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(54) **Title**: BMP INHIBITORS AND METHODS OF USE THEREOF

(57) **Abstract**: The present invention provides small molecule inhibitors of BMP signaling. These compounds may be used to modulate cell growth, differentiation, proliferation, and apoptosis, and thus may be useful for treating diseases or conditions associated with BMP signaling, including inflammation, cardiovascular disease, hematological disease, cancer, and bone disorders, as well as for modulating cellular differentiation and/or proliferation. These compounds may also be used to reduce circulating levels of ApoB-100 or LDL and treat or prevent acquired or congenital hypercholesterolemia or hyperlipoproteinemia; diseases, disorders, or syndromes associated with defects in lipid absorption or metabolism; or diseases, disorders, or syndromes caused by hyperlipidemia.

BMP INHIBITORS AND METHODS OF USE THEREOF

Related Applications

This application claims the benefit of priority to U.S. Provisional Application No. 61/772,465, filed March 4, 2013, the entire contents of which are hereby incorporated by reference herein in their entirety.

Statement Regarding Federally Sponsored Research or Development

This invention was supported in part by the United States Government under National Institutes of Health Grants NIH/NHLBI 5K08HL079943, NIH/NHLBI 5R01DK082971, and NIH/NIAMS 5R01AR057374. The Government may have certain rights in this invention.

Background of the Invention

Signaling involving the Transforming Growth Factor β (TGF-β) superfamily of ligands is central to a wide range of cellular processes, including cell growth, differentiation, and apoptosis. TGF-β signaling involves binding of a TGF-β ligand to a type II receptor (a serine/threonine kinase), which recruits and phosphorylates a type I receptor. The type I receptor then phosphorylates a receptor-regulated SMAD (R-SMAD; e.g., SMAD1, SMAD2, SMAD3, SMAD5, SMAD8 or SMAD9), which binds to SMAD4, and the SMAD complex then enters the nucleus where it plays a role in transcriptional regulation. The TGF superfamily of ligands includes two major branches, characterized by TGF-β/activin/nodal and Bone Morphogenetic Proteins (BMPs).

Signals mediated by bone morphogenetic protein (BMP) ligands serve diverse roles throughout the life of vertebrates. During embryogenesis, the dorsoventral axis is established by BMP signaling gradients formed by the coordinated expression of ligands, receptors, co-receptors, and soluble inhibitors (Massague et al. *Nat. Rev. Mol. Cell. Biol.* **1**:169-178, 2000). Excess BMP signaling causes ventralization, an expansion of ventral at the expense of dorsal structures,

while diminished BMP signaling causes dorsalization, an expansion of dorsal at the expense of ventral structures (Nguyen et al. *Dev. Biol.* **199**: 93-110, 1998; Furthauer et al. *Dev. Biol.* **214**:181-196, 1999; Mintzer et al. *Development* **128**:859-869, 2001; Schmid et al. *Development* **127**:957-967, 2000). BMPs are key regulators of gastrulation, mesoderm induction, organogenesis, and endochondral bone formation, and regulate the fates of multipotent cell populations (Zhao, *Genesis* **35**:43-56, 2003). BMP signals also play critical roles in physiology and disease, and are implicated in primary pulmonary hypertension, hereditary hemorrhagic telangiectasia syndrome, fibrodysplasia ossificans progressiva, and juvenile polyposis syndrome (Waite et al. *Nat. Rev. Genet.* **4**:763-773, 2003; Papanikolaou et al. *Nat. Genet.* **36**:77-82, 2004; Shore et al. *Nat. Genet.* **38**:525-527, 2006).

The BMP signaling family is a diverse subset of the TGF-β superfamily (Sebald et al. *Biol. Chem.* **385**:697-710, 2004). Over twenty known BMP ligands are recognized by three distinct type II (BMPRII, ActRIIa, and ActRIIb) and at least four type I (ALK1, ALK2, ALK3, and ALK6) receptors. Dimeric ligands facilitate assembly of receptor heteromers, allowing the constitutively-active type II receptor serine/threonine kinases to phosphorylate type I receptor serine/threonine kinases. Activated type I receptors phosphorylate BMP-responsive (BR-) SMAD effectors (SMADs 1, 5, and 8) to facilitate nuclear translocation in complex with SMAD4, a co-SMAD that also facilitates TGF signaling. In addition, BMP signals can activate intracellular effectors such as MAPK p38 in a SMAD-independent manner (Nohe et al. *Cell Signal* **16**:291-299, 2004). Soluble BMP inhibitors, such as noggin, chordin, gremlin, and follistatin, limit BMP signaling by ligand sequestration.

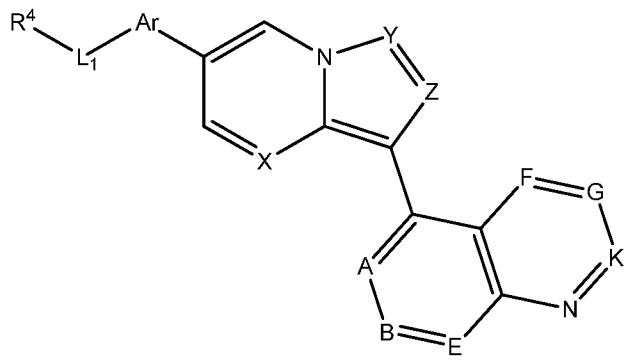
A role for BMP signals in regulating expression of hepcidin, a peptide hormone and central regulator of systemic iron balance, has also been suggested (Pigeon et al. *J. Biol. Chem.* **276**:7811-7819, 2001; Fraenkel et al. *J. Clin. Invest.* **115**:1532-1541, 2005; Nicolas et al. *Proc. Natl. Acad. Sci. U.S.A.* **99**:4596-4601, 2002; Nicolas et al. *Nat. Genet.* **34**:97-101, 2003). Hepcidin binds and promotes degradation of ferroportin, the sole iron exporter in vertebrates. Loss of ferroportin activity prevents mobilization of iron to the bloodstream from intracellular stores in enterocytes, macrophages, and hepatocytes (Nemeth et al. *Science* **306**:2090-2093,

2004). The link between BMP signaling and iron metabolism represents a potential target for therapeutics.

Given the tremendous structural diversity of the BMP and TGF-β superfamily at the level of ligands (>25 distinct ligands at present) and receptors (four type I and three type II receptors that recognize BMPs), and the heterotetrameric manner of receptor binding, traditional approaches for inhibiting BMP signals via soluble receptors, endogenous inhibitors, or neutralizing antibodies are not practical or effective. Endogenous inhibitors such as noggin and follistatin have limited specificity for ligand subclasses. Single receptors have limited affinity for ligand, whereas receptors heterotetramers exhibit more specificity for particular ligands. Neutralizing antibodies which are specific for particular ligands or receptors have been previously described, and are also limited by the structural diversity of this signaling system. Thus, there is a need in the art for pharmacologic agents that specifically antagonize BMP signaling pathways and that can be used to manipulate these pathways in therapeutic or experimental applications, such as those listed above.

Summary of the Invention

In one aspect, the invention provides compounds that inhibit BMP-induced phosphorylation of SMAD1/5/8 including compounds represented by general formula I:



Formula I

wherein

X and Y are independently selected from CR^{15} and N;

Z is selected from CR^3 and N;

Ar is selected from substituted or unsubstituted aryl and heteroaryl;

L_1 is absent or selected from substituted or unsubstituted alkyl and heteroalkyl; and

A, B, E, F, G and K, independently for each occurrence, are selected from CR^{16} and N;

provided that no more than two of A, B, E, F, G and K are N;

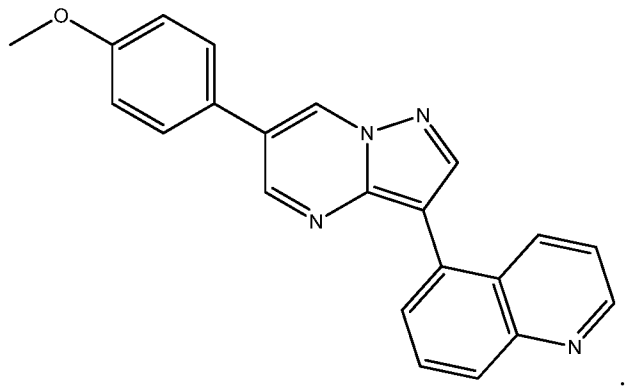
R^3 is selected from H and substituted or unsubstituted alkyl, cycloalkyl, halogen, acylamino, carbamate, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^4 is selected from H and substituted or unsubstituted alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^{15}, independently for each occurrence, is selected from H and substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acylamino, carbamate, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^{16}, independently for each occurrence, is absent or is selected from H and substituted or unsubstituted alkyl, alkenyl, alkynyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido,

with the proviso that the following compound is excluded:



In certain embodiments, either Y is N or Ar comprises a nitrogen atom in the ring.

In certain embodiments, Ar represents substituted or unsubstituted heteroaryl e.g., pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, quinoline, and pyrimidine. In certain embodiments, Ar represents substituted or unsubstituted aryl, such as phenyl. In certain embodiments, Ar is a 6-membered ring, such as a phenyl ring, e.g., in which L_1 is disposed on the para-position of Ar relative to the bicyclic core.

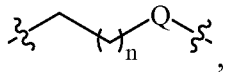
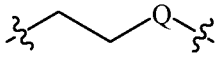
In certain embodiments, Ar represents a 6-membered aryl or heteroaryl ring.

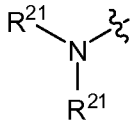
In certain embodiments as discussed above, substituents on Ar are selected from substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, or cyano).

In certain embodiments, A, B, E, F, G and K are CR^{16} or N, provided that no more than two of A, B, E, F, G and K are N;

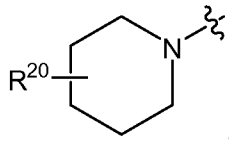
In certain embodiments, A, B, E, F, G and K are CH.

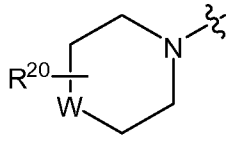
In certain embodiments, L_1 represents a linker M_k, wherein k is an integer from 1-8, preferably from 2-4, and each M represents a unit selected from $C(R^{18})_2$, NR^{19}, S, SO_2, or O, preferably selected so that no two heteroatoms occur in adjacent positions, more preferably with at least two carbon atoms between any nitrogen atom and another heteroatom; wherein R^{18}, independently for each occurrence, is selected from H and substituted or unsubstituted alkyl, heteroalkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido, preferably H or lower alkyl; and R^{19} is selected from H and substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, oxide, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfamoyl, or sulfonamido, preferably H or lower alkyl.

In certain embodiments, L_1 is absent. In certain embodiments, L_1 is selected from substituted or unsubstituted alkyl (e.g., C_1-C_8 chains, preferably C_2-C_4 chains) and heteroalkyl. In certain such embodiments, L_1 has a structure  , wherein n is an integer from 0 to 4, and Q is selected from $CR^{10}R^{11}$, NR^{12}, O, S, S(O), and SO_2; R^{10} and R^{11}, independently for each occurrence, are selected from H and substituted or unsubstituted alkyl, heteroalkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido, preferably H or lower alkyl; and R^{12} is selected from H and substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, oxide, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfamoyl, or sulfonamido, preferably H or lower alkyl. In certain embodiments, L_1 has a structure  , wherein Q is CH_2, NH, S, SO_2, or O, preferably O.

In certain embodiments, R^4 is  , wherein R^{21}, independently for each occurrence, is selected from H and substituted or unsubstituted alkyl, aralkyl,

cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, acyl, sulfonyl, sulfamoyl, or sulfonamido, preferably H or lower alkyl.

In certain embodiments, R^4 is heterocyclyl, e.g., comprising one or two heteroatoms, such as N, S or O (e.g., piperidine, piperazine, pyrrolidine, morpholine, lactone, or lactam). In certain such embodiments, R^4 is heterocyclyl comprising one nitrogen atom, e.g., piperidine or pyrrolidine, such as  , wherein R^{20} is absent or represents from 1-4 substituents on the ring to which it is attached, e.g., selected from substituted or unsubstituted alkyl, heteroaryl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, acyl, hydroxyl, alkoxyl, alkylthio, acyloxy, sulfonyl, sulfoxido, sulfamoyl, and sulfonamido, preferably H or lower alkyl. In certain embodiments, R^4 is heterocyclyl comprising two nitrogen atoms, e.g., piperazine. In certain embodiments, R^4 is heterocyclyl comprising a nitrogen and an oxygen atom, e.g., morpholine.

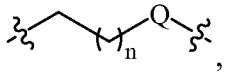
In certain embodiments, R^4 is a heterocyclyl or heteroaryl that includes an amine within the atoms of the ring, e.g., pyridyl, imidazolyl, pyrrolyl, piperidyl, pyrrolidyl, piperazyl, oxazolyl, isoxazolyl, thiazolyl, etc., and/or bears an amino substituent. In certain embodiments, R^4 is  , wherein R^{20} is as defined above; W represents a bond or is selected from $C(R^{21})_2$, O, or NR^{21}; and R^{21}, independently for each occurrence, is selected from H and substituted or unsubstituted alkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, acyl, sulfonyl, sulfamoyl, or sulfonamido, preferably H or lower alkyl.

In certain embodiments as discussed above, substituents on R^4 are selected from substituted or unsubstituted alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkylalkyl,

heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido (preferably substituted or unsubstituted alkyl, alkenyl, heteroalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, or cyano).

In certain embodiments, L_1 is absent and R^4 is directly attached to Ar. In embodiments wherein R^4 is a six-membered ring directly attached to Ar and bears an amino substituent at the 4-position of the ring relative to N, the N and amine substituents may be disposed *trans* on the ring.

In certain embodiments, L_1-R^4 comprises a basic nitrogen-containing group, e.g., either L_1 comprises nitrogen-containing heteroalkyl or an amine-substituted alkyl, or R^4 comprises a substituted or unsubstituted nitrogen-containing heterocyclyl or heteroaryl and/or is substituted with an amine substituent. In certain such embodiments, the pK_a of the conjugate acid of the basic nitrogen-containing group is 6 or higher, or even 8 or higher.

In certain embodiments, L_1 has a structure  , wherein n is an integer from 0 to 4, and R^4 is heterocyclyl.

In certain embodiments, L_1 is absent and R^4 is H and substituted or unsubstituted alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, ester, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfoxido, sulfamoyl, or sulfonamide.

In certain embodiments, L_1 is absent and R^4 is H and substituted or unsubstituted cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, amino, acylamino, carbamate, amido or amidino.

In certain embodiments, L_1 is absent and R^4 is heterocyclyl, especially a nitrogen-containing heterocyclyl. In certain embodiments, L_1 is absent and R^4 is piperidine, piperazine, pyrrolidine, or morpholine.

In certain of the embodiments disclosed above, if L_1 is absent, R^4 is cycloalkyl.

In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is heterocyclyl (especially a nitrogen-containing heterocycle), then Y is CR^{15}, wherein R^{15} is as defined above. In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is piperidine, then Y is CR^{15}, wherein R^{15} is as defined above. In certain embodiments wherein Y is CR^{15}, R^{15}, is selected from H, lower alkyl, heteroalkyl, and ester (e.g., lower alkyl ester, such as methyl ester).

In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is heterocyclyl (especially nitrogen-containing heterocyclyl), then X is CR^{15}, wherein R^{15} is as defined above. In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is piperidine, then X is CR^{15}, wherein R^{15} is as defined above. In certain embodiments wherein X is R^{15}, R^{15} is selected from H, lower alkyl, and heteroalkyl.

In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is heterocyclyl (especially nitrogen-containing heterocyclyl), Z is CR^3, wherein R^3 is as defined above. In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is piperidine, then Z is CR^3, wherein R^3 is as defined above. In certain embodiments wherein Z is CR^3, R^3 is selected from H, lower alkyl, and heteroalkyl.

In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is heterocyclyl (especially a nitrogen-containing heterocycle, such as piperidine), R^{13} represents 2 substituents on the ring to which it is attached and, independently for each occurrence, is selected from substituted or unsubstituted alkyl, heteroalkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, halogen, hydroxyl, alkoxyl, alkylthio, acyloxy, acylamino, carbamate, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido.

In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is heterocyclyl (especially a nitrogen-containing heterocycle, such as piperidine), Ar

represents substituted or unsubstituted heteroaryl (e.g., pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, quinoline, and pyrimidine). In certain such embodiments, Ar is substituted with one or more substituents selected from alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido.

In certain of the embodiments disclosed above, if L_1 is heteroalkyl and R^4 is heterocyclyl (e.g., piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like), R^4 is substituted with one or more substituents selected from alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido.

In certain of the embodiments disclosed above, compounds have one or more of the following features:

either Y is N or Ar comprises a nitrogen atom in the ring;

L_1 is absent;

R^4 is cycloalkyl, aryl, or heteroaryl;

X is CR^{15};

Y is CR^{15};

Z is CR^3;

A, B, E, F, G and K are CR^{16};

R^{13} represents 1-2 substituents on the ring to which it is attached and, independently for each occurrence, is selected from substituted or unsubstituted alkyl, heteroalkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl,

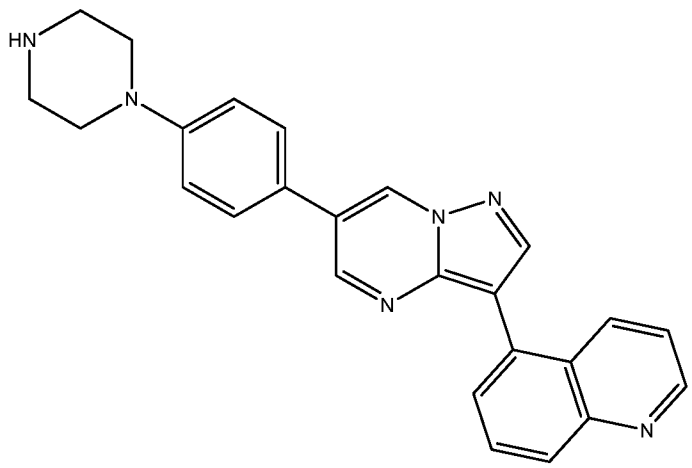
halogen, hydroxyl, alkoxyl, alkylthio, acyloxy, acylamino, carbamate, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

Ar represents substituted or unsubstituted aryl or heteroaryl (e.g., pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, quinoline, and pyrimidine);

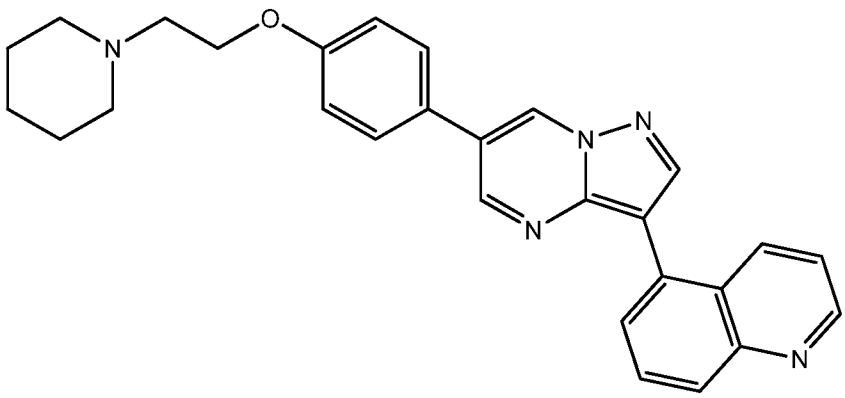
Ar is substituted with one or more substituents selected from alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl, aralkyl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido; and

R^4 is substituted with one or more substituents selected from alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, ester, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfoxido, sulfamoyl, or sulfonamide.

