

ANTI-FIBROTIC COMBINATION THERAPEUTICS

Field of the Invention

The present invention relates to combination therapies for use in methods of treatment. The treatments can have reparative effects on a range of progressive and/or degenerative diseases associated with fibrosis. In particular instances, the combination therapies can be used for the treatment of interstitial lung disease including, for example, idiopathic pulmonary fibrosis. In particular instances, the combination therapies comprise an anti-fibrotic compound, for example nintedanib or pirfenidone, and a therapeutic agent which can act by binding to and modulating mitochondrial complex I. The therapeutic agent which can act by binding mitochondrial complex I are referred to herein as mitochondrial complex I modulator compounds (or MCIM compounds).

Background

Mitochondrial Complex I (also known as NADH:ubiquinone oxidoreductase, Type I NADH dehydrogenase, respiratory Complex I, or simply 'Complex I') is the first enzyme complex of the respiratory chain (Yoga *et al*, 2021, which is hereby incorporated by reference in its entirety). Complex I is a very large protein complex comprising 45 subunits (Gutiérrez-Fernández, 2020) which is highly conserved in eukaryotes and prokaryotes. In eukaryotes, it couples the transfer of electrons from NADH to the coenzyme ubiquinone (Q) with the translocation of protons across the inner mitochondrial membrane, although the mechanism linking the spatially distinct proton translocation and electron transfer remains unknown (Gutiérrez-Fernández, 2020).

In humans, the most common form of ubiquinone is known as 'Q10' because it has ten isoprenyl subunits in its 'tail' region. Q10 is hydrophobic and it enters the Complex I enzyme from the mitochondrial inner membrane through a long binding channel (Bridges *et al*, 2020), which is often called the 'Q tunnel'. The Q-tunnel is long and heterogenous in nature. Various compounds are known to bind within the Q tunnel, but there is no single consensus site for compound binding. For example, piericidin A is reported to bind within the Q-tunnel as a 'short-form' ubiquinone, with interactions at multiple residues from the top of the Q-tunnel to its midway point (Gutiérrez-Fernández, 2020; Bridges *et al*, 2020; Chung *et al*, 2021, each of which is hereby incorporated by reference in its entirety). Aureothin and pyridaben, which are also quinone-like compounds, are also observed to bind at a similar site in *T. thermophilus* (Gutiérrez-Fernández, 2020; Chung *et al*, 2021). IACS-2858 and BAY-87-2243 act like a "cork in a bottle", binding a cluster of residues of subunits ND1 and NDUFS7 in the central charged region of the ubiquinone-binding pocket of mouse Complex I (Chung *et al*, 2021; Kurelac *et al*, 2022). Biguanides such as metformin are similarly thought to interact with Phe244 of ND1 and Arg77 of NDUFS7 in the Q-tunnel with a mode dependent on the

active or inactive state of the enzyme. Furthermore, evidence indicates that the biguanides may be non-selective in their binding, with a putative additional binding site on Complex IV, another subunit of the electron transport chain (LaMoia *et al*, 2021). The binding sites of several Complex I inhibitors are reviewed by Schiller and Zickermann (2022).

5 Classical Complex I inhibitors, such as those mentioned above, often cause cytotoxicity and cell death. For instance, piericidin A is insecticidal and antibacterial, pyridaben is an acaricide (killing ticks and mites) and aureothin exhibits antitumor, antifungal and insecticidal activity. IACS-010579 and IM156 (also known as HL156A) have been reported to possess anti-tumour effects due to profound impacts on cancer cell viability (Tsogbadrakh *et al*, 2018 and Izreig *et al*, 2020). However, despite
10 these properties, the known, classical, C1 inhibitors have not found use as approved therapeutics. Indeed, as well as showing mechanism-based toxicity (Yap, T *et al*, 2023), these agents have been used to elicit disease in mouse models of neurodegenerative conditions such as Parkinson disease (PD; Xiong *et al*, 2012, which is hereby incorporated by reference in its entirety) and Alzheimer's disease (AD; Joh *et al*, 2017, which is hereby incorporated by reference in its entirety). As such, for
15 classical complex I inhibitors, identifying a suitable approach for therapeutic use that provides a benefit without toxicity has proved to be a challenge. An alternative approach which might elicit a benefit without adverse effects may have potential benefits for the treatment of a variety of progressive diseases.

20 The goal of therapeutic tissue repair is to restore the tissue to its original state of structure and function (Krafts, 2010 and Paul and Sharma, 2021). Attaining this goal has proved elusive, with the only effective examples being organ transplantation or surgical implants using natural or biomimetic structures such as aortic valves or joint replacement). Further, whilst there has been a significant increase in research into strategies to achieve repair in different organ systems, these approaches
25 aim to remove the primary driver to tissue injury (e.g., the calcified heart valve) or to replace the dysfunctional matrix environment with an environment which favours homing of repair cells or local augmentation of soluble pro-repair factors (e.g., fibrin). Such examples include biomimetic scaffolds in orthopaedics, which simulate normal structural matrix for cell homing, and cellular products to simulate the soluble matrix microenvironment for cutaneous and ocular wound repair. As such, the
30 identification of a therapeutic agent(s) which both attenuates the drive to tissue injury and concurrently promotes a pro-repair microenvironment, has proved elusive.

Tissue repair (healing) is a highly orchestrated and complex process involving a sequence of overlapping events that are precisely timed. The process has three broad phases which were first delineated in the 19th Century (Virchow, 1859) and have been since augmented by the addition of
35 data on their cellular genotypes, phenotypes, and molecular mediators (Liehn, 2011, Takeo, 2015, and Somer *et al*, 2021). However, there are limited, if any, data available regarding the mechanisms

underpinning repair in most chronic disease settings (Peyrin-Biroulet, 2020) and current understanding of tissue repair processes has not led to substantial improvements in the clinical care of tissue damage (Eming *et al*, 2014).

As shown in Figure 1, the three phases of tissue repair are:

- 5 1. Inflammation
2. Proliferation (fibrogenesis and angiogenesis)
3. Tissue Remodelling
 (Lokmic *et al*, 2012.)

10 In the healthy, functional healing/repair process, these phases proceed in an overlapping sequence, with resolution of inflammation progressing alongside proliferation and matrix remodelling to restore tissue architecture. In conditions of chronic disease, this process is interrupted or dysregulated, resulting in persistent non-healing tissue damage, and which may lead to scarring (fibrosis) of the affected tissue.

15 Functional repair involves the resolution of the primary inflammatory response to injury and the simultaneous activation of the inflammatory response consequent upon repair. These phases show different qualitative and kinetic properties; the first phase induces a proliferative response in both infiltrating and resident cells whilst the second involves repolarisation of the infiltrating cells. Furthermore, the cells involved in the repair process show different responses to stress dependent on the progressing phase of repair. For example, immune cells of the myeloid lineage (short-lived cells
20 with high bioenergetic demand) respond to stress by reducing proliferation and activating apoptosis pathways such that the overall phenotype of the myeloid lineage is anti-inflammatory, and in the context of repair, a reduced population showing immunomodulatory effects. By contrast, mesenchymal and epithelial cells respond to similar microenvironmental stressors by activating effector pathways. For example, mesenchymal cells typically respond to low oxygen tension (hypoxia)
25 by the expansion and activation of a repair phenotype.

30 There is an urgent need for therapeutics which exert a dual pharmacology of reducing disease progression whilst supporting functional tissue repair (for example non-fibrotic tissue repair) in chronic progressive disease, medical conditions that worsen over time without medical intervention. In particular, there is an urgent need for therapeutics which inhibit progression of fibrosis and, ideally, repair fibrotic tissues. Prominent examples of progressive fibrotic diseases include interstitial lung disease including, including idiopathic pulmonary fibrosis, non-alcoholic fatty liver disease (NAFLD)/ non-alcoholic steatohepatitis (NASH) and chronic kidney disease. Progressive fibrotic diseases, and in particular pulmonary fibrotic diseases, may also be associated with autoimmune diseases such as rheumatoid arthritis (RA), psoriasis, lupus and inflammatory bowel disease (IBD). Without treatment
35 these conditions typically progress in severity over time and may, as in the case of pulmonary fibrosis, be fatal.

The current treatments for pulmonary fibrosis and related diseases inhibit the progression of the disease. However, these agents fail to promote non-fibrotic tissue repair by exerting a direct effector response on repair processes. Indeed, their suppression of the pro-inflammatory effector cell and molecular response proceeds cyclically, perhaps for months or years. This is exemplified in pathology of the distal lungs, where effective anti-inflammatory effects by a therapeutic do not result in effective, non-fibrotic repair. In fibrotic diseases of the distal lung, such as idiopathic pulmonary fibrosis, the suppression of inflammation does not halt the drive to fibroblastic expansion and emphysematous destruction, or result in the conservation of alveolar stem cells.

Furthermore, the kinetic interplay between the drive to injury and sequential activation of repair, often results in 'futile' repair, i.e., creation of maladapted restitution of tissue remodelling to a normal state. Such futile repair is seen in settings such as osteoarthritis and fibrosis.

The result of either failure to repair or futile repair is that most patients with chronic disease experience ongoing symptoms, and disease progression despite treatment. As such, there is a pressing need for therapeutics with a dual pharmacology that can act on both the inflammatory and repair arms of a lesion. Such a therapeutic would:

- display a pharmacology-dependent, differential transduction of microenvironmental stress signals.
- mitigate the drive to injury and simultaneously, augment and capitalise upon the stressor events that constitute the inflammatory to proliferative phase of tissue repair.
- orchestrate repair, in an anatomically appropriate manner *to restore key aspects of tissue function* (Eming *et al*, 2014).

In addition, the pharmacological intervention might elicit cellular changes consistent with those required to orchestrate a controlled repair response. For example, the approach might elicit production of the key basement membrane collagen IV in a controlled manner, alongside production of growth factors important for angiogenesis, epithelialisation and matrix remodelling, such as VEGF, FGF21 and GDF15. Were this approach to be successful it would elicit repair in a pathology agnostic manner, i.e. the repair response would be seen in multiple settings regardless of the nature of the original injury. In addition, it might alter the activation response of resident cells and de-sensitize the microenvironment to the effects of a pro-inflammatory cell infiltrate, resulting in a 'permission' to repair.

One proposed approach to eliciting a tissue repair response has been to repair the cell first (Fu, 2021). The induction of an integrated stress response (ISR) may be an effective means of achieving this goal. The ISR is a cytoprotective mechanism that maintains cellular proteostasis (i.e., protein homeostasis) in response to stress conditions. The ISR is highly conserved across cell types, and is

triggered in response to changes in mitochondrial function (Savu and Moiso, 2022). Activation of a controlled ISR has been shown to have beneficial effects in multiple disease settings. For example, in mouse models of multiple sclerosis the ISR can be harnessed to protect oligodendrocytes and myelin during inflammation (Way and Popko, 2016). In addition, the ISR has been shown to regulate the health of cardiac progenitor cells by removing unhealthy cells to prevent their differentiation and self-renewal (Searfoss *et al*, 2019), a property that may be shared across progenitor cells in other settings such as oligodendrocyte progenitors in the brain and spinal cord, alveolar type II epithelial cells (AT2) in the lung and mesenchymal stem cells and bone marrow progenitor cells. Roles for the ISR in obesity, neurodegeneration and heart failure have also been proposed. Importantly, to achieve these outcomes the ISR should be moderate and tightly regulated, like a rheostat, in order to avoid pushing cells towards apoptosis (Kaspar *et al*, 2021).

An agent which could both control symptoms and elicit tissue repair/healing may have greater benefits for patients than existing therapy and lead to improved treatment outcomes.

Effective treatments for fibrosis are urgently needed. To date, only two treatments, nintedanib and pirfenidone, are approved for use in treating idiopathic pulmonary fibrosis (IPF). Nintedanib is estimated to increase the median survival of patients from between 3.3-7 years to between 8.5-9.1 years, compared to patients not receiving treatment (Lancaster *et al* 2019). Likewise, pirfenidone also increases median survival of patients, with an increase in the 5-year survival rate of patients rising from approximately 31% in untreated patients to 56% in patients receiving pirfenidone (Zurkova *et al* 2019). While both approved treatments slow the progression of the IPF they are unable to completely arrest progression or reverse or repair the damage caused to the lungs. Thus, improved treatment options are urgently needed to achieve these goals and further increase the long-term survival of patients suffering from pulmonary fibrosis.

Nintedanib and pirfenidone are, to date, the only two approved drugs for the treatment of idiopathic pulmonary fibrosis. Nintedanib is a small molecule, competitive, triple angiokinase inhibitor which inhibits multiple receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (nRTKs). Nintedanib is described as a triple angiokinase inhibitor due to its inhibitory effects on platelet-derived growth factor receptors (PDGFR) α and β , fibroblast growth factor receptors (FGFR) 1-3, and vascular endothelial growth factor receptor (VEGFR) 1-3. However, nintedanib is also known to inhibit the RTKs colony stimulating factor 1 receptor (CSF1R), and Fms-like tyrosine kinase 3 (FLT3). Each of the aforementioned RTKs, with the exception of FLT3, has been implicated in pathogenesis of interstitial lung disease (ILD). Nintedanib binds to the ATP binding pocket of each target RTK and inhibits intracellular signalling cascades which have been implicated in pathogenesis of ILD. In addition, nintedanib inhibits the nRTKs Lck, Lyn and Src. While the role of Lck and Lyn are currently

unknown in the pathogenesis of ILD, inhibition of the Src pathway has been shown to reduce lung fibrosis.

The mechanism of effect of pirfenidone in inhibiting ILD progression is not well established. However, there are suggestions that pirfenidone is able to slow progression of ILD through regulation of the tumor necrosis factor (TNF) pathway and via its antioxidant effects (Kim et al 2015). In addition, some evidence suggests that pirfenidone attenuates production of the key profibrotic and pro-inflammatory cytokine, transforming growth factor β 1 (TGF β 1), which has been implicated in idiopathic pulmonary fibrosis (IPF).

Phosphodiesterase 4 (PDE4) is being investigated as potential new therapeutic target for treatment of IPF. Nerandomilast (previously known as BI 1015550) has been shown in a phase 2, double-blind, placebo-controlled trial to prevent a decrease in lung function of patient with IPF, both alone and in patients with background use of either nintedanib or pirfenidone (Richeldi *et al.*, 2022). However, as with nintedanib and pirfenidone, nerandomilast fails to promote tissue repair and disease regression.

A phase 2 safety and efficacy study of tulisokibart is currently underway for treatment of Systemic Sclerosis Associated with Interstitial Lung Disease (SSc-ILD). Tulisokibart is an inhibitor of TL1A, a ligand of death-receptor 3. TL1A and death receptor 3 have both been shown to be critical for homeostasis in the intestinal epithelium, and for post-injury repair (Jia *et al.*, 2016; Nakamura *et al.*, 2024). As such, despite positive clinical results showing the effects of inhibiting TL1A on control of inflammation, treatment with tulisokibart or other TL1A inhibitors, such as RVT-2101 and TEV-48574, are strongly expected to fail to promote tissue repair and disease regression.

Despite nintedanib and pirfenidone inhibiting progression of the decline in forced vital capacity in patients with IPF, the unmet need remains high, with most patients continuing to experience disease progression and/or exacerbation despite treatment (Spagnolo et al, 2018). In addition, a heterogeneous response and high adverse-effect burden of both treatments, including the potential for elevations in liver enzymes (Maher and Strek, 2019), provides scope for new agents (Somogyi et al, 2019).

The inventors have previously discovered that compounds which can bind to and modulate the activity of mitochondrial complex I (see European patent application No. 23162131.9, which is incorporated herein by reference) are beneficial in the treatment of inflammatory and/or progressive diseases such as interstitial lung disease or pulmonary fibrosis, inflammatory bowel disease (IBD), multiple sclerosis (MS), or rheumatoid arthritis (RA). The inventors demonstrated that such compounds could slow down and even prevent disease progression in mice with these conditions. Even more surprisingly, however, was the observation that these compounds additionally promoted repair of affected tissues thereby reversing disease progression.

While this newly discovered class of compounds provides an exciting new avenue for treatment of inflammatory and/or progressive disease, there is still an ongoing need to improve the efficacy of these nascent drug treatment regimens to further improve patient quality of life.

The present invention has been devised in light of the above considerations.

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Summary of the Invention

Current treatments for chronic, progressive conditions inhibit disease progression but fail to promote tissue repair. Consequently, patients with chronic disease experience ongoing symptoms and progression, and poor quality of life. Despite the attractiveness of restoring normal tissue architecture as a means to treat chronic disease, pharmacological interventions to achieve this have not been studied, and as a result practical applications of this approach do not yet exist. One potential approach to achieve pharmacological tissue repair is through changes in mitochondrial function. However, the literature currently teaches that eliciting changes in mitochondrial function suppresses repair and promotes inflammation (Cai *et al*, 2022), and indeed, alterations in mitochondrial function to alter disease progression through control of inflammation, or tissue remodelling, have not been extensively studied. The mitochondrial Complex I modulator (MCIM) compounds, which are disclosed in EP23162131.9, modulate the activity of mitochondrial Complex I in a manner that differs from that of conventional complex I inhibitors. This elicits an adaptive phase which directs cell fate choices and mimics a wound repair-like microenvironment. Phenotypically, this can control inflammation, alter the activation response of resident cells and desensitizes the microenvironment to the effects of a pro-inflammatory cell infiltrate, and concurrently initiate repair signals in affected tissues, such as the lung and joint. In addition, the MCIM compounds can stimulate the production of key growth factors such as VEGF, and collagen I, and the basement collagen IV. Together, these mechanisms support a reduction in inflammation and restoration of tissue architecture in multiple organ/tissue settings. In addition to the effects of MCIM compounds described in EP23162131.9, the inventors have further found that MCIM compounds can be used in combination with anti-fibrotic compounds to further improve treatment of diseases and disorders associated with progressive fibrosis of tissues, for example in a model mouse model for idiopathic lung fibrosis. Such combinations lead to a surprising and synergistic improvement in the levels of tissue repair observed compared to when either an MCIM compound or anti-fibrotic compound is administered alone. For example, a synergistic improvement in the levels of tissue repair may be observed in ILD mice models when mice are treated with an MCIM compound and the anti-fibrotic compound nintedanib. It is envisaged that these combination treatments will also be useful in the treatment of diseases associated with progressive fibrosis of tissues, for example, progressive fibrosing interstitial lung disease (pf-ILD), rheumatoid arthritis associated ILD (RA-ILD), systemic sclerosis (SSc), sclerodermatous graft vs. host disease, nephrogenic systemic fibrosis, radiation-induced fibrosis, cardiac fibrosis, liver fibrosis (Non-Alcoholic

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Steatohepatitis (NASH) and/or cirrhosis), kidney fibrosis, chronic kidney disease, endometriosis, arthrofibrosis, covid-induced fibrosis, familial pulmonary fibrosis.

At its broadest, the disclosure provides a pharmaceutical combination comprising an MCIM compound and an anti-fibrotic compound. The combination may be for use in therapy. A

5 pharmaceutical combination may comprise a single pharmaceutical composition comprising an MCIM compound and an anti-fibrotic compound. Alternatively, the pharmaceutical combination may comprise a first pharmaceutical composition comprising an MCIM compound and a second pharmaceutical composition comprising an anti-fibrotic compound. Thus, the disclosure further provides a pharmaceutical composition comprising an MCIM compound and an antifibrotic compound.

10 The disclosure further provides a pharmaceutical combination comprising a MCIM compound and an anti-fibrotic compound for use in therapy. The MCIM compound and the anti-fibrotic compound may be administered separately, sequentially, or simultaneously, and may be administered in any order. The combination therapy can achieve reparative effects when used to treat inflammatory and/or progressive diseases associated with tissue fibrosis. Preferably, the MCIM compounds bind complex

15 I and are able to modulate complex I function.

Accordingly, in a first aspect, the disclosure provides a pharmaceutical combination comprising a MCIM compound and an anti-fibrotic compound. The pharmaceutical combination may be formulated as a single pharmaceutical composition comprising the MCIM compound and the anti-fibrotic compound. Alternatively, the pharmaceutical combination may be formulated as two or more

20 separate compositions, wherein each of the two or more compositions comprise either an MCIM compound or an antifibrotic compound. In some embodiments, the combination may comprise two or more anti-fibrotic compounds. Where the combination comprises two or more anti-fibrotic compounds, the pharmaceutical combination may comprise a single composition comprising the two or more anti-fibrotic compounds or the two or more anti-fibrotic compounds may be comprised in

25 separate compositions. In a preferred embodiment, the pharmaceutical combination comprises HMC-C-01-A and nintedanib, pirfenidone, nerandomilast, tulisokibart, or a combination thereof. The pharmaceutical composition may comprise pharmaceutically acceptable excipients, stabilisers, processing aids and/or excipients which enhance the solubility, dissolution rate, permeability, absorption performance, site specific delivery of one or more of the active compounds contained

30 therein.

In a second aspect, the disclosure provides a pharmaceutical combination according to the first aspect for use in a method of treatment.

In a third aspect, the disclosure provides a pharmaceutical composition comprising a mitochondrial complex I modulator (MCIM) compound for use in the treatment of fibrosis in an inflammatory and/or

35 progressive disease in a subject. The treatment comprises the separate, sequential or simultaneous administration of the pharmaceutical composition and an anti-fibrotic compound to the subject to achieve disease control, regression, or tissue repair, or any combination of the foregoing.

In a related fourth aspect, the disclosure further provides a pharmaceutical composition comprising an anti-fibrotic compound for use in the treatment of fibrosis in an inflammatory and/or progressive disease in a subject. The treatment comprises the separate, sequential or simultaneous administration of the pharmaceutical composition and a mitochondrial complex I modulator (MCIM) compound to the subject to achieve disease control, regression, or tissue repair, or any combination of the foregoing.

In a related fifth aspect, the disclosure further provides a pharmaceutical composition comprising a mitochondrial complex I modulator (MCIM) compound and an anti-fibrotic compound for use in the treatment of fibrosis in an inflammatory and/or progressive disease in a subject. The treatment comprises administration of the composition to the subject to achieve disease control, regression, or tissue repair, or any combination of the foregoing.

In a sixth aspect, the disclosure provides a pharmaceutical combination comprising an MCIM compound and an anti-fibrotic compound for use in a method of treatment. The combination may be comprised in a single pharmaceutical composition. Alternatively, the MCIM compound and the anti-fibrotic compound may be comprised in separate pharmaceutical compositions. The method may comprise the separate, sequential or simultaneous administration of the pharmaceutical combination. That is, the MCIM and the anti-fibrotic compound may be administered separately, sequentially, or simultaneously.

In a seventh aspect, the disclosure further provides a method of treating fibrosis in an inflammatory and/or progressive disease in a subject, wherein the method comprises administering a pharmaceutical composition comprising a mitochondrial complex I modulator (MCIM) compound to the subject. The method further comprises the separate, sequential or simultaneous administration of an anti-fibrotic compound to the subject to achieve disease control, disease regression or tissue repair, or a combination of any of the foregoing.

In an eighth aspect, the disclosure further provides a method of treating fibrosis in an inflammatory and/or progressive disease in a subject, wherein the method comprises administering a pharmaceutical composition comprising an anti-fibrotic compound to the subject. The treatment further comprises the separate, sequential or simultaneous administration of a mitochondrial complex I modulator (MCIM) compound to the subject to achieve disease control, disease regression or tissue repair, or a combination of any of the foregoing.

