
TACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCC
ATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGGCAGCCGG
AGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCT
TCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAG

CCTCTCCCTGTCTCTGGGTGGCGGAGGCTCCGGGGGAGGGGGTAGCGGAGGA
GGGGGATCCCAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTG
GGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACTCATTCACTGACTAC
AACATGGTGTGGGTGCGACAGGCCCCTGGACAATGCCTTGAGTGGATGGGAT
ATATTGATCCTTACAATGGTGGTACTGGCTACAACCAGAAGTTCGAGGGCAG
AGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGG
AGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAACGAGGGATA
GATACGACGTCTGGTACTTCGATGTCTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCAGGAGGCGGAGGTTCCGGGGGAGGGGGCAGCGGAGGAGGCGGATCG
GGCGGAGGAGGAAGTGGAGGCGGAGGCAGCGACATCCAGATGACCCAGTCT
CCATCTTCCGTGTCTGCATCTGTTGGCGACAGAGTCACCATCACTTGTAAGGC
CAGTCAGGATGTGTATATTGCTGTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCTCCTGATCTATTGGGCATCCACCCGGGACACTGGGGTCCCATC
AAGGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATTTTGCAACTTACTATTGTCACCAATATAGCAGCTATCC
TCCTACGTTCGGCTGCGGGACCAAGGTGGAGATCAAA

SEQ ID NO: 28 (DNA of LC of antibody– Compound D)

GACATCCAGATGACCCAGTCTCCATCTTCTGTGTCTGCATCTGTAGGAGACAG
AGTCACCATCACTTGTCGGGCGAGTCAGGGTATTGACAACTGGTTAACGTGGT
ATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCGTCGAGGCATCCAG
TTTGCAATCAGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAT
TTCACTCTCACTATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTG
TCAACAGGCTAAGAGGTTTCCTCCCACTTTCGGCGGAGGGACCAAGGTGGAC
ATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA
GCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATC
CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
AGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA
ACAGGGGAGAGTGC

SEQ ID NO: 29 (DNA of HC of antibody/linker/scFv polypeptide – Compound E)

GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATAGCATGAAC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCGATTAGTA
GTAGTAGTAGTTACATATACTACGCAGACTCAGTGAAGGGCCGATTCACCAT
CTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGA
GCCGAGGACACGGCTGTGTATTACTGTGCGAGAGTCACAGATGCTTTTGATAT
CTGGGGCCAAGGGACACTGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCCA
TCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTC
CTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGT
GGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCA
CCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG
ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTG
AGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCG
TGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAG
TACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCC
ATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGGCAGCCGG
AGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCT
TCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAG
CCTCTCCCTGTCTCTGGGTGGCGGAGGCTCCGGGGGAGGGGGTAGCGGAGGA
GGGGGATCCCAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTG
GGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTGGTTACTCATTCACTGACTAC
AACATGGTGTGGGTGCGACAGGCCCCTGGACAATGCCTTGAGTGGATGGGAT

ATATTGATCCTTACAATGGTGGTACTGGCTACAACCAGAAGTTCGAGGGCAG
AGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGG
AGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAACGAGGGATA
GATACGACGTCTGGTACTTCGATGTCTGGGGCCAGGGAACCCTGGTCACCGTC
TCCTCAGGAGGCGGAGGTTCCGGGGGAGGGGGCAGCGGAGGAGGCGGATCG
GGCGGAGGAGGAAGTGGAGGCGGAGGCAGCGACATCCAGATGACCCAGTCT
CCATCTTCCGTGTCTGCATCTGTTGGCGACAGAGTCACCATCACTTGTAAGGC
CAGTCAGGATGTGTATATTGCTGTAGCCTGGTATCAGCAGAAACCAGGGAAA
GCCCCTAAGCTCCTGATCTATTGGGCATCCACCCGGGACACTGGGGTCCCATC
AAGGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGC
CTGCAGCCTGAAGATTTTGCAACTTACTATTGTCACCAATATAGCAGCTATCC
TCCTACGTTCGGCTGCGGGACCAAGGTGGAGATCAAA

SEQ ID NO: 30 (DNA of LC of antibody– Compound E)

GACATCCAGATGACCCAGTCTCCATCTTCTGTGTCTGCATCTGTAGGAGACAG
AGTCACCATCACTTGTCGGGCGAGTCAGGGTATTGACAACTGGTTAACGTGGT
ATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCGTCGAGGCATCCAG
TTTGCAATCAGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAT
TTCACTCTCACTATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTG
TCAACAGGCTAAGGCTTTTCCTCCCACTTTCGGCGGAGGGACCAAGGTGGAC
ATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA
GCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATC
CCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
AGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA
ACAGGGGAGAGTGC

SEQ ID NO: 31 (DNA of HC of antibody/linker/scFv polypeptide – Compound F)

GAGGTCCAGCTGGTGCAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATAGCATGCTT

TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCCATTAGTA
GTAGTAGTAGTTACATATACTACGCAGACTCAGTGAAGGGCCGATTCACCAT
CTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGA
GCCGAGGACACGGCTGTGTATTACTGTGCGAGAGTCACAGATGCTTTTGATAT
CTGGGGCCAAGGGACACTGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCCA
TCGGTCTTCCCGCTAGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGC
CCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGG
AACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTC
CTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGT
GGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCA
CCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG
ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTG
AGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGG
TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCG
TGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAG
TACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCC
ATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAA
GGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGCAATGGGCAGCCGG
AGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCT
TCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAG
CCTCTCCCTGTCTCTGGGTGGCGGAGGCTCCGGGGGAGGGGGTAGCGGAGGA
GGGGGATCCGACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCT
GGGCGAGAGGGCCACCATCAACTGCAAGGCCAGTCAGGATGTGTATATTGCT
GTAGCCTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGCTCATTTACT
GGGCATCCACCCGGGACACTGGGGTCCCTGACCGATTCAGTGGCAGCGGGTC
TGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCA
GTTTATTACTGTCACCAATATAGCAGCTATCCTCCTACGTTCGGCTGCGGGAC
CAAGGTGGAGATCAAAGGTGGCGGAGGATCTGGTGGAGGTGGCTCAGGAGG

TGGCGGAAGCGGCGGAGGTGGAAGTCAGGTGCAGCTGGTGCAGTCTGGGGCT
GAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGTT
ACTCATTCACTGACTACAACATGGTGTGGGTGCGACAGGCCCCTGGACAATG
CCTTGAGTGGATGGGATATATTGATCCTTACAATGGTGGTACTGGCTACAACC
AGAAGTTCGAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGC
CTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGT
GCGAGAACGAGGGATAGGTACGACGTCTGGTACTTCGATGTCTGGGGCCAGG
GAACCCTGGTCACCGTCTCCTCA

SEQ ID NO: 32 (human VEGFR2 ECD)

ASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQSGSEQRVE
VTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSD
QHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTI
PSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLV
LNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGV
TRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRIP
AKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTNPISKE
KQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQL
EEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIEVNKNQFALIEGKNKTVSTL
VIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCT
ADRSTFENLTWYKLGPQPLPIHVGELPTPVCKNLDTLWKLNATMFSNSTNDILIM
ELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIG
ESIEVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYTC
QACSVLGCAKVEAFFIIEGAQEKTNLE

SEQ ID NO: 33 (human Ang2)

YNNFRKSMDSIGKKQYQVQHGSCSYTFLLPEMDNCRSSSSPYVSNAVQRDAPLE
YDDSVQRLQVLENIMENNTQWLMKLENYIQDNMKKEMVEIQQNAVQNQTAVM
IEIGTNLLNQTAEQTRKLTDVEAQVLNQTTRLELQLLEHSLSTNKLEKQILDQTSEI
NKLQDKNSFLEKKVLAMEDKHIIQLQSIKEEKDQLQVLVSKQNSIIEELEKKIVTA
TVNNSVLQKQQHDLMETVNNLLTMMSTSNSAKDPTVAKEEQISFRDCAEVFKSG

HTTNGIYTLTFPNSTEEIKAYCDMEAGGGGWTIIQRREDGSVDFQRTWKEYKVGF
GNPSGEYWLGNEFVSQLTNQQRYVLKIHLKDWEGNEAYSLYEHFYLSSEELNYR
IHLKGLTGTAGKISSISQPGNDFSTKDGDNDKCICKCSQMLTGGWWFDACGPSNL
NGMYYPQRQNTNKFNGIKWYYWKGSGYSLKATTMMIRPADF

SEQ ID NO: 34
GGGGSGGGGS

SEQ ID NO: 35
GGGGSGGGGSGGGGS

SEQ ID NO: 36
GGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 37
GGGGSGGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 38 (HC of antibody/linker/scFv polypeptide - Compound A)
EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSS
SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQG
TMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYSFTD
YNMVWVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTMTTDTSTSTAYMEL
RSLRSDDTAVYYCARTRDRYDVWYFDVWGQGTLVTVSSGGGGSGGGGSGGGG
SGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQDVYIAVAWYQQKPGKA

PKLLIYWASTRDTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGC
GTKVEIK

SEQ ID NO: 39 (LC of antibody - Compound A and Compound B)

DIQMTQSPSSVSASIGDRVTITCRASQGIDNWLGWYQQKPGKAPKLLIYDASNLD
TGVPSRFSGSGSGTYFTLTISSLQAEDFAVYFCQQAKAFPPTFGGGTKVDIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 40 (HC of antibody/linker/scFv polypeptide - Compound B)

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSS
SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARVTDAFDIWGQG
TMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
PPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYSFTDYNM
VWVRQAPGQCLEWMGYIDPYNGGTGYNQKFEGRVTMTTDTSTSTAYMELRSLR
SDDTAVYYCARTRDRYDVWYFDVWGQGTLVTVSSGGGGSGGGGSGGGGSGGG
GSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQDVYIAVAWYQQKPGKAPKLLI
YWASTRDTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQYSSYPPTFGCGTKV
EIK