Exemplary compounds of Formula I include:



(Compound 1),



; and

their salts (including pharmaceutically acceptable salts).

In one aspect, the invention provides a pharmaceutical composition comprising a compound as disclosed herein and a pharmaceutically acceptable excipient or solvent. In certain embodiments, a pharmaceutical composition may comprise a prodrug of a compound as disclosed herein.

In another aspect, the invention provides a method of inhibiting BMP-induced phosphorylation of SMAD1/5/8, comprising contacting a cell with a compound as disclosed herein.

In certain embodiments, the method treats or prevents a disease or condition in a subject that would benefit by inhibition of Bone Morphogenetic Protein (BMP) signaling. In certain embodiments, the disease or condition is selected from pulmonary hypertension, hereditary hemorrhagic telangectasia syndrome, cardiac valvular malformations, cardiac structural malformations, fibrodysplasia ossificans progressiva, juvenile familial polyposis syndrome, parathyroid disease, cancer (*e.g.*, breast carcinoma, prostate carcinoma, renal cell carcinoma, bone metastasis, lung metastasis, osteosarcoma, and multiple myeloma), anemia, vascular calcification, atherosclerosis, valve calcification, renal osteodystrophy, inflammatory disorders (*e.g.*, ankylosing spondylitis), infections with viruses, bacteria, fungi, tuberculosis, and parasites.

In certain embodiments, the method reduces the circulating levels of ApoB-100 and/or LDL and/or total cholesterol in a subject that has levels of ApoB-100 and/or LDL and/or total cholesterol that are abnormally high or that increase a

patient's risk of developing a disease or unwanted medical condition. In certain embodiments, the method of reducing circulating levels of ApoB-100 and/or LDL and/or total cholesterol in a subject reduces the risk of primary or secondary cardiovascular events. In certain embodiments, the method treats or prevents a disease or condition in a subject that would benefit by inhibition of Bone Morphogenetic Protein (BMP) signaling. In certain embodiments, the disease or condition is selected from pulmonary hypertension; hereditary hemorrhagic telangectasia syndrome; cardiac valvular malformations; cardiac structural malformations; fibrodysplasia ossificans progressive; juvenile familial polyposis syndrome; parathyroid disease; cancer (*e.g.*, breast carcinoma, prostate carcinoma, renal cell carcinoma, bone metastasis, lung metastasis, osteosarcoma, and multiple myeloma); anemia; vascular calcification; vascular inflammation; atherosclerosis; acquired or congenital hypercholesterolemia or hyperlipoproteinemia; diseases, disorders, or syndromes associated with defects in lipid absorption or metabolism; diseases, disorders, or syndromes caused by hyperlipidemia; valve calcification; renal osteodystrophy; inflammatory disorders (*e.g.*, ankylosing spondylitis); infections with viruses; bacteria; fungi; tuberculosis; and parasites.

In another aspect, the invention provides a method of treating hypercholesterolemia, hyperlipidemia, hyperlipoproteinemia or hepatic steatosis in a subject comprising administering an effective amount of a compound as disclosed herein. In certain such embodiments, the hypercholesterolemia, hyperlipidemia, hyperlipoproteinemia or hepatic steatosis is acquired hypercholesterolemia, hyperlipidemia, hyperlipoproteinemia or hepatic steatosis. In certain such embodiments, the hypercholesterolemia, hyperlipidemia, hyperlipoproteinemia, or hepatic steatosis is associated with diabetes mellitus, hyperlipidemic diet and/or sedentary lifestyle, obesity, metabolic syndrome, intrinsic or secondary liver disease, biliary cirrhosis or other bile stasis disorders, alcoholism, pancreatitis, nephrotic syndrome, endstage renal disease, hypothyroidism, iatrogenesis due to administration of thiazides, beta-blockers, retinoids, highly active antiretroviral agents, estrogen, progestins, or glucocorticoids.

In another aspect, the invention provides a method of reducing primary and secondary cardiovascular events arising from coronary, cerebral, or peripheral vascular disease in a subject, comprising administering an effective amount of a compound as disclosed herein.

In another aspect, the invention provides a method of preventing and treating hepatic dysfunction in a subject associated with nonalcoholic fatty liver disease (NAFLD), steatosis-induced liver injury, fibrosis, cirrhosis, or non-alcoholic steatohepatitis (NASH) in a subject comprising administering an effective amount of a compound as disclosed herein.

In another aspect, the invention provides a method of inducing expansion or differentiation of a cell, comprising contacting the cell with a compound as disclosed herein. In certain embodiments, the cell is selected from an embryonic stem cell and an adult stem cell. In certain embodiments, the cell is *in vitro*.

In certain embodiments, a method of the invention may comprise contacting a cell with a prodrug of a compound as disclosed herein.

Brief Description of the Figures

Figure 1a shows the IC_{50} values of various BMP inhibitors for ALK1, ALK2, ALK3, ALK4 and ALK5.

Figures 1b and 1c show the fold selectivity of various BMP inhibitors over ALK2.

Figures 2a and 2b show the selectivity of various BMP inhibitors for ALK2 and ALK 5.

Figures 3a, 3b, 4a and 4b show the selectivity of various BMP inhibitors for caALK1, caALK2, caALK3, caALK4 and caALK5 in a BMP responsive (BRE-Luc C2C12) and TGF-β responsive (CAGA-Luc 293T) cell-based luciferase reporter assay system.

Figure 5a shows the inhibition profile of LDN-212854 corresponding to compound 1 for ALK1, ALK2, ALK3, ALK4 and ALK5.

Figures 5b and 5c show the improved selectivity of LDN-212854 corresponding to compound 1 versus LDN-193189 using BMP7 induced pSMAD1/5/8 in $BMPR2^{-/-}$ and TGF- β1 induced pSMAD2.

Figures 6a and 6b show the selectivity of LDN-212854 corresponding to compound 1 and LDN-193189 for caALK2 and caALK3, and the resulting inhibition curves for BMP6 and BMP4 induced alkaline phosphatase (ALP).

Figure 7a shows the effect of LDN-212854 corresponding to compound 1 and LDN-193189 on Hepcidin expression.

Figures 8a and 8b include x-ray images and alizarin red /alcian blue staining to visualize heterotopic bone formation and GFP expression to confirm $ALK2^{Q207D}$ expression at the site of Ad. Cre injection, to show the effect of LDN-212854 corresponding to compound 1 in fibrodysplasia ossificans progressiva (FOP) mutant mice.

Detailed Description of the Invention

The invention provides for compounds that inhibit the BMP signaling pathway, as well as methods to treat or prevent a disease or condition in a subject that would benefit by inhibition of BMP signaling.

I. Compounds

Compounds of the invention include compounds of Formula I as disclosed above and their salts (including pharmaceutically acceptable salts). Such compounds are suitable for the compositions and methods disclosed herein.

II. Definitions

The term "acyl" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term "acylamino" is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-, preferably alkylC(O)NH-.

The term "acyloxy" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term "aliphatic", as used herein, includes straight, chained, branched or cyclic hydrocarbons which are completely saturated or contain one or more units of unsaturation. Aliphatic groups may be substituted or unsubstituted.

The term "alkoxy" refers to an oxygen having an alkyl group attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term "alkenyl", as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated. In preferred embodiments, a straight chain or branched chain alkenyl has 1-12 carbons in its backbone, preferably 1-8 carbons in its backbone, and more preferably 1-6 carbons in its backbone. Examplary alkenyl groups include allyl, propenyl, butenyl, 2-methyl-2-butenyl, and the like.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, and branched-chain alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C_1-C_{30} for straight chains, C_3-C_{30} for branched chains), and

more preferably 20 or fewer. In certain embodiments, alkyl groups are lower alkyl groups, *e.g.* methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl and *n*-pentyl.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. In certain embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C_1-C_{30} for straight chains, C_3-C_{30} for branched chains). In preferred embodiments, the chain has ten or fewer carbon (C_1-C_{10}) atoms in its backbone. In other embodiments, the chain has six or fewer carbon (C_1-C_6) atoms in its backbone.

Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, an alkylthio, an acyloxy, a phosphoryl, a phosphate, a phosphonate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aryl or heteroaryl moiety.

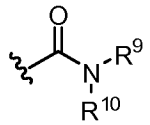
The term "C_{x-y}" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. For example, the term "C_{x-y}alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-tirfluoroethyl, etc. C_0 alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y}alkenyl" and "C_{2-y}alkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "alkylamino", as used herein, refers to an amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

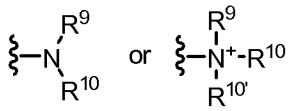
The term "alkynyl", as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated. In preferred embodiments, an alkynyl has 1-12 carbons in its backbone, preferably 1-8 carbons in its backbone, and more preferably 1-6 carbons in its backbone. Exemplary alkynyl groups include propynyl, butynyl, 3-methylpent-1-ynyl, and the like.

The term "amide", as used herein, refers to a group



wherein R^9 and R^{10} each independently represent a hydrogen or hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, *e.g.*, a moiety that can be represented by



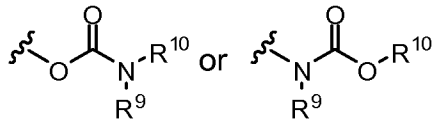
wherein R^9, R^{10}, and $R^{10'}$ each independently represent a hydrogen or a hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "aminoalkyl", as used herein, refers to an alkyl group substituted with an amino group.

The term "aralkyl", as used herein, refers to an alkyl group substituted with one or more aryl groups.

The term "aryl", as used herein, include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. Aryl groups include phenyl, phenol, aniline, and the like.

The term "carbamate" is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group.

The terms "carbocycle", "carbocyclyl", and "carbocyclic", as used herein, refers to a non-aromatic saturated or unsaturated ring in which each atom of the ring is carbon. Preferably a carbocycle ring contains from 3 to 10 atoms, more preferably from 5 to 7 atoms.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbonate" is art-recognized and refers to a group $-OCO_2-R^9$, wherein R^9 represents a hydrocarbyl group, such as an alkyl group.

The term "carboxy", as used herein, refers to a group represented by the formula $-CO_2H$.

The term "cycloalkyl", as used herein, refers to the radical of a saturated aliphatic ring. In preferred embodiments, cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably from 5-7 carbon atoms in the ring structure. Suitable cycloalkyls include cycloheptyl, cyclohexyl, cyclopentyl, cyclobutyl and cyclopropyl.

The term "ester", as used herein, refers to a group -C(O)OR^9 wherein R^9 represents a hydrocarbyl group, such as an alkyl group or an aralkyl group.

The term "ether", as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include "alkoxyalkyl" groups, which may be represented by the general formula alkyl-O-alkyl.

The terms "halo" and "halogen", as used herein, means halogen and includes chloro, fluoro, bromo, and iodo.

The term "heteroalkyl", as used herein, refers to a saturated or unsaturated chain of carbon atoms including at least one heteroatom (e.g., O, S, or NR^{50}, such as where R^{50} is H or lower alkyl), wherein no two heteroatoms are adjacent.

The terms "hetaralkyl" and "heteroaralkyl", as used herein, refers to an alkyl group substituted with a hetaryl group.

The terms "heteroaryl" and "hetaryl" include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom (*e.g.*, O, N, or S), preferably one to four or one to 3 heteroatoms, more preferably one or two heteroatoms. When two or more heteroatoms are present in a heteroaryl ring, they may be the same or different. The terms "heteroaryl" and "hetaryl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls,

cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Preferred polycyclic ring systems have two cyclic rings in which both of the rings are aromatic. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, quinoline, and pyrimidine, and the like.

The term "heteroatom", as used herein, means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The terms "heterocyclyl", "heterocycle", and "heterocyclic" refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term "heterocyclylalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocycle, alkyl, alkenyl, alkynyl, and combinations thereof.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. Examples of straight chain or branched chain lower alkyl include methyl, ethyl, isopropyl, propyl, butyl, tertiary-butyl, and the

like. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitation aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, *e.g.*, the rings are "fused rings". Preferred polycycles have 2-3 rings. Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

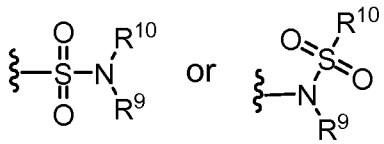
The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of the invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, an alkylthio, an acyloxy, a phosphoryl, a phosphate, a phosphonate, an amino, an amido, an amidine, an imine, a cyano, a

nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety.

Unless specifically stated as "unsubstituted," references to chemical moieties herein are understood to include substituted variants. For example, reference to an "aryl" group or moiety implicitly includes both substituted and unsubstituted variants.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt or ester thereof.

The term "sulfonamide" is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl, such as alkyl.

The term "sulfoxide" is art-recognized and refers to the group $-S(O)-R^9$, wherein R^9 represents a hydrocarbyl, such as alkyl, aryl, or heteroaryl.

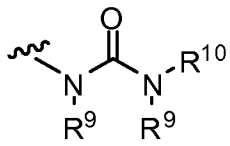
The term "sulfonate" is art-recognized and refers to the group $-SO_3H$, or a pharmaceutically acceptable salt or ester thereof.

The term "sulfone" is art-recognized and refers to the group $-S(O)_2-R^9$, wherein R^9 represents a hydrocarbyl, such as alkyl, aryl, or heteroaryl.

The term "thioester", as used herein, refers to a group $-C(O)SR^9$ or $-SC(O)R^9$ wherein R^9 represents a hydrocarbyl, such as alkyl.

The term "thioether", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term "urea" is art-recognized and may be represented by the general formula



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl, such as alkyl.

At various places in the present specification substituents of compounds of the invention are disclosed in groups or in ranges. It is specifically intended that the invention include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_1-C_6 alkyl" is specifically intended to individually disclose methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, isobutyl, etc.

For a number qualified by the term "about", a variance of 2%, 5%, 10% or even 20% is within the ambit of the qualified number

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term "prodrug" is intended to encompass compounds which, under physiologic conditions, are converted into the therapeutically active agents of the present invention (e.g., a compound of Formula I or Formula II). A common method for making a prodrug is to include one or more selected moieties which are hydrolyzed under physiologic conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal. For example, esters (e.g., esters of alcohols or carboxylic acids) are preferred prodrugs of the present invention. In various embodiments disclosed herein (e.g., the various compounds, compositions, and methods), some or all of the compounds of formula A, compounds of any one of Formula I or Formula II, all or a portion of a

compound of Formula I or Formula II in a formulation represented above can be replaced with a suitable prodrug, e.g., wherein a hydroxyl or carboxylic acid present in the parent compound is presented as an ester.

As used herein, the term "treating" or "treatment" includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to improve or stabilize a subject's condition. As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "small molecule" refers to an organic molecule having a molecular weight less than about 2500 amu, preferably less than about 2000 amu, even more preferably less than about 1500 amu, still more preferably less than about 1000 amu, or most preferably less than about 750 amu. Preferably a small molecule contains one or more heteroatoms.

The phrase "activity of ALK2" means ALK-2 enzymatic activity (e.g., such as kinase activity; the ability of ALK-2 to phosphorylate BMP-responsive SMAD proteins) and/or ALK-2-mediated signaling (e.g., such as the ability of ALK-2 to mediate downstream signal transduction and transcriptional activity following activation of ALK-2 by binding of BMP ligands). In some embodiments, "activity of ALK2" means ALK2-mediated BMP signaling. In some embodiments, "activity of ALK2" means ALK2-mediated BMP-responsive gene transcription (e.g., transcriptional activity mediated by BMP/ALK2 signal transduction). The assays described in Examples 1-3 permit the measurement of ALK2 activity.

The phrase "activity of ALK5" means ALK-5 enzymatic activity (e.g., such as kinase activity; the ability of ALK-5 to phosphorylate TGF-β responsive SMAD

proteins; the ability of ALK-5 to phosphorylate SMAD2 or SMAD3) and/or ALK-5-mediated signaling (e.g., such as the ability of ALK-5 to mediate downstream signal transduction and transcriptional activity following activation of ALK-5 by binding of TGF-β ligands). I n some embodiments, "activity of ALK5" means ALK5-mediated TGF-β signaling. In some embodiments, "activity of ALK5" means ALK5-mediated TGF-β -responsive gene transcription (e.g, transcriptional activity mediated by TGFβ/ALK5 signal transduction). The assays described in Examples 1-3 permit the measurement of ALK5 activity.

The phrase "activity of ALK1" means ALK-1 enzymatic activity (e.g., such as kinase activity; the ability of ALK-1 to phosphorylate BMP-responsive SMAD proteins) and/or ALK-1-mediated signaling (e.g., such as the ability of ALK-1 to mediate downstream signal transduction and transcriptional activity following activation of ALK-1 by binding of BMP ligands). In some embodiments, "activity of ALK1" means ALK1-mediated BMP signaling. In some embodiments, "activity of ALK1" means ALK1-mediated BMP-responsive gene transcription (e.g., transcriptional activity mediated by BMP/ALK1 signal transduction). The assays described in Examples 1-3 permit the measurement of ALK1 activity.

The phrase "activity of ALK3" means ALK-3 enzymatic activity (e.g., such as kinase activity; the ability of ALK-3 to phosphorylate BMP-responsive SMAD proteins) and/or ALK-3-mediated signaling (e.g., such as the ability of ALK-3 to mediate downstream signal transduction and transcriptional activity following activation of ALK-3 by binding of BMP ligands). In some embodiments, "activity of ALK3" means ALK3-mediated BMP signaling. In some embodiments, "activity of ALK3" means ALK3-mediated BMP-responsive gene transcription (e.g., transcriptional activity mediated by BMP/ALK3 signal transduction). The assays described in Examples 1-3 permit the measurement of ALK3 activity.

The phrase "activity of ALK4" means ALK-4 enzymatic activity (e.g., such as kinase activity; the ability of ALK-4 to phosphorylate activin-responsive SMAD proteins; the ability of ALK-4 to phosphorylate SMAD 2 or SMAD 3) and/or ALK-

4-mediated signaling (e.g., such as the ability of ALK-4 to mediate downstream signal transduction and transcriptional activity following activation of ALK-4 by binding of activin ligands). In some embodiments, "activity of ALK4" means ALK4-mediated activin signaling. In some embodiments, "activity of ALK4" means ALK4-mediated activin-responsive gene transcription (e.g., transcriptional activity mediated by activin/ALK4 signal transduction). The assays described in Examples 1-3 permit the measurement of ALK4 activity.