In a ninth aspect, the disclosure further provides a method of treating fibrosis in an inflammatory and/or progressive disease in a subject, wherein the method comprises administering a pharmaceutical composition comprising a mitochondrial complex I modulator (MCIM) compound and an anti-fibrotic compound to a subject to achieve disease control, disease regression or tissue repair, or a combination of any of the foregoing. In some embodiments of the above aspects of the disclosure, the treatment elicits disease control, disease regression or tissue repair, or a combination

of any of the foregoing. In some embodiments, the treatment achieves disease control, disease regression or tissue repair, or a combination of any of the foregoing. In some embodiments, the treatment initiates an adaptive response in certain cell types that leads to pharmacodynamic evidence of disease control, disease regression or tissue repair, or any combination of the foregoing. In some

5 embodiments, the adaptive response leads to tissue repair and/or disease regression. In some embodiments, the tissue repair or disease regression induces a restoration of tissue architecture towards its healthy state, which is characterised by anatomically normal architecture. In some embodiments, disease control comprises inhibition of disease progression. In some embodiments, inhibition of disease progression includes a prevention of disease progression. In some

10 embodiments, inhibition of disease progression includes a reduced rate of disease progression. In some embodiments, disease control comprises the prevention of a loss of anatomically normal tissue architecture, or a reduction in the speed of the loss of anatomically normal tissue architecture. In some embodiments, inhibition of disease progression comprises inhibition of fibrosis. In some embodiments, inhibition of disease progression comprises repair of fibrotic lesions.

15 The inflammatory and/or progressive disease may be any condition in which an imbalance is seen between cellular pathology and cellular repair, and wherein the disease is associated with tissue fibrosis. In some embodiments, the disease or disorder may be, pulmonary fibrosis, idiopathic pulmonary fibrosis, idiopathic non-specific interstitial pneumonia, rheumatoid arthritis interstitial lung disease (RA-ILD), systemic sclerosis, systemic sclerosis-associated interstitial lung disease (SSc-

20 ILD), sarcoidosis, COVID-19-related pulmonary fibrosis, drug-induced pulmonary fibrosis, endometriosis, radiation-induced fibrosis, cardiac fibrosis, liver fibrosis (Non-Alcoholic Steatohepatitis (NASH) and/or cirrhosis), chronic kidney disease, or hypersensitivity pneumonitis. In some embodiments, the inflammatory and/or progressive disease may be an interstitial lung disease (ILD). In some embodiments, the ILD comprises pulmonary fibrosis. In some embodiments, the ILD is

25 idiopathic pulmonary fibrosis, idiopathic non-specific interstitial pneumonia, rheumatoid arthritis interstitial lung disease (RA-ILD), systemic sclerosis, sarcoidosis, COVID-19-related pulmonary fibrosis, drug-induced pulmonary fibrosis, familial pulmonary fibrosis, progressive fibrosing ILD, or hypersensitivity pneumonitis. In a preferred embodiment, the progressive disease is idiopathic pulmonary fibrosis. In some embodiments, the inflammatory and/or progressive disease is non-

30 alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH). In some embodiments, the inflammatory and/or progressive disease is kidney disease. In some embodiments, the inflammatory and/or progressive disease is chronic kidney disease. In some embodiments, the tissue repair and/or disease regression is characterised by an increased clinical repair score and/or comprises increased wound healing.

35 In some embodiments, the inflammatory and/or progressive disease is an autoimmune disease associated with fibrosis. In some embodiments, the disease or disorder may be RA, psoriatic arthritis, inflammatory arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, reactive arthritis,

osteoarthritis, inflammatory bowel disease, Crohn's disease, fistulising Crohn's disease, or atopic dermatitis eczema. In some embodiments, the inflammatory and/or progressive disease may be rheumatoid arthritis-associated interstitial lung disease (RA-ILD) and systemic sclerosis-associated interstitial lung disease (SSc-ILD). In some embodiments, the tissue repair and/or disease regression
5 is characterised by an increased clinical repair score and/or comprises increased wound healing.

Clinical repair scores can be used to objectively assess clinical repair in inflammatory and/or progressive diseases. Increased clinical repair score can be indicated by a decreased disease score. In some embodiments, disease control is characterised by a decreased rate of change in the disease score. In some embodiments, disease control is characterised by no change in disease score
10 following treatment – that is, disease control may be characterised by an inhibition of disease progression associated with an increase in disease score.

In some embodiments, the disease control, regression or tissue repair, or any combination thereof, comprises an increased cell count of reparative cells and/or a decreased cell count of pathology driving cells. In some embodiments, the disease control, regression, or tissue repair, or any
15 combination thereof, regression comprises an increased count of mesenchymal and/or epithelial cells. In some embodiments, the adaptive response is characterised by an increased count of mesenchymal and/or epithelial cells. In some embodiments, the adaptive response is characterised by an increase in the differentiation of mesenchymal and/or epithelial cells. For instance, when the inflammatory and/or progressive disease is an interstitial lung disease, an increase in the number of
20 alveolar type 1, alveolar type 2, or club cells, or any combination thereof, may be seen in the lung. In addition to, or alternatively, in the lung, a decrease in the number or activity of fibrosis facilitating fibroblasts, active myofibroblasts, or dysfunctional epithelial cells, or any combination thereof, may be seen. In the gastrointestinal tract, when the disease is an IBD, the reparative cells may comprise epithelial cells and/or mucus cells. For instance, in the gastrointestinal tract the cells may comprise
25 fibroblasts or epithelial cells, or alternatively repair may be indicated by an increase in the overall number of mucus cells which indicate that epithelial differentiation is proceeding normally. In addition to, or alternatively, when the inflammatory and/or progressive disease is an IBD, the adaptive response is characterised by a decreased count of lymphocytes, macrophages, or granulocytes, or any combination thereof.

In some embodiments, the adaptive response comprises a change in the function of mesenchymal or epithelial cells. For instance, in IBD the cells may comprise PAS positive cells, or surfactant producing epithelial cells. In some embodiments, the tissue repair and/or disease regression comprises a decreased cell count or decreased function of activated immune cell subtypes. For instance, when the inflammatory and/or progressive disease is RA-ILD, the disease control, tissue
35 repair, or disease regression, or any combination thereof, may comprise a decrease in the number of myeloid cells such as macrophages, osteoclasts, lymphocytes such as T-, B-, or Th17 cells, or fibroblasts such as FLS cells, or any combination thereof. Preferably, the decrease in immune cell

activity and/or activated immune cell numbers is not accompanied by a decrease in the activity, function or numbers of adapting mesenchymal or epithelial cells.

In some embodiments, the tissue repair and/or disease regression induces a restoration of tissue architecture towards its healthy state, which is characterised by anatomically normal architecture. In

- 5 some embodiments, the disease control, tissue repair, or disease regression, or any combination thereof, comprises a reduction in inflammatory cytokine production from pro-inflammatory myeloid cells. In some embodiments, the disease control, tissue repair, or disease regression, or any combination thereof, comprises an increase in growth factors that are important for angiogenesis, epithelialisation and matrix remodelling, such as VEGF, FGF21 or GDF15, or a combination thereof.
- 10 Cytokine and growth factor levels can be measured by any suitable method, e.g. via ELISA as described herein, or via ELISpot. In some embodiments, the disease control, tissue repair, or disease regression, or any combination thereof, comprises an increase in basement collagen IV. Collagen IV levels can be measured by any suitable method, e.g. via ELISA as described herein and/or by immunohistochemical analysis.
- 15 Relatedly, the disclosure also provides a pharmaceutical composition comprising an MCIM compound for use in combination with an anti-fibrotic compound to increase reparative cells and/or decrease destructive cells in a subject with an inflammatory and/or progressive disease, to achieve disease control and/or tissue repair and, as a result, disease regression or resolution, or improved symptom control and quality of life. Relatedly, the disclosure also provides a pharmaceutical composition
- 20 comprising an anti-fibrotic compound for use in combination with an MCIM compound to increase reparative cells and/or decrease destructive cells in a subject with an inflammatory and/or progressive disease, to achieve disease control and/or tissue repair and, as a result, disease regression or resolution, or improved symptom control and quality of life. Relatedly, the disclosure also provides a pharmaceutical composition comprising an MCIM compound and an anti-fibrotic compound to
- 25 increase reparative cells and/or decrease destructive cells in a subject with an inflammatory and/or progressive disease, to achieve disease control and/or tissue repair and, as a result, disease regression or resolution, or improved symptom control and quality of life. In some embodiments, the pharmaceutical compositions trigger an adaptive response in a subject with an inflammatory and/or progressive disease. The disclosure also provides methods for increasing reparative cells and/or
- 30 decreasing destructive cells in a subject with an inflammatory and/or progressive disease, comprising administering the pharmaceutical composition to achieve disease control, tissue repair, disease regression, resolution, or improved symptom control and quality of life, or any combination thereof. In some embodiments, the method increases the adaptive response of mesenchymal and/or epithelial cells. In some embodiments, the method decreases the activation of and/or decreases the numbers of
- 35 pro-inflammatory, fibrotic, or erosive cells, or any combination thereof, in a subject with an inflammatory and/or progressive disease. Relatedly, the disclosure also provides pharmaceutical compositions for use in reducing cytokine production from pro-inflammatory myeloid cells in a subject

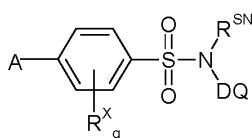
with an inflammatory and/or progressive disease, to achieve disease control, tissue repair and/or disease regression. The disclosure also provides methods for reducing cytokine production from pro-inflammatory myeloid cells in a subject with an inflammatory and/or progressive disease, comprising administering the pharmaceutical composition to achieve disease control, tissue repair, or disease regression, or any combination thereof. The compounds and inflammatory and/or progressive diseases are defined herein.

Preferably, the compound binds to Complex I and modulates Complex I activity. The modulation of Complex I activity may be determined by detecting a reduction in cellular O₂ consumption. In some embodiments, the reduction in cellular O₂ consumption is not associated with a reduction of cell viability. O₂ consumption may be measured by any standard technique known in the art, for example, using a real-time cell metabolic analyser (e.g. a Seahorse Analyzer). The modulation of Complex I activity may also lead to a reversible reduction of cell proliferation. The reversibility of the reduction of cell proliferation means that the reduction of proliferation is reversed when the compound is removed. This contrasts with the reduction of proliferation that is observed following treatment of the same cell type with a classical complex I binder, where reduced cell proliferation is not reversed by removal of the classical complex I binder. To assess the reversibility of the reduction of cell proliferation, an MCIM is applied to a cell culture, at a concentration to substantially reduce cell proliferation, for 24 hours at 37°C / 5% CO₂. After 24 hours, the cell culture is washed and cultured under conditions conducive to cell growth and proliferation. Recovery of cell proliferation is measured after 24-hours incubation in these 'growth' conditions (37°C / 5% CO₂, without MCIM present). Recovery of cell proliferation is observed. This contrasts to observations made on the same cells that have undergone the same culture/wash/grow cycle with a classical complex I binder in the culture step. The reversible reduction of cell proliferation may be assessed using human primary lung fibroblasts, e.g. as described in Example 3 (as illustrated in Figure 5). Preferably, the MCIM compound interacts with Complex I at a binding site at the top of or outside the Q tunnel. In some embodiments, the binding site comprises one or more amino acid residues from NDUSF2 (SEQ ID NO: 1) and/or NDUSF7 (SEQ ID NO: 2). In some embodiments, the compound interacts with at least one amino acid residues in NDUSF2 (SEQ ID NO: 1), for instance His92, Gly85, Tyr141, His88, Leu95, Asp193, or Phe458. In some embodiments, the compound interacts with one or more amino acid residues in NDUSF2 (SEQ ID NO: 1) selected from Tyr141, His92 and Asp193.

In some embodiments, the response may include promotion of a repair phenotype during the same time course as control of inflammation. The control of inflammation induced by MCIM compounds of the disclosure may be distinct from the control of inflammation induced by other anti-inflammatory drugs which rely on the suppression of inflammation before activation of tissue repair as a secondary effect.

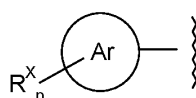
In some embodiments, the compound comprises four or more of the pharmacophore features of the pharmacophore model represented in Figure 15. The three-dimensional arrangement of the pharmacophore features may be as described in Tables 8-A, 8-B and 8-C.

In some embodiments, the compound is a compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:



as defined in claim 1 of WO2010/032009, wherein:

-A is independently:



-Ar is independently phenyl, pyridinyl, or pyrimidinyl; and
p is independently an integer from 0 to 3;

and wherein:

q is independently an integer from 0 to 3;

and wherein:

-R^{SN} is independently -H or saturated aliphatic C₁₋₄alkyl;

and wherein:

-DQ is independently -D¹-Q¹ or -D²=O;

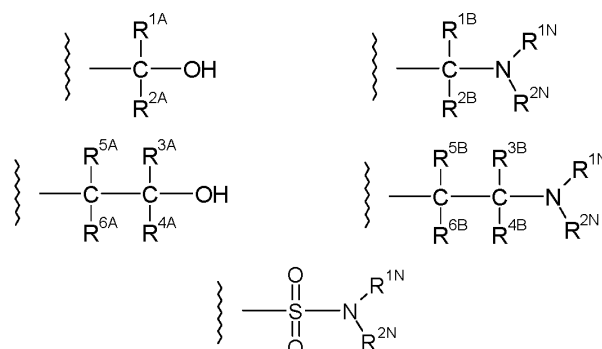
-D¹- is independently cyclopentane-di-yl, cyclohexane-di-yl, cycloheptane-di-yl, bicyclo[3.1.1]heptane-di-yl, or bicyclo[3.2.1]octane-di-yl, and is optionally substituted with one or more groups -R^D;

-D²= is independently cyclopentane-yl-ylidene, cyclohexane-yl-ylidene, cycloheptane-yl-ylidene, bicyclo[3.1.1]heptane-yl-ylidene, or bicyclo[3.2.1]octane-yl-ylidene, and is optionally substituted with one or more groups -R^D;

each -R^D is independently selected from -F, -Cl, -Br, -I, -R^{DD}, -CF₃, -OH, -OR^{DD}, -NH₂, -NHR^{DD}, and -NR^{DD}₂; and
each -R^{DD} is independently saturated aliphatic C₁₋₄alkyl;

and wherein -Q¹ is independently selected from:





wherein:

5

each -R^{1N} is independently -H, -R^{CN}, or -R^{CF};
 each -R^{2N} is independently -H, -R^{CN}, or -R^{CF};
 each -R^{CN} is independently saturated aliphatic C₁₋₄alkyl; and
 each -R^{CF}, if present, is independently saturated aliphatic C₁₋₄fluoroalkyl;
 or:

10

-NR^{1N}R^{2N} is independently azetidino, pyrrolidino, imidazolidino, pyrazolidino,
 piperidino, piperazino, morpholino, thiomorpholino, azepino, or diazepino, each
 optionally substituted with one or more groups independently selected from saturated
 aliphatic C₁₋₄alkyl;

15

-R^{1A} is independently -H, -R^C, or -R^F; and
 -R^{2A} is independently -H, -R^C, or -R^F;
 or -R^{1A} and -R^{2A} together form a saturated aliphatic C₂₋₄alkylene group;

20

-R^{1B} is independently -H, -R^C, or -R^F; and
 -R^{2B} is independently -H, -R^C, or -R^F;
 or -R^{1B} and -R^{2B} together form a saturated aliphatic C₂₋₄alkylene group;
 or -R^{1B} and -R^{2B} together form =O;

25

-R^{3A} is independently -H, -R^C, or -R^F; and
 -R^{4A} is independently -H, -R^C, or -R^F;
 or -R^{3A} and -R^{4A} together form a saturated aliphatic C₂₋₄alkylene group;

30

-R^{5A} is independently -H, -R^C, -R^F, or -R^J; and
 -R^{6A} is independently -H, -R^C, or -R^F;
 or -R^{5A} and -R^{6A} together form a saturated aliphatic C₂₋₄alkylene group;

35

-R^{3B} is independently -H, -R^C, or -R^F; and
 -R^{4B} is independently -H, -R^C, or -R^F;
 or -R^{3B} and -R^{4B} together form a saturated aliphatic C₂₋₄alkylene group;

-R^{5B} is independently -H, -R^C, -R^F, -OH, or -OR^O; and
 -R^{6B} is independently -H, -R^C, or -R^F;
 or -R^{5B} and -R^{6B} together form a saturated aliphatic C₂₋₄alkylene group;

5

each -R^C is independently saturated aliphatic C₁₋₄alkyl;
 each -R^F is independently saturated aliphatic C₁₋₄fluoroalkyl;
 -R^O is independently saturated aliphatic C₁₋₄alkyl;
 -R^J is independently -NH₂, -NHR^{JN1}, -NR^{JN1}₂, or -NR^{JN2}R^{JN3};

10

each -R^{JN1} is independently saturated aliphatic C₁₋₄alkyl; and
 -NR^{JN2}R^{JN3} is independently azetidino, pyrrolidino, imidazolidino, pyrazolidino,
 piperidino, piperazino, morpholino, thiomorpholino, azepino, or diazepino, each
 optionally substituted with one or more groups independently selected from saturated
 aliphatic C₁₋₄alkyl;

15

and wherein each -R^X is independently:

-F, -Cl, -Br, -I,

-R^{XX},

-OH, -OR^{XX},

20

-SH, -SR^{XX},

-CF₃, -OCF₃, -SCF₃,

-NH₂, -NHR^{XX}, -NR^{XX}₂, -NR^{YY}R^{ZZ},

-C(=O)R^{XX}, -OC(=O)R^{XX},

-C(=O)OH, -C(=O)OR^{XX},

25

-C(=O)NH₂, -C(=O)NHR^{XX}, -C(=O)NR^{XX}₂, -C(=O)NR^{YY}R^{ZZ},

-OC(=O)NH₂, -OC(=O)NHR^{XX}, -OC(=O)NR^{XX}₂, -OC(=O)NR^{YY}R^{ZZ},

-NHC(=O)R^{XX}, -NR^{XX}C(=O)R^{XX},

-NHC(=O)OR^{XX}, -NR^{XX}C(=O)OR^{XX},

-NHC(=O)NH₂, -NHC(=O)NHR^{XX}, -NHC(=O)NR^{XX}₂, -NHC(=O)NR^{YY}R^{ZZ},

30

-NR^{XX}C(=O)NH₂, -NR^{XX}C(=O)NHR^{XX}, -NR^{XX}C(=O)NR^{XX}₂, -NR^{XX}C(=O)NR^{YY}R^{ZZ},

-CN,

-NO₂,

-S(=O)₂NH₂, -S(=O)₂NHR^{XX}, -S(=O)₂NR^{XX}₂, -S(=O)₂NR^{YY}R^{ZZ},

-S(=O)R^{XX}, -S(=O)₂R^{XX}, -OS(=O)₂R^{XX}, -S(=O)₂OH, or -S(=O)₂OR^{XX};

35

wherein:

each -R^{XX} is independently saturated aliphatic C₁₋₆alkyl, phenyl, or benzyl,
 wherein said phenyl and benzyl are optionally substituted with one or more groups
 selected from: -F, -Cl, -Br, -I, -CF₃, -OCF₃, -R^{XXX}, -OH, -OR^{XXX}, or -SR^{XXX}, wherein

each -R^{XXX} is independently saturated aliphatic C₁₋₄alkyl; and

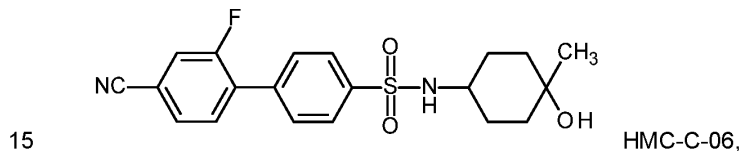
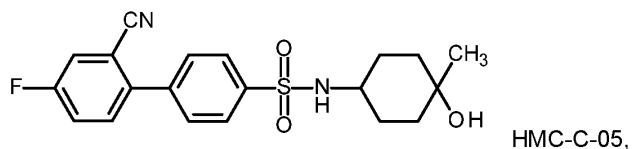
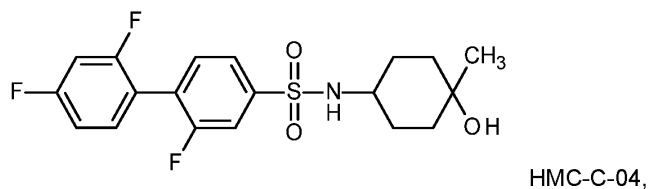
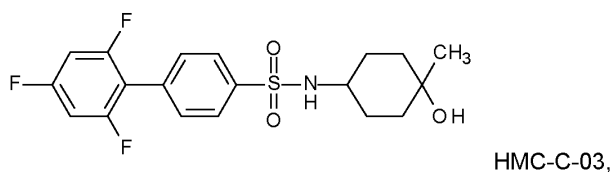
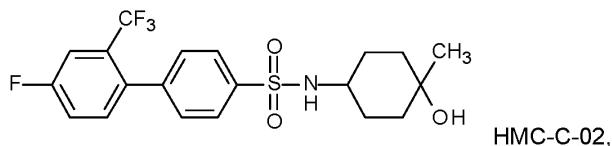
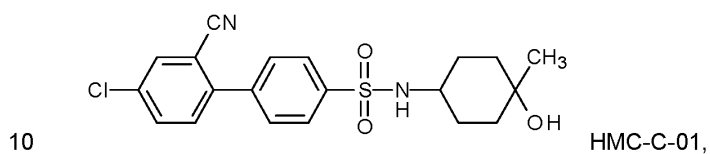
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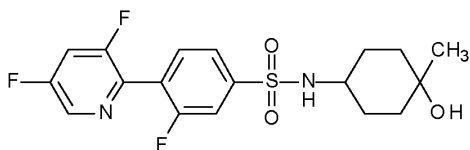
each -NR^{YY}R^{ZZ} is independently azetidino, pyrrolidino, imidazolidino,
 pyrazolidino, piperidino, piperazino, morpholino, thiomorpholino, azepino, or

diazepino, each optionally substituted with one or more groups independently selected from saturated aliphatic C₁₋₄alkyl.

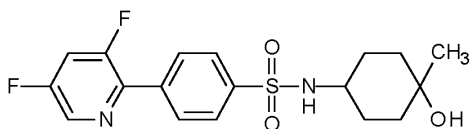
Claim 1 of WO2010/032009 is hereby incorporated by reference. Furthermore, WO2010/032009 is hereby incorporated by reference in its entirety.

- 5 In one embodiment, the compound is a compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof as defined in WO2014/207445 A1, which is incorporated herein by reference in its entirety. For example, in some embodiments, the compound is a compound selected from compounds of the following formulae, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:

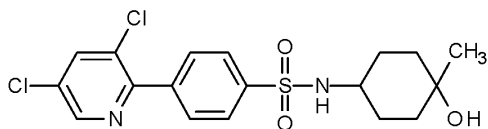




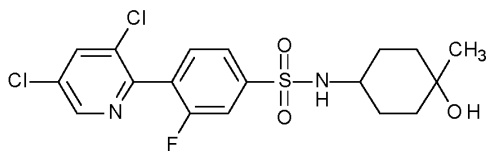
HMC-N-01,



HMC-N-02,

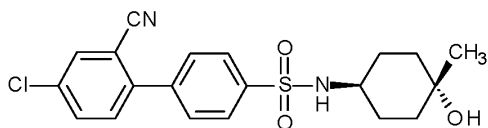


HMC-N-03, and



HMC-N-04.

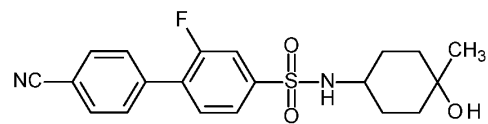
- 5 In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



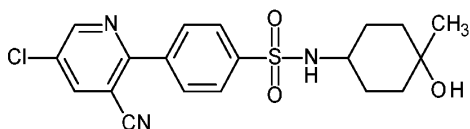
HMC-C-01-A.

In one embodiment, the compound is a compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof as defined in WO2016/097001 A1, which is incorporated herein by reference in its

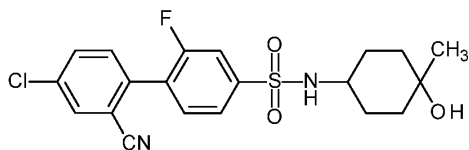
- 10 entirety. For example, in some embodiments, the compound is a compound selected from compounds of the following formulae, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



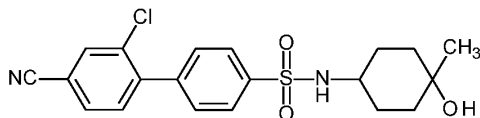
HMC-C-07.