WE CLAIM:

1. A compound, comprising an antibody fused by two linkers to two single chain fragment variable (scFv) polypeptides, wherein:
   a) the antibody comprises two identical heavy chains (HCs) and two identical light chains (LCs), wherein each HC comprises a heavy chain variable region (HCVR) whose amino acid sequence is given in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, and wherein each LC comprises a light chain variable region (LVCR) whose amino acid sequence is given in SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15,
   b) the two scFv polypeptides are identical and each comprise an HCVR operably linked to an LCVR, wherein each HCVR has the amino acid sequence given in SEQ ID NO: 21, or SEQ ID NO: 22, and wherein each LCVR has the amino acid sequence given in SEQ ID NO: 23, or SEQ ID NO: 24, and
   c) the two linkers are identical glycine-rich linkers that each operably link the carboxy-terminus of one HC of the antibody to the amino-terminus of one of the scFv polypeptides.
2. The compound of Claim 1, wherein the two scFv polypeptides each comprise the carboxy-terminus of the LCVR of one scFv polypeptide operably linked to the amino-terminus of the HCVR of one scFv polypeptide.
3. The compound of Claims 1 or 2, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 1, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 13, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.
4. The compound of Claims 1 or 2, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 2, each LCVR of the

antibody has the amino acid sequence given in SEQ ID NO: 14, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.

5. The compound of Claims 1 or 2, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 3, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 15, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 21, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 23.

6. The compound of Claims 1 or 2, wherein each HCVR of the antibody has the amino acid sequence given in SEQ ID NO: 4, each LCVR of the antibody has the amino acid sequence given in SEQ ID NO: 13, the HCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 22, and the LCVR of each of the scFv polypeptides has the amino acid sequence given in SEQ ID NO: 24.

7. The compound of Claims 1 or 2, wherein the antibody comprises two heavy chains (HCs) and two light chains (LCs), wherein each HC has the amino acid sequence given in one of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 8, and each LC has the amino acid sequence given in one of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

8. The compound of Claim 7, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 5, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 16.

9. The compound of Claim 7, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 6, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 17.

10. The compound of Claim 7, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 7, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 18.

11. The compound of Claim 7, wherein each HC of the antibody has the amino acid sequence given in SEQ ID NO: 8, and each LC of the antibody has the amino acid sequence given in SEQ ID NO: 16.

12. The compound of Claim 1 or 7, wherein each scFv polypeptide has the identical amino acid sequence given in one of SEQ ID NO: 19 or SEQ ID NO: 20.

13. The compound of Claim 12, wherein each scFv polypeptide has the amino acid sequence given in SEQ ID NO: 19.

14. The compound of Claim 12, wherein each scFv polypeptide has the amino acid sequence given in SEQ ID NO: 20.

15. A compound comprising two first polypeptides and two second polypeptides wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16, SEQ ID NO: 17, or SEQ ID NO: 18.

16. The compound of Claim 15, wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 9, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16.

17. The compound of Claim 15, wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 10, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 17.

18. The compound of Claim 15, wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 11, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 18.

19. The compound of Claim 15, wherein each of the first polypeptides has the amino acid sequence of SEQ ID NO: 12, and each of the second polypeptides has the amino acid sequence of SEQ ID NO: 16.

20. The compound of any one of Claims 15-19, wherein each of the first polypeptides forms an inter-chain disulfide bond with each of the second polypeptides, and the first polypeptide forms two inter-chain disulfide

bonds with the other first polypeptide, and each of the first polypeptides forms an intra-chain disulfide bond.

21. A mammalian cell comprising a DNA molecule comprising a polynucleotide sequence a encoding a polypeptide selected from the group consisting of a polypeptide consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 and a polynucleotide sequence encoding a polypeptide selected from the group consisting of a polypeptide consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, wherein the cell is capable of expressing a compound comprising a first polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 and a second polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.

22. A process for producing a compound comprising two first polypeptides selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12 and two second polypeptides selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, comprising cultivating the mammalian cell of Claim 21 under conditions such that the compound is expressed, and recovering the expressed compound.

23. A compound obtainable by the process of Claim 22.

24. A pharmaceutical composition, comprising the compound of any one of Claims 1-20, and an acceptable carrier, diluent, or excipient.

25. A method of treating cancer, comprising administering to a patient in need thereof, an effective amount of the compound of any one of Claims 1-20.

26. The method of Claim 25, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, biliary tract cancer, or hepatocellular carcinoma.

27. The method of Claim 25, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, or hepatocellular carcinoma.

28. The compound of any one of Claims 1-20, for use in therapy.
29. The compound of any one of Claims 1-20, for use in the treatment of cancer.
30. The compound for use of Claim 29, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, biliary tract cancer, or hepatocellular carcinoma.
31. The compound for use of Claim 29, wherein the cancer is breast cancer, ovarian cancer, gastric cancer, colorectal cancer, or hepatocellular carcinoma.