The phrase "activity of ALK6" means ALK-6 enzymatic activity (e.g., such as kinase activity; the ability of ALK-6 to phosphorylate BMP-responsive SMAD proteins) and/or ALK-6-mediated signaling (e.g., such as the ability of ALK-6 to mediate downstream signal transduction and transcriptional activity following activation of ALK-6 by binding of BMP ligands). In some embodiments, "activity of ALK6" means ALK6-mediated BMP signaling. In some embodiments, "activity of ALK6" means ALK6-mediated GDF5 signaling. In some embodiments, "activity of ALK6" means ALK6-mediated BMP-responsive gene transcription (e.g., transcriptional activity mediated by BMP/ALK6 signal transduction). The assays described in Examples 1-3 permit the measurement of ALK6 activity.

Human ALK2 is a 509 amino acid protein. The protein sequence is published, for example, as GenBank accession number NP_001104537.1, (with corresponding nucleotide sequence at NM_001111067.2) UniProt entry Q04771.

Human ALK5 has, at least, two isoforms: a 503 amino acid protein (isoform 1) and a 426 amino acid protein. The protein sequence for human ALK5 isoform 1 is published, for example, as GenBank accession number NP_004603.1 (with corresponding nucleotide sequence at NM_004612.2) The protein sequence for the 426 amino acid isoform is published, for example, as GenBank accession number NP_001124388.1 (with corresponding nucleotide sequence at NM_001130916.1). Information regarding both isoforms is also published as UniProt entry P36897.

Human ALK1 is a 503 amino acid protein. The protein sequence is published, for example, as GenBank accession number NP_001070869.1 (with corresponding nucleotide sequence at NM_001077401.1; transcript variant 2) and NP_000011.2 (with corresponding nucleotide sequence at NM_000020.2; transcript variant 1), UniProt entry P37023.

Human ALK3 is a 532 amino acid protein. The protein sequence is published, for example, as GenBank accession number NP_004320 (with corresponding nucleotide sequence at NM_004329.2), UniProt entry P36894.

Human ALK4 has at least three isoforms. Isoform a is a 505 amino acid protein. The protein sequence is published, for example, as GenBank accession number NP_004293 (with corresponding nucleotide sequence at NM_004302), UniProt entry P36896.

Isoform a of human ALK6 is a 532 amino acid protein and isoform b is a 502 amino acid protein. The protein sequence for human ALK6 isoform a is published, for example, as GenBank accession number NP_001243722 (with corresponding nucleotide sequence at NM_001256793.1). The protein sequence for human ALK6 isoform b is published, for example, as GenBank accession number NP_001194 (with corresponding nucleotide sequence at NM_001203.2).

Note that each of the foregoing proteins are further processed in vivo, such as by the cleaving of a signal sequence, to yield a mature form.

III. Pharmaceutical Compositions

Compounds of the present invention may be used in a pharmaceutical composition, e.g., combined with a pharmaceutically acceptable carrier, for administration to a patient. Such a composition may also contain diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with compounds of the invention, or to minimize side effects caused by the compound of the invention.

The pharmaceutical compositions of the invention may be in the form of a liposome or micelles in which compounds of the present invention are combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The terms "pharmaceutically effective amount" or "therapeutically effective amount", as used herein, means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., treatment, healing, prevention, inhibition or amelioration of a physiological response or condition, such as an inflammatory condition or pain, or an increase in rate of treatment, healing, prevention, inhibition or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

Each of the methods of treatment or use of the present invention, as described herein, comprises administering to a mammal in need of such treatment or use a pharmaceutically or therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt or ester form thereof. Compounds of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies.

Administration of compounds of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways. Exemplary routes of administration that can be used include oral, parenteral, intravenous, intra-arterial, cutaneous, subcutaneous, intramuscular, topical, intracranial, intraorbital, ophthalmic,

intravitreal, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, central nervous system (CNS) administration, or administration by suppository.

When a therapeutically effective amount of a compound(s) of the present invention is administered orally, compounds of the present invention may be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder may contain from about 5 to 95% compound of the present invention, and preferably from about 10% to 90% compound of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oils, phospholipids, tweens, triglycerides, including medium chain triglycerides, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition typically contains from about 0.5 to 90% by weight of compound of the present invention, and preferably from about 1 to 50% compound of the present invention.

When a therapeutically effective amount of a compound(s) of the present invention is administered by intravenous, cutaneous or subcutaneous injection, compounds of the present invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to compounds of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers,

preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of compound(s) of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments the patient has undergone. Ultimately, the practitioner will decide the amount of compound of the present invention with which to treat each individual patient. Initially, the practitioner may administer low doses of compound of the present invention and observe the patient's response. Larger doses of compounds of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 μg to about 100 mg (preferably about 0.1 mg to about 50 mg, more preferably about 1 mg to about 2 mg) of compound of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the compounds of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the practitioner will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

IV. Use with polymers

The compounds as disclosed herein may be conjugated to a polymer matrix, e.g., for controlled delivery of the compound. The compound may be conjugated via a covalent bond or non-covalent association. In certain embodiments wherein the compound is covalently linked to the polymer matrix, the linkage may comprise a moiety that is cleavable under biological conditions (e.g., ester, amide, carbonate, carbamate, imide, etc.). In certain embodiments, the conjugated compound may be a

pharmaceutically acceptable salt, ester, or prodrug of a compound disclosed herein. A compound as disclosed herein may be associated with any type of polymer matrix known in the art for the delivery of therapeutic agents.

V. Synthetic Preparation

The compounds disclosed herein can be prepared in a variety of ways known to one skilled in the art of organic synthesis, and in analogy with the exemplary compounds whose synthesis is described herein. The starting materials used in preparing these compounds may be commercially available or prepared by known methods. Preparation of compounds can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Greene and Wuts, *Protective Groups in Organic Synthesis*, 44th. Ed., Wiley & Sons, 2006, which is incorporated herein by reference in its entirety.

The reactions of the processes described herein can be carried out in suitable solvents which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, i.e., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

VI. Uses

BMPs and TGF-beta signaling pathways are essential to normal organogenesis and pattern formation, as well as the normal and pathological remodeling of mature tissues. Defects in the BMP signaling pathway are implicated in a number of congenital and acquired disease processes, including Hereditary Hemorrhagic Telangectasia syndrome, Primary Pulmonary Hypertension or

Pulmonary Arterial Hypertension, Juvenile Familial Polyposis, as well as sporadic renal cell and prostate carcinomas. It has been suggested that in certain disease states associated with defective signaling components, attenuated BMP signaling might be a cause, while our findings have suggested that in some contexts excess BMP signaling might be pathogenic (Waite et al. *Nat. Rev. Genet.* **4**:763-773, 2005; Yu et. *J. Biol. Chem.* **280**:24443-24450, 2003). The ability to modulate BMP signaling experimentally would provide a means for investigating therapy, and for determining the root causes of these conditions.

A. Treatment of anemia, including iron deficiency and anemia of chronic disease

For a review, see Weiss et al. *N. Engl. J. Med.* **352**:1011-1023, 2005. Anemia of inflammation (also called anemia of chronic disease) can be seen in patients with chronic infections, autoimmune diseases (such as systemic lupus erythematosis and rheumatoid arthritis, and Castleman's disease), inflammatory bowel disease, cancers (including multiple myeloma), and renal failure. Anemia of inflammation is often caused by maladaptive expression of the peptide hormone hepcidin. Hepcidin causes degradation of ferroportin, a critical protein that enables transport of iron from intracellular stores in macrophages and from intestinal epithelial cells. Many patients with renal failure have a combination of erythropoietin deficiency and excess hepcidin expression. BMP signaling induces expression of hepcidin and inhibiting hepcidin expression with BMP inhibitors increases iron levels. Compounds as described herein can be used to treat anemia due to chronic disease or inflammation and associated hyperhepcidinemic states.

The inflammatory cytokine IL-6 is thought to be the principal cause of elevated hepcidin expression in inflammatory states, based upon the elevation of IL-6 in anemia of inflammation of diverse etiologies, the effects of chronic IL-6 administration in vivo, and the protection against anemia in rodents deficient in IL-6 (Weiss et al. *N. Engl. J. Med.* **352**:1011-1023, 2005). It has been shown that stimulating hepatoma cell lines with IL-6 induces hepcidin expression, while treatment with a BMP inhibitor abrogates IL-6-induced hepcidin expression (Yu et al. *Nat. Chem. Biol.* **4**:33-41, 2008). Moreover, we have found that BMP inhibitors

can inhibit hepcidin expression induced by injection of pathogenic bacteria in vivo. It has also been shown that systemic iron administration in mice and zebrafish rapidly activates BMP-responsive-SMADs and hepcidin expression in the liver, and that BMP antagonism effectively blocks these responses (Yu et al. *Nat. Chem. Biol.* **4**:33-41, 2008). The functional importance of BMP signaling in iron regulation is supported by our finding that BMP inhibitors can inhibit hepcidin expression and raise serum iron levels in vivo. Taken together these data suggest that iron- and inflammation-mediated regulation of hepcidin and circulating iron levels require BMP signaling. Compounds as described herein may be used to alter iron availability in diverse circumstances for therapeutic benefit.

Compounds as described herein may be used in anemic states to (i) augment the efficacy of dietary iron or oral iron supplementation (which is safer than intravenous administration of iron) to increase serum iron concentrations; (ii) augment build up of hemoglobin in the blood in anticipation of surgery or to enable blood donation for self in anticipation of surgery; (iii) enhance the efficacy of erythropoietin and its relatives, thereby enabling lower doses of erythropoietin to be administered for anemia while minimizing known toxicities and side effects of erythropoietin (i.e., hypertension, cardiovascular events, and tumor growth), amd (iv) inhibit the hepcidin expression to help correct the anemia associated with inflammatory bowel disesease (Wang et al., Inflamm. Bowel Dis. 2012 Jan;18(1):112-9.. Epub 2011 Feb 23).

B. Treatment of fibrodysplasia ossificans progressiva (FOP)

FOP is caused by the presence of a constitutively-active mutant form of ALK2 in affected individuals (Shore et al. *Nat. Genet.* **38**:525-527, 2006). A specific inhibitor of BMP signaling such as a compound as described herein can be used to prevent excessive bone formation in response to trauma, musculoskeletal stress or inflammation. Such a compound could also be used to aid in regression of pathologic bone. The BMP inhibitor could be administered systemically or locally to concentrate or limit effects to areas of trauma or inflammation.

A BMP inhibitor as described herein may be used as chronic therapy to suppress spontaneous bone formation in individuals who are highly susceptible. Transient therapy may be used to prevent abnormal bone formation in FOP individuals who develop osteomas or pathologic bone most frequently in association with trauma by administration before, during, or even after the traumatic incident. Transient therapy with BMP inhibitors as described herein could be used before, during or immediately after necessary or emergent medical or surgical procedures (and even important immunizations and tooth extractions) in individuals with FOP, to prevent pathologic calcification. Combination therapy with other bone inhibiting agents, immune modulatory or anti-inflammatory drugs (such as NSAIDs, steroids, cyclosporine, cyclophosphamide, azathioprine, methotrexate, rituxumab, etanercept, or similar drugs) may increase the effectiveness of BMP inhibitors in inhibiting heterotopic bone formation in this disorder.

A mouse model of FOP has been developed in which expression of a constitutively-active mutant form of ALK2 is induced by injecting the popliteal fossa of a genetically-modified mouse with an adenovirus directing expression of Cre recombinase. This model reproduces the ectopic calcification and disability seen in FOP patients.

C. Treatment of cancers

Excessive BMP signaling, which could arise due to over-expression of BMPs, or, paradoxically, as a result of loss of BMP type II receptor expression, may contribute to the oncogenesis, growth or metastasis of certain solid tumors, including breast, prostate carcinomas, bone, lung, and renal cell carcinomas (Yu et al. *J. Biol. Chem.* **280**:24443-24450, 2008; Waite et al. *Nat. Rev. Genet.* **4**:763-773, 2003; Alarmo et al. *Genes, Chromosomes Cancer* **45**:411-419, 2006; Kim et al. *Cancer Res.* **60**:2840-2844, 2000; Kim et al. *Clin. Cancer Res.* **9**:6046-6051, 2003; Kim et al. *Oncogene* 23:7651-7659, 2004). Inhibition of BMP9 signaling can prevent ovarian cancer cell growth (Herrera et al. Cancer Res. 2009 Dec 15;69(24):9254-62). Ovarian cancer growth is promoted by ALK2-SMAD signaling and could be inhibited by selective ALK2 inhibitors (Tsai et al. Cell Rep. 2012 Aug 30;2(2):283-93. Epub 2012 Aug 9), such as with the compounds described herein.

If increased BMP activity associated with BMP over-expression or BMP type II receptor deficiency contributes to the pathogenesis of disease, then inhibiting BMP signaling activity using compounds as described herein at the level of BMP type I receptors (downstream of both ligands and type II receptor) could be an effective means of normalizing BMP signaling activity and potentially inhibiting tumor growth or metastasis.

Compounds as described herein can be used to slow or arrest the growth or metastasis of such tumor cells (as well as other tumor constituent cell types) for clinical benefit, either as adjunctive or primary chemotherapy. Also, BMP inhibitors as described herein may be used to interfere with the bone metastatic properties of certain types of cancers (e.g., adenocarcinoma, such as prostate and breast carcinomas). In addition, compounds as described herein can be used to inhibit osteoblastic activity in tumors that either form bone or are bone-derived, such as osteosarcomas (as adjunctive or primary chemotherapy). Further, compounds as described herein can be used to inhibit osteoclastic activity (also regulated by BMPs through the action of its target gene RANKL), which is pathologically increased in conditions such as multiple myeloma and other bone-targeted tumors. Application of BMP inhibitors in these conditions may reduce the presence of osteolytic lesions and bone fractures due to tumor involvement.

D. Immune modulation via BMP inhibitors

BMPs have been reported to attenuate the inflammatory or immune response (Choi et al. *Nat. Immunol.* **7**:1057-1065, 2006; Kersten et al. *BMC Immunol.* **6**:9, 2005), which can impair an individual's ability to fight infections (i.e., viral, bacterial, fungal, parasitic, or tuberculosis). Inhibitors of BMP signaling as described herein may thus augment the inflammatory or immune response enabling individuals to clear infections more rapidly.

Lymphocytes and other immune cells express BMP receptors on their cell surfaces, and there is growing evidence that BMPs regulate the development and maturation of various humoral and cellular immunologic compartments, and regulate humoral and cellular immune responses in mature organisms. The effects

of BMP signals on immune cells are likely to be context-specific, as is commonly known for the effects of numerous cytokines of immunologic importance, and thus whether they augment or diminish the development or function of particular lymphocyte populations must be empirically determined. BMP antagonism using compounds as described herein may be an effective strategy for intentionally biasing the development of cellular, innate, or humoral immune compartments for therapy, or a strategy for the therapeutic deviation of immune responses in mature immune systems. These strategies may target inborn disorders of cellular, innate, or humoral immunity, or target disorders in which immune responses are inappropriately weak (e.g., as an adjuvant to promote successful antigen sensitization when immunization is difficult or ineffective by other means), or target disorders in which immune responses are excessive or inappropriate (e.g., autoimmunity and autosensitization). BMP inhibitors as described herein may also be effective in some contexts for the intentional induction of immune tolerance (i.e., in allotransplantation or autoimmunity) and for indications such as autoimmune diseases and inflammatory bowel disease (IBD) (Wang et al., Inflamm. Bowel Dis. 2012 Jan;18(1):112-9.. Epub 2011 Feb 23). BMP inhibitors as described herein may also attenuate macrophage-mediated inflammation in response to *Salmonella typhimurium* in a model of inflammatory colitis (Wang L et al, J Clin Invest. 2009; 119(11):3322).

E. Treatment of pathologic bone formation

Compounds as described herein can be used to ameliorate pathologic bone formation/bone fusion in inflammatory disorders, such as ankylosing spondylitis or other "seronegative" spondyloarthropathies, in which autoimmunity and inflammation in such disorders appear to stimulate bone formation. One application of the compounds would be to prevent excess bone formation after joint surgery, particularly in patients with ankylosing spondylitis or rheumatoid arthritis. Compounds as described herein can also be used to prevent calcinosis (dystrophic soft-tissue calcification) in diseases such as systemic lupus erythematosus, scleroderma, or dermatomyositis.

Blunt traumatic injury to muscles can cause abnormal bone formation within muscle in certain individuals, resulting in a disorder called myositis ossificans

traumatica (Cushner et al. *Orthop. Rev.* **21**:1319-1326, 1992.). Head trauma and burn injury can also induce heterotopic bone formation markedly impairing patient rehabilitation and recovery. Treatment with a BMP inhibitor as described herein, optionally in addition to anti-inflammatory medications usually prescribed for such a condition (eg. non-steroidal anti-inflammatory drugs such as indomethacin or ibuprofen) may help to prevent the formation of pathologic bone in predisposed individuals, or to help lessen or regress lesions in individuals recently or remotely affected. Very rarely other muscles have been described to develop ossification in the presence of injury or trauma, including heart muscle, and similar treatment with a BMP inhibitor as described herein could be helpful in those circumstances.

F. Treatment of ectopic or maladaptive bone formation

BMP signals and their transcriptional targets are implicated in intimal and medial vascular remodeling and calcification in Monckeberg's vascular calcification disease and in atheromatous vascular disease (Bostrom et al. *J. Clin. Invest.* **91**:1800-1809, 1993; Tyson et al. *Arterioscler. Thromb. Vasc. Biol.* **23**:489-494, 2003). BMPs and BMP-induced osteodifferentation are also implicated in cardiac valvular calcification. Native cardiac valves can calcify particularly when they are already abnormal. A classic example is bicuspid aortic valve—these valves typically become calcified leading to stenosis. Patients with calcific aortic valve stenosis often require cardiac surgery for valve replacement. Abnormal calcification can adversely affect the function of prosthetic vascular grafts or cardiac valves. For example, prosthetic heart valves become calcified leading to narrowing and often leakage.

Compounds as described herein can be used to inhibit vascular or valvular calcific disease alone or in combination with atheromatous disease, renal disease, renal osteodystrophy or parathyroid disease.

Compounds as described herein can be used to inhibit calcification of prosthetic vascular or valvular materials by systemic or local administration or direct incorporation into prosthesis materials or other implants (e.g., in admixture with a polymer that coats or constitutes all or part of the implant or prosthesis).

In some instances, it is desired to delay fracture healing following a bone fracture, or to purposely inhibit fracture healing in certain locations to prevent impairment of function by maladaptive bone formation. For example, if a fracture occurs and for medical or practical reasons surgery cannot be performed immediately, fracture healing may be temporarily "suspended" by use of a BMP inhibitor as described herein, until definitive surgery or manipulation can be performed. This could prevent the need for subsequent intentional re-fracture in order to ensure correct apposition of bone fragments, for example. It is expected that upon stopping a BMP inhibitor normal fracture healing processes would ensue if the period of treatment is relatively short. In other cases, any amount of novel bone growth might impair function, such as when fracture affects a joint directly. In these cases, global or local inhibition of BMP activity (by systemic or local delivery of a BMP inhibitor as described herein via diffusion from a local implant or matrix) may be used to inhibit fracture healing or prevent fracture calluses at the critical areas.