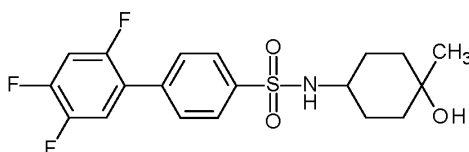
HMC-N-05,



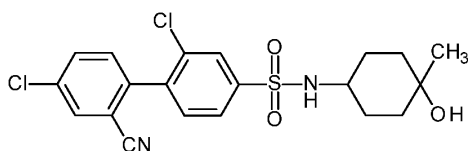
HMC-C-08,



HMC-C-09,



HMC-C-10, and



HMC-C-11.

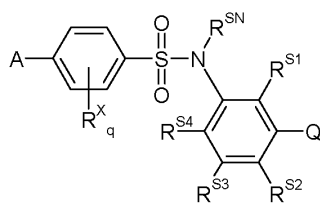
- 5 Note for the “HMC” compounds as defined in WO2014/207445 A1 and WO2016/097001 A1, and as described above, the substituents on one side of the cyclohexyl ring (i.e., OH and CH₃ on the right-hand side) may be positioned “trans” / “cis” or “cis” / “trans” with respect to the rest of the molecule (that is, on the cyclohexyl ring to which they attached, with respect to the rest of the compound which is attached at the para position of the cyclohexyl ring).

10

rest of compound “cis-OH”	rest of compound “cis-OH”
rest of compound “trans-OH”	rest of compound “trans-OH”

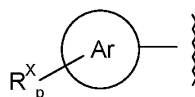
Unless otherwise indicated, it is intended that all such conformations are encompassed by a reference to a compound that does not specify a particular conformation.

In some embodiments, the compound is a compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:



as defined in claim 1 of WO2010/032010, wherein:

-A is independently:



-Ar is independently phenyl, pyridinyl, or pyrimidinyl; and
p is independently an integer from 0 to 3;

and wherein:

q is independently an integer from 0 to 3;

and wherein:

-R^{SN} is independently -H or saturated aliphatic C₁₋₄alkyl;

and wherein:

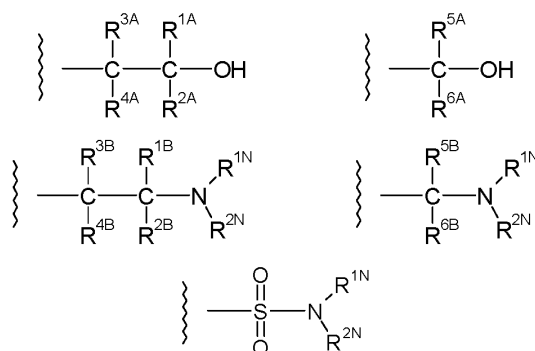
-R^{S1} is independently -H or saturated aliphatic C₁₋₄alkyl;

-R^{S2} is independently -H or saturated aliphatic C₁₋₄alkyl;

-R^{S3} is independently -H or saturated aliphatic C₁₋₄alkyl;

-R^{S4} is independently -H or saturated aliphatic C₁₋₄alkyl;

and wherein -Q is independently selected from:



wherein:

each -R^{1N} is independently -H or -R^{CN};

each -R^{2N} is independently -H or -R^{CN};

each -R^{CN} is independently saturated aliphatic C₁₋₄alkyl;

or:

-R^{1N}R^{2N} is independently azetidino, pyrrolidino, imidazolidino, pyrazolidino, piperidino, piperazino, morpholino, thiomorpholino, azepino, or diazepino, each optionally substituted with one or more groups independently selected from saturated aliphatic C₁₋₄alkyl;

-R^{1A} is independently -H, -R^C, or -R^F; and
 -R^{2A} is independently -H, -R^C, or -R^F;
 or -R^{1A} and -R^{2A} together form a saturated aliphatic C₂₋₄alkylene group;

-R^{3A} is independently -R^C, -R^F, or -R^J; and
 -R^{4A} is independently -H, -R^C, or -R^F;
 or -R^{3A} and -R^{4A} together form a saturated aliphatic C₂₋₄alkylene group;

-R^{5A} is independently -R^C or -R^F; and
 -R^{6A} is independently -H, -R^C, or -R^F;
 or -R^{5A} and -R^{6A} together form a saturated aliphatic C₂₋₄alkylene group;

-R^{1B} is independently -H, -R^C, or -R^F; and
 -R^{2B} is independently -H, -R^C, or -R^F;
 or -R^{1B} and -R^{2B} together form a saturated aliphatic C₂₋₄alkylene group;

-R^{3B} is independently -H, -R^C, -R^F, -OH, or -OR^O; and
 -R^{4B} is independently -H, -R^C, or -R^F;
 or -R^{3B} and -R^{4B} together form a saturated aliphatic C₂₋₄alkylene group;

-R^{5B} is independently -H, -R^C, or -R^F; and
 -R^{6B} is independently -H, -R^C, or -R^F;
 or -R^{5B} and -R^{6B} together form a saturated aliphatic C₂₋₄alkylene group;

each -R^C is independently saturated aliphatic C₁₋₄alkyl;
 each -R^F is independently saturated aliphatic C₁₋₄fluoroalkyl;
 -R^O is independently saturated aliphatic C₁₋₄alkyl;
 -R^J is independently -NH₂, -NHR^{JN1}, -NR^{JN1}₂, or -NR^{JN2}R^{JN3};
 each -R^{JN1} is independently -R^{J1}, -R^{J2}-OH, -R^{J2}-O-R^{J1};
 each -R^{J1} is independently saturated aliphatic C₁₋₄alkyl;
 each -R^{J2} is independently saturated aliphatic C₂₋₄alkylene;

-R^{JN2}R^{JN3} is independently azetidino, pyrrolidino, imidazolidino, pyrazolidino, piperidino, piperazino, morpholino, thiomorpholino, azepino, or diazepino, each optionally substituted with one or more groups independently selected from saturated aliphatic C₁₋₄alkyl;

and wherein each $-R^X$ is independently:

- $-F$, $-Cl$, $-Br$, $-I$,
 $-R^{XX}$,
 $-OH$, $-OR^{XX}$,
 $-SH$, $-SR^{XX}$,
 $-CF_3$, $-OCF_3$, $-SCF_3$,
 $-NH_2$, $-NHR^{XX}$, $-NR^{XX}_2$, $-NR^{YY}R^{ZZ}$,
 $-C(=O)R^{XX}$, $-OC(=O)R^{XX}$,
 $-C(=O)OH$, $-C(=O)OR^{XX}$,
 $-C(=O)NH_2$, $-C(=O)NHR^{XX}$, $-C(=O)NR^{XX}_2$, $-C(=O)NR^{YY}R^{ZZ}$,
 $-OC(=O)NH_2$, $-OC(=O)NHR^{XX}$, $-OC(=O)NR^{XX}_2$, $-OC(=O)NR^{YY}R^{ZZ}$,
 $-NHC(=O)R^{XX}$, $-NR^{XX}C(=O)R^{XX}$,
 $-NHC(=O)OR^{XX}$, $-NR^{XX}C(=O)OR^{XX}$,
 $-NHC(=O)NH_2$, $-NHC(=O)NHR^{XX}$, $-NHC(=O)NR^{XX}_2$, $-NHC(=O)NR^{YY}R^{ZZ}$,
 $-NR^{XX}C(=O)NH_2$, $-NR^{XX}C(=O)NHR^{XX}$, $-NR^{XX}C(=O)NR^{XX}_2$, $-NR^{XX}C(=O)NR^{YY}R^{ZZ}$,
 $-CN$,
 $-NO_2$,
 $-S(=O)_2NH_2$, $-S(=O)_2NHR^{XX}$, $-S(=O)_2NR^{XX}_2$, $-S(=O)_2NR^{YY}R^{ZZ}$,
 $-S(=O)R^{XX}$, $-S(=O)_2R^{XX}$, $-OS(=O)_2R^{XX}$, $-S(=O)_2OH$, or $-S(=O)_2OR^{XX}$;

wherein:

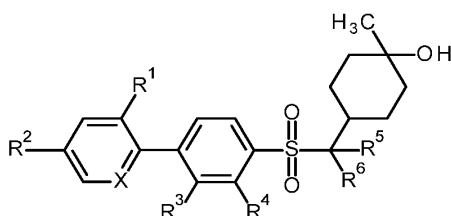
each $-R^{XX}$ is independently saturated aliphatic C_{1-6} alkyl, phenyl, or benzyl, wherein said phenyl and benzyl are optionally substituted with one or more groups selected from: $-F$, $-Cl$, $-Br$, $-I$, $-CF_3$, $-OCF_3$, $-R^{XXX}$, $-OH$, $-OR^{XXX}$, or $-SR^{XXX}$, wherein

each $-R^{XXX}$ is independently saturated aliphatic C_{1-4} alkyl; and

each $-NR^{YY}R^{ZZ}$ is independently azetidino, pyrrolidino, imidazolidino, pyrazolidino, piperidino, piperazino, morpholino, thiomorpholino, azepino, or diazepino, each optionally substituted with one or more groups independently selected from saturated aliphatic C_{1-4} alkyl.

- Claim 1 of WO2010/032010 is hereby incorporated by reference. Furthermore, WO2010/032010 is hereby incorporated by reference in its entirety.

In some embodiments, the compound is a compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:



as defined in claim 1 of WO2020/035560 A1, wherein:

=X- is independently -CH= or -N=;

-R¹ is independently -H or -R^{1X};

-R^{1X} is independently -F, -Cl, -R^{1C}, -R^{1F}, or -CN;

5 -R^{1C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{1F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R² is independently -H or -R^{2X};

-R^{2X} is independently -F, -Cl, -R^{2C}, -R^{2F}, or -CN;

10 -R^{2C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{2F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R³ is independently -H or -R^{3X};

-R^{3X} is independently -F, -Cl, -R^{3C}, -R^{3F}, or -CN;

15 -R^{3C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{3F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R⁴ is independently -H or -R^{4X};

-R^{4X} is independently -F, -Cl, -R^{4C}, -R^{4F}, or -CN;

20 -R^{4C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{4F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R⁵ is independently -H or -R^{5X};

-R^{5X} is independently -F, -R^{5C}, or -R^{5F};

25 -R^{5C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{5F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R⁶ is independently -H or -R^{6X};

-R^{6X} is independently -F, -R^{6C}, or -R^{6F};

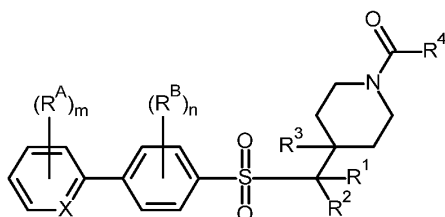
30 -R^{6C} is independently saturated linear or branched C₁₋₃alkyl; and

-R^{6F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

or -R⁵ and -R⁶, taken together with the carbon atom to which they are attached, form saturated C₃₋₆cycloalkyl.

35 Claim 1 of WO2020/035560 A1 is hereby incorporated by reference. Furthermore, WO2020/035560 A1 is hereby incorporated by reference in its entirety.

In some embodiments, the compound is a compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:



as defined in claim 1 of WO2020/212581 A1, , wherein:

-X= is independently -CH= or -N=;

"m" is independently 0, 1, 2, or 3;

each -R^A is independently -F, -Cl, -R^{AC}, -R^{AF}, or -CN;

5 -R^{AC} is independently saturated linear or branched C₁₋₃alkyl;

-R^{AF} is independently saturated linear or branched C₁₋₃fluoroalkyl;

"n" is independently 0, 1, or 2;

each -R^B is independently -F, -Cl, -R^{BC}, -R^{BF}, or -CN;

10 -R^{BC} is independently saturated linear or branched C₁₋₃alkyl;

-R^{BF} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R¹ is independently -H or -R^{1X};

-R^{1X} is independently -F, -R^{1C}, or -R^{1F};

15 -R^{1C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{1F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

-R² is independently -H or -R^{2X};

-R^{2X} is independently -F, -R^{2C}, or -R^{2F};

20 -R^{2C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{2F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

or -R¹ and -R², taken together with the carbon atom to which they are attached, form saturated C₃₋₆cycloalkyl;

25

-R³ is independently -H or -R^{3X};

-R^{3X} is independently -R^{3C} or -R^{3F};

-R^{3C} is independently saturated linear or branched C₁₋₃alkyl;

-R^{3F} is independently saturated linear or branched C₁₋₃fluoroalkyl;

30

-R⁴ is independently -R^{4C}, -R^{4CC}, or -N(R^{4N1})(R^{4N2});

-R^{4C} is independently saturated linear or branched C₁₋₆alkyl;

-R^{4CC} is independently saturated C₃₋₆cycloalkyl;

$-R^{4N1}$ is independently -H or $-R^{4N1C}$;

$-R^{4N1C}$ is independently saturated linear or branched C_{1-4} alkyl;

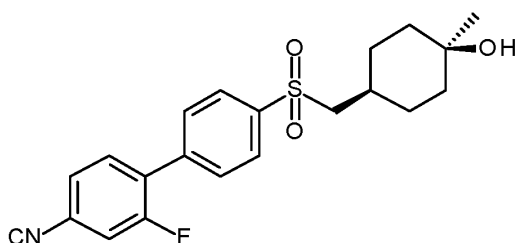
$-R^{4N2}$ is independently -H or $-R^{4N2C}$; and

$-R^{4N2C}$ is independently saturated linear or branched C_{1-4} alkyl,

- 5 or $-N(R^{4N1})(R^{4N2})$ is independently azetidiny, pyrrolidiny, piperidiny, piperaziny, or morpholiny, and is optionally substituted with one or more saturated linear or branched C_{1-4} alkyl groups.

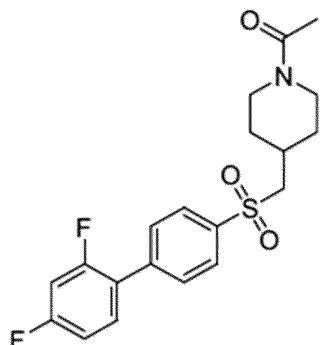
Claim 1 of WO2020/212581 A1 is hereby incorporated by reference. Furthermore, WO2020/212581 A1 is hereby incorporated by reference in its entirety.

- 10 In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



CHMSA-01-A.

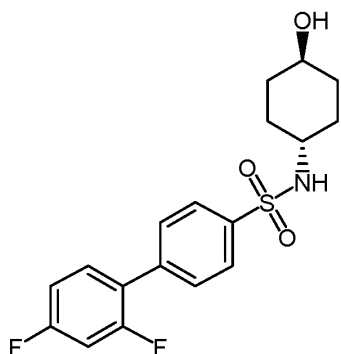
In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



15

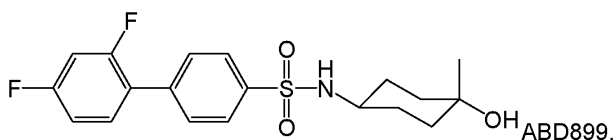
NASMP-01-A.

In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



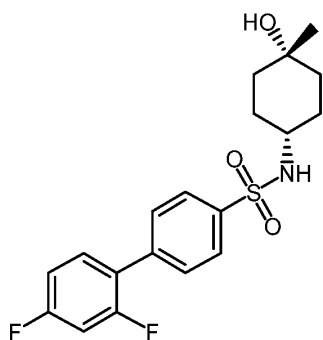
ABD599.

In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



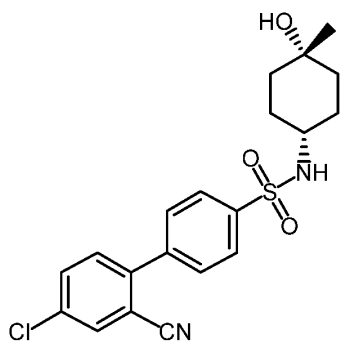
ABD899.

- 5 In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



ABD900.

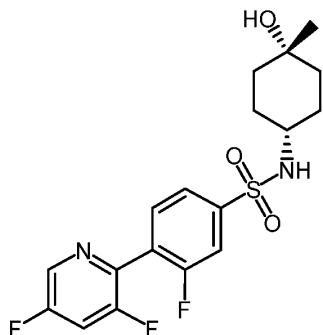
In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



10

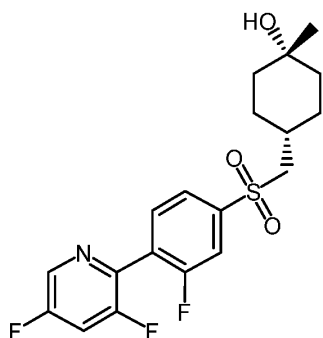
HMC-C-01-B.

In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



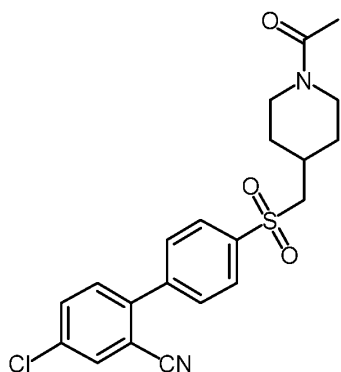
HMC-N-01-B.

- 5 In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



CHMSA-03-A.

In some embodiments, the compound is a compound of the following formula, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



NASMP-06.

- 10 In some embodiments of the above aspects, fibrosis may be associated with or caused by overstimulation of PDGFR α , PDGFR β , FGFR1, FGFR2, FGFR3, VEGFR1, VEGFR2, VEGFR3, CSF1R, Lck, Lyn, Src, TNF, TGB β 1, phosphodiesterase 4B (PDE4B), or a combination of any of the foregoing.

In some embodiments, the anti-fibrotic compounds inhibits PDGFR α , PDGFR β , FGFR1, FGFR2, FGFR3, VEGFR1, VEGFR2, VEGFR3, CSF1R, Lck, Lyn, Src, TNF, TGB β 1, phosphodiesterase 4B (PDE4B), Death receptor 3 (DR3), or a combination of any of the foregoing. In some embodiments, the antifibrotic compound comprises a triple angiokinase inhibitor. In some embodiments of the
5 above aspects, the anti-fibrotic compound comprises nintedanib. In some embodiments of the above aspects, the anti-fibrotic compound comprises pirfenidone. In some embodiments of the above aspects, the anti-fibrotic compound comprises nintedanib and pirfenidone. In some embodiments of the above aspects, the anti-fibrotic compound comprises tulusokibart, RVT-2101, or TEV-48574. In some embodiments of the above aspects, the anti-fibrotic compound comprises nerandomilast. In
10 some embodiments of the above aspects, the anti-fibrotic compound comprises nintedanib, pirfenidone, tulusokibart, or nerandomilast, or a combination of any of the foregoing.

In some embodiments, the MCIM compound and the anti-fibrotic compound are uniformly dispersed in a solution, suspension, semi-solid or solid mixture within a single dosage form. In some embodiments, the single dosage form comprises one or more pharmaceutically acceptable excipients.
15 In some embodiments, the MCIM compound and the anti-fibrotic compound are heterogeneously dispersed and separated from each other in different regions within a single dosage form.

The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

20 **Summary of the Figures**

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures in which:

Figure 1. A graph of response over time of each of the three phases of repair (Inflammation, proliferation and tissue remodelling) which are proposed to be broadly similar across tissues. Cell
25 types and soluble factors involved in each phase are shown below the graph along with changes in the extracellular matrix.

Figure 2. High throughput integrative biology platform (BioMAP®) profile of the effects of MCIM compounds on multiple disease-associated regulatory pathways identified that these compounds have the potential to regulate inflammatory responses and the tissue remodelling collagen, collagen
30 type IV, for ABD599 (A) and HMC-C-01-A (B) and (C).

Figure 3. High throughput integrative biology platform (BioMAP®) profile of the effects of approved anti-fibrotic, nintedanib (A) and HMC-C-01-A (B) on multiple disease-associated regulatory pathways identified that the compounds show specific and differentiated effects on inflammatory responses, and the tissue remodelling collagen, collagen Type I.

Figure 4. Electron micrographs of human primary myeloid cells (osteoclasts) treated with MCIM compounds show an adaptive response by changing mitochondrial morphology, with an increase in mitochondrial area without an overt increase in mitochondrial mass.

Figure 5. A) Three graphs showing the change in intracellular ATP levels (top left), nuclei count (top middle) and ATP readout per cell (top right) in response to increasing MCIM compound dose in standard, glucose supplemented media (squares), media supplemented with glucose and L-glutamine (open circles), or media supplemented with glucose, L-glutamine, and pyruvate (close circles). B) A graph showing the change in VEGF secretion in response to increasing MCIM compound concentration in standard, glucose and L-glutamine supplemented media containing pyruvate (closed circles), or medium containing glucose and L-glutamine but without pyruvate (open circles). MCIM compounds promote an adaptive response by increasing VEGF secretion in metabolically restricted human primary lung fibroblasts without a change in cell number.

Figure 6. Cell proliferation inhibition measured by BrdU incorporation (A) and nucleic count (B) as a function of increasing dosage of either Rotenone (squares), IACS-010759 (closed circles) or MCIM compound (open circles). Comparison of cells without washout vs washout of test compounds on cell proliferation measure by BrdU incorporation (C) and nuclei count (D). Non-washout and washout of rotenone are shown in grey and open grey, respectively. Non-washout and washout of IACS-010759 are shown in black and open black, respectively. Non-washout and washout of MCIM compound are shown in checkboard fill and open fill pattern, respectively. MCIM compounds reversibly inhibit cell proliferation whereas cells treated with typical Complex I inhibitors IACS-010759 and rotenone do not recover cell proliferative capacity after compound washing out.

Figure 7. Two graphs showing the change in (A and B) lung hydroxyproline levels following treatment of mice with bleomycin-induced lung fibrosis with vehicle control, nintedanib or MCIM compounds. MCIM compounds reduce lung hydroxyproline levels in mice (panels A and B) which is indicative of reduced collagen and fibrosis in lung. Data are mean $\pm$ s.e.m.. ** $p < 0.01$, *** $p < 0.005$ vs vehicle.

Figure 8. H&E-stained bleomycin-induced fibrotic lung tissue from mice treated with Vehicle, nintedanib and MCIM compounds. MCIM compounds (middle panels) reduce fibrosis to at least the same extent as nintedanib (bottom panels), and significantly vs vehicle (top panels).

Figure 9. H&E-stained bleomycin-induced fibrotic lung tissue from mice treated with Vehicle, nintedanib and MCIM compound. (A) Compound elicits a cuboid phenotype in the AEC2 cells (lower panel, arrows), compared to vehicle which shows a flattened appearance (top panel, arrows). This indicates adaptive repair changes, which are accompanied by increased surfactant production ((B); magnified image with circles).

Figure 10. (A) MCIM compounds in combination with nintedanib reduce the histopathological presentation of fibrosis (as measured by the Ashcroft scoring system) compared to vehicle controls,

or MCIM alone or nintedanib alone, reducing the Ashcroft score from ~5 in vehicle control and ~4 when either is administered alone to <2 indicating MCIM compounds in combination with nintedanib reduce the pathological presentation of lung fibrosis. (B) MCIM compounds, nintedanib and a combination of MCIM compound and nintedanib reduce vascular inflammation compared to vehicle control treated mice. The reduction of inflammation between treated groups is not significantly different indicating the reduction in the histopathological presentation of fibrosis is not due to an increase the control of inflammation. Data are mean $\pm$ s.e.m.. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs vehicle.

Figure 11. Two graphs showing changes in (A) AT2 cell hyperplasia (cell number) and (B) AT2 cell hypertrophy (cell size) in mice with bleomycin-induced lung fibrosis treated with vehicle control, MCIM compounds, nintedanib, or a combination of MCIM compound and nintedanib. MCIM compounds increase alveolar AT2 cell number (A) and cell size (B). Increased AT2 cell size and cell number is seen with the MCIM compound, with highly significant increases seen at 20 mg/kg/day indicating mobilisation of a repair response. No significant increase in AT cell size or number is seen following treatment with nintedanib at 30 mg/kg/ given twice daily. Surprisingly, however, treatment with a combination of MCIM compound and nintedanib leads to a synergistic increase in both AT2 cell size and number. AT2 cells maintain alveolar homeostasis by secreting pulmonary surfactant and serve as alveolar stem cells with the potential to self-renew and differentiate into AT1 cells. Loss of these cells contributes to the development of lung fibrosis. Data are mean $\pm$ s.e.m.. *** $p < 0.005$ vs vehicle, §§§ $p < 0.005$ vs nintedanib.