G. Treatment of skin diseases

Expansion of cultured keratinocytes — In vitro, BMPs inhibit keratinocyte proliferation and promote differentiation (reviewed in Botchkarev et al. *Differentiation* **72**:512-526, 2004). In patients in need of skin grafting (eg. after burns), skin grafts are made from cultured keratinocytes. The keratinocytes may be derived from other animals (xenografts), but these are only temporary as they will be rejected by the immune system. Keratinocytes can be derived from the patient themselves and can be grown into sheets of cells in the laboratory (cultured epithelial autografts). The patient will not reject keratinocytes derived from his/her own body. Addition of BMP inhibitors as described herein to keratinocyte cultures can be used to facilitate keratinocyte proliferation enabling patients to receive grafts sooner.

Improved epithelialization — BMP6 is highly expressed in skin injury, and high levels of BMP6 are detected in chronic human wounds of different etiologies (Kaiser et al. *J. Invest. Dermatol.* **111**:1145-1152, 1998). In mice overexpressing BMP6 in their skin, reepithelialization and healing skin wounds were significantly

delayed (Kaiser et al. *J. Invest. Dermatol.* **111**:1145-1152, 1998). Improved epithelialization can reduce scar formation. Topical or systemic administration of BMP inhibitors as described herein can be used to augment epithelialization of skin wounds, for example, in the treatment of pressure ulcers (bed sores) or non-healing or poorly-healing skin ulcers (e.g., in patients with peripheral vascular disease, diabetes mellitus, venous incompetence). Compounds would also be expected to decrease scar formation.

Promotion of hair growth — Growth of hair follicles on the scalp is cyclic with three phases: anagen (the growth phase), catagen (the involutional phase), and telogen (resting phase). Recent evidence suggests that BMP signals delay the transition from telogen to anagen (Plikus et al. *Nature* **451**:340-344, 2008). Inhibition of BMP signaling using compounds as described herein can shorten the telogen phase and increase the number of follicles in the anagen phase. Compounds as described herein can be used to treat circumstances wherein hair follicles are insufficient or when hairs are being lost more frequently than they are grown. These circumstances include androgenetic alopecia (male pattern balding), alopecia areata, and telogen effluvium.

Treatment of psoriasis — Psoriasis is an inflammatory skin disorder which sometimes occurs following skin trauma and the ensuing repair and inflammation (Koebner phenomenon). BMPs may participate in repair and inflammatory mechanisms that cause psoriasis, since over-expression of BMP6 in the skin of mice leads to skin lesions similar to those seen in patients with psoriasis (Blessing et al. *J. Cell. Biol.* **135**:227-239, 1996). Compounds as described herein may be administered topically or systemically to treat established psoriasis or prevent its development after skin injury.

Treatment of corneal scarring — BMP6 expression is associated with conjunctival scarring (Andreev et al. *Exp. Eye Res.* **83**:1162-1170, 2006). Compounds as described herein can be used to prevent or treat corneal scarring and the resulting blindness.

H. Treatment of systemic hypertension

Infusion of BMP4 induces systemic hypertension in mice (Miriyala et al. *Circulation* **113**:2818-2825, 2006). Vascular smooth muscle cells express a variety of BMP ligands. BMPs increase the expression of voltage gated potassium channels and thereby increase constriction of vascular smooth muscle (Fantozzi et al. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **291**:L993-1004, 2006). Compounds as described herein that inhibit BMP signaling can be used to reduce blood pressure. Sustained reduction of blood pressure in patients with hypertension would be expected to prevent myocardial infarction, congestive heart failure, cerebrovascular accidents, and renal failure. BMP inhibitors as described herein can be used to target the hypertension in specific vascular beds, such as in pulmonary hypertension via local delivery (e.g., via aerosol).

I. Treatment of pulmonary hypertension

BMP signaling contributes to the pathogenesis of pulmonary hypertension. For example, mice with decreased BMP4 levels are protected from the pulmonary hypertension and pulmonary vascular remodeling induced by breathing low oxygen concentrations for prolonged periods (Frank et al. *Circ. Res.* **97**:496-504, 2005). Moreover, mutations in the gene encoding the type II BMP receptor (BMPRII) are frequently found in patients with sporadic and familial pulmonary arterial hypertension. It might be anticipated that decreased BMP signaling might cause pulmonary hypertension. However, Yu and colleagues (Yu et al. *J. Biol. Chem.* **280**:24443-24450, 2008) reported that BMPRII deficiency paradoxically increases BMP signaling by subsets of BMP ligands, and thus increased BMP signaling using compounds as described herein may actually contribute to the development of pulmonary hypertension.

Compounds as described herein can be used to prevent the development of pulmonary arterial hypertension in patients at risk for the disease (e.g., patients with BMPRII mutations) or to treat patients with idiopathic or acquired pulmonary arterial hypertension. Decreased pulmonary hypertension in individuals treated with the compounds described herein would be expected to decrease shortness of breath, right ventricular hypertrophy, and right ventricular failure.

J. Treatment of ventricular hypertrophy

BMP-10 levels are increased in the hypertrophied ventricles of rats with hypertension, and this BMP ligand induces hypertrophy in cultured neonatal rat ventricular myocytes (Nakano et al. *Am. J. Physiol. Heart. Circ. Physiol.* **293**:H3396-3403, 2007). Sun et al. (Hypertension 2013 Feb;61(2):352-60)suggest that small molecule BMP inhibitors can reduce adverse left ventricular remodeling (hypertrophy). Inhibition of BMP-10 signaling with compounds as described herein can to prevent/treat ventricular hypertrophy. Ventricular hypertrophy can lead to congestive heart failure due to diastolic dysfunction. Compounds described herein would be expected to prevent/treat congestive heart failure.

K. Treatment of neurologic disorders

Treatment of spinal cord injury and neuropathy — BMPs are potent inhibitors of axonal regeneration in the adult spinal cord after spinal cord injury (Matsuura et al. *J. Neurochem.* 2008). Expression of BMPs is reported to be elevated in oligodendrocytes and astrocytes around the injury site following spinal cord contusion. Intrathecal administration of noggin, a BMP inhibitor, led to enhanced locomotor activity and significant regrowth of the corticospinal tract after spinal cord contusion.

RGMa inhibits axonal growth and recovery after spinal cord injury, as well as synapse re-formation, effects which are blocked by an antibody directed against RGMa (Hata et al. *J. Cell. Biol.* **173**:47-58, 2006; Kyoto et al. *Brain Res.* **1186**:74-86, 2007). RGMa enhances BMP signaling (Babitt et al. *J. Biol. Chem.* **280**:29820-29827, 2005) suggesting that BMP signaling may be responsible for preventing axonal growth and recovery.

Based on these considerations, compounds as described herein would be expected to increase axonal growth and recovery after spinal cord injury. Compounds as described herein would be expected to prevent/treat neuropathies associated with a wide spectrum of disorders including diabetes mellitus. Compounds as described herein would be expected to treat both the pain and motor dysfunction associated with neuropathies.

Treatment of neurologic disorders associated with central nervous system inflammation — BMP4 and 5 have been detected in multiple sclerosis and Creutzfeldt-Jakob disease lesions (Deininger et al. *Acta Neuropathol.* **90**:76-79, 1995). BMPs have also been detected in mice with experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (Ara et al. *J. Neurosci. Res.* **86**:125-135, 2008). Compounds as described herein may be used to prevent or treat multiple sclerosis as well as other neurologic disorders associated with central nervous system inflammation, or maladaptive injury repair processes mediated by BMP signals.

Treatment of dementias — Inhibitors of BMP signaling can promote neurogenesis in mouse neural precursor cells (Koike et al. *J. Biol. Chem.* **282**:15843-15850, 2007). Compounds as described herein can be used to augment neurogenesis in a variety of neurologic disorders associated with accelerated loss of neurons including cerebrovascular accidents and Alzheimer's Disease, as well as other dementias.

Altering memory and learning — BMP signaling has an important role in the development and maintenance of neurons involved in memory and cognitive behavior. For example, mice deficient in the BMP inhibitor, chordin, have enhanced spatial learning but less exploratory activity in a novel environment (Sun et al. *J. Neurosci.* **27**:7740-7750, 2007). Compounds as described herein can be used to alter or prevent memory or learning, for example, inducing amnesia for anesthesia or in other situations likely to cause distress, or to prevent Post-Traumatic Stress Disorder.

L. Treatment of atherosclerosis

Abundant evidence suggests that BMP ligands are pro-inflammatory and pro-atherogenic in the blood vessel wall (Chang et al. *Circulation* **116**:1258-1266, 2007). Knocking-down expression of BMP4 decreased inflammatory signals, whereas knocking-down BMP inhibitors (eg follistatin or noggin) increased inflammatory signals. Compounds as described herein can be used to reduce vascular inflammation associated with atherosclerosis, automimmune disease, and

other vasculitides. By decreasing atherosclerosis, it would be anticipated that compounds as described herein would decrease the incidence and/or severity of acute coronary syndromes (angina pectoris and heart attack), transient ischemic attacks, stroke, peripheral vascular disease, and other vascular ischemic events. Moreover, in so far as atherosclerosis contributes to the pathogenesis of aneurysm formation, compounds as described herein can be used to slow the progression of aneurysm formation decreasing the frequency of aneurismal rupture and the requirement for surgery.

As BMPs and many of the BMP-induced gene products that affect matrix remodeling are overexpressed in early atherosclerotic lesions, BMP signals may promote atherosclerotic plaque formation and progression (Bostrom et al. J Clin Invest. 91: 1800-1809. 1993; Dhore et al. Arterioscler Thromb Vasc Biol. 21: 1998-2003. 2001). BMP signaling activity in the atheromatous plaque may thus represent a form of maladaptive injury-repair, or may contribute to inflammation. Over time, BMP signals may also induce resident or nascent vascular cell populations to differentiate into osteoblast-like cells, leading to intimal and medial calcification of vessels (Hruska et al. Circ Res. 97: 105-112. 2005). Calcific vascular disease, or arteriosclerosis, is associated with decreased vascular distensibility, and increased risk of cardiovascular events and mortality, and is particularly problematic when associated with underlying atherosclerotic disease (Bostrom et al. Crit Rev Eukaryot Gene Expr. 10: 151-158. 2000). Both atherosclerotic and calcific lesions may be amenable to regression, however, if signals which contribute to their progression can be intercepted (Sano et al. Circulation. 103: 2955-2960. 2001). In certain aspects, inhibitor of BMP type I receptor activity may be used to limit the progression of atheromatous plaques and vascular calcification *in vivo* (Derwall et al. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012; 32: 613-622).

M. Treatment of Hypercholesterolemia or Hyperlipoproteinemia

Treatment with small molecule or recombinant BMP inhibitors reduces vascular inflammation (via macrophage accumulation and cathepsin activity), atheroma formation, and vascular calcification in mice deficient in low-density lipoprotein receptor ($LDLR^{-/-}$). Without wishing to be bound by theory, as potential

explanations for impact on vascular inflammation, oxidized LDL (oxLDL) has been found to increase BMP2 expression and induce the production of reactive oxygen species (ROS) in human aortic endothelial cells. ROS production induced by oxLDL appears to require BMP signaling, based on inhibition by small molecule or recombinant BMP inhibitors. Treatment with small molecule BMP inhibitors reduces plasma low-density lipoprotein levels without inhibiting HMG-CoA reductase activity, suggesting a role of BMP signaling in the regulation of LDL cholesterol biosynthesis. Small molecule BMP inhibitors have also been found to inhibit hepatosteatosis seen in LDLR-deficient mice fed a high-fat diet. Small molecule or recombinant BMP inhibitors inhibit the synthesis of ApoB-100 in hepatoma cells in vitro. These findings implicate BMP signaling in vascular calcification and atherogenesis and provide at least two novel mechanisms by which BMP signaling may contribute to the pathogenesis of atherosclerosis. These studies highlight the BMP signaling pathway as a therapeutic target in the treatment of atherosclerosis while identifying several novel functions of BMP signaling in the regulation of vascular oxidative stress, inflammation and lipid metabolism.

In certain embodiments, BMP inhibitors as described herein may be used for the reduction of circulating levels of ApoB-100 in patients. In certain embodiments, BMP inhibitors as described herein may be used for the reduction of circulating levels of LDL in patients. Accordingly, BMP inhibitors as described herein may be used for the treatment of hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia, including congenital or acquired hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia.

In certain embodiments, the congenital hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia is autosomal dominant hypercholesterolemia (ADH), familial hypercholesterolemia (FH), polygenic hypercholesterolemia, familial combined hyperlipidemia (FCHL), hyperapobetalipoproteinemia, or small dense LDL syndrome (LDL phenotype B).

In certain embodiments, the acquired hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia is associated with diabetes mellitus, hyperlipidemic diet and/or sedentary lifestyle, obesity, metabolic syndrome, intrinsic or secondary liver

disease, primary biliary cirrhosis or other bile stasis disorders, alcoholism, pancreatitis, nephrotic syndrome, endstage renal disease, hypothyroidism, iatrogenesis due to administration of thiazides, beta-blockers, retinoids, highly active antiretroviral agents, estrogen, progestins, or glucocorticoids.

In certain embodiments, BMP inhibitors as described herein may be used for the treatment of diseases, disorders, or syndromes associated with defects in lipid absorption or metabolism, such as sitosterolemia, cerebrotendinous xanthomatosis, or familial hypobetalipoproteinemia.

In certain embodiments, BMP inhibitors as described herein may be used for the treatment of diseases, disorders, or syndromes caused by hyperlipidemia, such as coronary artery disease and its manifestations (e.g., myocardial infarction; angina pectoris; acute coronary artery syndromes, such as unstable angina pectoris; cardiac dysfunction, such as congestive heart failure, caused by myocardial infarction; or cardiac arrhythmia associated with myocardial ischemia/infarction), stroke due to occlusion of arteries supplying portions of the brain, cerebral hemorrhage, peripheral arterial disease (e.g., mesenteric ischemia; renal artery stenosis; limb ischemia and claudication; subclavian steal syndrome; abdominal aortic aneurysm; thoracic aortic aneurysm, pseudoaneurysm, intramural hematoma; or penetrating aortic ulcer, aortic dissection, aortic stenosis, vascular calcification, xanthoma, such as xanthoma affecting tendons or scleral and cutaneous xanthomas, xanthelasma, or hepatosteatosis. In certain embodiments, BMP inhibitors as described herein may be used for the treatment of the foregoing diseases, disorders, or syndromes regardless of circulating lipid levels, such as in individuals exhibiting normal circulating lipid levels or metabolism.

In certain embodiments, BMP inhibitors as described herein may be used for the reduction of secondary cardiovascular events arising from coronary, cerebral, or peripheral vascular disease. In certain such embodiments, BMP inhibitors as described herein may be used to treat individuals regardless of lipid levels, such as used in the treatment of individuals exhibiting normal circulating cholesterol and lipid levels. In certain such embodiments, BMP inhibitors as described herein are administered conjointly with a HMG-CoA reductase inhibitor.

In certain embodiments, BMP inhibitors as described herein may be used for the prevention of cardiovascular disease, such as in individuals with elevated markers of cardiovascular risk (e.g., C-reactive protein) or, for example, an elevated Framingham Risk Score. In certain such embodiments, BMP inhibitors as described herein may be used to prevent cardiovascular disease in individuals exhibiting normal circulating cholesterol and lipid levels.

In certain embodiments wherein one or more BMP inhibitors as described herein are used in the treatment or prevention of the foregoing diseases, disorders, or syndromes, the patient being treated is not diagnosed with and/or is not suffering from one or more of the following conditions: vascular inflammation associated with atherosclerosis, automimmune disease, and other vasculitides; atherosclerotic disease, atheromatous plaques, and/or vascular calcification; an aneurysm and/or aneurysm formation; acute coronary syndromes (angina pectoris and heart attack), transient ischemic attacks, stroke, peripheral vascular disease, or other vascular ischemic events.

In other embodiments wherein one or more BMP inhibitors as described herein are used in the treatment or prevention of the foregoing diseases, disorders, or syndromes (e.g., for the reduction of circulating levels of ApoB-100 and/or LDL in patients; for the treatment of hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia, including congenital or acquired hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia; for the treatment of diseases, disorders, or syndromes associated with defects in lipid absorption or metabolism; for the treatment of diseases, disorders, or syndromes caused by hyperlipidemia; for the reduction of secondary cardiovascular events arising from coronary, cerebral, or peripheral vascular disease; or for the reduction of secondary cardiovascular events arising from coronary, cerebral, or peripheral vascular disease), the patient being treated is also diagnosed with and/or is also suffering from one or more of the following conditions: vascular inflammation associated with atherosclerosis, automimmune disease, and other vasculitides; atherosclerotic disease, atheromatous plaques, and/or vascular calcification; an aneurysm and/or aneurysm formation;

acute coronary syndromes (angina pectoris and heart attack), transient ischemic attacks, stroke, peripheral vascular disease, or other vascular ischemic events.

N. Propagation, engraftment and differentiation of progenitor cells including embryonic and adult stem cells in vitro and in vivo

BMP signals are crucial for regulating the differentiation and regeneration of precursor and stem cell populations, in some contexts and tissues preventing (while in other contexts directing) differentiation towards a lineage. Compounds as described herein can be used to (i) maintain a pluripotential state in stem cell or multipotent cell populations in vivo or in vitro; (ii) expand stem cell or multipotent cell populations in vivo or in vitro; (iii) direct differentiation of stem cell or multipotent cell populations in vivo or in vitro; (iv) manipulate or direct the differentiation of stem cell or multipotent cell populations in vivo or in vitro, either alone or in combination or in sequence with other treatments; and (v) modulate the de-differentiation of differentiated cell populations into multipotent or progenitor populations.

Numerous stem cell and precursor lineages require BMP signals in order to determine whether they will expand, differentiate towards specific tissue lineages, home in and integrate with particular tissue types, or undergo programmed cell death. Frequently BMP signals interact with signals provided by growth factors (bFGF, PDGF, VEGF, HBEGF, PlGF, and others), Sonic Hedgehog (SHH), notch, and Wnt signaling pathways to effect these changes (Okita et al. *Curr. Stem Cell Res. Ther.* **1**:103-111, 2006). Compounds as described herein can be used to direct the differentiation of stem cells (e.g., embryonic stem cells) or tissue progenitor cells towards specific lineages for therapeutic application (Park et al. *Development* **131**:2749-2762, 2004; Pashmforoush et al. *Cell* **117**:373-386, 2004). Alternatively for certain cell populations, BMP inhibitors as described herein may be effective in preventing differentiation and promoting expansion, in order to produce sufficient numbers of cells to be effective for a clinical application. The exact combination of BMP inhibitor and growth factor or signaling molecule may be highly specific to each cell and tissue type.