Figure 12. Homology model of the complete Complex I constructed from publicly available structures for 5 different organisms. The putative targets were resolved in all 5 structures.

Figure 13. *In silico* homology model of NDUFS2. Druggability assessment was performed using SiteMap and identified two binding pockets in the Complex I subunit NDUFS2 (spheres).

Figure 14. *In silico* SiteFinder model of NDUFS2 when in Complex I. A narrow channel was identified for Q10 and drug-like compound binding in the Q-tunnel. Spheres are used to illustrate the space/channels around NDUFS2 when in the Complex I structure.

Figure 15. *In silico* model of a MCIM compound docked in the Q-site of Complex I. This model reveals interactions between a MCIM compound and NDUFS2 and additional interactions with the neighbouring Complex I subunit NDUFS7.

Figure 16. 3D representation of a pharmacophore model built from *in silico* modelling of drug docking in Complex I.

Figure 17. Overlay of a MCIM compound on the ligand-protein pharmacophore model illustrating successful docking of the MCIM compound in the Q-tunnel of Complex I.

Figure 18. Overlay of a MCIM compound, CHMSA-02-A, on the ligand-protein pharmacophore model (top panel) and the chemical structure of CHMSA-02-A (bottom panel). This illustrates successful docking of CHMSA-02-A in the Q-tunnel of Complex I.

Figure 19. Graph showing the quantitative structure-activity relationship (QSAR) model used to identify further MCIM compounds, which bind Complex I, and to predict their activity in vivo. Predicted pAct of MCIM compounds correlates well with their experimentally validated pAct, with a coefficient of determination (R^2) value of 0.8322 demonstrating that this QSAR model can be used to accurately identify novel MCIM compounds which bind Complex I.

Figure 20. Comparison of the profile of conventional complex I inhibitor, IACS-010759 and an MCIM of the invention, ABD900, in the short bleomycin-induced lung fibrosis mouse model. (A) Ashcroft score, (B) Bronchoalveolar lavage (BAL) cell counts, (C) AT2 cell size (hypertrophy), (D) AT1 cell metaplasia, (E) apoptosis/necrosis score and (F) pyknotic foci. Naïve controls are shown in spotted white bars, vehicle is shown in black bars, IACS-010759 dosed at 0.01, 0.1 and 1 mg/kg/day in white, light grey or dark grey bars respectively, and ABD900 in striped bars. Data are mean $\pm$ s.e.m.. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ vs vehicle. §§§ $p < 0.005$ vs 1 mg/kg/day IACS-010759.

Detailed Description of the Invention

Aspects and embodiments of the present invention will now be discussed with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art.

All documents mentioned in this text are incorporated herein by reference.

The present disclosure provides a pharmaceutical combination and a pharmaceutical composition comprising an MCIM compound and a TNF inhibitor compound. The combination or composition may be for use as a medicament. The present disclosure also provides an MCIM compound for use in a method of treating or preventing fibrosis in an inflammatory and/or progressive disease, wherein the method further comprises administering an anti-fibrotic compound. Also provided is an anti-fibrotic compound for use in a method of treating or preventing fibrosis in an inflammatory and/or progressive disease, wherein the method further comprises administering an MCIM compound. Also provided is the use of an MCIM compound in the manufacture of a medicament for use in a method of treatment, for example for use in treating or preventing fibrosis in an inflammatory and/or progressive disease, wherein the method further comprises administering an anti-fibrotic compound. Also provided is the use of an anti-fibrotic compound in the manufacture of a medicament for use in a method of treatment, for example for use in a method of treating or preventing fibrosis in an inflammatory and/or progressive disease, wherein the method further comprises administering an MCIM compound.

The present disclosure also provides an MCIM compound for use in a method of treatment comprising administering an MCIM compound and an anti-fibrotic compound to a subject. The present

disclosure also provides an anti-fibrotic compound for use in a method of treatment comprising administering an anti-fibrotic compound and an MCIM compound to a subject. The present disclosure further provides a pharmaceutical combination comprising an MCIM compound and an antifibrotic compound for use in a method of treatment. The MCIM compound and the anti-fibrotic compound
5 may be administered separately, simultaneously, or sequentially. The mode of administration of the MCIM compound and the anti-fibrotic compound may be the same or different. In some embodiments, the anti-fibrotic compound comprises nintedanib. In some embodiments, the anti-fibrotic compound comprises pirfenidone. In some embodiments, the anti-fibrotic compound comprises tulisokibart. In some embodiments, the anti-fibrotic compound comprises nerandomilast.

- 10 Further provided is a method of treating or preventing fibrosis in an inflammatory and/or progressive disease, the method comprising administering a therapeutically- or prophylactically-effective amount of (i) an MCIM compound and (ii) an anti-fibrotic compound to a subject in need of treatment.

The present disclosure also provides an MCIM compound and an anti-fibrotic compound (e.g. in the form of a pharmaceutical combination or pharmaceutical composition comprising an MCIM compound
15 and an anti-fibrotic compound) for use in a method of treating or preventing fibrosis in an inflammatory and/or progressive disease. Also provided is the use of an MCIM compound and an anti-fibrotic compound (e.g. in the form of a pharmaceutical combination or pharmaceutical composition comprising an MCIM compound and an anti-fibrotic compound) in the manufacture of a medicament for use in a method of treating or preventing fibrosis in an inflammatory and/or progressive disease.

- 20 Also provided is a method of treating or preventing fibrosis in an inflammatory and/or progressive disease, the method comprising administering a therapeutically- or prophylactically-effective amount of an MCIM compound and an anti-fibrotic compound (e.g. in the form of a pharmaceutical combination or pharmaceutical composition comprising an MCIM compound and an anti-fibrotic compound) to a subject in need of treatment.

- 25 In some aspects and embodiments, the MCIM compound and the anti-fibrotic compound may be provided as a combination therapy. In some embodiments, the MCIM compound and the anti-fibrotic compound may be administered simultaneously or sequentially. Simultaneous administration refers to administration of the two or more agents together, for example as a pharmaceutical composition containing both agents (i.e. as a combined preparation), or immediately after one another (e.g. within
30 1, 4, 6, 8 or 12 hours), and optionally via the same route of administration, e.g. to the same artery, vein or other blood vessel. Sequential administration refers to administration of one of the agents followed after a given time interval by separate administration of another agent. It is not required that the agents are administered by the same route, although this is the case in some embodiments. The time interval may be any time interval.

Also provided is the use of an MCIM compound in the manufacture of a medicament for use in treating or preventing inflammatory and/or progressive disease, wherein treating or preventing the inflammatory and/or progressive disease further comprises administering an anti-fibrotic compound.

The anti-fibrotic compound may be nintedanib, pirfenidone, tulisokibart, or nerandomilast. The inflammatory and/or progressive disease may be a progressive fibrotic disease. For example, the inflammatory and/or progressive disease may be a pulmonary fibrotic disease. For example, the pulmonary fibrotic disease may be idiopathic pulmonary fibrosis, interstitial lung disease (ILD), or scleroderma-associated interstitial lung disease (SSc-ILD).

Fibrosis and dysregulated metabolism

Fibrosis is the result of abnormal, pathogenic deposition of extracellular matrix components which can result in organ damage and dysfunction. This uncontrolled deposition of extracellular matrix components can ultimately lead to organ failure and death.

Dysregulated mitochondria and metabolism may be a contributing factor to fibrosis progression in various resident cells of the affected organs. For example, in the lung, the major cell types responsible for pulmonary fibrosis may be myofibroblasts, alveolar epithelial cells, macrophages, and endothelial cells. Metabolic reprogramming and dysregulation of these cells can lead to pathological mechanisms being activated, such as autophagy, apoptosis and inflammatory responses, which may contribute to progression of fibrosis. In some embodiments, fibrosis is associated with mitochondrial dysfunction and/or dysregulated metabolism.

Anti-fibrotic drugs

Anti-fibrotic compounds include drugs which are capable of inhibiting progression of fibrosis in a tissue and/or reversing fibrosis in a tissue.

Anti-fibrotic compounds may inhibit signalling through one or more of the following receptor tyrosine kinases: platelet-derived growth factor receptors (PDGFR) α , PDGFR β , fibroblast growth factor receptors (FGFR) 1, FGFR2, FGFR3, vascular endothelial growth factor receptor (VEGFR) 1, VEGFR2, VEGFR3, and colony stimulating factor 1 receptor (CSF1R). In addition to, or alternatively, the anti-fibrotic compound may inhibit one or more of the non-receptor tyrosine kinases selected from: Lck, Lyn and Src. In addition, or alternatively, the anti-fibrotic compound may regulate (e.g. inhibit) tumor necrosis factor pathway signalling, inhibit transforming growth factor β 1 (TGF β 1) production, and/or exhibit an antioxidant effect. In addition, or alternatively, the anti-fibrotic compound may inhibit Phosphodiesterase 4B (PDE4B). In addition, or alternatively, the anti-fibrotic compound may inhibit DR3. In some embodiments, the antifibrotic compound comprises nintedanib. In some embodiments, the antifibrotic compound comprises pirfenidone. In some embodiments, the antifibrotic compound comprises tulisokibart. In some embodiments, the antifibrotic compound comprises nerandomilast. In

some embodiments, the antifibrotic compound comprises a combination of nintedanib and pirfenidone.

Pharmacophores

The term “pharmacophore” is used herein as defined in Wermuth, C.G., Ganellin, C.R., Lindberg, P., Mitscher, L.A.; Glossary of Terms Used in Medicinal Chemistry (IUPAC Recommendations 1998); *Pure & Appl. Chem.* 70:5 (1998) 1129–1143: A pharmacophore is an ensemble of aromatic steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response. The ensemble of aromatic steric and electronic features represent so-called “pharmacophoric features”. Typical pharmacophoric features include, for example, hydrogen bond donor, hydrogen bond acceptor, hydrophobic, aromatic, and positively and negatively ionized areas.

As used herein, the term “pharmacophore model” relates to a pharmacophore hypothesis for the binding interactions in a particular active site. A pharmacophore model is made up of a set of annotation points which are interrelated in 3D space. The annotation points show the location and type of biologically important atoms and groups, i.e., each annotation point relates to a pharmacophore feature of the model. Each annotation point is associated with a radius that describes the permissible variation in 3D space for the location of the given pharmacophoric feature.

As used herein, the phrase “conform to a pharmacophore model” means that a compound described herein binds to the target binding site (i.e., the NDUSF2 and/or NDUSF7 binding site) in a 3D conformation (i.e., “pose”) whereby, 4 or more of the annotation points of the pharmacophore model described herein are occupied by corresponding features of the MCIM compound described herein, as determined the unified annotation scheme in *Molecular Operating Environment (MOE)*, 2022.02 Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2022. In some embodiments, four or more of the annotation points of the pharmacophore model described herein are occupied by corresponding features of the MCIM compound described herein. In some embodiments, five or more, six or more, seven or more, eight or more, or all nine of the annotation points of the pharmacophore model described herein are occupied by corresponding features of the MCIM compound described herein. The conformance of a compound described herein with the pharmacophore features is as determined using the unified annotation scheme in *Molecular Operating Environment (MOE)*, 2022.02 Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2022

Annotation points can be broadly divided into three categories: *atom*, *projected*, and *centroid*. Annotation points are determined for a given compound by the unified annotation scheme in *Molecular Operating Environment (MOE)*, 2022.02 Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2022.

The annotation points used herein are described below.

Atom

Don: H-bond Donor

Acc: H-bond Acceptor

- 5 Atom annotations are located directly on an atom of a molecule and typically indicate a function related to protein-ligand binding.

Don annotates an H-bond donor heavy atom. A **Don** annotation is added to all oxygen and nitrogen atoms with at least one (possibly implicit) hydrogen attached.

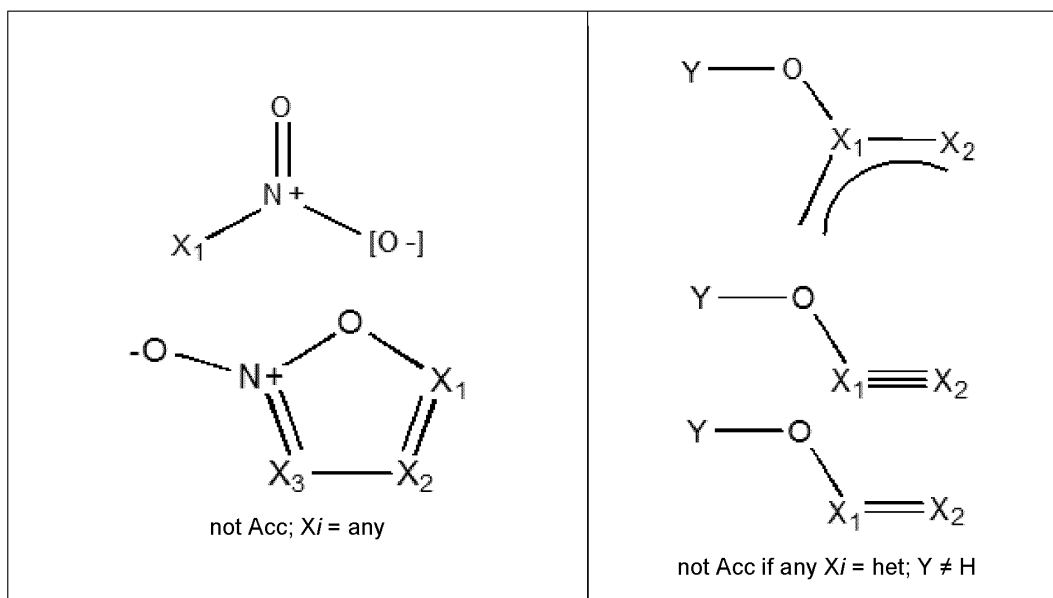
- 10 **Acc** annotates an H-bond acceptor heavy atom. O, S, and N elements can be hydrogen bond acceptors provided that they conform to the following rules:

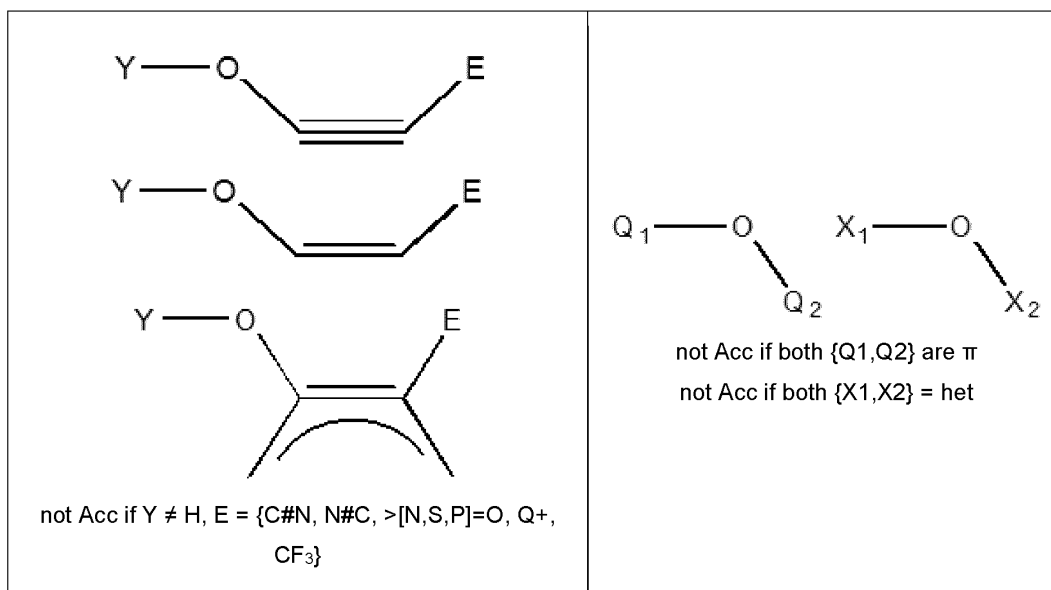
1. Sulfur atoms are not acceptors except that sulfur in S=C groups and anionic sulfurs are acceptors and are given **Acc** annotations.

2. Nitrogen atoms are acceptors and given **Acc** annotations provided that they are not buried. A buried nitrogen is one of {=N=, -N#, >N=, >N<} or a non-3-ring conjugated nitrogen of the form
15 {>N- π , >N-[C+], >N-B, >N-S=O, >N-P=O}.

3. Oxygen atoms are acceptors and are given **Acc** annotations provided that they are not buried and provided that they are not in certain exception groups. A buried oxygen is one of {=O=, -O#, >O=, >O<} or a non-3-ring conjugated oxygen of the form {>O- π , >O-B, >O-[C+]}. Non-buried oxygen atoms are acceptors unless they are part of the following exception groups:

20

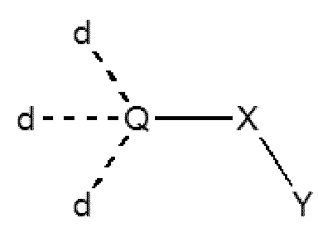
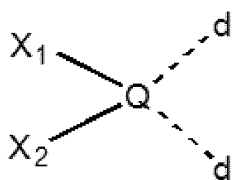
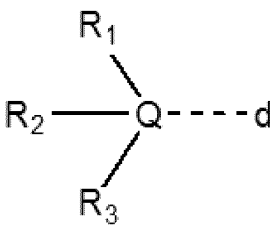


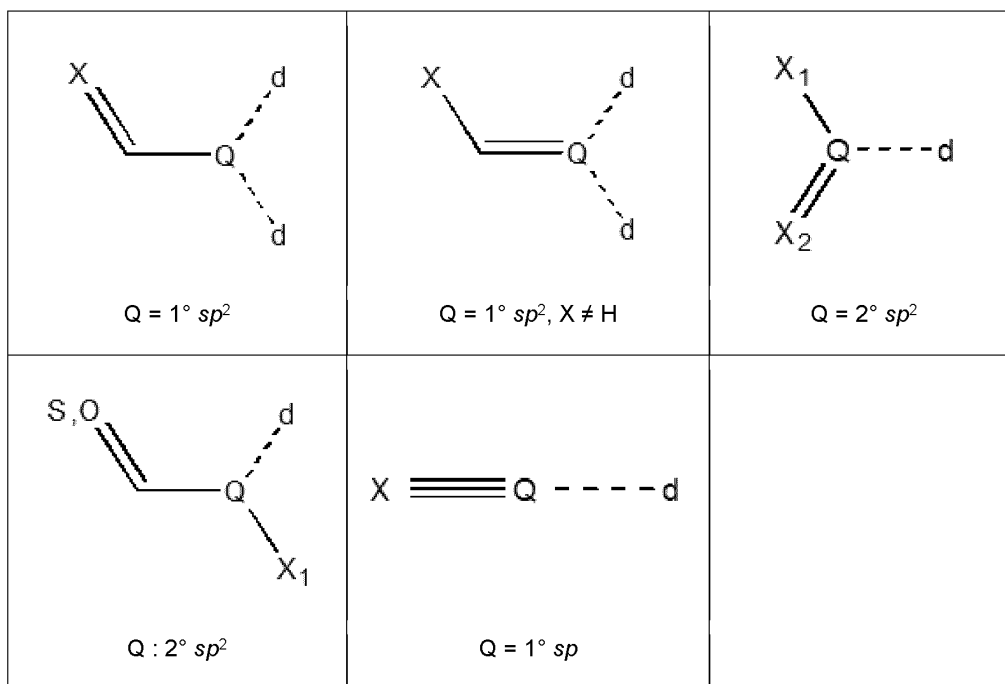
Projected**Don2:** Projected Donor**Acc2:** Projected Acceptor

- 5 *Projected* annotations are (typically) located along implicit lone pair or implicit hydrogen directions and are used to annotate the location of possible hydrogen bond or metal ligation partners, or possible R-group atom locations.

Projected **Don2** annotations are added according to the hybridization and the heavy atom coordination of the donor. In the following table the **d** denotes a **Don2** feature.

10

Table 1 Projected donors		
 $Q = 1^\circ sp^3$, $X > 1\text{-coord}$, $Y \neq H$	 $Q = 2^\circ sp^3$	 $Q = 3^\circ sp^3$



Hydrogen bond **Acc2** projected annotations are added to those heavy atoms that qualify as H-bond acceptors (see above) and are given **Acc** annotations. The **Acc2** projected annotations are added in the same locations as those for the **Don2** projected annotations and according to the same rules.

- 5 (That is, the donors and the acceptors are projected using the same angles and the same distances.)
An atom that is both a **Don** and an **Acc** will be annotated with “Don2&Acc2” projected annotations.

- Projected annotations such as **Don2**, and **Acc2** are located at potential heavy atom positions. For example, **Don2** indicates a potential hydrogen bond partner heavy atom. Realistically, this partner atom cannot have too much overlap with any of the atoms of the molecule generating the projected
10 annotation. This condition depends on the particular conformation of a molecule and cannot be reliably predicted by topological means. Consequently, a *solvent exposure test* must be applied to validate any hits resulting from a Pharmacophore Search, *i.e.*, a test to verify that applicable projected features are not covered by other parts of the conformation (that would prevent the putative projection atom from occupying the intended position).

- 15 Centroid

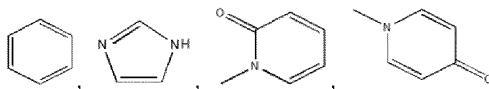
Aro Aromatic

Hyd Hydrophobic

Centroid annotations (Aro, Hyd) are located at the geometric center of a subset of the atoms of a molecule.

Aro annotation centroids are used for aromatic and pseudo aromatic rings. The **Aro** annotation centroid is placed at the centroid of each aromatic ring (e.g. two centroids in naphthalene).

The definition of aromaticity is generous (a Daylight-style definition) in which each ring is treated in isolation and a Hückel $4n+2$ rule is applied. C=O carbons count 0 electrons, otherwise C=R count as 1; N=X nitrogens count 1 and >N- nitrogens and -O- oxygens count 2 electrons. Thus, the following are treated as (pseudo) aromatic and are given an **Aro** annotation centroid in the center of the ring:



Hydrophobic atoms are annotated with **HydA** and hydrophobic centroids are annotated with **Hyd**.

Hydrophobic groups are determined by graph theoretic algorithms.

The fundamental rules for deciding whether an atom is hydrophobic are summarized below:

1. Nitro nitrogen atoms (not in nitrate anions) are hydrophobic.
2. Divalent sulfur atoms with two heavy neighbors bonded only to carbon or sulfur are hydrophobic.
3. Halogens are hydrophobic.
4. Carbon atoms are hydrophobic except
 - a) aliphatic carbons π bonded to non-carbon atoms; or
 - b) π carbon atoms adjacent to univalent oxygen; or
 - c) aromatic carbon adjacent to aromatic oxygen in 5-rings; or
 - d) carbon atoms adjacent to two or more {N,O} atoms; or
 - e) anionic carbons in c1cccc1;

The assignment of hydrophobic annotations proceeds by first applying the preceding hydrophobic atom typing rules but leaving out fluorine atoms on the grounds that they are small and should not affect annotation placement.

The Unified scheme provides an atom-centered hydrophobic annotation, **HydA** and a centroid hydrophobic feature **Hyd**. The **HydA** annotation is used for hydrophobic atoms that are deemed to have sufficiently high (potential) exposure to a potential receptor. This means that, for example, *sp*³ carbons with 4 heavy neighbors are not marked (since they are buried) and aromatic carbons with two heavy neighbors and two *ortho* substituents are not annotated.

The **Hyd** annotations are assigned by a procedure that groups connected hydrophobic atoms and assigns centroids weighted by an estimate of the likely exposed surface area of each hydrophobic atom; that is, the **Hyd** centroid will be placed closer to more exposed hydrophobic atoms in a hydrophobic group.

5 The following grouping algorithm is used:

1. **Strip Fluorines.** Remove all fluorines that are not bonded to {H,F} from further consideration. Note that estimates of exposed surface area use a fluorine suppressed molecule.

2. **Rings.** Find all 5-, 6-, 7-, and 8-member rings that are not composed of smaller rings. For each such ring, extract each contiguous stretch of hydrophobic atoms with at least three atoms that
10 have a sufficiently high total exposed surface area, and generate a surface area weighted centroid annotation. Remove all annotated ring atoms from further consideration.

3. **Components.** Find all connected components (single-linkage clusters) among the remaining hydrophobes and remove from consideration those clusters with an exposed surface area sum deemed too small (less than a -CH₂- group).

15 4. **Small Components.** For the remaining hydrophobic components with three or fewer atoms, generate an exposed surface area weighted centroid annotation.

5. **Large Components.** Identify the center of the component graph and generate a weighted centroid at the center including its neighbors. Remove the annotated atoms, splitting the component and apply the Small Components step and/or the Large Components step (recursively) to group the
20 hydrophobes.

The MCIM compounds described herein conform to a pharmacophore model as described herein. That is, the MCIM compound as annotated by *Molecular Operating Environment (MOE) 2022* unified annotation scheme comprises 4 or more pharmacophore features which conform to the pharmacophore model represented in Figure 16, and Tables 8-A, 8-B and 8-C. Compounds which do
25 not conform to the pharmacophore model, e.g. rotenone, IACS-010579, are not MCIM compounds.

The MCIM compounds for use in combination with an anti-fibrotic compound described herein conform to a pharmacophore model as described herein.

Inflammatory and/or progressive diseases

30 As described herein, a combination of the mitochondrial Complex I modulator (MCIM) compound with an anti-fibrotic compound is suitable for use in treating inflammatory and/or progressive diseases associated with fibrosis.

Fibrosis is the result of excess deposition of extracellular matrix components in affected tissues. Excess deposition of extracellular matrix components may result in the overgrowth, hardening and/or

scarring of the affected tissue. Major tissues which may be affected by fibrosis include lung, liver, kidney, heart, vasculature, eye, skin, intestine, pancreas, brain and the bone marrow.

Following tissue damage, which may result from various stimuli including, for example, infection, autoimmune reactions, and mechanical injury, typically the repair process involves replacing the damaged cells with cells of the same lineage. The repair phase also includes fibroplasia, a process in which connective tissues replace normal parenchymal tissue. While fibroplasia is normally beneficial, at least in the early stages, this process becomes pathogenic when it is not controlled adequately and excess extracellular matrix is deposited in the affected tissue/organ. Pathogenic fibroplasia (fibrosis), for example as seen in idiopathic pulmonary fibrosis, liver cirrhosis (e.g. as may be seen in NAFLD/NASH), cardiovascular fibrosis, systemic sclerosis and nephritis (e.g. as a result of chronic kidney disease) can lead to organ failure and ultimately death.

Fibrosis is associated with a number of inflammatory diseases including, for example, rheumatoid arthritis (RA), psoriatic arthritis, inflammatory arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, reactive arthritis, osteoarthritis, inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis. Arthritic diseases, for example, may be associated with pulmonary fibrosis and, as such, in some embodiments, the compositions may be used to treat pulmonary fibrosis associated with arthritis.

Inflammatory bowel diseases (IBD), for example Crohn's disease and ulcerative colitis, may be associated with fibrosis of gastrointestinal tissues. In some embodiments, the compositions may be used to treat fibrosis of gastrointestinal tissues. IBD may be associated with pulmonary fibrosis and, as such, in some embodiments, the compositions may be used to treat pulmonary fibrosis associated with IBD and/or fibrosis of gastrointestinal tissues.

Fibrosis of the liver is associated with a number of conditions including, for example, NAFLD/NASH, alcohol related liver disease, chronic hepatitis (hepatitis B and C), and autoimmune liver disease. Late-stage fibrosis of the liver, known as cirrhosis, is characterised by extensive scar tissue accumulation throughout the liver, with liver cells proliferating in pockets (nodules). Untreated, cirrhosis can be fatal. In some embodiments, the compositions may be used to treat liver fibrosis, including cirrhosis.

Chronic kidney disease (CKD) may be associated with kidney fibrosis. Kidney fibrosis may be characterised by tubulointerstitial fibrosis and glomerulosclerosis. In some embodiments, the compositions may be used to treat chronic kidney disease, tubulointerstitial fibrosis and/or glomerulosclerosis.

The disease may be a chronic progressive disease associated with fibrosis of the affected tissue(s), such as: interstitial lung disease (ILD), idiopathic pulmonary fibrosis (IPF); progressive fibrosing ILD; pulmonary fibrosis, systemic scleroderma associated ILD (SSc-ILD); liver fibrosis; non-alcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD); kidney fibrosis; chronic kidney

disease (CKD); cardiac fibrosis; ischaemia reperfusion injury; heart failure with reduced ejection fraction, heart failure with preserved ejection fraction; myelofibrosis; retroperitoneal fibrosis; atherosclerosis; myocardial infarction; stroke; neurodegenerative disease; multiple sclerosis; fronto-temporal dementia (FTD); amyotrophic lateral sclerosis (ALS), Alzheimer's disease

5 (AD); osteoarthritis; endometriosis.

The disease may be an autoimmune disease in which fibrosis is a symptom, such as: rheumatoid arthritis (RA); psoriatic arthritis; ankylosing spondylitis; spondyloarthritis; reactive arthritis; infectious arthritis; systemic lupus erythematosus; scleroderma; juvenile idiopathic arthritis; psoriasis; systemic lupus erythematosus; lupus nephritis; uveitis; systemic sclerosis; scleroderma; hepatitis; Sjogren's

10 syndrome; inflammatory bowel disease; ulcerative colitis; Crohn's disease; multiple sclerosis; atherosclerosis; chronic obstructive pulmonary disease (COPD); uveitis; allergic disease (including, e.g., atopy, allergic rhinitis, atopic dermatitis, anaphylaxis, allergic bronchopulmonary aspergillosis, allergic gastroenteritis, hypersensitivity pneumonitis); type I diabetes; celiac disease; oophoritis; primary biliary cirrhosis; insulin-resistant diabetes; Behçet's disease; myasthenia gravis; autoimmune

15 polyneuritis; pemphigus; rheumatic carditis; Goodpasture's syndrome; postcardiotomy syndrome; polymyositis; dermatomyositis; irritable bowel syndrome; pancreatitis; gastritis, chronic pulmonary inflammation; pulmonary alveolitis; polycystic kidney disease; cryopyrin-associated periodic syndrome (CAPS); Muckle-Wells Syndrome; Guillain-Barre syndrome; chronic inflammatory demyelinating polyneuropathy; dermatitis; atopic dermatomyositis; Graves' disease; autoimmune

20 (Hashimoto's) thyroiditis; bronchitis; cystic fibrosis; pulmonary embolism; sarcoidosis; emphysema; respiratory failure; acute respiratory distress syndrome; BENTA disease; or polymyositis; SSC-ILD, hidradenitis suppurative, alopecia areata, atopic dermatitis.

Evaluating disease repair in humans

Chronic autoimmune diseases are amenable to treatment with a combination of the Complex I

25 modulator (MCIM) compound as described herein with an anti-fibrotic compound. Such diseases include fibrosis associated with RA, IBD, Ulcerative colitis (UC), multiple sclerosis (MS), Psoriatic arthritis (PsA), and psoriasis, and fibrotic conditions such as pulmonary fibrosis, liver fibrosis and chronic kidney disease. As described herein, the MCIM compound can elicit tissue repair and disease healing.

30 In IBD, repair/healing can be clinically assessed using gut transit time, occult blood, endoscopy, histopathology, electrolytes and/or by measuring biomarkers such as pANCA, ASCA, GP2, CUZD1, CHI3L1, GM-CSF, ACA, PS/PT, ALCA, ACCA, AMCA, OmpC, I2, CBir1, Laminarin, Chitin, IFI16, IL-1 β , IL-6, IL-8, IL-9, IFN- γ , TNF, CCL2, IL-22, IL-2, and/or IL-6, as disclosed in Chen *et al* (2020) which is incorporated herein in its entirety by reference. Repair/healing can also be clinically assessed

35 using ultrasound elastography, diffusion weighted MRI, or cross-sectional imaging (by ultrasonography, computed tomography, magnetic resonance or enterography) as disclosed in Sousa

and Magro (2023) which is incorporated by reference in its entirety. In some embodiments, repair/healing is characterised by a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% reduction in total lesion area.

5 In pulmonary conditions, repair/healing can be clinically assessed using high-resolution computed tomography (HRCT), MRI, exacerbation frequency, measuring biomarkers, or pulmonary surfactant proteins, or using any combination of the foregoing. For example, in pulmonary fibrosis biomarkers which may be measured include ATF3, PPP1R15A, ZFP36, SOCS3, NAMPT, GADD45B, COL15A1, GIMPAP6, JAM2, LMO7, TSPAN13, LAMA3, GDF15, FGF-21, MUC1 and ANXA3, as disclosed in Maghsoudloo *et al* (2020) which is incorporated herein in its entirety by reference. In some
10 embodiments, repair/healing is assessed using HRCT or MRI. In some embodiments, repair/healing is characterised by a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% reduction in total lesion area. In some embodiments, repair/healing is assessed using HRCT or MRI.

15 In liver fibrosis and steatohepatitis such as NASH and NAFLD, repair/healing can be clinically assessed using imaging to measure liver distension, biopsy (Ishak score, Metavir score and/or Knodell score) and histopathology, liver function tests such as measures of albumin, total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), bilirubin, lactate dehydrogenase (LD) or prothrombin time (PT) or by measuring biomarkers, or using any combination of the foregoing. For example, in liver fibrosis biomarkers which may be measured
20 include Type IV collagen, laminin, MMPs, TIMPs, YKL-40, P3NP, TGF- β 1, MFAP-4, or combinatorial biomarkers such as APRI, AST/ALT ratio, ELF index and/or fibro index, among others (Nallagangula KS et al, 2018 which is incorporated herein in its entirety by reference). In some embodiments, repair/healing is characterised by a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% reduction in total lesion area.

25 In renal fibrosis and chronic kidney disease, repair/healing can be clinically assessed using histological assessment of biopsy samples, kidney functional tests such as glomerular filtration rate (GFR), estimated GFR (eGFR), or by measuring biomarkers, or using any combination of the foregoing. Non-invasive imaging techniques may also be used to assess repair/healing, such
30 ultrasonography, strain elastography, shear wave elastography, and MRI (Jian et al, 2023, which is incorporated by reference in its entirety). For example, in kidney fibrosis biomarkers which may be measured include creatinine, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), *N*-acetyl- β -D-glucosaminidase (NAG), liver-type fatty acid binding protein (L-FABP), and uromodulin (UMOD) (Lousa, I et al, 2021 which is incorporated herein in its entirety by reference). In some embodiments, repair/healing is characterised by a 10%, 15%, 20%, 25%, 30%,
35 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% reduction in total lesion area.

In some embodiments, tissue repair, disease regression, disease control (which includes, for example, preventing or slowing disease progression), increase in reparative cells, decrease in destructive cells and/or reduction in cytokine production from peripheral mononuclear cells or lymphoid cells (such as T-, B- or NK-cells) can be determined as defined herein. For instance, in
5 some embodiments, the MCIM compound reduces the levels of inflammatory mediators such as $\text{TNF}\alpha$, eSEL, CD38, CD40, CD69, sIgG, sIL-17A, sIL-17F, sIL-2 and/or sIL-6 that are produced by a target cell. In some embodiments, the MCIM increases collagen type IV production. In some
10 embodiments, the MCIM compound increases ETC efficiency, without an increase in biomass. In some embodiments, the MCIM compound decreases ETC efficiency. In some embodiments, the MCIM compound reduces cellular proliferation without reducing ATP concentration/cell and viability, dependent on the environmental composition such as in the absence of pyruvate. In some
15 embodiments, the MCIM compound induces an adaptive/repair response under conditions of metabolic stress conditions. For instance, the MCIM compound modulates Complex I activity and attenuates high energy processes such as proliferation and/or differentiation and concurrently induces an adaptive/repair response by increasing the production of pro-angiogenic/repair factors such as VEGF to restore tissue metabolic homeostasis, particularly under metabolic stress conditions. In
20 some embodiments, the MCIM compound reduced cell viability, particularly in cell types which are heavily dependent on Complex I metabolism and lack the metabolic flexibility to adapt. Each of these effects on tissue repair, disease control disease regression, increase in reparative cells, decrease in destructive cells and/or reduction in cytokine production from pro-inflammatory myeloid cells can be determined by comparing the effect in the presence and absence of the MCIM compound.

In some embodiments, lung fibroblasts are used as a target cell to determine the effect of the MCIM compound. For example, cellular adaptation may be determined in human primary lung fibroblasts, e.g. by measuring vascular endothelial growth factor (VEGF) secretion. (VEGF can be induced in a
25 cell that is not receiving enough oxygen or nutrients to support ATP production. This can indicate how metabolic signalling pathways interact and integrate with angiogenic signalling events and repair). In some embodiments, VEGF secretion can be measured by plating primary human lung fibroblasts at 2×10^3 cells/well in 96-well plates in 100 μL DMEM complete medium with: 1 g/L glucose and 110 mg/L pyruvate; or 1 g/L glucose without pyruvate (each supplemented with 1% penicillin-
30 streptomycin and 10% heat inactivated foetal bovine serum), and incubating in a humidified 37°C incubator with 5% CO_2 for 24 hours. The test compound is prepared as a 10x final concentration solution in culture medium and added to final concentrations before further incubating the cells at 37°C / 5% CO_2 for 24 hours. VEGF secretion is measured in the cell supernatants using Quantikine® ELISA Human VEGF kits according to the manufacturer's instructions. Absorbance at 450 nm is
35 measured on a BMG Plate reader (CLARIOstar plus) using pathlength correction. Background absorbance is measured at 540 nm. Preferably, treatment with the test compound results in an increase in VEGF secretion in the absence of pyruvate, but not in the presence of pyruvate as a

metabolic substrate for the cell. This indicates the induction of an adaptive/repair response under conditions of metabolic stress conditions via the attenuation of highly energy-consuming processes such as proliferation and concurrent production of pro-angiogenic/repair factors such as VEGF to restore tissue metabolic homeostasis.

- 5 Disease control may be achieved by treatment with the compositions disclose herein. Disease control can comprise inhibition of disease progression and/or control of disease symptoms. Inhibition of disease progression includes prevention of disease progression and slowing down disease progression. Standard methods in the art may be employed to determine disease progression.

- 10 In some embodiments, a histopathological assessment can be used to determine the effect of the MCIM compound in combination with an anti-fibrotic compound on lung fibrosis: Histopathological assessment of the biological effects of a combination of an MCIM compound and an anti-fibrotic compound on fibrosis may be performed on lung sections stained with Masson's Trichome (MT) to assess fibrosis using the Ashcroft score, and Haematoxylin and Eosin (H&E) to assess parameters of vascular inflammation, bronchiolar inflammation, neutrophil inflammation, lymphoid infiltration,
15 monocyte/macrophage infiltration, pneumonitis, apoptosis/necrosis, AT2 cell hypertrophy and AT2 cell hyperplasia. The histology outputs are assessed on a scale of 0-5 as follows:

0: normal. 1: minimal, focal. 2: moderate, focal. 3: moderate, multi-focal or diffuse. 4: marked, focal. 5: marked, multi-focal or diffuse. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound reduces the average score compared with subject treated with a negative
20 control. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound prevents disease progression and can be indicated by no further increase in the average score compared following treatment.

- In some embodiments, to assess fibrosis in the lung, lung sections can be graded from 0 (normal lung) to 8 (total fibrous obliteration of the section) as described in Hubner *et al* (2008). The Ashcroft
25 score is assessed on a scale of 0-8 as follows:

- 0: Normal lung. 1: Minimal fibrous thickening of alveolar or bronchiolar walls. 2. 3 Moderate thickening of walls without obvious damage to lung architecture. 4. 5: Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses. 6. 7: Severe distortion of structure and large fibrous areas; "honeycomb lung" is placed in this category. 8: Total
30 fibrous obliteration of the field. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound reduces the Ashcroft score in a subject relative to the Ashcroft score in a patient before receiving treatment. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound reduces the area of fibrotic lesions by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% in a subject.

- 35 In some embodiments, a histopathological assessment can be used to determine the effect of the MCIM compound in combination with an anti-fibrotic compound on IBD: Biopsy tissue sections can

be stained with Haematoxylin and Eosin (H&E) and parameters of inflammation, mucosal erosion, epithelial hyperplasia, epithelial metaplasia, mucus cell metaplasia, and fibroplasia are assessed on a scale of 0-5 as follows:

0: normal. 1: minimal, focal. 2: moderate, focal. 3: moderate, multi-focal or diffuse. 4: marked, focal.

- 5 5: marked, multi-focal or diffuse. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound reduces the average score compared with subject treated with a negative control, or either an MCIM compound or an anti-fibrotic compound alone. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound prevents disease progression and can be indicated by no further increase in the average score compared following treatment. In some
- 10 embodiments, the combination of an MCIM compound with an anti-fibrotic compound prevents, inhibits or reverses fibrosis of gastrointestinal tissue in patients with IBD. In some embodiments, the MCIM compound in combination with an anti-fibrotic compound reduces the area of fibrotic lesions by 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% in a subject.
- 15 Inhibition of disease progression, for example in subjects suffering from IBD, may be determined by the histopathological methods described above, or any other suitable method known in the art. For example, inhibition of disease progression may be determined by comparing histopathological results from a subject before and after treatment with the compositions disclosed herein. Inhibition of disease progression may be indicated by the histopathological score being stable (i.e. not changing)
- 20 over time following treatment. Inhibition of disease progression may be indicated by the histopathological score progressing at a slower rate following treatment compared to disease progression before treatment.

Pharmaceutical combinations

- 25 A pharmaceutical combination as disclosed herein refers to a combination comprising two or more active compounds. A pharmaceutical combination may comprise two or more compositions each comprising at least one active compound. In such embodiments, the two or more compositions may be administered separately, sequentially or simultaneously. For example, the pharmaceutical combination may comprise a first composition comprising an MCIM compound, such as HMC-C-01-A,
- 30 and a second composition comprising an anti-fibrotic compound, such as nintedanib, pirfenidone, tulisokibart, or nerandomilast. The first and second composition may be administered separately, sequentially or simultaneously.

- Alternatively, a pharmaceutical combination may comprise a single composition comprising two or more active compounds. For example, the combination may comprise a single composition
- 35 comprising an MCIM compound and an anti-fibrotic compound. In some embodiments, the MCIM compound comprises HMC-C-01-A. In some embodiments, the anti-fibrotic compound comprises one

or more compounds selected from nintedanib, pirfenidone, tulisokibart, and nerandomilast. In some embodiments, the composition comprises the MCIM compound HMC-C-01-A and the anti-fibrotic compound nintedanib. In some embodiments, the composition comprises the MCIM compound HMC-C-01-A and the anti-fibrotic compound pirfenidone. In some embodiments, the composition
5 comprises the MCIM compound HMC-C-01-A and the anti-fibrotic compounds nintedanib and pirfenidone.

Pharmaceutical compositions

Medicaments and pharmaceutical compositions according to the aspects disclosed herein may be formulated for administration by a number of routes, including but not limited to, parenteral i.e. non-oral route (for example, by injection: sub-cutaneous, intravenous, intra-arterial, intramuscular, or intratumoural; by topical or intradermal; by inhalation or intranasal; or by rectal), and peroral i.e. oral route. The medicaments and compositions may be formulated in a solid, semi-solid, or liquid dosage form. The pharmaceutical compositions according to this invention may be delivered by a route which facilitates exposure in the systemic circulation or by a route or method which gives rise to localised,
15 targeted, delivery of the active compounds to a selected region in the body. The pharmaceutical compositions according to this invention may also be administered to humans or animals.

Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. For example, the rate of release of the active compounds from the pharmaceutical composition may be immediate, sustained, extended, controlled, pulsatile or follow a pattern that is optimal for the intended therapeutic application. The frequency of dosing can be fixed or variable depending on the rate of drug release, the required level in the systemic circulation or at the target site to provide the desired therapeutic effect. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of physicians and other medical
20 doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 23rd Edition, 2020, pub. Lippincott, Williams & Wilkins.

Pharmaceutical compositions may be prepared using a pharmaceutically acceptable "carrier" composed of materials, also known as excipients, that are generally considered safe and pharmacologically inert. "Pharmaceutically acceptable" refers to molecular entities and compositions that are "generally regarded as safe - GRAS", e.g., that are physiologically tolerable and do not typically produce adverse or untoward effects when administered to a human. In some embodiments, this term refers to molecular entities and compositions approved by a regulatory agency of the US
30 federal or a state government, as the GRAS list under section 204(s) and 409 of the Federal Food, Drug and Cosmetic Act, that is subject to premarket review and approval by the FDA or similar lists,

the U.S. Pharmacopeia or another generally recognised pharmacopeia for use in animals, and more particularly in humans.

The term "carrier" refers to excipients that serve one or more functions in the pharmaceutical compositions, for example, as diluents, binders, lubricants, glidants, disintegrants, solubilisers or solubility enhancers, stabilisers, preservatives, taste-masking or taste-modifying agents, flavourant, and/or colourant. The pharmaceutical compositions, when formulated as a solid dosage form, may further be uncoated or coated with an outer layer to impart aesthetic features, physical protection, enhance the physical and/or chemical stability of the active compounds or other constituents of the pharmaceutical compositions, and/or alter the rate of dissolution and release of the active compounds from the pharmaceutical compositions. The pharmaceutical compositions, when formulated as a parenteral injectable dosage form, may additionally contain aqueous or non-aqueous solvents, co-solvent mixtures, buffering agents, surfactants, tonicity modifying agents, chelating agents, pH modifiers, viscosity modifiers, and/or suspending agents. The pharmaceutical compositions, when formulated as a parenteral dosage form for inhalation, may additionally contain particle carriers for the pulmonary delivery of the active compounds, absorption penetration enhancers, and/or propellants, dependent on the inhalation delivery device to be used. Those with skill in the art are familiar with such pharmaceutical carriers, and excipients therein, and methods of compounding these carriers and excipients into pharmaceutical compositions. Suitable excipients for use in the pharmaceutical compositions can be found in standard pharmaceutical texts, for example, Handbook of Pharmaceutical Excipients, 9th edition, Pharmaceutical Press, American Pharmaceutical Association, 2020.

The choice of the pharmaceutical carrier is dependent on the dosage form type, the dose, intended route of delivery, the physicochemical properties of the active compounds, and the required bioavailability and/or exposure profile. When used, the excipients of the pharmaceutical compositions should not adversely affect the stability, bioavailability, safety, and/or therapeutic efficacy of the active compounds, i.e. the MCIM compound and/or the anti-fibrotic compound used in the pharmaceutical compositions. Thus, the skilled person will appreciate that pharmaceutical compositions are provided wherein there is no significant incompatibility between any of the components of the dosage form.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound(s). The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g., active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

Unless otherwise specified, a reference to a particular compound also includes the solvate forms thereof.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound(s), for example, a pharmaceutically-acceptable salt of an MCIM compound, for example HMC-C-01-A, nintedanib, pirfenidone, tulisokibart, and/or nerandomilast. Examples of pharmaceutically acceptable salts are discussed in Berge et al., 1977, "Pharmaceutically Acceptable Salts," J. Pharm. Sci., Vol. 66, pp. 1-19.

For example, if the compound is anionic, or has a functional group which may be anionic (e.g., COOH may be COO⁻), then a salt may be formed with a suitable cation. If the compound is cationic, or has a functional group which may be cationic (e.g., NH₂ may be NH₃⁺), then a salt may be formed with a suitable anion.

Unless otherwise specified, a reference to a particular compound also include salt forms thereof.

For example, nintedanib may be present in a salt form wherein the anion is esilate.