For example, certain embryonic stem cell lines require co-culture with leukemia inhibitory factor (LIF) to inhibit differentiation and maintain the pluripotency of certain cultured embryonic stem cell lines (Okita et al. *Curr. Stem Cell Res. Ther.* **1**:103-111, 2006). Use of a BMP inhibitor as described herein may be used to maintain pluripotency in the absence of LIF. Other ES cell lines require coculture with a specific feeder cell layer in order to maintain pluripotency. Use of a BMP inhibitor as described herein, alone or in combination with other agents, may be effective in maintaining pluripotency when concerns of contamination with a feeder cell layer, or its DNA or protein components would complicate or prevent use of cells for human therapy.

In another example, in some circumstances antagonizing BMP signals with a protein such as noggin shortly before cessation of LIF in culture is able to induce differentiation into a cardiomyocyte lineage (Yuasa et al. *Nat. Biotechnol.* **23**:607-611, 2005). Use of a pharmacologic BMP inhibitor as described herein may achieve similar if not more potent effects. Such differentiated cells could be introduced into diseased myocardium therapeutically. Alternatively, such treatment may actually be more effective on engrafted precursor cells which have already homed in to diseased myocardium. Systemic therapy with a protein inhibitor of BMP such as noggin would be prohibitively expensive and entail complicated dosing. Delivery of a BMP inhibitor as described herein, systemically or locally, could bias the differentiation of such precursor cells into functioning cardiomyocytes in situ.

O. Treatment of cartilage defects

The selective inhibition of specific BMP receptors enables cartilage formation by preventing calcification and mineralization of scaffolds produced by mesenchymal stem cells (Hellingman et al. Tissue Eng Part A. 2011 Apr;17(7-8):1157-67. Epub 2011 Jan 17.) Accordingly, compounds of the invention may be useful to promote cartilage repair/regeneration in patients with cartilage injuries or defects, as well as in the ex vivo or in vitro production of cartilage tissue, e.g., for implantation, from appropriate cells, such as mesenchymal stem cells.

P. Application of compounds with varying degrees of selectivity: Compounds which inhibit BMP signaling via particular BMP type I receptors, or compounds which also affect signaling via TGF-β, Activin, AMP kinase, or VEGF receptors

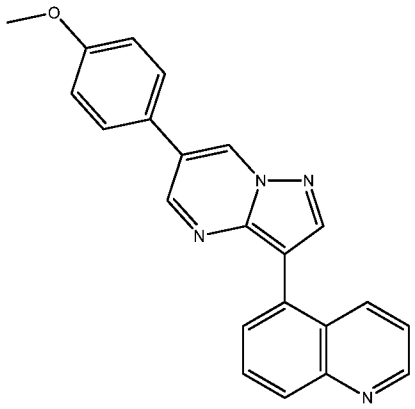
ALK-specific inhibitors — Dorsomorphin inhibits the activity of the BMP type I receptors, ALK2, ALK3, and ALK6. Dorsomorphin inhibits ALK2 and ALK3 to a greater extent than it does ALK6 (Yu et al. *Nat. Chem. Biol.* **4**:33-41, 2008). Several of the compounds described herein will have relative greater selectivity for particular BMP type I receptors. The pathogenesis of certain diseases might be attributed to the dysfunctional signaling of one particular receptor. For example, fibrodysplasia ossificans progressiva is a disease caused by aberrant (constitutively active) ALK2 function (Yu et al. *Nat. Chem. Biol.* **4**:33-41, 2008). In such instances, compounds as described herein which specifically antagonize the function a subset of the BMP type I receptors may have the advantage of reduced toxicity or side effects, or greater effectiveness, or both.

Some compounds as described herein may have a high degree of selectivity for BMP vs. TGF-β, Activin, AMP kinase, and VEGF receptor signaling. Other compounds may be less specific and may target other pathways in addition to BMP signaling. In the treatment of tumors, for example, agents which inhibit BMP signaling as well as one or more of the above pathways can have beneficial effects (e.g. decrease tumor size), when molecular phenotyping of specific patients' tumors reveals dysregulation of multiple pathways.

Some compounds as described herein have a high degree of selectivity for ALK2 versus ALK1 or ALK3 or ALK4 or ALK5 or ALK6. Selective inhibition of ALK2 versus ALK1 or ALK3 or ALK4 or ALK5 or ALK6 may minimize unwanted effects or toxicity. Chronic ALK3 inhibition might impair normal mucosal epithelial turnover due to known importance in intestinal crypt stem cell recycling, and implication of ALK3 function in juvenile familial polyposis. ALK1 inhibition might impair normal vascular remodeling and lead to complications similar to human hereditary telangiectasia syndrome type 2 (HHT2), such as leaky capillaries, AV malformations, and bleeding. Accordingly, compounds that selectively inhibit ALK2

relative to ALK3 and ALK1 may help avoid toxicities of this type that might be encountered through the use of an unselective inhibitor.

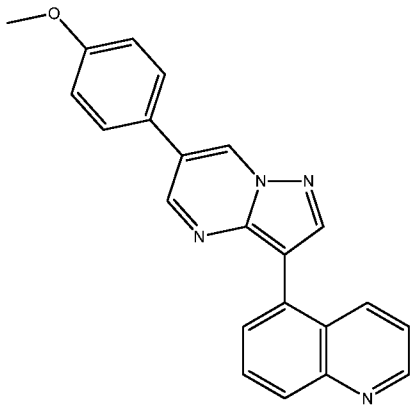
In certain embodiments, the invention provides a method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK1. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of about 2 than its IC_{50} for inhibiting the activity of human ALK1. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 5 than its IC_{50} for inhibiting the activity of human ALK1. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 10 than its IC_{50} for inhibiting the activity of human ALK1. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 15 or 20 or 30 or 40 or 50 or 100 or 200 or 300 or 400 or 500 or 600 or 800 or 1000 or 1500 or 2000 or 5000 or 10000 or 15,000 or 20,000 or 40,000 or 50,000 or 60,000 or 70,000 or 80,000 or 90,000 or 100,000 than its IC_{50} for inhibiting the activity of human ALK1. In some such embodiments, the small molecule is not



or a pharmaceutically acceptable salt thereof. In certain embodiments, the small molecule has a structure of Formula I as described herein.

In certain embodiments, the invention provides a method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity

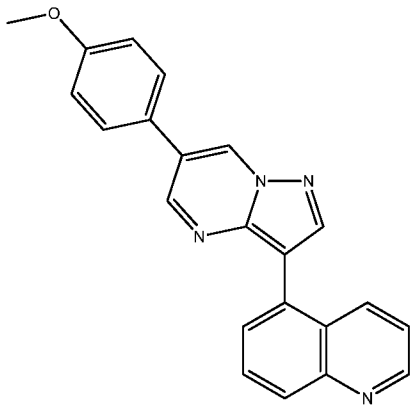
of human ALK3. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 15 than its IC_{50} for inhibiting the activity of human ALK3. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 20 than its IC_{50} for inhibiting the activity of human ALK3. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 30 than its IC_{50} for inhibiting the activity of human ALK3. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 50 or 100 or 200 or 300 or 400 or 500 or 600 or 800 or 1000 or 1500 or 2000 or 5000 or 10000 or 15,000 or 20,000 or 40,000 or 60,000 or 70,000 or 80,000 or 90,000 or 100,000 than its IC_{50} for inhibiting the activity of human ALK3. In some such embodiments, the small molecule is not



or a pharmaceutically acceptable salt thereof. In certain embodiments, the small molecule has a structure of Formula I as described herein.

In certain embodiments, the invention provides a method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK4. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 1000 than its IC_{50} for inhibiting the activity of human ALK4. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 2000 than its IC_{50} for inhibiting the activity of human ALK4. In some such

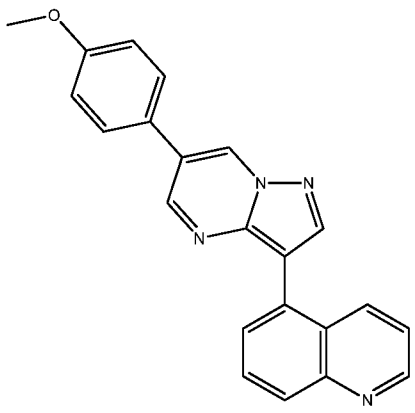
embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 3000 than its IC_{50} for inhibiting the activity of human ALK4. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 4000 or 5000 or 6000 or 7000 or 8000 or 9000 or 10,000 or 12,000 or 14,000 or 16,000 or 18,000 or 20,000 or 25,000 or 30,000 or 40,000 or 50,000 or 60,000 or 70,000 or 80,000 or 90,000 or 100,000 than its IC_{50} for inhibiting the activity of human ALK4. In some such embodiments, the small molecule is not



or a pharmaceutically acceptable salt thereof. In certain embodiments, the small molecule has a structure of Formula I as described herein.

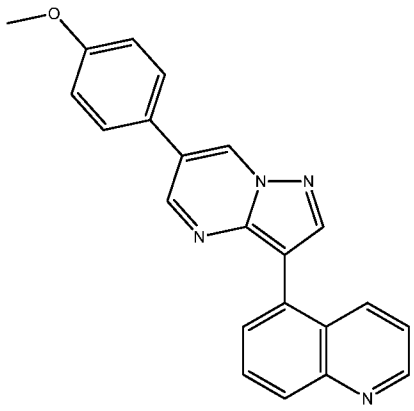
In certain embodiments, the invention provides a method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK6. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 2 than its IC_{50} for inhibiting the activity of human ALK6. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 5 than its IC_{50} for inhibiting the activity of human ALK6. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 10 than its IC_{50} for inhibiting the activity of human ALK6. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 15 or 20 or 30 or 40 or 50 or 100 or 200 or 300 or 400 or 500 or 600 or 800 or 1000 or 1500 or 2000 or 5000 or

10000 or 15,000 or 20,000 or 40,000 or 50,000 or 60,000 or 70,000 or 80,000 or 90,000 or 100,000 than its IC_{50} for inhibiting the activity of human ALK6. In some such embodiments, the small molecule is not



or a pharmaceutically acceptable salt thereof. In certain embodiments, the small molecule has a structure of Formula I as described herein.

In one aspect, the invention provides a method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK5. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 1000 than its IC_{50} for inhibiting the activity of human ALK5. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 2000 than its IC_{50} for inhibiting the activity of human ALK5. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 3000 than its IC_{50} for inhibiting the activity of human ALK5. In some such embodiments, the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 4000 or 5000 or 6000 or 7000 or 8000 or 9000 or 10,000 or 12,000 or 14,000 or 16,000 or 18,000 or 20,000 or 25,000 or 30,000 or 40,000 or 50,000 or 60,000 or 70,000 or 80,000 or 90,000 or 100,000 than its IC_{50} for inhibiting the activity of human ALK5. In some such embodiments, the small molecule is not



or a pharmaceutically acceptable salt thereof. In certain embodiments, the small molecule has a structure of Formula I as described herein.

Compounds as described herein can be used to treat subjects (e.g., humans, domestic pets, livestock, or other animals) by use of dosages and administration regimens that are determined to be appropriate by those of skill in the art, and these parameters may vary depending on, for example, the type and extent of the disorder treated, the overall health status of the subject, the therapeutic index of the compound, and the route of administration. Standard clinical trials can be used to optimize the dose and dosing frequency for any particular pharmaceutical composition of the invention. Exemplary routes of administration that can be used include oral, parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, topical, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or administration by suppository. Methods for making formulations that can be used in the invention are well known in the art and can be found, for example, in Remington: The Science and Practice of Pharmacy (20th edition, Ed., A.R. Gennaro), Lippincott Williams & Wilkins, 2000.

Q. Combination therapies

In certain instances BMP inhibitors as described herein may be used in combination with other current or future drug therapies, because the effects of inhibiting BMP alone may be less optimal by itself, and/or may be synergistic or more highly effective in combination with therapies acting on distinct pathways which interact functionally with BMP signaling, or on the BMP pathway itself. In

certain instances, conjoint administration of a BMP inhibitor as described herein with an additional drug therapy reduces the dose of the additional drug therapy such that it is less than the amount that achieves a therapeutic effect when used in a monotherapy (e.g., in the absence of a BMP inhibitor as described herein). Some examples of combination therapies could include the following.

In certain embodiments, BMP inhibitors as described herein may be administered conjointly with other antihyperlipidemic agents or antilipidemic agents including, but not limited to, HMG-CoA reductase inhibitors (e.g., atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastain, pravastatin, rosuvastatin, or simvastatin), fibrates (e.g., bezafibrate, ciprofibrate, clofibrate, gemfibrozil, or fenofibrate), ezetimibe, niacin, cholesteryl ester transfer protein (CETP) inhibitors (e.g., torcetrapib, anacetrapib, or dalcetrapib), cholestyramine, colestipol, probucol, dextrothyroxine, bile acid sequestrants, or combinations of the above.

In certain embodiments, BMP inhibitors as described herein may be administered conjointly with a treatment for diabetes including, but not limited to, sulfonyl ureas (*e.g.*, chlorpropamide, tolbutamide, glyburide, glipizide, or glimepiride), medications that decrease the amount of glucose produced by the liver (*e.g.*, metformin), meglitinides (*e.g.*, repaglinide or nateglinide), medications that decrease the absorption of carbohydrates from the intestine (*e.g.*, alpha glucosidase inhibitors such as acarbose), medications that effect glycemic control (*e.g.*, pramlintide or exenatide), DPP-IV inhibitors (*e.g.*, sitagliptin), insulin treatment, thiazolidinones (e.g., troglitazone, ciglitazone, pioglitazone, or rosiglitazone), oxadiazolidinediones, alpha-glucosidase inhibitors (e.g., miglitol or acarbose), agents acting on the ATP-dependent postassium channel of the beta cells (e.g., tolbutamide, glibenclamide, glipizide, glicazide, or repaglinide), nateglinide, glucagon inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis and/or glycogenolysis, or combinations of the above.

In certain embodiments, BMP inhibitors as described herein may be administered conjointly with a treatment for obesity including, but not limited to, orlistat, sibutramine, phendimetrazine, phentermine, diethylpropion, benzphetamine, mazindol, dextroamphetamine, rimonabant, cetilistat, GT 389-255, APD356, pramlintide/AC137, PYY3-36, AC 162352/PYY3-36, oxyntomodulin, TM 30338,

AOD 9604, oleoyl-estrone, bromocriptine, ephedrine, leptin, pseudoephedrine, or pharmaceutically acceptable salts thereof, or combinations of the above.

In certain embodiments, BMP inhibitors as described herein may be administered conjointly with an antihypertensive agent including, but not limited to, beta-blockers (e.g., alprenolol, atenolol, timolol, pindolol propranolol and metoprolol), ACE (angiotensin converting enzyme) inhibitors (e.g., benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril and ramipril), calcium channel blockers (e.g., nifedipine, felodipine, nicardipine, isradipine, nimodipine, diltiazem and verapamil), and alpha-blockers (e.g., doxazosin, urapidil, prazosin and terazosin), or combinations of the above.

In certain embodiments, BMP inhibitors as described herein may be administered conjointly with a treatment for anemia (e.g., anemia of inflammation ssociated with renal failure and hemodialysis), including but not limited to erythopoiesis-stimulating agents (e.g. erythropoietin).

Tyrosine kinase receptor inhibitors, such as SU-5416, and BMP inhibitors as described herein may have synergistic effects at inhibiting angiogenesis, particularly for anti-angiogenic therapy against tumors. BMP signals (BMP-4) are thought to be critical for the commitment of stem or precursor cells to a hematopoietic/endothelial common progenitor, and may promote the proliferation, survival, and migration of mature endothelial cells necessary for angiogenesis (Park et al. *Development* **131**:2749-2762, 2004). Thus antagonism of BMP signals using compounds as described herein may provide additional inhibition of angiogenesis at the level of endothelial precursors and cells. Similarly, co-treatment with BMP inhibitors as described herein and other tyrosine kinase receptor inhibitors such as imatinib (Gleevec) could be used to inhibit vascular remodeling and angiogenesis of certain tumors.

The combination of a sonic hedgehog agonist and a BMP inhibitor as described herein may be particularly useful for promoting hair growth, as SHH activity is known to stimulate the transition of follicles out of telogen (resting) phase (Paladini et al. *J. Invest. Dermatol.* **125**:638-646, 2005), while inhibiting the BMP pathway shortens the telogen phase (Plikus et al. *Nature* **451**:340-344, 2008). The

use of both would be expected to cause relatively increased time in the anagen or growth phase.

Combined use of Notch modulators (e.g., gamma-secretase inhibitors) and BMP inhibitors as described herein may be more effective than either agent alone in applications designed to inhibit vascular remodeling or bone differentiation, because increasing evidence suggests both pathways function cooperatively to effect cell differentiation, and vascular cell migration (Kluppel et al. *Bioessays* **27**:115-118, 2005). These therapies may be synergistic in the treatment of tumors in which one or both pathways is deranged (Katoh, *Stem Cell Rev.* **3**:30-38, 2007).

Combined use of an Indian Hedgehog (IHH) antagonist and a BMP inhibitor as described herein may inhibit pathologic bone formation. IHH is responsible for the commitment of bone precursors to chondrocyte or cartilage forming cells. Endochondral bone formation involves coordinated activity of both chondrogenesis (promoted by BMP signals and IHH signals) and their subsequent calcification by mineralization programs initiated by BMP signals (Seki et al. *J. Biol. Chem.* **279**:18544-18549, 2004; Minina et al. *Development* **128**:4523-4534, 2001). Coadministration of an IHH antagonist with a BMP inhibitor as described herein, therefore, may be more effective in inhibiting pathological bone growth due to hyperactive BMP signaling (such as in FOP), or in any of the inflammatory or traumatic disorders of pathologic bone formation described above.

Strong experimental evidence exists for an effect of both Smo antagonism and BMP antagonism for treating glioblastoma. Compounds as described herein may be used in combination with Smo antagonists to treat glioblastoma.

R. Inhibition of BMP signaling in insects

Some of the compounds as described herein may have activity against, and perhaps even selectivity for the BMP receptors of arthropods versus those of chordates. Inhibiting BMP signaling in arthropod larvae or eggs is likely to cause severe developmental abnormalities and perhaps compromise their ability to reproduce, e.g., via the same dorsalization that is observed in zebrafish and drosophila when this pathway is inhibited. If BMP inhibitors as described herein

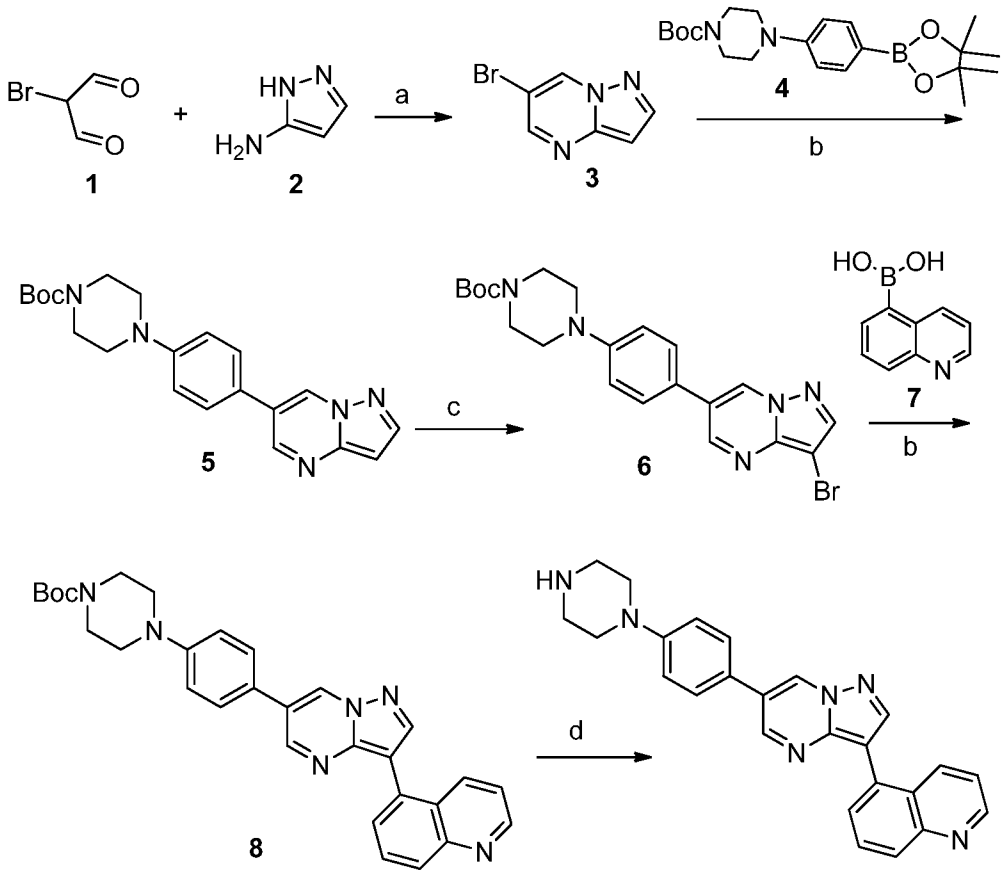
have very strong selectivity for arthropod BMP receptors versus those of humans, they may be used as insecticides or pest control agents that are demonstrably less toxic or more environmentally sound than current strategies.

In addition to being administered to patients in therapeutic methods, compounds as described herein can also be used to treat cells and tissues, as well as structural materials to be implanted into patients (see above), ex vivo. For example, the compounds can be used to treat explanted tissues that may be used, for example, in transplantation.

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Exemplification

The synthesis and in vitro and in vivo evaluation of certain BMP inhibitors disclosed herein is set forth in WO 2009/114180, which is herein incorporated by reference in its entirety.



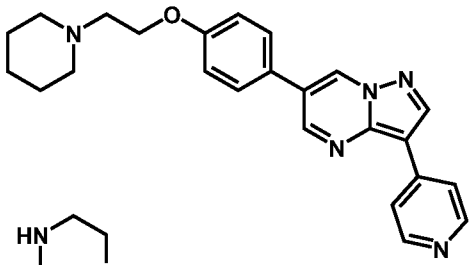
Scheme 1. Reagents and conditions: (a) AcOH, MeOH, 80 °C, (61%); (b) $Pd(PPh_3)_4$, 2.0 M Na_2CO_3, dioxane, 101 °C, (82-95%); (c) NBS, DCM, (63%); (d) TFA, DCM, sat. $NaHCO_3$, (55%).

Example 1: Kinase Assay

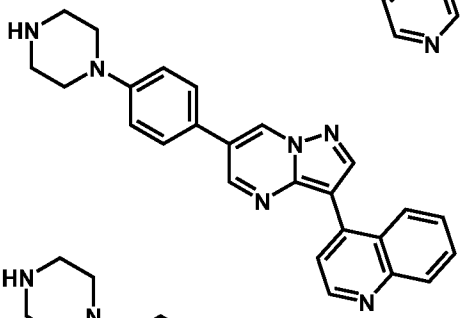
Equal 8μL fractions of purified kinase (Invitrogen), ATP (Sigma), ATP [γ-^{32}P] (Perkin Elmer), and dephosphorylated casein (Sigma) diluted in kinase buffer (Cell Signaling) containing 0.2% bovine serum albumin and supplemented with 10mM $MnCL_2$ to a final concentration of 2.5 nM, 6 μM, 0.05 μCi/μL, and 0.5 mg/mL respectively were added to a 96-well plate containing compounds diluted in kinase buffer at final concentrations ranging from .01 nM to 10 μM in triplicate. Positive controls were generated by replacing compounds with an 8 μL of just kinase buffer and negative controls were generated by replacing both the purified kinase and compounds with two 8 μL aliquots of kinase buffer. The reaction was allowed to proceed at room temperature for 45 minutes and quenched with the

addition of 10 μL of 10% phosphoric acid. A multi-channel pipette was used to transfer the entire reaction volume (50 μL) to 96-well P81 phosphocellulose filter plates (Millipore) and allowed to rest for 5 minutes. A vacuum manifold system was then used to filter the reaction liquid as well as 20 repeated washings of 150 μL of 1% phosphoric acid washing solution per well. The filter plates were then dried at RT for 1 hour and the back sealed with the corresponding opaque tape (Millipore). A multi-channel pipette was used pipette 200 μL of Microscint 20 scintillation fluid (Perkin Elmer) per well and the plate was sealed using optically clear adhesive QPCR seals (Thermo Scientific). Light output was measured using a Spectramax L luminometer (Molecular Devices) using the photon counting setting with an integration time of one second per well. Data was normalized to positive controls at 100% enzyme activity with negative controls being subtracted as background. GraphPad Prism® software was used for graphing and regression analysis by sigmoidal dose-response with variable Hill coefficient. Figures 1a and 2a show some of the compounds tested and their respective selectivity profile. Corresponding structures follow:

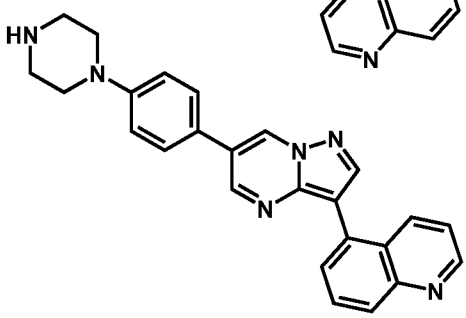
Dorsomorphin:



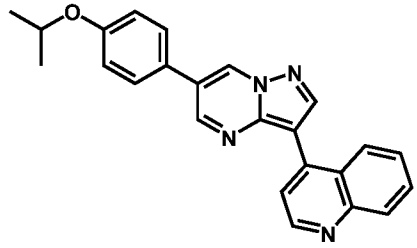
LDN-193189:



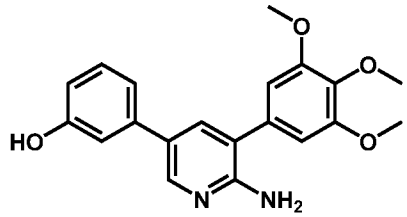
LDN-212854:



DMH1:



K02288a:



Example 2: Cell Culture

C2C12 myofibroblasts cells stably transfected with BMP responsive element from the Id1 promoter fused to luciferase reporter gene (BRE-Luc) and human embryonic kidney 293T cells stably transfected with the TGF-β responsive element from the PAI-1 promoter fused to luciferase reporter gene (CAGA-Luc) were cultured in DMEM (Life Technologies) supplemented with 10% FBS, L-glutamine, and pen/strep at 37°C and 10% CO_2. HepG2 human hepatoma cells (ATCC) were cultured in EMEM (Life Technologies) supplemented with 10% FBS, L-glutamine, and pen/strep at 37°C and 10% CO_2. C2C12 myofibroblasts (ATCC) were cultured in DMEM (Life Technologies) supplemented with 10% FBS, L-glutamine, and pen/strep at 37°C and 10% CO_2. Pulmonary arterial smooth muscle cells (PASMCs) were isolated from both wild type and $BMPR2^{flox/flox}$ mice and the latter exposed to adenovirus specifying Cre recombinase (Ad. Cre) to generate BMP type II receptor deficient ($BMPR2^{del/del}$) cells, as previously described (Yu; JBC, 2005). PASMCs were cultured in RPMI medium (Life Technologies) supplemented with 10% FBS, L-glutamine, and pen/strep at 37°C and 5% CO_2. Results for several compounds are shown in Figures 3a, 3b, 4a and 4b.

Example 3: Luciferase Assay (BRE-luc and CAGA-luc)

C2C12 Bre-Luc and 293T CAGA-Luc cells were seeded at 20,000 cells in 80 μL DMEM supplemented with 2% FBS per well in tissue culture treated 96-well plates (Costar® 3610; Corning). The cells were incubated for 1 hour at 37°C and 10% CO_2 and allowed to settle and attach. The compounds of interest were diluted in DMEM at 10-fold the final concentrations ranging from 1nM to 10 μM and added in

10 μL aliquots. Positive controls were generated by replacing the compound aliquot with just 10 μL of DMEM. The cells were then incubated for 30 min at 37°C and 10% CO_2. Finally 10 μL aliquots of adenovirus expressing constitutively active BMP and TGF-β type 1 receptors (caALK1-5) were added to achieve a multiplicity of infection (MOI) of 100. The negative controls were generated by replacing both the compound and adenovirus aliquots with just 20 μL of DMEM. Plates were left to incubate overnight for 16 to 24 hours at 37°C and 10% CO2. After determining cell viability using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay (CellTiter 96®; Promega) per the manufacturer's instructions, the media was discarded, 30 μL of passive lysis buffer (Promega) added, and the plates allowed to incubate at RT on a shaker for 15 minutes. A multichannel pipette was used to add 15 μL of luciferase assay system (Promega) solution to each well and the plate was shaken gently for 15 seconds. Light output was measured using a Spectramax L luminometer (Molecular Devices) using the auto-range setting with an integration time of one second per well. Data was normalized to positive controls at 100% BMP or TGF-β signaling activity with negative controls being subtracted as background. GraphPad Prism® software was used for graphing and regression analysis by sigmoidal dose-response with variable Hill coefficient. Results for several compounds are shown in Figures 3a, 3b, 4a and 4b.

Example 4: Western Blot (BMP7 vs TGF-beta induced pSMAD)

Both WT and $BMPR2^{del/del}$ PASMCs were seeded in 12-well plates (Falcon®; BD Biosciences) at 75% confluency (~375,000 cells in 480 μL per well). The cells were incubated for 1 hour at 37°C and 5% CO_2 and allowed to settle and attach. Compounds of interest were diluted in RPMI at 50 fold the final concentrations ranging from 1 nM to 25.6 μM and added in 10 μL aliquots. Positive controls were generated by replacing the compound aliquot with just 10 μL of RPMI. The cells were then incubated for 30 min at 37°C and 10% CO_2. Cells were then stimulated with 10 μL aliquots of BMP7 and TGF-β1 ligands at a final concentration of 20 ng/mL and 5 ng/mL respectively. Negative controls were generated by replacing both the compound and ligand aliquots with just 20 μL of

RPMI. The phosphorylation state of downstream effector proteins (pSMAD1/5/8 and pSMAD2 for BMP and TGF- β respectively) was measured by western blotting performed 30 minutes after ligand stimulation. Western blots were analyzed using ImageJ with positive controls at 100% ligand induced phospho-SMAD and negative controls being subtracted as background. GraphPad Prism® software was used for graphing and regression analysis by sigmoidal dose-response with variable Hill coefficient. Results for LDN-193189 and LDN-212854 are shown in figures 5a, 5b and 5c.

Example 5: BMP4/6 induced ALP Activity

C2C12 myofibroblasts cells were seeded in clear tissue culture treated 96-well plates (Costar® 3596;Corning) at 2,000 cells in 40 μL per well in DMEM supplemented with 2% FBS. Compounds diluted in DMEM at 5-fold the final concentrations ranging from 1nM to 10 μM were added in 10 μL aliquots in quadruplicate. Positive controls were generated by replacing the compound aliquot with just 10 μL of DMEM. BMP4 and BMP6 ligands diluted in DMEM at 5-fold the final concentration of 20 ng/mL were added in 10 μL aliquots. Negative controls were generated by replacing both the compound and ligand aliquots with just 20 μL of DMEM. Cells were incubated for 6 days at 37°C and 5% CO_2 and subsequently harvested in 50 μL of 1% Triton X-100. A 20 μL extract from each well was incubated at RT for 30 minutes with 100 μL of alkaline phosphatase (ALP) yellow (pNPP) liquid substrate for ELISA (Sigma-Aldrich), and ALP activity was measured by absorbance at 405 nM per the manufacturer's instructions. Absorbance data was analyzed with positive controls as 100% ALP activity and negative controls being subtracted as background. GraphPad Prism® software was used for graphing and regression analysis by sigmoidal dose-response with variable Hill coefficient. Results for LDN-193189 and LDN-212854 are shown in figures 6a and 6b.

Example 6: IL-6 Induced Hepcidin Expression:

HepG2 cells were seeded in a 12-well plate (Falcon®; BD Biosciences) at 75% confluency or approximately 100,00 cells per well in 985 μL of EMEM supplemented with 0.1% FBS and starved for 6 hours at 37°C and 5% CO_2. Cells

were pretreated for 30 minutes by adding compounds diluted in EMEM at 200-fold the final concentrations ranging from 1 nM to 125 nM in 5 μL aliquots in quadruplicate. Positive controls were generated by replacing the compound aliquot with just 5 μL of EMEM. Human recombinant Interleukin-6 (IL-6) (R&D Systems) was then added at a final concentration of 100 ng/mL in 10 μL aliquots. After 90 minutes, the media was removed, and each well washed twice with PBS. Both RNA isolation using TRIzol® (Life Technologies) and cDNA synthesis using M-MLV-reverse transcriptase (Promega) and the Mastercyler® ep gradient S (Eppendorf) were conducted per the manufacturer's instructions. The expression of hepcidin transcripts was measured using SYBR® FAST real-time qPCR kit (Kapa Biosystems), human primers (Forward 5'-CTGACCAGTGGCTCTGTTTTC-3', Reverse 5'-GAAGTGGGTGTCTCGCCTC-3') and Mastercyler® ep gradient S realplex^2 (Eppendorf) per the manufacturer's instructions. The relative expression of hepcidin was normalized to 18S human RNA (Forward 5'-GCTGGAATTACCGCGGCT-3', Reverse 5'- CGGCTACCACATCCAAGGAA -3') with negative controls as baseline expression and positive controls as maximal expression. Excel® (Microsoft) software was used for data analysis and graphing. Results for LDN-193189 and LDN-212854 are shown in Figure 7a.

Example 7: Q207D caALK2 Mouse Model of FOP

Heterotopic ossification was induced in mice containing a single allele of the gene encoding a conditionally-expressed constitutively-active ALK2 (ALK2^{Q207D} or caALK2) by postnatal (P7) retropopliteal injection of Ad. Cre ($1x10^8$ plaque-forming units) as previously described (Yu, Nat Med. 2008; Fukuda, Genesis 2006). Mice (n=6 per group) were treated for 4 weeks with both LDN-193189 and LDN-212854 at 6 mg/kg or vehicle control twice daily (BID) with weights measured daily. Impaired mobility, which correlates to the degree of bone formation, was quantified daily by passive range of motion analysis by dorsiflextion of the left ankle joint. A score was given based on the dorsiflex angle of 0 (normal flexion, 0° – 20°), 1 (mildly impaired, 20° – 90°), 2 (moderately impaired, 90° – 135°), 3 (severely impaired, >135°). Mice were sacrificed, imaged by X-ray (Carestream), and soft tissues fixed and stained by the Alizarin red and Alcian blue method as previously

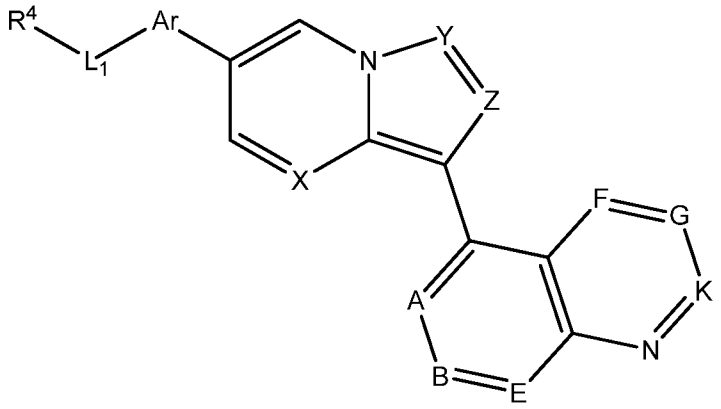
described (Komori, T. et al.). Results for LDN-212854 are shown in figures 8a and 8b.

All publications and patents cited herein are hereby incorporated by reference in their entirety.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A compound having a structure of Formula I or a pharmaceutically acceptable salt, ester, or prodrug thereof;



Formula I

wherein

X and Y are independently selected from CR^{15} and N;

Z is selected from CR^3 and N;

Ar is selected from substituted or unsubstituted aryl and heteroaryl;

L_1 is absent or selected from substituted or unsubstituted alkyl and heteroalkyl; and

A, B, E, F, G and K, independently for each occurrence, are selected from CR^{16} and N;

provided that no more than two of A, B, E, F, G and K are N;

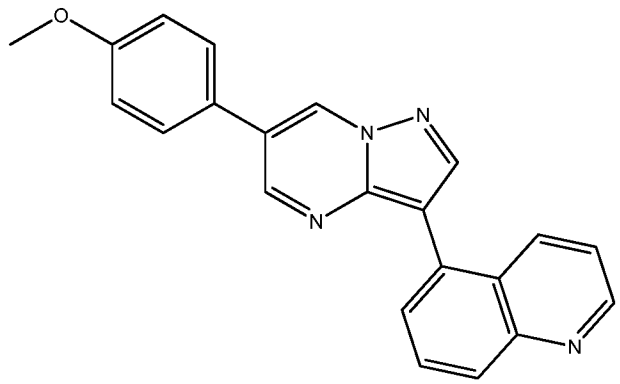
R^3 is selected from H and substituted or unsubstituted alkyl, cycloalkyl, halogen, acylamino, carbamate, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^4 is selected from H and substituted or unsubstituted alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^{15}, independently for each occurrence, is selected from H and substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acylamino, carbamate, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^{16}, independently for each occurrence, is absent or is selected from H and substituted or unsubstituted alkyl, alkenyl, alkynyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, halogen, acyl, carboxyl, ester, hydroxyl, alkoxyl, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido,

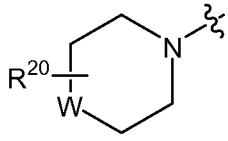
with the proviso that the following compound is excluded:



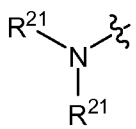
2. The compound of claim 1, wherein A, B, E, F, G and K are each CR^{16}, preferably CH.

3. The compound of claim 1 or 2, wherein R^4 is selected from H and substituted or unsubstituted cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, amino, acylamino, carbamate, amido, amidino, or sulfonamide.