SPECIFIC COFORMULATIONS

The pharmaceutical compositions according to this invention include, but are not limited to, dosage forms wherein the active compounds are co-formulated uniformly in a common excipient base, such as a conventional single-layer tablet or conventional powder-in-capsule for oral administration; a solution, suspension or dispersed system for parenteral administration; a lotion, cream, ointment for topical administration; a transdermal patch, microneedle or autoinjector system for transdermal administration; a dry powder, solution or suspension for inhalation administration; and a solution or suspension for intranasal administration.

Alternative pharmaceutical compositions, particularly useful for solid dosage forms for oral administration, are provided according to this invention wherein the active compounds are physically separated within a single pharmaceutical composition to avoid the potential for physical and chemical interactions and incompatibilities. Such pharmaceutical compositions include, but are not limited to, a bi-/multi-layer tablet, wherein the formulation of each layer is optimised for each active compound, which can be combined in a single pharmaceutical composition by conventional tablet compression. Such pharmaceutical compositions may also include a single-layer or bi-/multi-layer tablet wherein one of the active compounds is contained within a layer that is coated on the outside of the single-layer or bi-/multi-layer compressed tablet core, thus, providing a physical separation of the active compounds and their associated excipient systems. A further alternative pharmaceutical composition according to this invention is a multi-particulate capsule, wherein the formulation of each active compound in a particulate/granular form can be optimised, then combined and encapsulated into a conventional capsule as a single pharmaceutical composition.

The methods for the preparation of the pharmaceutical compositions accordingly to this invention are well known to the skilled person in the art and are well described in standard pharmaceutical formulation textbooks, such as Remington, The Science and Practice of Pharmacy, Editor: Adeboye

Adejare, 23rd edition, 2020, publisher: Elsevier, Aulton's Pharmaceutics, The Design and Manufacture of Medicines, editors: Kevin Taylor and Michael Aulton, 6th edition, 2021 publisher: Elsevier, and Lachman/Liebermans: The Theory and Practice of Industrial Pharmacy, Editors: Roop Khar, SP Vyas, Farnham Ahmad, Gaurav Jain, 4th edition, 2014, publisher: CBS.

5

Sequence Listing

Human NDUFS2 amino acid sequence SEQ ID NO: 1:

MAALRALCGFRGVAAQVLRPGAGVRLPIQPSRGVRQWQPDVEWAQQFGGAVMYPKETAHWKPP
PWNDVDPPKDTIVKNITLNFPGQHPAAHGVLRLVMELSGEMVRKCDPHIGLLHRGTEKLEIYKTYLQA
10 LPYFDRLDYVSMMCNEQAYSLAVEKLLNIRPPPRAQWIRVLFGEITRLNHIMAVTTHALDLGAMTPFF
WLFEEREKMFEFYERVSGARMHAAYIRPGGVHQDLPLGLMDDIYQFSKNFSLRLDELEELLTNNRIW
RNRTIDIGVVTAEALNYGFSGVMLRGSGIQWDLRKTQPYDVYDQVEFDVPVGSRGDCYDRYLCRV
EEMRQSLRIIAQCLNKMPPEGIKVDDAKVSPPKRAEMKTSMESLIHHFKLYTEGYQVPPGATYTAIEA
PKGEFGVYLVSDGSSRPYRCKIKAPGFAHLAGLDKMSKGHMLADVVAIIGTQDIVFGEVD R

15 Human NDUFS7 amino acid sequence SEQ ID NO:2:

MAVLSAPGLRGRILGLRSSVGPVQARGVHQSVATDGPSTQPALPKARAVAPKPSSRGEYVVAK
LDDLNVWARRSSLWPMTFGLACCAVEMMHMAAPRYDMDRFGVVFRASPRQSDVMIVAGTLTNKMA
PALRKVYDQMPEPRYVSMGSCANGGGYYHYSYSVVRGCDRIVPVDIYIPGCPPTAEALLYGILQLQ
RKIKRERRRLQIWYRR

20 The features disclosed in the foregoing description, or in the following claims, or in the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for obtaining the disclosed results, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

While the invention has been described in conjunction with the exemplary embodiments described
25 above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention.

For the avoidance of any doubt, any theoretical explanations provided herein are provided for the
30 purposes of improving the understanding of a reader. The inventors do not wish to be bound by any of these theoretical explanations.

Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise” and “include”, and variations such as “comprises”, “comprising”, and “including” will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

- 5 It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of
- 10 the antecedent “about,” it will be understood that the particular value forms another embodiment. The term “about” in relation to a numerical value is optional and means for example +/- 10%.

Examples

EXAMPLE 1 – BioMAP profiling

- 15 A high-throughput integrative biology platform (BioMAP®) was used to profile the effect of MCIM compounds on multiple disease-associated regulatory pathways. BioMAP® has been developed as a method to assess efficacy, safety and the mechanism of action of drugs in multiple human cell types stimulated with inflammatory challenges as described in US6656695, which is incorporated herein in its entirety. The BioMAP system reflects human disease pathology and has the ability to detect and
- 20 distinguish the effects of approved drugs and investigational human therapeutic compounds. BioMAP technology enables rapid determination of efficacy, side effects and mechanism of action of drug candidates.

BioMAP® provides an unbiased, target-agnostic and data-driven approach to understanding compound or combination therapy impact on human disease models and translational biomarkers.

- 25 The system is validated with clinically approved drugs and known test agents. The principle of the assay is to test compounds in human primary cell-based disease systems, and compare the data with a Reference Database of over 4,500 compounds. The profile of a compound can be compared against a reference compound to see if the biological activity of the test item is differentiated from the reference.

- 30 The activity of MCIM compounds was determined in three BioMAP systems; Fibrosis panel, Autoimmune panel HDFSAg and Diversity Plus.

Thirteen primary human cell and co-culture assays were used to assess the effects of MCIM compounds on clinically relevant protein biomarkers of inflammation, cell growth, and fibrosis as part of the quality controlled BioMAP® Diversity PLUS, commercially available service (Eurofins DiscoverX

- 35 Corporation, Freemont, CA, USA; for full details <https://www.discoverx.com>). Briefly, the BioMAP®

panels consist of human primary cell-based systems designed to model different aspects of the human body in an in vitro format. The 12 cell assays utilised in the Diversity PLUS panel allow characterisation of test agent responses in an unbiased way across a broad set of systems modelling various human disease states compared to historical controls. BioMAP® panels are constructed with primary cell types from healthy human donors, with stimuli (such as cytokines or growth factors) added to capture relevant signalling networks that naturally occur in human tissue or pathological conditions.

MCIM Compounds were tested in these assays at four concentrations: 4000 nM, 1300 nM, 400 nM & 150 nM. Human primary cells employed in the BioMAP® systems were used at passage 4 or earlier, derived from multiple donors (n = 2–6), commercially purchased and handled according to the recommendations of the manufacturers. Human blood derived CD14 + monocytes were differentiated into macrophages in vitro before being added to the LPS system (Eurofins DiscoverX Corporation).

The human cell types and stimuli used in each assay system were as follows: 3 C system [human umbilical vein endothelial cells (HUVEC) + (IL-1 β , TNF α and IFN γ)], 4H system [HUVEC + (IL-4 and histamine)], lipopolysaccharide (LPS) system [peripheral blood monocyte cells (PBMC) and HUVEC + LPS (TLR4 ligand)], Sag system [peripheral blood mononuclear cells, PBMC and HUVEC + TCR ligands], HDFSAg system [peripheral blood mononuclear cells, PBMC and human neonatal dermal fibroblasts + TCR ligands], BT system [CD19 + B cells and PBMC + (α -IgM and TCR ligands)], BF4T system [bronchial epithelial cells and human neonatal dermal fibroblasts, HDFn, + (TNF α and IL-4)], BE3C system [bronchial epithelial cells + (IL-1 β , TNF α and IFN γ)], CASM3C system [coronary artery smooth muscle cells + (IL-1 β , TNF α and IFN γ)], HDF3CGF system [HDFn + (IL-1 β , TNF α , IFN γ , EGF, bFGF and PDGF-BB)], KF3CT system [keratinocytes and HDFn + (IL-1 β , TNF α , IFN γ and TGF β)], MyoF system [differentiated lung myofibroblasts + (TNF α and TGF β)], SAEMyoF system [small airway epithelial cells and differentiated lung myofibroblasts + (TNF α and TGF β)], ReMyoF system [renal epithelial cells and differentiated lung myofibroblasts + (TNF α and TGF β)] and IMphg system [HUVEC and M1 macrophages + Zymosan (TLR2 ligand)].

Assays were derived from either single cell types or co-culture systems. Adherent cell types were cultured in 96 or 384-well plates until confluence, followed by the addition of PBMC (Sag and LPS systems). The BT system consisted of CD19 + B cells co-cultured with PBMC and stimulated with a BCR activator and low levels of TCR stimulation. Test agents prepared in either DMSO (small molecules; final concentration $\leq$ 0.1%) or PBS (biologics) were added at the indicated concentrations 1 h before stimulation, and cells remained in culture for 24 h or as otherwise indicated [48 h, MyoF system; 72 h, BT system (soluble readouts); 168 h, BT system (secreted IgG)]. Each assay plate contained negative controls (e.g., non-stimulated conditions) and vehicle controls (e.g., 0.1% DMSO) appropriate for each system. Direct ELISA was used to measure biomarker levels of cell-associated and cell membrane targets. Soluble factors from supernatants were quantified using either HTRF® detection, bead-based multiplex immunoassay, or capture ELISA. Overt adverse effects of

(compounds) on cell proliferation and viability (cytotoxicity) were detected by sulforhodamine B (SRB) staining for adherent cells, and alamarBlue® reduction for cells in suspension. For proliferation assays, individual cell types were cultured at subconfluence and measured at time points optimised for each system (48 h: 3 C and CASM3C systems; 72 h: BT and HDF3CGF systems; 96 h: HDFSAg and Sag system). Cytotoxicity for adherent cells was measured by SRB (24 h: 3 C, 4H, LPS, Sag, HDFSAg, BF4T, BE3C, CASM3C, HDF3CGF, KF3CT, and IMphg systems; 48 h: MyoF system), and by alamarBlue® staining for cells in suspension (24 h: HDFSAg and Sag system; 42 h: BT system) at the time points indicated.

Biomarker measurements in treated samples were divided by the average of the control samples (at least 6 vehicle controls from the same plate) to generate a ratio that was then log10 transformed. Significance prediction envelopes were calculated using proprietary historical vehicle control data at a 95% confidence interval. Biomarker activities were annotated when two or more consecutive concentrations change in the same direction relative to vehicle controls were outside of the significance envelope and had at least one concentration with an effect size > 20% (log10 ratio > 0.1). Biomarker key activities were described as modulated if these activities increase in some systems but decrease in others. Cytotoxic conditions were noted when total protein levels decreased by more than 50% (log10 ratio of SRB or alamar Blue® levels < -0.3) and were indicated by a thin black arrow above the X-axis. A compound was considered to have broad cytotoxicity when cytotoxicity was detected in 3 or more systems. Concentrations of test agents with detectable broad cytotoxicity were excluded from biomarker activity annotation and downstream benchmarking, similarity search and cluster analysis. Antiproliferative effects were defined by an SRB or alamar Blue® log10 ratio value < -0.1 from cells plated at a lower density and were indicated by grey arrows above the X-axis. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation.

Figure 2 shows the BioMAP profile of several MCIM compounds. (A) ABD599 tested in the full bioMAP profile shows decreased inflammation and immune modulation, and tissue remodelling with increased collagen IV levels. (B) HMC-C-01-A shows anti-inflammatory and immune modulation activity in the BT and Sag systems and (C) HMC-C-01-A in the fibrosis panels increases Collagen type IV.

Figure 3 shows the BioMAP profile of approved anti-fibrotic, nintedanib (A) and HMC-C-01-A (B) in the HDFSAg system, modelling inflammation, fibrosis and wound healing biology using fibroblasts and peripheral blood mononuclear cells. (A) nintedanib shows decreased inflammation and immune modulation, and tissue remodelling with reduced VEGF but, with no effect on the matrix Type I collagen. (B) HMC-C-01-A shows more pronounced effects on inflammation and immune modulation, and tissue remodelling with decreased MMP-1 and sVEGF, but increases in the matrix Type I collagen.

Table 2 shows the key differences between the activities regulated by the MCIM compound HMC-C-01-A and the anti-fibrotic drugs nintedanib and pirfenidone, and the experimental anti-fibrotic drug nerandomilast. HMC-C-01-A reduces inflammation (decreased M-CSF, I-TAC, sIL-6, and MCP-1), myofibroblast activation (decreased αSMA), fibrosis (decreased MMP-1, collagen I, and MMP-9), and tissue remodelling/wound healing (decreased sVEGF). Several of these activities are in common with nintedanib (decreased ITAC, sIL6, αSMA, Coll I and MMP9), pirfenidone (decreased ITAC, sIL6, αSMA, Coll I, MMP9) and tulisokibart (decreased MCP1). However, importantly, HMC-C-01-A increases the key epithelial basement membrane collagen IV which plays a critical role in restitution of the alveolar barrier (Loscertales, 2016). This is a clear point of difference between HMC-C-01-A and either of the approved anti-fibrotic agents, nintedanib or pirfenidone, or tulisokibart and points to a potential reparative effect of HMC-C-01-A on the alveolar barrier in the lung.

Table 2. Key activities regulated by various MCIM compounds and anti-fibrotic compounds in BioMAP experiments.				
	HMC-C-01-A	Nintedanib	Pirfenidone	Nerandomilast
Inflammation	decreased M-CSF , ITAC, MCP-1 , sIL6	decreased ITAC, sIL6, IL8	decreased ITAC, MCP-1, sIL6	decreased E-selectin , MCP-1 , sTNFα, IL8, sIL-10, CD69
Myofibroblast activation-related activities	decreased αSMA	decreased αSMA	decreased αSMA	Not tested
Fibrosis matrix related activities	MMP1 , MMP9	MMP9, TIMP1	Decreased PAI-1 , MMP9, TIMP1	Not tested
Tissue remodeling/wound healing activities	Decreased sVEGF Increased Coll IV	Decreased sVEGF		<i>Decreased PAI-1</i>

EXAMPLE 2 – Mitochondrial phenotype assessment in osteoclasts

The ability of mitochondria to undergo fusion and fission processes is essential to mitochondrial function and cellular health. Qualitative and/or quantitative changes in the mitochondrial reticulum are also observed under pathological conditions that are caused by inherited mutations in mitochondrial DNA or in nuclear OXPHOS genes and suggest a tight relationship between mitochondrial structure

and function. In addition, several lines of evidence suggest that the damage response of injured cells can be ameliorated by the presence of healthy mitochondria (Jin, *et al*, 2019, which is hereby incorporated by reference in its entirety). Studying mitochondrial form and function may therefore yield important insights into the potential of cells and tissues to recover from damage. Osteoclasts are a highly energetic cell type sensitive to changes in mitochondrial metabolism that are suitable for evaluating such relationships.

Peripheral blood mononuclear cells were isolated from human whole blood by differential centrifugation over Ficoll-Paque PLUS (GE Healthcare Biosciences). CD14⁺ monocytes were purified from the freshly isolated PBMCs using the CD14⁺ selection kit (StemCells. UK) by positive magnetic selection according to manufacturer's instructions. Cells were differentiated to osteoclasts by adding 1×10^6 ml⁻¹ cells in complete minimum essential medium- α supplemented with 10% heat inactivated fetal bovine serum (FBS, Invitrogen, UK), 2mM glutamine (Invitrogen, UK) 20U ml⁻¹ penicillin, 100ug/ml streptomycin (Sigma Aldrich, UK) along with 25ng ml⁻¹ recombinant human M-CSF (Peprotech, UK) and 25 ng ml⁻¹ RANKL for 6 days. On the 6th day, the cells were treated with test compound (final concentration 0.03-1 μ M, 0.05 % DMSO) or a control, rotenone (100 nM).

Adherent cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h, post fixed with 1% osmium tetroxide (Electron Microscopy Science), dehydrated in a graded series of ethanol, and embedded in Epon (Electron Microscopy Science). The embedded samples were sectioned by an ultramicrotome (Ultracut E, Richert-Jung, Leica Microsystem). Thin sections (90 nm thick) were collected on 300 mesh nickel grids and stained with uranyl acetate (Electron Microscopy Science) and lead citrate. Samples were observed by using a Zeiss EM 109 apparatus (Zeiss). Images were captured using a Nikon digital camera Dmx 170 1200F and ACT-1 software.

Representative images are shown in Figure 4. Top left panel; control cells show a heterogeneous and dynamic population of mitochondria with a good balance of fusion and fission (mitochondria (M) and endoplasmic reticulum (ER)). Top right panel; cells treated with the archetypal Complex I inhibitor, rotenone shows increased numbers of abnormal mitochondria which were more rounded and had condensed cristae with evidence of fragmentation, and evidence of lysosomes (L) close by. Middle left panel; 0.03 μ M ABD900 showed increased tubular mitochondrial with evidence of extrusion budding, consistent with an adaptive change in mitochondrial structure to increase mitochondrial area without an overt increase in organelle biomass. Dumbbell morphology, consistent with the formation of electron transport chain (ETC) super-complexes, were also observed. Middle right panel; 0.1 μ M ABD900 showed a similar profile with evidence of cristae refraction. Bottom left panel; 0.3 μ M ABD900 shows filamentous extensions in many mitochondria consistent with an attempt to enlarge cristae volume. Bottom right panel; 1 μ M ABD900 shows a heterogeneous mitochondrial population with rounded morphology and condensed cristae.

The results demonstrate that, in contrast to the classical Complex 1 blocker, rotenone, the MCIM compounds disclosed herein elicit a mitochondrial phenotype consistent with differentiation based on maturity. For example, older mitochondria, which are usually cleared by mitophagy, are retained as part of an integrated stress response. To increase ETC efficiency, without an overt increase in biomass, structural adaptation occurs to maintain cellular free energy.

EXAMPLE 3 – Cellular metabolism and viability

The *in vitro* effects of test compounds on cellular metabolism and viability were determined by incubation with human primary lung fibroblasts followed by measurement of cellular ATP concentrations and cell counts.

ATP is an organic compound which can be produced by several cellular processes such as glycolysis and oxidative phosphorylation. However, if levels of oxygen, or substrates to fuel oxidative phosphorylation are insufficient, cells can reprogramme their metabolism towards glycolysis or other pathways to maintain their availability of ATP. Depending on the environment of the cell, such changes in metabolism may be accompanied by adaptive changes in gene expression. For example, under certain culture conditions, cells may upregulate an 'adaptive response' gene known as vascular endothelial growth factor (VEGF); VEGF encodes a proangiogenic protein (VEGF), whose function to induce new blood vessel formation to seek out new sources of oxygen and nutrients. This is critical to eliciting a functional repair response. By modulating the activity of Complex I, the MCIM compounds disclosed herein modulate ATP production, and thereby induce a cellular adaptation response.

The effects of the MCIM compounds on cellular adaptation was assessed *in vitro* in human primary lung fibroblasts (HLF). Cells were cultured with different metabolic substrates- glucose, L-glutamine, and pyruvate- to explore potential changes according to the microenvironment of the cell. Cells cultured in these conditions were treated with compound at various concentrations, and intracellular ATP concentrations, nucleus counts, and VEGF secretion were measured to quantify cell viability, metabolism, and indications of adaptive response.

Human primary lung fibroblasts were plated at a concentration of 2×10^3 cells/well in 96-well plates in 100 μ L DMEM complete media (5.5 mM glucose, 2 mM L-glutamine, and 1 mM pyruvate) containing 1% penicillin-streptomycin and 10% heat inactivated foetal bovine serum. Cells were incubated at 37°C / 5% CO₂ overnight to allow cell attachment. On the day of the treatment, cell culture medium was removed, and the cells were washed three times with Gibco™ DMEM, no glucose, no L-glutamine, no pyruvate, no phenol red + 1% penicillin-streptomycin and 10% heat inactivated foetal bovine serum (termed 'basal' culture medium). Fresh cell culture supplemented with 5.5 mM glucose alone, 5.5 mM glucose and 2 mM L-glutamine, or 5.5 mM glucose, 2 mM L-glutamine, and 1 mM pyruvate was added to the wells. Test compounds were prepared as 10x final concentration solutions in each appropriate culture medium. Compounds were added to the cultures at 1x final concentration

and incubated at 37°C / 5% CO₂ for 1 or 3 days. On day 1, VEGF secretion was measured from cell culture supernatants; 72 hours post treatment, cells were assayed for nucleus counts and ATP generation.

5 To assess nucleus counts, formaldehyde was added to a final concentration of 4%. Following a further 20-minute incubation at room temperature, the medium was aspirated, the cells were washed twice with 200 µL TBS-T (1x TBS + 0.1% Tween 20) and the nuclei were stained with 50 µL of PBS containing 10% heat inactivated foetal bovine serum, 0.1% Triton-X-100 and 2 µM Hoechst dye. Following a 30-minute incubation at room temperature, protected from light, the cells were washed
10 twice with TBS-T, and cells were counted in 100 µL PBS solution using an ImageXpress Pico system with stitched plate acquisition (4x magnification), DAPI channel (50ms exposure, -3 digital confocal setting).

To assess ATP concentration, 50 µL of reconstituted ATPlite substrate solution (ATPlite 1step Luminescence Assay System, Perkin Elmer) was added to the cells. After a 5-minute incubation at room temperature on a plate shaker, luminescence was measured on a BMG Plate reader
15 (PHERAstar) using LUM plus module, gain 3000, CR 96/384 aperture spoon (type A3).

The average values across the concentrations tested were then plotted and the half-maximal inhibitory concentration (IC₅₀) for the effect on ATP or nuclei counts was calculated by fitting the data to a four-parameter IC₅₀ equation using GraphPad Prism software (v9). ATP readout per nuclei count was calculated by dividing ATPlite luminescence readout values by the Hoechst-stained nuclei count
20 number per test concentration.

To assess VEGF secretion, cell supernatants of three replicate wells were combined and VEGF secretion was measured following the Quantikine® ELISA Human VEGF kit manufacturer instructions. Absorbance at 450 nm was measured on a BMG Plate reader (CLARIOstar plus) using pathlength correction. Background absorbance was measured at 540 nm.

25 Data were normalised to a VEGF standard curve and expressed as the average of the control wells in pg/mL. Data were plotted and the IC₅₀ for the effect on VEGF secretion was calculated by fitting the data to a four-parameter IC₅₀ equation using GraphPad Prism software (v9).

The results are shown in Figure 5.

Figure 5A consists of 3 panels showing intracellular ATP (left panel), nucleus counts (middle panel)
30 and ATP readout per cell (right panel) after 72 hrs incubation with MCIM compound. In cells cultured in glucose-supplemented media (squares), there was no effect of the MCIM compound on intracellular ATP levels, nuclei count or ATP readout per cell. In cells cultured in media supplemented with glucose and glutamine (open circles), there was an increase in intracellular ATP levels and nucleus counts given vehicle compared to cells cultured with glucose alone. Treatment of cells cultured in
35 medium supplemented with glucose and glutamine with the MCIM compound reduced intracellular

ATP levels and nucleus counts, in a concentration-dependent manner, with no effect on ATP levels per cell. When the basal culture media supplemented with glucose, L-glutamine was enriched with pyruvate (filled circles), the effect of the MCIM compound on intracellular ATP and nucleus counts seen in the medium supplemented with glucose and L-glutamine alone was eliminated.

- 5 The results show that in cells cultured in basal medium containing glucose, the MCIM compound showed no cytotoxicity and had no effect on cell viability. When the cells were cultured in medium supplemented with glucose and L-glutamine, there was an increase in intracellular ATP levels and nucleus counts reflecting that L-glutamine is a precursor amino acid essential for cellular proliferation. The reduction in intracellular ATP and nucleus counts seen in the cells cultured with glucose and L-
- 10 glutamine, and lack of effect on ATP levels per cells shows that the cells were adapting to the metabolic effects of Complex I modulation by the MCIM compound by limiting highly energy-demanding processes, such as cellular proliferation, in order to maintain their intracellular levels of ATP. In addition, the reversal of this effect with the medium was supplemented further with pyruvate (glucose + L-glutamine + pyruvate condition) shows that the adaptive response observed (reduction
- 15 of cell proliferation) is dependent on nutrient availability to the cell.