4. The compound of any preceding claim, wherein R^4 is selected from



and



, wherein

W is absent or is $C(R^{21})_2$, O, or NR^{21};

R^{20} is absent or is selected from substituted or unsubstituted alkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, acyl, sulfonyl, sulfoxido, sulfamoyl, and sulfonamido; and

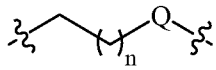
R^{21}, independently for each occurrence, is selected from H and substituted or unsubstituted alkyl, aralkyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, heteroaralkyl, cycloalkylalkyl, heterocyclylalkyl, acyl, sulfonyl, sulfamoyl, or sulfonamido.

5. The compound of any preceding claim, wherein Ar is a 6-membered aryl or heteroaryl ring.

6. The compound of any preceding claim, wherein L_1 is disposed on the para-position of Ar relative to the bicyclic core.

7. The compound of any preceding claim, wherein L_1 is not absent.

8. The compound of any preceding claim, wherein L_1 has a structure



wherein

Q is selected from $CR^{10}R^{11}$, NR^{12}, O, S, S(O), and SO_2; and

R^{10} and R^{11}, independently for each occurrence, are selected from H and substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, cycloalkylalkyl, heterocyclylalkyl, amino, acylamino, carbamate, amido, amidino, cyano, sulfonyl, sulfoxido, sulfamoyl, or sulfonamido;

R^{12} selected from H and substituted or unsubstituted alkyl, cycloalkyl, heterocyclyl, heterocyclylalkyl, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfamoyl, or sulfonamido and

n is an integer from 0-4.

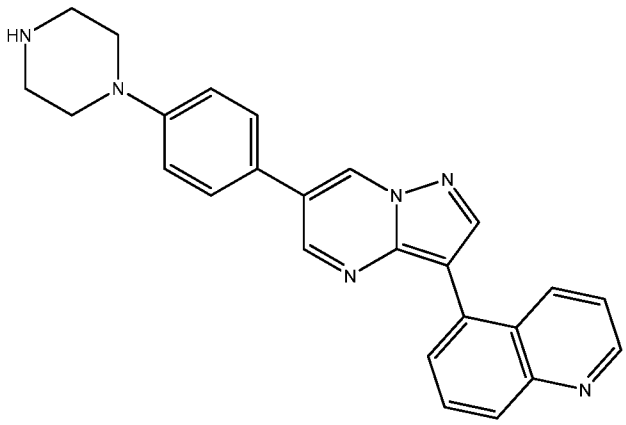
9. The compound of any one of claims 1-6, wherein, when L_1 is absent,

R^4 is selected from H and substituted or unsubstituted alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, heteroaryl, acyl, carboxyl, ester, alkylthio, acyloxy, amino, acylamino, carbamate, amido, amidino, sulfonyl, sulfoxido, sulfamoyl, or sulfonamide.

10. The compound of any one of claims 1-6, wherein, when L_1 is absent,

R^4 is selected from H and substituted or unsubstituted cycloalkyl, heterocyclyl, aryl, heteroaryl, amino, acylamino, carbamate, amido or amidino.

11. The compound of claim 1 having the structure:



or a pharmaceutically acceptable salt thereof.

12. A pharmaceutical composition comprising a compound of any preceding claim and a pharmaceutically acceptable excipient or solvent.

13. A method of reducing circulating levels of ApoB-100 or LDL in a subject, comprising administering an effective amount of a compound of any one of claims 1-11.

14. A method of treating hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia in a subject, comprising administering an effective amount of a compound of any one of claims 1-11.

15. The method of claim 14, wherein the hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia is congenital hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia.

16. The method of claim 15, wherein the hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia is autosomal dominant hypercholesterolemia (ADH), familial hypercholesterolemia (FH), polygenic hypercholesterolemia, familial combined hyperlipidemia (FCHL), hyperapobetalipoproteinemia, or small dense LDL syndrome (LDL phenotype B).

17. The method of claim 14, wherein the hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia is acquired hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia.

18. The method of claim 17, wherein the hypercholesterolemia, hyperlipidemia, or hyperlipoproteinemia is associated with diabetes mellitus, hyperlipidemic diet and/or sedentary lifestyle, obesity, metabolic syndrome, intrinsic or secondary liver disease, primary biliary cirrhosis or other bile stasis disorders, alcoholism, pancreatitis, nephrotic syndrome, endstage renal disease, hypothyroidism, iatrogenesis due to administration of thiazides, beta-blockers, retinoids, highly active antiretroviral agents, estrogen, progestins, or glucocorticoids.

19. A method of treating diseases, disorders, or syndromes associated with defects in lipid absorption or metabolism or caused by hyperlipidemia in a subject, comprising administering an effective amount of a compound of any one of claims 1-11.

20. A method of reducing secondary cardiovascular events arising from coronary, cerebral, or peripheral vascular disease in a subject, comprising administering an effective amount of a compound of any one of claims 1-11.

21. A method of preventing cardiovascular disease in a subject with elevated markers of cardiovascular risk, comprising administering an effective amount of a compound of any one of claims 1-11.

22. A method of inhibiting BMP-induced phosphorylation of SMAD1/5/8, comprising contacting the cell with a compound of any one of claims 1-11.

23. The method of claim 22, wherein the method treats or prevents a disease or condition in a subject that would benefit by inhibition of Bone Morphogenetic Protein (BMP) signaling.

24. The method of claim 23, wherein the disease or condition is selected from pulmonary hypertension, hereditary hemorrhagic telangectasia syndrome, cardiac valvular malformations, cardiac structural malformations, fibrodysplasia ossificans progressiva, juvenile familial polyposis syndrome, parathyroid disease, cancer, anemia, vascular calcification, atherosclerosis, valve calcification, renal osteodystrophy, inflammatory disorders, and infections with viruses, bacteria, fungi, tuberculosis, and parasites.

25. The method of claim 24, wherein the cancer is selected from breast carcinoma, prostate carcinoma, renal cell carcinoma, bone metastasis, lung metastasis, osteosarcoma, and multiple myeloma.

26. The method of claim 24, wherein the inflammatory disorder is ankylosing spondylitis.

27. A method of inducing expansion or differentiation of a cell, comprising contacting the cell with a compound of any of claims 1-11.

28. The method of claim 27, wherein the cell is selected from an embryonic stem cell and an adult stem cell.

29. The method of claim 27 or 28, wherein the cell is *in vitro*.

30. A method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK5.

31. The method of claim 30, wherein the small molecule inhibits the activity of human ALK2 with an IC_{50} that is lower by a factor of 1000 than its IC_{50} for inhibiting the activity of human ALK5.

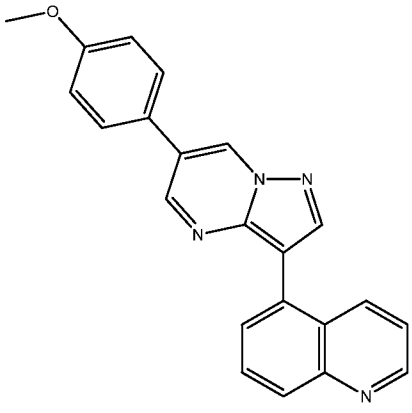
32. A method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK1.

33. A method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK3.

34. A method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK4.

35. A method of inhibiting the activity of ALK2 in a human, comprising administering to the human a small molecule that selectively inhibits the activity of human ALK2 relative to the activity of human ALK6.

36. The method of any of claims 30-35, wherein the small molecule is not



or a pharmaceutically acceptable salt thereof.

a

Compound	IC_{50} (nM) ALK1	ALK2	ALK3	ALK4	ALK5
Dorsomorphin	19.5	9.76	222	3,080	7,829
LDN-193189	1.48	0.67	14.3	108	117
LDN-212854	2.40	1.30	85.8	2,133	9,276
DMH1	77.9	12.62	241	11,023	5,971
K02288a	3.65	1.20	25.8	232	236

b

Compound	Fold Selectivity over ALK2 ALK1	ALK2	ALK3	ALK4	ALK5
Dorsomorphin	2	1	23	316	802
LDN-193189	2	1	21	161	175
LDN-212854	2	1	66	1,641	7,135
DMH1	6	1	19	873	473
K02288a	3	1	22	193	197

c

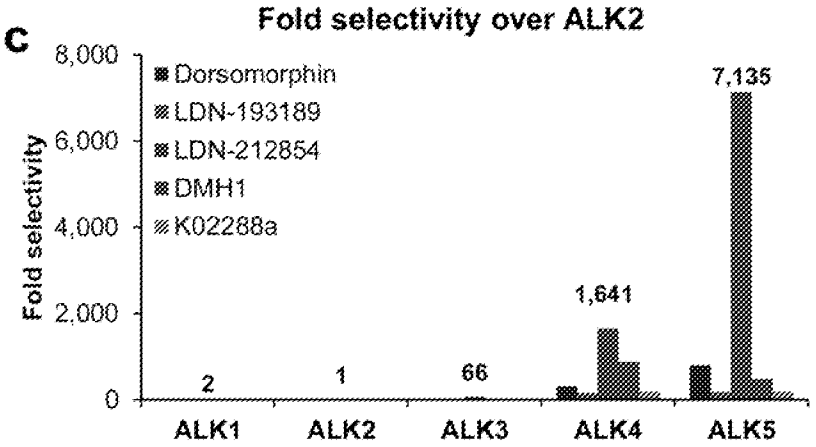


FIGURE 1

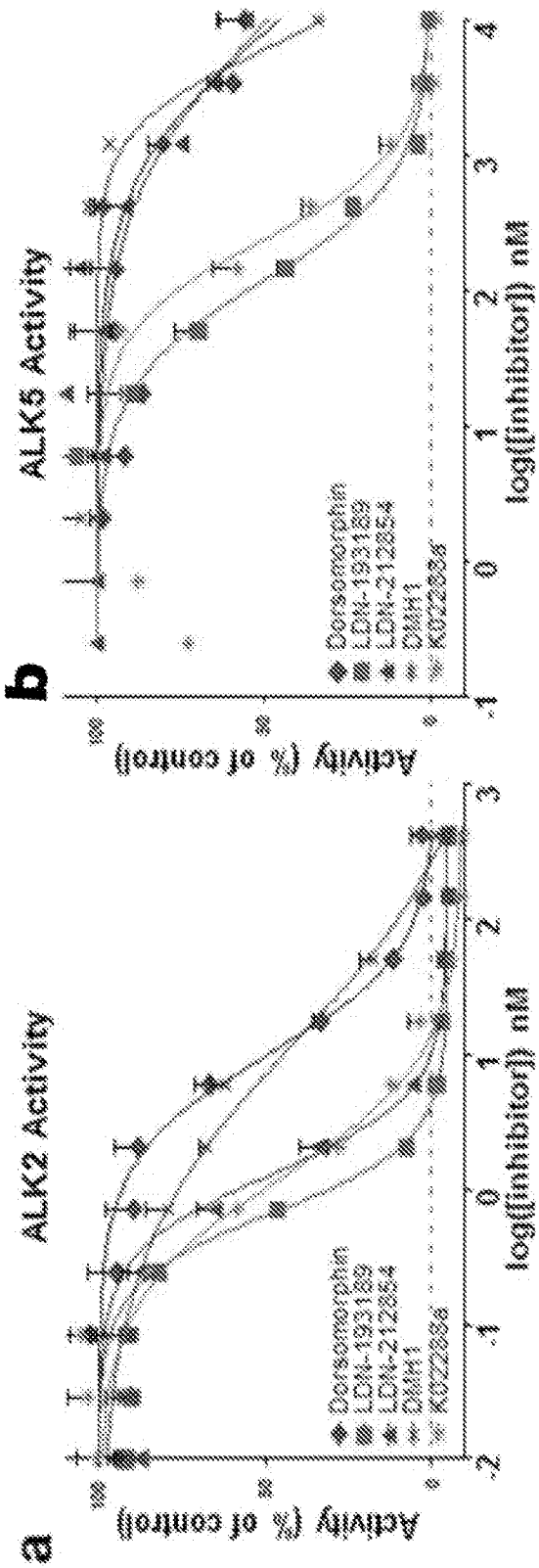

a
ALK2 Activity
Activity (% of control)
log([inhibitor]) nM
Dorsomorphin
LDN-193189
LDN-212854
DMH1
K02288
b
ALK5 Activity
Activity (% of control)
log([inhibitor]) nM
Dorsomorphin
LDN-193189
LDN-212854
DMH1
K02288

FIGURE 2

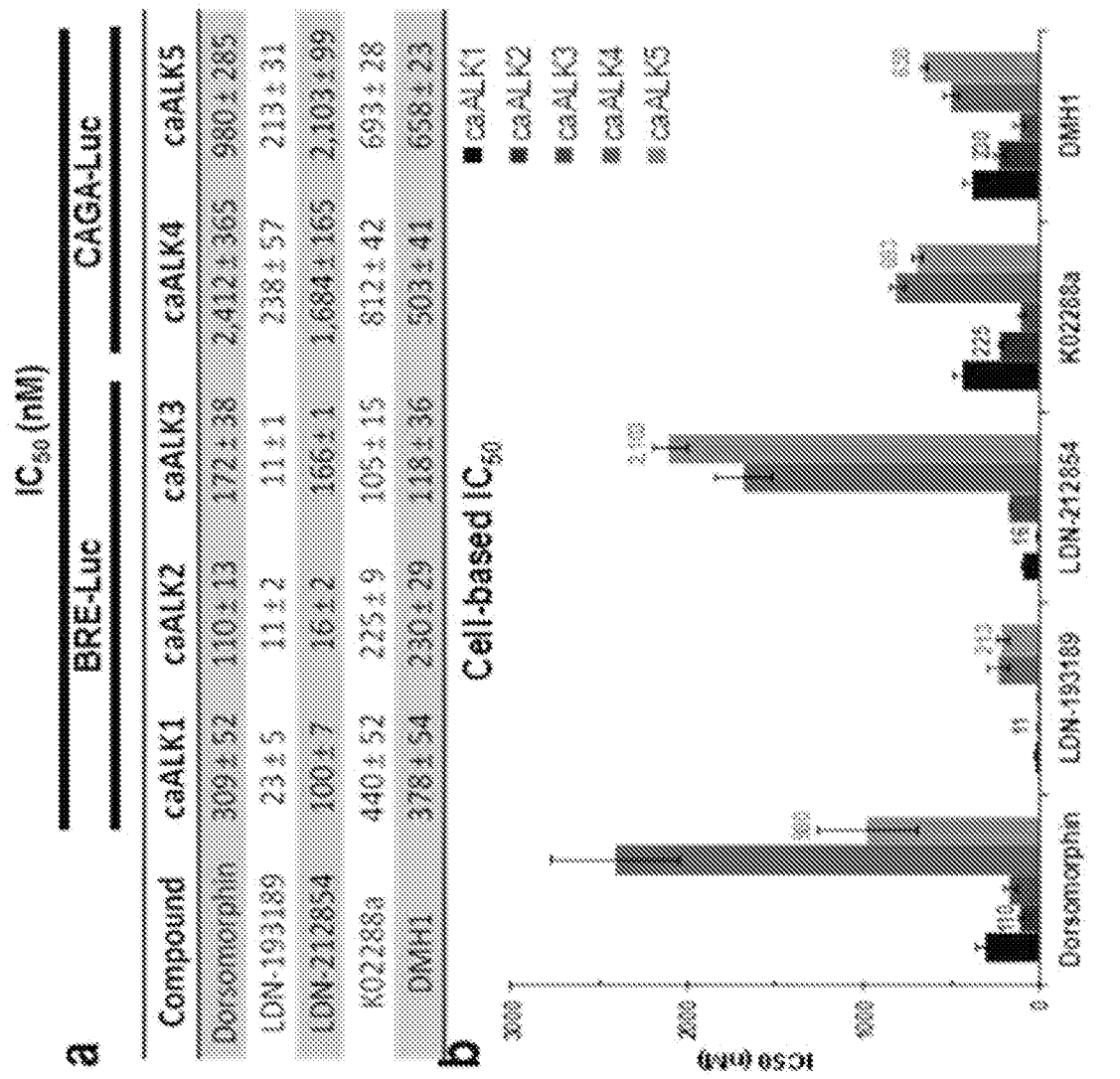

a
IC50 (nM)
BRE-Luc
CAGA-Luc
Compound caALK1 caALK2 caALK3 caALK4 caALK5
Dorsomorphin 309 ± 52 110 ± 13 172 ± 38 2,412 ± 369 980 ± 285
LDN-193189 23 ± 5 11 ± 2 11 ± 1 238 ± 57 213 ± 31
LDN-212854 100 ± 7 16 ± 2 166 ± 1 1,684 ± 169 2,103 ± 99
K02288a 440 ± 52 225 ± 9 105 ± 15 812 ± 42 693 ± 28
DMH1 378 ± 54 230 ± 29 116 ± 36 503 ± 41 658 ± 23
b
Cell-based IC50
caALK1
caALK2
caALK3
caALK4
caALK5
IC50 (nM)
3000
2000
1000
0
Dorsomorphin
LDN-193189
LDN-212854
K02288a
DMH1