Figure 5B shows that in complete medium, there is no effect of the MCIM compound on VEGF secretion (filled circles (●)), but in the absence of pyruvate in the media (open circles (O)), the MCIM compound elicited a concentration-dependent increase in VEGF secretion (half-maximal inhibitory concentration (IC_{50}) = 112 nM).

- 20 The results show that by modulating Complex I, in the absence of an excess of substrate, cells trigger an adaptive response to cause growth and repair by upregulating VEGF. The absence of an effect when pyruvate was present in the medium, shows that the adaptive repair response is also dependent on nutrient availability.

- In summary, the results show that treatment with MCIM compounds disclosed herein induce an
- 25 adaptive response to enable cells to maintain their supply of ATP. When cells are treated with MCIM compound, they attempt to restore tissue homeostasis by reducing energy-intensive activities such as proliferation and increasing production of growth factors such as VEGF. This occurs without any effect on cell viability.

30 EXAMPLE 4

- Classical Complex I inhibitors often cause cytotoxicity and cell death and despite reports of anti-tumour effects for compounds such as IACS-010579 the known inhibitors have not found use as approved therapeutics, largely due to mechanism-based toxicity (Yap, T *et al*, 2023). As such, identifying a suitable approach for complex I inhibition that provides a benefit without toxicity has
- 35 proved to be a challenge. An alternative approach which does not elicit adverse effects may have

potential benefits for the treatment of a variety of progressive diseases. In this study the inventors evaluated whether the MCIM compounds disclose herein showed differences in their cellular behaviour compared with known Complex I inhibitors, IACS-010579 and rotenone.

Human primary lung fibroblasts were plated at a concentration of 5×10^3 cells/well in 96 well plates in 100 μ L DMEM complete media (5.5 mM glucose, 2 mM L-glutamine, and 1 mM pyruvate) containing 1% penicillin-streptomycin and 10% heat-inactivated foetal bovine serum. Cells were incubated overnight at 37°C / 5% CO₂ to allow cell attachment. On the day of the treatment, cell culture medium was removed, and the cells were washed three times with Gibco™ DMEM, no glucose, no L-glutamine, no pyruvate, no phenol red + 1% penicillin-streptomycin and 10% heat inactivated foetal bovine serum (termed 'basal' culture medium). Fresh cell culture media (without pyruvate) was added to the wells. Test compounds were prepared as 10x final concentration solutions in pyruvate-free medium. Following 24-hours compound treatment (in 5% CO₂ 37°C cell incubator), spent medium was removed from all wells and were thoroughly washed thrice with basal cell culture medium. For washout assay condition, 100 μ L of pyruvate-free media was added per well. For No Washout, 90 μ L of pyruvate-free media and 10 μ L of 10x final assay concentration prepared test agents (or assay media) were added per well. Recovery of cell proliferation was measured after 24-hours incubation at 37°C / 5% CO₂ with either pyruvate-free media (without re-addition of the compound) or re-addition of compounds in pyruvate-free media.

To assess nucleus counts, formaldehyde was added to a final concentration of 4%. Following a further 20-minute incubation at room temperature, the medium was aspirated, the cells were washed twice with 200 μ L TBS-T (1x TBS + 0.1% Tween 20) and the nuclei were stained with 50 μ L of PBS containing 10% heat inactivated foetal bovine serum, 0.1% Triton-X-100 and 2 μ M Hoechst dye. Following a 30-minute incubation at room temperature, protected from light, the cells were washed twice with TBS-T, and cells were counted in 100 μ L PBS solution using an ImageXpress Pico system with stitched plate acquisition (4x magnification), DAPI channel (50 ms exposure, -3 digital confocal setting).

When cells grow and divide (termed proliferation), cells progress through the cell cycle, a tightly regulated process that consists of two main activities: DNA replication and mitosis/cell division. As a method of accurately quantifying cellular proliferation rate, the pyrimidine analogue, BrdU can be utilised to measure DNA replication. BrdU can be incorporated into the newly synthesized DNA in place of thymidine. Following incubation of proliferating cells with BrdU for 20 hours, labelling solution was removed thoroughly, and cells were fixed with 70 μ L of fix solution for 30 min at room temperature. Fix solution was aspirated, and anti-BrdU-Eu antibodies were added for 2 hours at room temperature in the dark. Wells were washed thrice with wash solution and 70 μ L of DELFIA inducer per well was added and incubated for 30 min, in the dark. Eu-fluorescence was measured in a time-

resolved manner using the PHERAstar FSX (Ex337/ Em620 nm). Data were plotted and a curve was fitted using a 4-parameter equation with GraphPad Prism software.

The results are shown in Figure 6. The figure shows 4 panels: cell proliferation inhibition measured by BrdU incorporation (A) and nucleus counts (B), and effects of compound washout on cell proliferation by BrdU incorporation (C) and nucleus counts (D) at the highest tested concentration. In panels A and B, ABD900 is shown in filled circles (●), rotenone is shown in grey squares (■) and IACS-010759 in open circles (○). In panel C-D, cells without washout are shown in solid black (IACS-010759) and grey (Rotenone) fill, and washout in open black (IACS-010759) and grey (rotenone) bars. For ABD900, washout cells are shown with an open fill pattern and cells not washed out are shown in a checkerboard fill. Data are mean $\pm$ s.e.m..

The results show that typical Complex I inhibitors such as IACS-010759 and rotenone reduce cell proliferation (BrdU incorporation and nucleus counts) when cells are cultured in pyruvate-restricted conditions. For known Complex 1 inhibitors such as IACS-010759 and rotenone, there is no recovery from these effects when the compounds are washed out. However, for the MCIM compound, there is a recovery in the proliferative capacity (BrdU incorporation and nucleus counts) of the cells after the compound is washed out. Overall, the data together show that the MCIM compounds disclosed herein display different cellular effects to the known, archetypal, inhibitors of Complex I.

EXAMPLE 5 – Behaviour of anti-Fibrotic drugs and MCIM compounds in Mouse bleomycin-induced lung fibrosis

Eight- to ten-week-old female C57Bl/6 mice were used for all procedures. Animals were housed in groups of 4 and were maintained at 22°C $\pm$ 3°C on a 12-hour light/dark cycle with food and water ad libitum. Bleomycin was prepared by dissolving in saline at 1.5 mg/mL. All mice were administered intranasally with 3 mg/kg bleomycin.

Seven days after bleomycin administration, vehicle control, nintedanib or MCIM compound administration was initiated. Nintedanib was administered twice daily (b.d.) by oral gavage at a total daily dose of 30 mg/kg/day for 14 days. MCIM compounds were administered once daily (q.d.) by oral gavage at a total daily dose of either 10 or 20 mg/kg/day for 14 days.

On the 14th day, lungs were collected after perfusion. The left lung lobe was assessed for fibrosis by measuring total lung hydroxyproline levels. The right lung lobes were fixed and sectioned for histopathology.

For histopathological assessment of the biological effects of MCIM compounds on fibrosis, lung sections were stained with Masson's Trichome (MT) to assess fibrosis using the Ashcroft score, and Haematoxylin and Eosin (H&E) to assess parameters of vascular inflammation, bronchiolar inflammation, neutrophil inflammation, lymphoid infiltration, monocyte/macrophage infiltration,

- pneumonitis, apoptosis/necrosis, alveolar type 2 (AT2) cell hypertrophy and AT2 cell hyperplasia, as described in Hubner RH *et al* (2008) which is hereby incorporated by reference in its entirety. The latter parameters were assessed on a scale of 0-5 as follows:
- 5

0: normal

1: minimal, focal

2: moderate, focal

3: moderate, multi-focal or diffuse

4: marked, focal

5: marked, multi-focal or diffuse
- 10

To assess fibrosis in the lung, 10 successive fields of view of a lung section was visually graded from 0 (normal lung) to 8 (total fibrous obliteration of the area) as described in Hubner *et al* (2008). The Ashcroft score is assessed on a scale of 0-8 as follows:

0: Normal lung

1: Minimal fibrous thickening of alveolar or bronchiolar walls

2

3 Moderate thickening of walls without obvious damage to lung architecture

4

5: Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses

6

7: Severe distortion of structure and large fibrous areas; "honeycomb" lung" is placed in this category

8: Total fibrous obliteration of the field
- The data were analysed by generating an average histopathology score across each treatment group.

Test groups were compared to the vehicle and positive control groups using two-way ANOVA with multiple comparisons (Prism 9.2.0). Data shown as mean ± s.e.m.. * p<0.05, ** p<0.01, *** p<0.005 vs vehicle. The data are summarised in Table 3 and Table 4.
- | |
|--|
| <div>Table 3</div> <div>Inhibition of Fibrosis and support of repair</div> |
|--|

Compound	Dose (mg/kg/ day)	Lung hydroxyproline, ug/g		Ashcroft Score		Vascular Inflammation	
Study		Vehicle value	Treatment value	Vehicle value	Treatment value	Vehicle value	Treatment value
Nintedanib	30 b.d.	109 ± 15	82 ± 10***	4.4 ± 0.7	3.3 ± 0.8	2.3 ± 0.3	1.7 ± 0.4
Nintedanib	30 b.d.	56 ± 16	26 ± 8***	5.1 ± 0.6	1.3 ± 0.3**	3.0 ± 0.2	1.1 ± 0.2***
ABD900	20 q.d.	109 ± 15	86 ± 16**	4.4 ± 0.7	2.8 ± 0.8	2.3 ± 0.3	1.2 ± 0.4
HMC-C-01-A	10 q.d.	56 ± 16	36 ± 6*	5.1 ± 0.6	1.6 ± 0.4*	3.0 ± 0.2	1.4 ± 0.3***

Table 4					
Inhibition of Fibrosis and support of repair					
Compound	Dose (mg / kg / day)	AT2 cell size		AT2 cell number	
Study		Vehicle value	Treatment value	Vehicle value	Treatment value
Nintedanib	30 b.d.			1.9 ± 0.3	1.6 ± 0.2
Nintedanib	30 b.d.	1.2 ± 0.1	1.6 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
ABD900	20 q.d.	Not measured	Not measured	1.9 ± 0.3	3.3 ± 0.2***, \$\$\$
HMC-C-01-A	10 q.d.	Not measured	3.6 ± 0.2***, \$\$\$	1.1 ± 0.1	3.4 ± 0.2***, \$\$\$

Figure 7 shows 4 graphs, showing the anti-fibrotic effects of the MCIM compounds and control drug nintedanib in mice with bleomycin-induced lung fibrosis. Quantification of lung hydroxyproline levels is an established and widely used method for biochemical quantification of collagen content in the lung, with an increase in hydroxyproline being indicative of an increase in collagen deposition and fibrosis. The effect on lung hydroxyproline levels following treatment with ABD900 (A), HMC-C-01-A (B), or nintedanib (A) was measured. As demonstrated in Figures 7A and B, MCIM compounds

reduce hydroxyproline levels in mice with established lung fibrosis indicating a decrease in total collagen and fibrosis in these mice.

Effects of MCIM compounds on lung fibrosis was also assessed using an established scoring system known as the Ashcroft score first described in Ashcroft et al., 1988, which is incorporated herein in its entirety and refined by Hubner *et al* (2008). The Ashcroft score grades lung fibrosis on a scale of 0 to 8, described above. Following treatment with nintedanib or ABD900 (Figure 6C) or HMC-C-01-A (Figure 7D), histological analysis was performed, and Ashcroft scores generated. ABD900 can lead to a modest reduction in the Ashcroft score, comparable to nintedanib. However, HMC-C-01-A can significantly reduce the Ashcroft score of mice with bleomycin-induced lung fibrosis compared to mice treated with vehicle.

Figures 8 and 9 show H&E-stained sections of lung in mice treated with either vehicle control, 30 mg/kg/day nintedanib (Ofev) or 10 mg/kg/day HMC-C-01-A. As can be seen in Figure 8, in mice treated with vehicle control extensive fibrosis in the lung is seen. In contrast, mice treated with nintedanib show a much-reduced level of fibrosis compared to control. Surprisingly, treatment with an MCIM compound alone leads to an even more striking decrease in fibrosis in the lung compared to either vehicle control or nintedanib.

Figure 9 further demonstrates that in mice treated with MCIM compounds, a cuboid phenotype is elicited in lung AEC2 cells (A, lower panel, arrows), compared to vehicle which shows a flattened appearance (top panel, arrows). This indicates that MCIM compound induce adaptive repair, which are accompanied by increased surfactant production ((B); magnified image with circles).

Together, the above data indicate that the MCIM compounds show excellent activity in inhibiting the key fibrosis outcomes of lung hydroxyproline levels and Ashcroft Score. Importantly, administration also elicited an effect consistent with protection of the alveolar epithelial response, indicative of an adaptive repair phenotype, with preservation of AT2 cell size and number. Nintedanib was effective in ameliorating fibrosis but showed no effect on AT2 cells and thus no protection of the alveolar epithelial response.

In summary, the data in Figures 7-9 shows that overall HMC-C-01-A outperforms nintedanib in preventing inflammation in the lungs of mice with bleomycin-induced lung fibrosis and preventing fibrotic lesions developing. Surprisingly, this data also demonstrates that the MCIM compounds promote repair responses of alveolar epithelial cell hypertrophy and hyperplasia which are not changed compared with vehicle controls by nintedanib.

EXAMPLE 6 - Behaviour of 'classical' Complex I inhibitors vs MCIM compounds in the bleomycin-induced lung fibrosis model

Various compounds are known to bind within the Q tunnel of Complex I. For example, IACS-2858, a close relative of the development candidates IAC-010759 and BAY-87-2243, acts like a “cork in a bottle”, to inhibit Complex I (Chung et al, 2021; Kurelac et al, 2022), whilst biguanides such as metformin and a biguanide derivative IM-156 bind Complex I and may have putative additional sites, for example in Complex IV. To investigate whether conventional Complex I inhibitors could elicit the same profile of repair as the MCIM compounds, the activity of the Complex I inhibitor IACS-010759 was profiled in the bleomycin model.

- 10 Eight- to ten-week-old female C57Bl/6 mice were used for all procedures. Animals were housed in groups of 4 and were maintained at 22°C ± 3°C on a 12-hour light/dark cycle with food and water ad libitum. Bleomycin was prepared by dissolving in saline at 1.5 mg/mL. All mice were administered intranasally with 3 mg/kg bleomycin. Following intranasal administration of 3 mg/kg bleomycin, approximately 1 hr later mice were administered vehicle control, IACS-010759 or MCIM compound.
- 15 IACS-10759 was administered once daily (q.d.) by oral gavage at a total daily dose of 0.01, 0.1 or 10 mg/kg/day for 7 days. MCIM compounds were administered once daily (q.d.) by oral gavage at a total daily dose of 10 mg/kg/day for 7 days.

- On the 7th day, Bronchoalveolar lavage (BAL) fluid was collected under anaesthesia and processed for white blood cell counts, and the right lung lobes were fixed and sectioned for histopathology, which
- 20 was conducted according to the methods provided in Example 5, above. The data are presented as mean s.e.m. across the group. Statistical analysis was performed using a two-way ANOVA with multiple comparisons (GraphPad Prism v 9.2.0). Naïve controls are shown in spotted white bars, vehicle is shown in black bars, IACS-010759 dosed at 0.01, 0.1 and 1 mg/kg/day in white, light grey or dark grey bars respectively, and ABD900 in striped bars. Data are mean ± s.e.m.. * p<0.05, ** p<0.01, *** p<0.005 vs vehicle. §§§ p<0.005 vs 1 mg/kg/day IACS-010759.
- 25

The results are shown in Figure 20, which contains 6 panels: (A) Ashcroft score. (B) BAL cell counts, (C) AT2 cell size, (D) AT1 metaplasia, (E) apoptosis/necrosis, and (F) pyknotic foci (indicative of cells undergoing necrosis). Vehicle is shown in black bars and animals dosed with ABD900 at 10 mg/kg/day in grey bars. Data are mean ± s.e.m..

- 30 The results show that IACS- 010759 (1 mg/kg/day) reduced Ashcroft scores (Figure 20A), and BAL cell counts (Figure 20B) to a similar degree to ABD900 (10 mg/kg/day). However, in contrast to ABD900, which increased AT2 cell size (hypertrophy), IACS-010759 decreased AT2 hypertrophy with a reduction seen even at low doses of 0.01 mg/kg/day (Figure 20C). In addition, IACS-010759 administration resulted in a dose-dependent increase in AT1 metaplasia, contrasting with the effect of
- 35 ABD900, which significantly reduced AEC1 metaplasia compared with vehicle, and showed a profile consistent with that of naïve animals (Figure 20D). The change in AT1 metaplasia observed with

IACS-010759 was accompanied by increases in apoptosis/necrosis, and pyknotic foci (Figure 20E and F), again contrasting with ABD900 which reduced both apoptosis/necrosis, and pyknotic foci compared with vehicle and again showed a profile consistent with that of naïve animals.

Overall, the data indicate a difference in the phenotypic pharmacology elicited by MCIM compounds as opposed to classical complex I inhibitors such as IACS-010759, the latter showing a profile of increased apoptosis and pyknosis (an indicator of necrosis) consistent with the expectations of a chemotherapeutic agent, whilst ABD900 and HMC-C-01-A elicit an adaptive phenotype consistent with a primary repair response with decreased levels of apoptosis and necrosis.

EXAMPLE 7 – Combination therapy in mouse bleomycin-induced lung fibrosis model

Given the effective repair response elicited by MCIM compounds, the inventors next investigated whether an improved response could be demonstrated in mice with bleomycin-induced lung fibrosis treated with a combination of the MCIM compound and the approved anti-fibrotic drug nintedanib. Mice were treated and tissues processed as described in Example 5. Seven days after bleomycin administration, compound administration was initiated. All compounds were administered once daily by oral gavage for 14 days.

The data are summarised in Tables 5 and 6.

Table 5 Inhibition of Fibrosis				
Compound	Dose (mg / kg / day)	Lung hydroxyproline, ug/g	Ashcroft Score	Vascular Inflammation
Vehicle	-	50 ± 12	5.3 ± 0.5	3.5 ± 0.2
Nintedanib	30 b.d.	35 ± 4***	3.7 ± 0.8	1.6 ± 0.3***
HMC-C-01-A	3 q.d.	40 ± 10*	3.8 ± 0.5	2.0 ± 0.2***
Combination	20 b.d (nintedanib) + 3 q.d. (HMC-C-01-A).	33 ± 4***	1.9 ± 0.5**	1.6 ± 0.2***

*** p<0.01 vs vehicle

§§§ p<0.01 vs nintedanib alone

Figures 10 shows 2 graphs demonstrating the anti-fibrotic effects of the MCIM compound, nintedanib or a combination of MCIM compound and nintedanib vs controls. Figure 10A and B shows the effect on Ashcroft score and vascular inflammation, respectively.

These data indicate that the compounds when administered alone show excellent activity in inhibiting the key fibrosis outcomes as indicated by the Ashcroft Score and vascular inflammation score, with effects equivalent to the approved drug, nintedanib. Importantly, however, when the two agents are

combined, the effect on fibrosis is augmented, with a more pronounced decrease in the Ashcroft score being apparent.

EXAMPLE 8 – Effects of combination treatment on alveolar cells

5 To maintain alveolar sac integrity, AT2 cells produce a range of surfactants, which in turn play a role in regulating hydration of the alveolar wall surface. Different surfactants serve different roles in this regard, and in controlling surface tension of the alveolar wall and thus facilitating patency of the airspaces. For example, surfactant protein C (SpC) is a continually synthesized ‘surveillance’ surfactant, important in regulating the absorption of lipid films in the alveolar air-liquid interface, which
10 covers a significant area of the AT1 surface. SpC expression and area are reduced in a range of lung pathologies, such as pulmonary fibrosis or ARDS. By contrast, surfactant protein A (SpA) is a ‘fast response’ surfactant induced in stress conditions, such as infection and, as such, is a useful register of AT2 ‘health’. Reduced SpA levels are correlate with degeneration of the AT2 population. Like SpA, surfactant protein D (SpD) is involved in the acute response of the alveolar sac to injury and
15 possessed anti-microbial and anti-inflammatory effects. SpD is both secreted and re-absorbed by the AT2, which allows the AT2 to ‘sample’ the local microenvironment and thus allow early detection of injury. This surfactant has a wider expression than either SpA or SpC and is expressed on a range of progenitor lineages in the transitional zone stem cell niche, notably on the pluripotent club cell. Therefore, by monitoring surfactant protein expression in the lung it is possible to monitor the repair
20 responses of the lung in relation to the stem cell niche.

In this study, bleomycin was used to induce fibrosis, and mice were treated with HMC-C-01-A as described above once daily for 4 or 7 days. Lungs were collected, inflated with 0.5mL 10% Neutral Buffered Formalin and then fixed in 10% Neutral Buffered Formalin for 48 hours. Following sectioning, slides were blocked to prevent non-specific binding, followed by immunostaining for the proliferation
25 marker, Ki67, surfactant protein A (SP-A), pro-surfactant protein C (pSP-C) and surfactant protein D (SP-D), and counterstaining with Harris Haematoxylin and Eosin. 30 randomly selected fields were examined at x20 magnification using a 400 x 400 um grid with 50 µm Mertz lines for counting according to the following grading system.

Grade	Definition
0	No abnormalities
1	Minimal change; focal
2	Moderate change, focal
3	Moderate change, multi-focal or diffuse across >3 fields
4	Marked change, focal
5	Marked change, multi-focal or diffuse across > 3 fields

30

For AT2 cells, the data were analysed by generating an average histopathology score across each treatment group. For the AT2 staining, the % of positive cells in 200 AT2 cells was counted. For the

epithelial lining, the % of positive alveoli (showing > 30% surface staining) in 100 alveoli was counted. The count grid was from the largest terminal bronchiole as the mid-point, then overlaying transects moving clockwise out. Any grid with greater than 25% blood vessel or air space (or edge of tissue) was rejected.

- 5 The data are presented as mean s.e.m. across the group. Statistical analysis was performed using a two-way ANOVA with multiple comparisons (GraphPad Prism v 9.2.0). *p<0.05, ***p <0.005 vs vehicle.

At the early phases (Days 4 to 7) the bleomycin model shows a pneumonitis (lung inflammation) with the beginnings of fibroplasia (the development of fibrous tissue). During this phase, alveolar epithelial

- 10 Type 2 cells (AT2), the 'stem cells' of the lung, activate to maintain alveolar sac patency by augmenting a surfactant response, and initiate differentiation to AT1 cells to maintain alveolar wall structure and spatial organisation with respect to the pulmonary microvasculature. AT2 differentiation is a highly organised process which occurs in the so-called 'transitional zone' at the ends of the terminal bronchioles, and in the alveolar pores. In these areas, a variety of cell forms (a stem cell
15 reservoir) can be seen which reflects the differentiation responses of AT2, AT1 and transitional 'club' cell forms. The AT2 can de-differentiate to club cells, and club cells can differentiate into AT2 and directly into AT1 cells. Hence, repair, in the context of AT2 responses, is a complex equilibrium between these three transitional forms.

- In this example, AT2 cell hypertrophy and hyperplasia were assessed. The hyperplasia was
20 constrained and localised to areas of disorganised Type I alveolar epithelial cells (AT1) and in areas adjacent to transitional zones. The hypertrophy was associated with maintenance of a 'vacuolated' phenotype in AT2 and distinct from the flattened 'basaloid' forms associated with active pneumonitis/fibroplasia. Figure 11 shows 2 graphs illustrating the effects of the MCIM compound HMC-C-01-A, nintedanib or a combination of the MCIM compound and nintedanib on AT2 cell sizer (A) and AT2 cell
25 number (B) in mice with bleomycin-induced lung fibrosis. Compared to mice receiving nintedanib or vehicle control, mice treated with an MCIM compound have a significant increase in AT2 cell hypertrophy (Figure 11A) and hyperplasia (Figure 11B) indicating that HMC-C-01-A promotes an adaptive response in the lung, consistent with repair of the tissue. Figure 11 also demonstrates that treatment with a combination of an MCIM compound and nintedanib leads to a further increase in AT2
30 hypertrophy and hyperplasia compared to treatment with either compound alone, which is surprising given that nintedanib alone does not lead to a significant change in either AT cell hypertrophy or hyperplasia. These data are also summarised in Table 6.