FIGURE 3

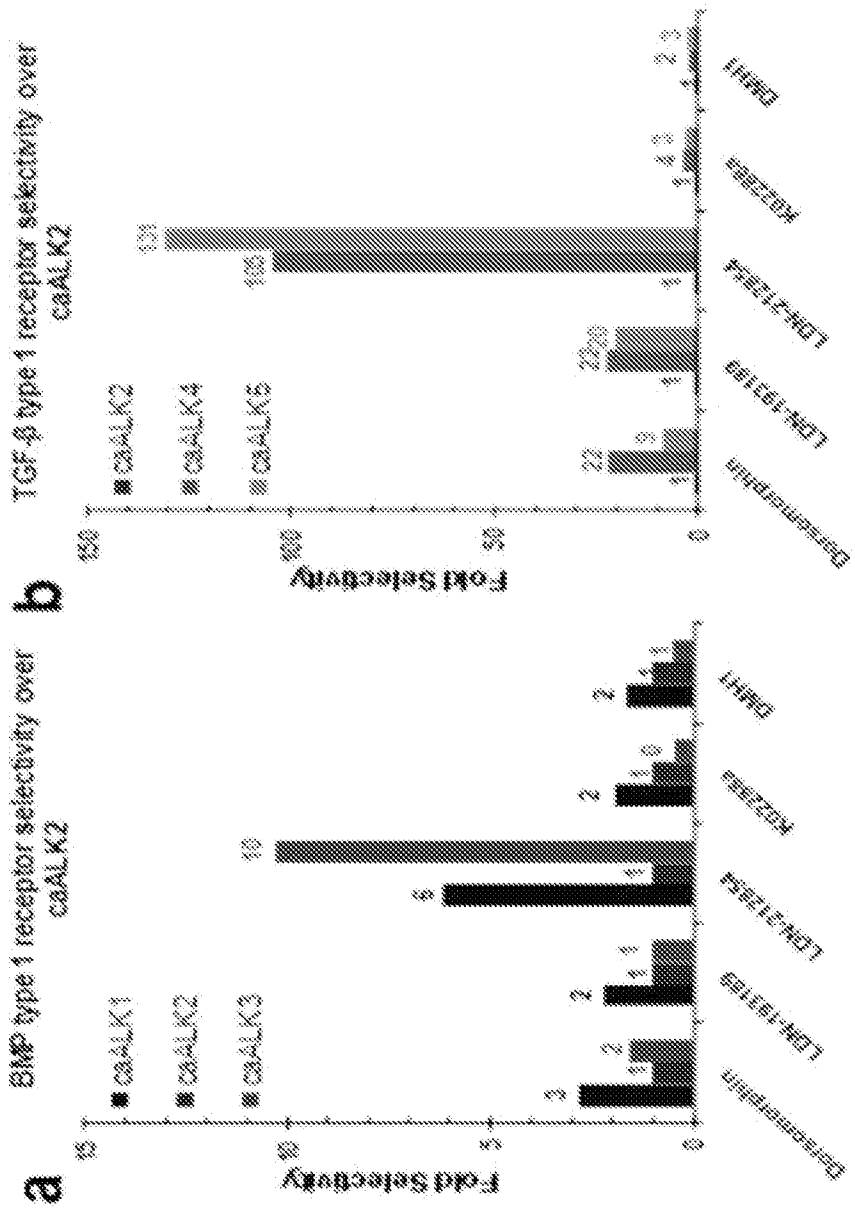

a
BMP type 1 receptor selectivity over caALK2
caALK1
caALK2
caALK3
Fold Selectivity
15
10
5
0
3
2
2
1
1
9
10
2
1
0
2
1
1
Dorsomorphin
LDN-193189
LDN-212854
K02288
DMH1
b
TGF-β type 1 receptor selectivity over caALK2
caALK2
caALK4
caALK5
Fold Selectivity
150
100
50
0
22
3
1
2000
1
100
1
1
1
2
2
Dorsomorphin
LDN-193189
LDN-212854
K02288
DMH1

FIGURE 4

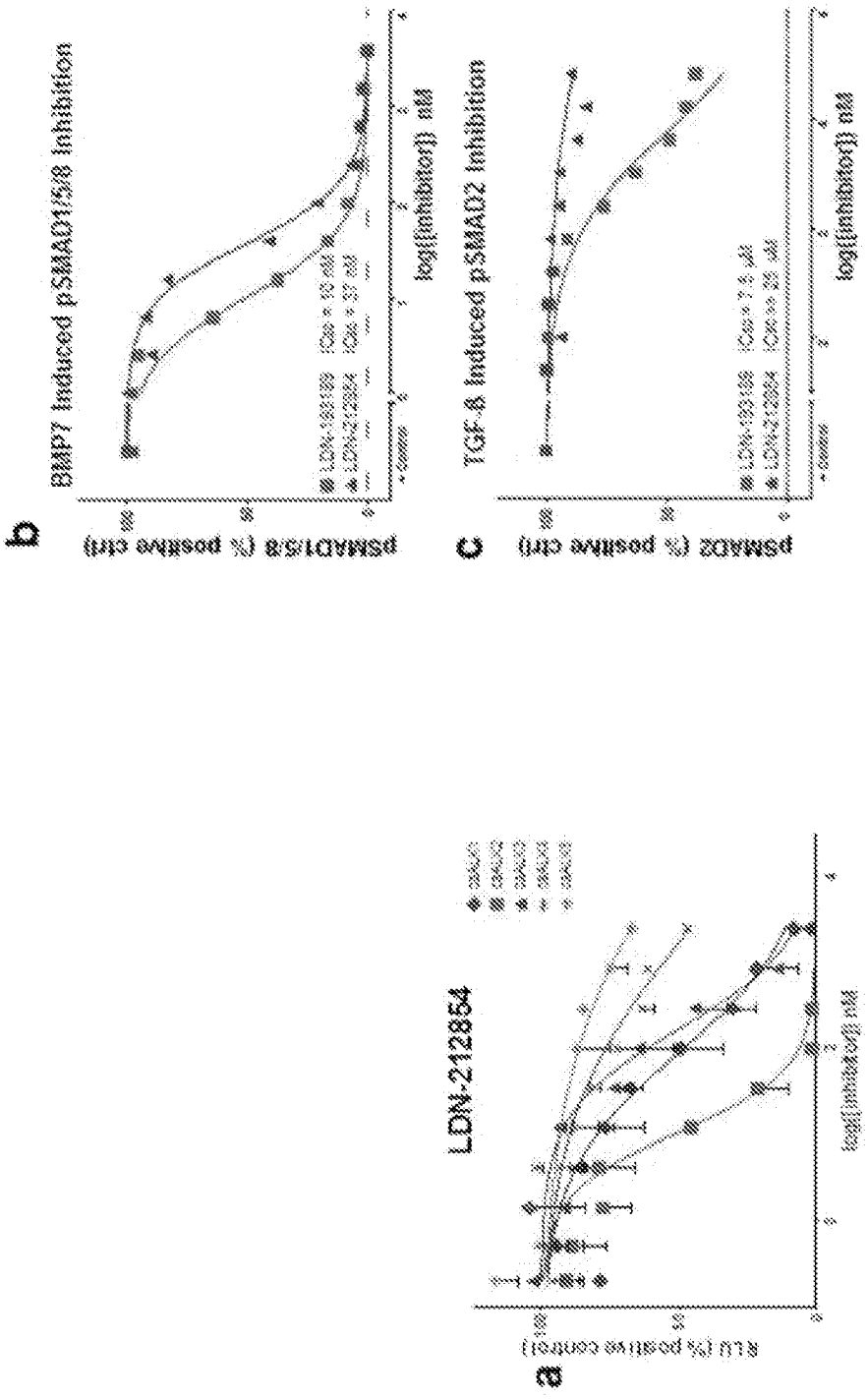

a
LDN-212854
BLU (% positive control)
log[inhibitor]} nM
b
BMP7 Induced pSMAD1/5/8 Inhibition
pSMAD1/5/8 (% positive ctrl)
log[inhibitor]} nM
c
TGF-ß Induced pSMAD2 Inhibition
pSMAD2 (% positive ctrl)
log[inhibitor]} nM

FIGURE 5

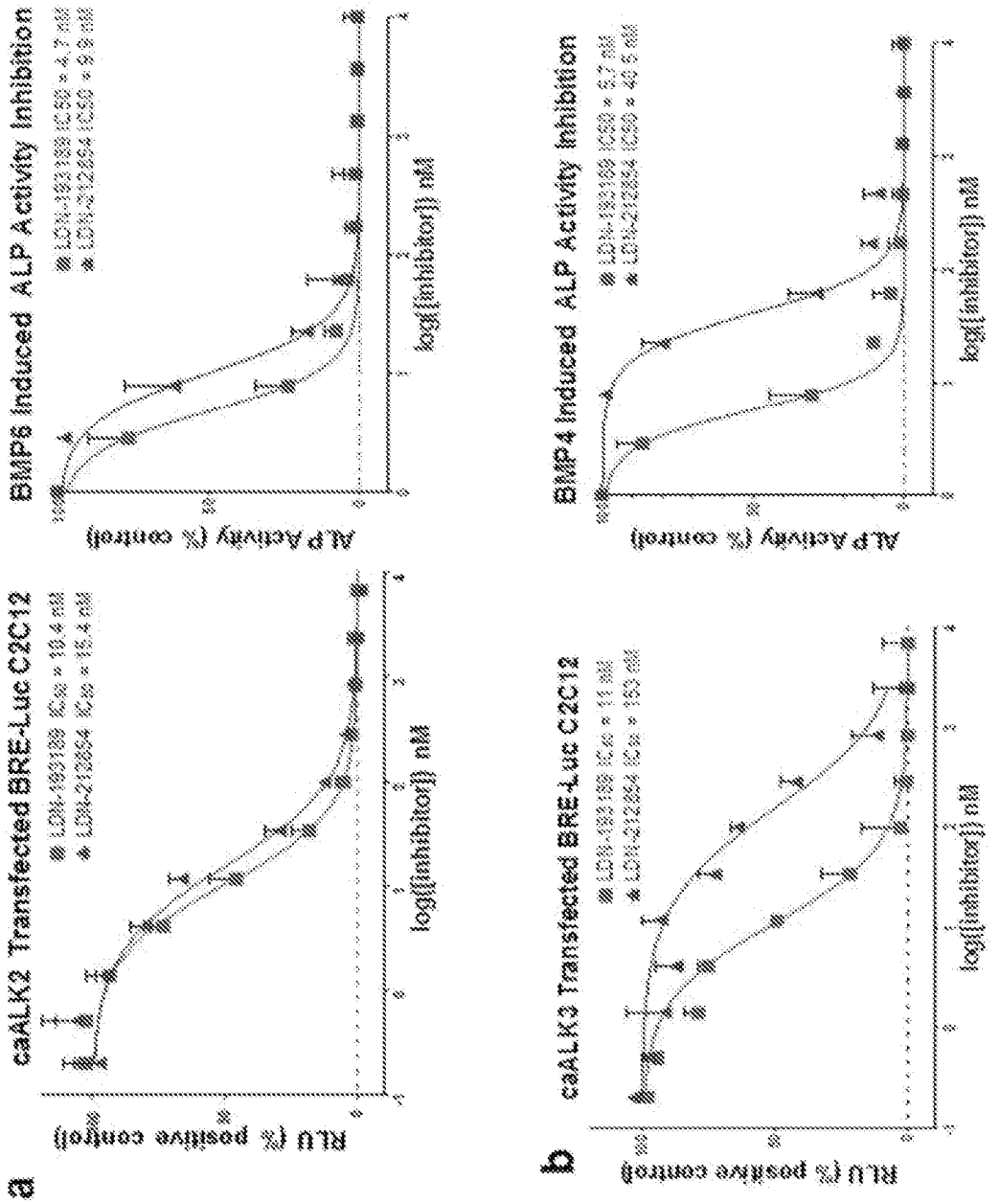

a
caALK2 Transfected BRE-Luc C2C12
LDN-193189 IC50 = 10.4 nM
LDN-212854 IC50 = 16.4 nM
RLU (% positive control)
log([inhibitor]) nM
BMP6 Induced ALP Activity Inhibition
LDN-193189 IC50 = 4.7 nM
LDN-212854 IC50 = 9.9 nM
ALP Activity (% control)
log([inhibitor]) nM
b
caALK3 Transfected BRE-Luc C2C12
LDN-193189 IC50 = 11 nM
LDN-212854 IC50 = 183 nM
RLU (% positive control)
log([inhibitor]) nM
BMP4 Induced ALP Activity Inhibition
LDN-193189 IC50 = 5.7 nM
LDN-212854 IC50 = 49.5 nM
ALP Activity (% control)
log([inhibitor]) nM

FIGURE 6

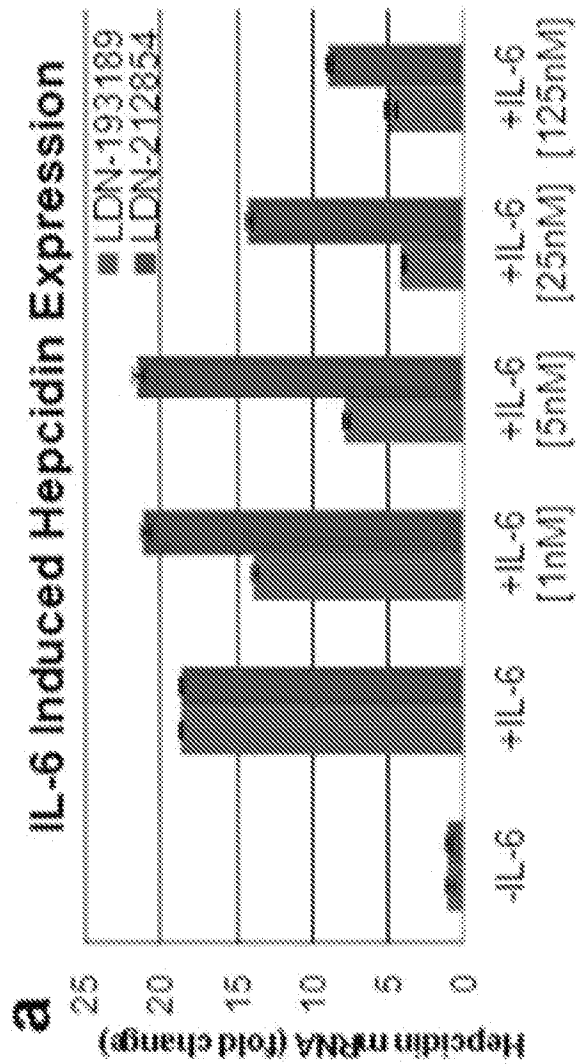

a
IL-6 Induced Hepcidin Expression
LDN-193189
LDN-212854
Hepcidin mRNA (fold change)
25
20
15
10
5
0
-IL-6
+IL-6
+IL-6 [1nM]
+IL-6 [5nM]
+IL-6 [25nM]
+IL-6 [125nM]

FIGURE 7

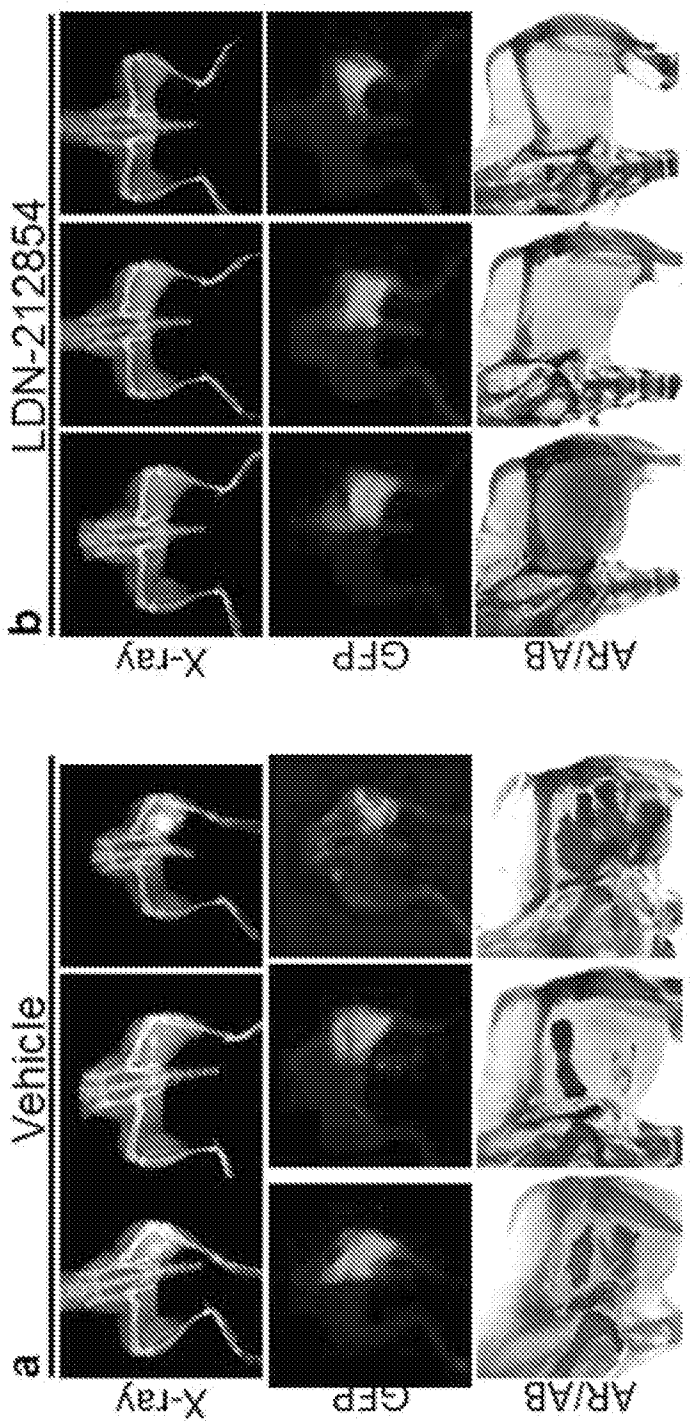


FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/020360

A. CLASSIFICATION OF SUBJECT MATTER

(see extra sheet)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D 487/04, A61K 31/519, A61P 3/00, 3/04, 3/06, 9/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, PatSearch (RUPTO internal), RUPAT, EAPATIS, Espacenet, PAJ, USPTO, CIPO, DEPATISnet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CUNY, Gregory D. et al. Structure-activity relationship study of bone morphogenetic protein (BMP) signaling inhibitors, Bioorganic & Medicinal Chemistry Letters, 2008, 18, pp. 4388-4392, especially, p. 4391, Table 2, compound 50	1-36
X	WO 2009/114180 A1 (THE GENERAL HOSPITAL CORPORATION et al.) 17.09.2009, p. 31, first compound, p. 87, example 9, p. 88, Table 1, compound 3 from the bottom	1-36
A	WO 2008/033408 A2 (THE GENERAL HOSPITAL CORPORATION et al.) 20.03.2008, claims 8-10, fig. 17	1-36
P,X	MOHEDAS, Agustin H. et al. Development of an ALK2-Biased BMP Type I Receptor Kinase Inhibitor. ACS Chemical Biology, 2013, 8, pp. 1291-1302, abstract, fig. 2-8, compound LDN-212854	1-36
P,X	ENGERS, Darren W. et al. Synthesis and structure-activity relationships of a novel and selective bone morphogenetic protein receptor (BMP) inhibitor derived from the pyrazolo [1.5-a]pyrimidine scaffold of Dorsomorphin: The discovery of ML347 as an ALK2 versus ALK3 selective MLPCN probe. Bioorganic & Medicinal Chemistry Letters, 2013, pp. 3248-3252, p. 3250, Table 2, p. 3251, Table 3, compound 13m	1-36

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 May 2014 (27.05.2014)	21 August 2014 (21.08.2014)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer O. Zavarzina Telephone No. 8(495)531-64-81

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT
Classification of subject matter

International application No.
PCT/US 2014/020360

C07D 487/04 *(2006.01)*
A61K 31/519 *(2006.01)*
A61P 3/00 *(2006.01)*
A61P 3/04 *(2006.01)*
A61P 3/06 *(2006.01)*
A61P 9/00 *(2006.01)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2014/020360

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-10, 12-36 (all partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 1 refers to an extreme large number of possible compounds due to the huge breadth of radical definitions and a meaningful search over the whole breadth of the claims is impossible. Moreover support and disclosure in the sense of Articles 5 and 6 is to be found for a very small proportion of the compounds claimed, namely, only two compounds that fall within the formula (I) are disclosed in the description (see pp. 11, 12, structural formulae; p. 60, scheme 1; p. 61, compound LDN-212854). Consequently, the search for claims 1-36 have been restricted for compounds of the formula (I), wherein X and Y are N; Z is CR^3; Ar is a substituted or unsubstituted phenyl; A, B, E, F, G and K are CR^{16}.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.