Table 6 Inhibition of Fibrosis			
Compound	Dose (mg / kg / day)	AT2 cell size	AT2 cell number
Vehicle	-	1.1 ± 0.1	1.5 ± 0.2
Nintedanib	30 b.d.	1.5 ± 0.2	1.2 ± 0.1
HMC-C-01-A	3 q.d.	2.6 ± 0.3***	2.3 ± 0.3***
Combination	20 q.d.	3.8 ± 0.1***§§§	3.0 ± 0.2***§§§

*** p<0.01 vs vehicle

§§§ p<0.01 vs nintedanib alone

These data further show that the MCIM compounds show excellent activity in inhibiting the key
5 fibrosis outcomes in the bleomycin-induced lung fibrosis model. In contrast to nintedanib, the MCIM
compounds alone increase alveolar epithelial type 2 cell size and number, consistent with an alveolar
epithelial response. Importantly, however, when MCIM compounds are combined with nintedanib, the
effect on fibrosis is augmented, with a more pronounced decrease in the Ashcroft score (see Example
5) and a synergistic increase in the alveolar epithelial response being apparent (Figure 11 and Table
10 6). The results show that adding MCIM compound to the approved anti-fibrotic agent further reduces
fibrosis, but importantly, adds an alveolar epithelial repair response which is absent from the
approved agent when used alone.

EXAMPLE 9 – Cellular thermal shift assay (CETSA) and multiplexes quantitative mass spectrometry

15 Following the observation that MCIM compounds can affect mitochondrial morphology and modulate
cellular metabolism it was next investigated whether the reparative properties of the MCIM
compounds could be attributed to binding to/modulation of mitochondrial proteins and/or complexes.
To this end, MCIM compounds were assessed using a cellular thermal shift assay (CETSA) coupled
with quantitative mass spectrometry (MS) to determine what pathways are modulated by the MCIM
20 compounds.

Thp-1 cells were incubated in the presence of 2 µM MCIM compound (ABD900) or DMSO (vehicle
control) for 4 hours. Following the incubation period, samples were heated to one of the following
temperatures: 40.0, 42.9, 46.0, 49.6, 53.2, 56.8, 60.8, 64.0, 67.1, 70.0°C. Each test condition was
performed in duplicate.

25 Following heating, cells were lysed, and the samples digested with trypsin. The digested soluble
peptide fractions corresponding to individual temperatures were then labelled with a different isobaric
tag, using the TMT10plex system as described in Bantscheff, M., et al (2007), Bantscheff, M., et al
(2012) and Franken, H., et al (2015), each of which are incorporated by reference in their entirety.

Labelling of fractions from individual temperatures with an individual TMT10Plex tag allows the samples to be pooled and analysed by mass spectroscopy in a single run.

Following TMT10Plex labelling and pooling, the samples were fractionated using hydrophilic strong anion exchange (hSAX) (24 fractions per sample) and Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) performed. The LC-MS/MS data was analysed to identify proteins whose thermal stability was shifted in the presence of the MCIM compound using the TPP R Package (Franken, H., et al (2015)) with a procedure described in Savitski., MM et al (2014). Generally, upon compound binding, proteins become more stable and thus more resistant to thermal denaturation. Therefore, a shift in thermal stability of a protein in the described CETSA assay can indicate direct binding of a compound to the protein with shifted thermal stability. Alternatively, a shift on thermal stability may indicate the protein is involved in a downstream event from the bound protein, for example, a post-translation modification as a result of an altered metabolic or signalling pathway.

Briefly, criteria for target candidate selection were as follows:

- Min p-value <0.4 (Benjamini-Hochberg corrected)
- DTm of Run 1 (R1) and Run 2 (R2) have same sign
- DTm (drug vs DMSO) > DTm (DMSO R1 vs DMSO R2)
- Minimum slope less than -0.06

Using the above criteria, 105 proteins were identified as showing a thermal shift when incubated with the MCIM compound indicating that they were either stabilised or destabilised. Of these 105 proteins, 73 were classified as high confidence and 32 as medium confidence hits.

Functional protein interactions and/or associations of the 105 identified candidate proteins were retrieved using the freely available STRING software (<https://string-db.org>). STRING analysis identified candidates involved in oxidative phosphorylation (e.g. NDUFA6 and SDHB), mitochondrial function and the ER to Golgi apparatus interface.

In addition, thermal shifts were observed in proteins involved in adaptive stress responses (e.g. YME1L1, OXSR1, MKNK1) and other proteins which play a role in NF- κ B signalling (e.g. OXSR1, RASA1 and BIRC2).

It was therefore concluded, in line with the data in Example 3, that incubation with MCIM compounds alters the metabolic activity of cells specifically through modulation of oxidative phosphorylation, as indicated by the thermal shift of NDUFA6 (a Respiratory Complex 1 component). It was hypothesised that such modulation of Complex 1 and the oxidative phosphorylation pathway would alter NF κ B and adaptive response pathways in cells, as indicated by the thermal shift observed for proteins such as YME1L1, OXSR1, MKNK1, RASA1 and BIRC2.

EXAMPLE 10 - Photoaffinity labelling (PAL) and quantitative stable isotope labelling by amino acids in cell culture (SILAC)

To investigate the binding partner(s) of the MCIM compounds, and the cellular activities modulated by them, Photoaffinity labelling (PAL) and quantitative stable isotope labelling by amino acids in cell culture (SILAC) was performed. A clickable linker probe MCIM compound was generated to do this. This identified NDUFS2, a subunit of Mitochondrial Complex I, as a binder of the clickable linker probe MCIM compound. Together, the CETSA and PAL/SILAC-MS results strongly indicate that MCIM compounds can modulate oxidative phosphorylation and stress response pathways by binding and modulating the activity of the Mitochondrial Complex I.

EXAMPLE 11 - Computational modelling to identify binding sites in NDUFS2

Given that both CETSA and PAL/SILAC-MS methods identified Complex I subunits as targets of MCIM compounds, an *in silico* computer modelling approach was used to determine the binding site of MCIM compounds with the Complex I subunits. Initially, homology models were built from publicly available structures of mitochondrial Complex I from five organisms, including human (Table 7). The putative targets were fully resolve in 5 structures. Mitochondrial Complex 1 used for the following modelling approaches is show in Figure 12.

Table 7							
Publicly available Complex I structures. FMN = Flavin mononucleotide.							
Protein	Size	Type	Method	Liganded?		Ref	Protein database ID
				Y/N	Site		
Y. Lipolytica	466 KD	Fungal	X-Ray	N		Zickerman et al Science, 2015, 247, 44-49	4WZ7
T. Thermophilus		Bacterial	X-Ray	FMN	NADH-FMN	Efremov et al Nature, 2010, 465, 441-445	3M9S

T. Thermophilus	536 KD	Bacterial	X-Ray	FMN, Y	NADH-FMN, Quinone	Sazanov et al Nature, 2013, 2013, 494, 443-450	4HEA
T. Thermophilus	570 KD	Bacterial	X-Ray	NADH, FMN	NAD-FMN	Sazanov et al JBC, 2009, 284, 29773	3IAM
Bos Taurus	517 KD	Mammalian	Cryo-EM	N		Hirst et al Nature, 2014, 515, 80-84	4UQ8
Bos Taurus	891 KD	Mammalian	Cryo-EM	NADH, FMN	NADH-FMN	Hirst et al Nature, 2016, 536, 354	5LDW, 5LDX, 5LC5
Ovis Aries	978 KD	Mammalian	Cryo-EM	N		Sazanov et al Nature, 2016, 538, 406	5LNK
Ovis Aries	1.28 MD	Mammalian	Cryo-EM	N		Sazanov et al Nature, 2016, 537, 644	5J4Z, 5J7Y, 5J8K
Homo Sapiens		Mammalian	Cryo-EM	NADP, FMN	NADH-FMN	Guo et al Cell, 2017, 170, 1247-1257	5XTD, 5XTH, 5XTI

The homology modelling revealed a lid pocket in the NDUF52 subunit of Complex I which is in contact with the Q-tunnel. In four of the models the lid pocket was seen to be in an “open” conformation while in the remaining model it was in a “closed” conformation.

- 5 A homology model of the “open” confirmation was constructed and then SiteFinder was used to map NDUF52 in the open and closed conformations. In particular, SiteFinder (Halgren T. A., 2009) was used to build a model of the “drugability” of NDUF52, as measured by the volume of buried non-polar

available surface area (ASA; Figure 13). This model identified two binding sites on NDUFS2. The first binding site ("Pocket A") is located on the lid pocket in the open conformation and is represented on Figure 13 by a cluster of spheres. Pocket A has a percentage buried non-polar available surface area of 72%. The second binding site ("Pocket B") is located at the "back" of the mitochondrial Complex I, relative to the position of the mitochondrial Complex I subunits NDUFS7 and ND1 and is represented by a second cluster of spheres. Pocket B has a percentage buried non-polar available surface area of 71%.

Further modelling of Q10, the natural ligand of the Q-tunnel, within the Q-site of Complex 1 using SiteFinder indicated that the space for binding in this pocket is limited (Figure 14). The spheres in Figure 14 illustrate the space and channels around NDUFS2 when NDUFS2 is associated with Complex 1. This modelling approach also revealed a further ligand binding site at the junction between NDUFS2 and NDUFS7 (Figure 15). In particular, the MCIM compound is predicted to interact with His38 and Tyr141 of NDUFS2.

Using these protein models it was determined that the optimal binding site of each of ABD900, HMC-C-01-A and HMC-N-01-A was the NDUFS2 lid pocket (as illustrated in Figure 13, "Pocket A"), specifically the lid pocket in the open conformation (Glide score ~6), which is close to the Q-site. Even more particularly, the inventors were able to predict which amino acid residues in NDUFS2 contributed to the binding of the compounds:

- H-bonding with backbone carbonyl of Gly85, backbone amine of Leu95 and carboxylic acid of Asp193
- H-bonding to Tyr141 and His38
- π - π stacking with Phe458 and His88

In conclusion, analysis of the Q-site of NDUFS2 indicates that this site needs to be in the open confirmation to accommodate Q10 or small drug-like compounds.

EXAMPLE 12 - Virtual Screening to assess structure-activity relationship against the NDUFS2 pocket

As discussed in Example 11 it has been determined that the optimal binding site for MCIM compounds is in the lid pocket of NDUFS2 which is in close proximity to the Q-tunnel. From the data in Example 11, a ligand-protein pharmacophore model was built which identified 9 pharmacophoric features (Figure 16 and Tables 8A to 8C). Table 8-A describes the relationship between the type of pharmacophoric feature and the permissible variation in 3D space for the location of the given pharmacophoric feature. Table 8-B shows a distance matrix describing the 3D relationship between the centre of each pharmacophoric feature. Table 8-C describes the angle between each triplet combination of pharmacophoric features, wherein column "Y" describes the vertex of each angle. Using the parameters described in Tables 8-A to 8-C a visual representation of the pharmacophore

model has been produced using the unified annotation scheme in *Molecular Operating Environment (MOE)* software tool (Figure 16). Using *Molecular Operating Environment (MOE)*, the parameters can also be used to determine if a molecule conforms to the pharmacophore model – i.e. if 4 or more of the annotation points of the pharmacophore model are occupied by a corresponding annotation point located on a test molecule.

Table 8-A		
Pharmacophore feature annotations, and radii		
<i>Feature Number</i>	<i>Feature Annotation</i>	<i>Radius (Å)</i>
F1	Acc	1.2
F2	Acc	1.2
F3	Acc	1.2
F4	Acc2	1.4
F5	Aro	1.4
F6	Hyd	1.4
F7	Hyd	1.4
F8	Hyd	1.4
F9	Don2	1.4

Table 8-B										
Pharmacophore feature distances (Å)										
<i>Dist.</i> (Å)	<i>Feature</i> <i>Number</i>	<i>F1</i>	<i>F2</i>	<i>F3</i>	<i>F4</i>	<i>F5</i>	<i>F6</i>	<i>F7</i>	<i>F8</i>	<i>F9</i>
<i>Feature</i> <i>Number</i>	<i>Feature</i> <i>Ann</i>	Acc	Acc	Acc	Acc2	Aro	Hyd	Hyd	Hyd	Don2
<i>F1</i>	Acc	0.00	2.64	8.50	8.16	8.11	4.07	4.77	3.13	4.82
<i>F2</i>	Acc	2.64	0.00	7.21	9.80	8.48	4.60	3.85	3.85	3.46
<i>F3</i>	Acc	8.50	7.21	0.00	16.55	14.19	10.57	4.37	10.95	9.58
<i>F4</i>	Acc2	8.16	9.80	16.55	0.00	6.76	6.87	12.41	6.05	8.88
<i>F5</i>	Aro	8.11	8.48	14.19	6.76	0.00	4.12	9.96	6.41	5.82
<i>F6</i>	Hyd	4.07	4.60	10.57	6.87	4.12	0.00	6.26	3.31	3.22
<i>F7</i>	Hyd	4.77	3.85	4.37	12.41	9.96	6.26	0.00	7.12	5.90
<i>F8</i>	Hyd	3.13	3.85	10.95	6.05	6.41	3.31	7.12	0.00	3.77
<i>F9</i>	Don2	4.82	3.46	9.58	8.88	5.82	3.22	5.90	3.77	0.00

Table 8-C			
Pharmacophore feature angles between features X-Y-Z (°),			
X	Y	Z	Angle (°)
F1	F2	F3	110.39
F1	F2	F4	45.38
F1	F2	F5	72.94
F1	F2	F6	61.46
F1	F2	F7	92.58
F1	F2	F8	53.87
F1	F2	F9	103.51
F1	F3	F4	6.61
F1	F3	F5	30.48
F1	F3	F6	21.30
F1	F3	F7	22.60
F1	F3	F8	11.59
F1	F3	F9	30.18
F1	F4	F5	65.03
F1	F4	F6	29.85
F1	F4	F7	12.32
F1	F4	F8	18.96
F1	F4	F9	32.52
F1	F5	F6	7.99
F1	F5	F7	28.34
F1	F5	F8	21.01
F1	F5	F9	35.98
F1	F6	F7	49.67
F1	F6	F8	48.90
F1	F6	F9	81.99
F1	F7	F8	20.48
F1	F7	F9	52.40
F1	F8	F9	88.17

Table 8-C			
Pharmacophore feature angles between features X-Y-Z (°),			
X	Y	Z	Angle (°)
F2	F3	F4	15.65
F2	F3	F5	27.53
F2	F3	F6	20.78
F2	F3	F7	26.84
F2	F3	F8	5.82
F2	F3	F9	17.47
F2	F4	F5	58.21
F2	F4	F6	24.99
F2	F4	F7	14.75
F2	F4	F8	6.62
F2	F4	F9	20.61
F2	F5	F6	14.35
F2	F5	F7	22.35
F2	F5	F8	25.41
F2	F5	F9	18.14
F2	F6	F7	37.84
F2	F6	F8	55.34
F2	F6	F9	48.68
F2	F7	F8	22.45
F2	F7	F9	34.01
F2	F8	F9	54.05
F3	F4	F5	58.15
F3	F4	F6	22.97
F3	F4	F7	5.71
F3	F4	F8	18.05
F3	F4	F9	27.40
F3	F5	F6	24.17
F3	F5	F7	5.20

Table 8-C			
Pharmacophore feature angles between features X-Y-Z (°),			
X	Y	Z	Angle (°)
F3	F5	F8	47.65
F3	F5	F9	29.69
F3	F6	F7	5.20
F3	F6	F8	87.80
F3	F6	F9	63.56
F3	F7	F8	143.73
F3	F7	F9	137.11
F3	F8	F9	59.09
F4	F5	F6	73.80
F4	F5	F7	93.94
F4	F5	F8	54.64
F4	F5	F9	89.42
F4	F6	F7	142.01
F4	F6	F8	61.79
F4	F6	F9	118.85
F4	F7	F8	17.94
F4	F7	F9	41.28
F4	F8	F9	127.89
F5	F6	F7	146.38
F5	F6	F8	118.68
F5	F6	F9	104.33
F5	F7	F8	39.90
F5	F7	F9	31.63
F5	F8	F9	63.74
F6	F7	F8	27.72
F6	F7	F9	30.48
F6	F8	F9	53.57
F7	F8	F9	55.96

Using the *Molecular Operating Environment (MOE)*, 2022.02 Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2022 MOE docking using the pharmacophore algorithm for ligand placement and the GBVI/WSA dG scoring function, 117 compounds were assessed for their ability to dock in the predicted binding site using the pharmacophore model described above. To be treated as a successful docking in the predicted binding site, it is required that a molecule hits at least 4 features of the pharmacophore model shown in Figure 16 and has a half-maximal inhibitory concentration (IC_{50}) $\leq 1 \mu M$.

Figure 17 shows a MCIM compound that conforms to the pharmacophore model and satisfies 7 out of the 9 annotation points determined to be important for binding to Complex I. Surprisingly, it was also found that out of the 117 compounds assessed for their ability to bind in the pharmacophore model, only 13 compounds had an $IC_{50} > 1 \mu M$, which indicates that these compounds would fail to dock in the predicted binding sites. The remaining 104 compounds hit at least 4 of the pharmacophore features and are predicted to have an $IC_{50} \leq 1 \mu M$. Figure 18 shows representative compound CHMSA-02-A, which fulfils the pharmacophore model and is predicted to have a $pAct$ ($-\log(IC_{50})$) of ~ 7 .

EXAMPLE 13 – Ultra-high throughput virtual screening (uHTVS)

Following building of the pharmacophore model, the inventors next wanted to identify additional NDUFS2 binders using this model.

The 3D model of the entire Complex 1 described in Example 11 (Figure 12) was validated by docking Q10 and active MCIM compounds. This allowed establishment of the bioactive conformation of the MCIM compounds when docked in the Q-tunnel of Complex I. This further allowed a structure-based hypothesis to be generated and to rationalise the structure-activity relationship (SAR) of the compounds and to build the pharmacophore model described in Example 12 and a QSAR model (Figure 19) for activity prediction. The QSAR model is a linear regression model which includes docking scores and parameters related to ligand energies and electrostatics.

For uHTVS, a library of compounds was screened against the pharmacophore model described in Example 11. The model identified 37.6 million compounds from the library of compounds which generally hit 3-6 of the pharmacophore features. Of these 37.6 million compounds, those up to a molecular weight of ~ 350 Da were then virtually docked in the 3D Complex I model without imposing pharmacophore constraints. 67,000 compounds were predicted to dock in Complex I and were retained for further screening.

The retained compounds were then filtered through a more theoretically rigorous docking method and evaluated with a QSAR model to predict biological activity of each compound (Figure 19). The GBVI/WSA ΔG forcefield-based scoring function (Naim *et al.* 2007) on the MOE dock (*Molecular Operating Environment (MOE)*, 2022.02 Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7) was used. Using the screening process described, 756 compounds were identified as potential drug candidates for targeting NDUFS2/NDUFS7 of Complex 1.

References

A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. The entirety of each of these references is incorporated herein.

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Claims

1. A pharmaceutical combination comprising a mitochondrial complex I modulator (MCIM) compound and an anti-fibrotic compound.
2. The pharmaceutical combination according to claim 1, wherein the pharmaceutical combination is formulated as a single pharmaceutical composition.
3. A pharmaceutical combination according to claim 1 or claim 2 for use in medicine.
4. A pharmaceutical composition comprising a mitochondrial complex I modulator (MCIM) compound for use in the treatment of fibrosis in an inflammatory and/or progressive disease in a subject in need thereof, wherein the treatment comprises the separate, sequential or simultaneous administration of the pharmaceutical composition and an anti-fibrotic compound to the subject.
5. A pharmaceutical composition comprising an anti-fibrotic compound for use in the treatment of fibrosis in an inflammatory and/or progressive disease in a subject in need thereof, wherein the treatment comprises the separate, sequential or simultaneous administration of the pharmaceutical composition and a mitochondrial complex I modulator (MCIM) compound to the subject.
6. A pharmaceutical composition comprising a mitochondrial complex I modulator (MCIM) compound and an anti-fibrotic compound for use in the treatment of fibrosis in an inflammatory and/or progressive disease in a subject in need thereof, wherein treatment comprises administration of the composition to the subject.
7. The composition for the use according to any one of claims 4 to 6, wherein the treatment achieves disease control, tissue repair, or disease regression, or any combination thereof.
8. The composition for the use according to claim 7, wherein disease control comprises supporting tissue repair and/or inhibition of disease progression.
9. The composition for the use according to claim 7 or 8, wherein disease control comprises the prevention of a loss of anatomically normal tissue architecture, or a reduction in the speed of the loss of anatomically normal tissue architecture.
10. The composition for the use according to any one of claims 7 to 9, wherein the disease control, regression, or tissue repair, or any combination thereof, comprises an increased repair score and/or increased wound healing.
11. The composition for the use according to any one of claims 7 to 10, wherein the disease control, regression, or tissue repair, or any combination thereof, comprises an increased cell count of reparative cells and/or a decreased cell count of pathology driving cells.
12. The composition for the use according to any one of claims 7 to 11, wherein the disease control, regression, or tissue repair, or any combination thereof, induces a restoration of anatomically normal tissue architecture.

13. The composition for the use according to anyone of claims 4 to 12, wherein the fibrosis is associated with or caused by overstimulation of platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), transforming growth factor receptor beta receptor (TGBF- β R), colony stimulating factor 1 receptor (CSF1R), Lck, Lyn, Src, or phosphodiesterase 4B (PDE4B), or any combination of the foregoing.

14. The composition for the use according to any one of claims 4 to 13, wherein the inflammatory and/or progressive disease is an interstitial lung disease (ILD), progressive fibrosing ILD (pf-ILD), systemic sclerosis-associated interstitial lung disease (SSc-ILD), liver fibrosis, non-alcoholic fatty acid liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, cardiac fibrosis, kidney disease, kidney fibrosis, endometriosis, arthrofibrosis, covid-induced fibrosis, or familial pulmonary fibrosis.

15. The composition for the use according to claim 14, wherein the ILD comprises pulmonary fibrosis.

16. The composition for use according to claim 14 or claim 15, wherein the ILD is idiopathic pulmonary fibrosis, idiopathic non-specific interstitial pneumonia, progressive fibrosing ILD, rheumatoid arthritis interstitial lung disease (RA-ILD), systemic sclerosis, sarcoidosis, COVID-19-related pulmonary fibrosis, drug-induced pulmonary fibrosis, systemic sclerosis (SSc), systemic sclerosis-associated interstitial lung disease (SSc-ILD), sclerodermatous graft vs. host disease, nephrogenic systemic fibrosis, radiation-induced fibrosis, or hypersensitivity pneumonitis.

17. The composition for the use according to any one of claims 4 to 16, wherein the inflammatory and/or progressive disease is an autoimmune disease.

18. The composition for the use according to any one of claims 4 to 13, wherein the inflammatory and/or progressive disease is rheumatoid arthritis (RA), psoriatic arthritis, inflammatory arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, reactive arthritis, osteoarthritis, systemic sclerosis, inflammatory bowel disease (IBD), Crohn's disease, fistulising Crohn's disease, or ulcerative colitis.

19. The composition for the use according to any one of claims 4 to 18, wherein the disease control, regression, or tissue repair, or any combination thereof, comprises a reduction in cytokine production from pro-inflammatory myeloid cells.

20. The combination according to any one of claims 1 to 3 or the composition for the use according to any one of claims 4 to 19, wherein the binding of the MCIM compound to complex I modulates complex I activity, wherein complex I modulation is determined by detecting a reduction in cellular O₂ consumption without a reduction of cell viability.

21. The combination according to any one of claims 1 to 3 or 20 or the composition for the use according to any one of claims 4 to 20, wherein the binding of the MCIM compound to complex I modulates complex I activity, leading to a reversible reduction in cell proliferation.

22. The combination according to any one of claims 1 to 3 or 20 to 21 or the composition for the use according to any one of claims 4 to 21, wherein the MCIM compound interacts with complex I at a binding site outside of the Q tunnel.

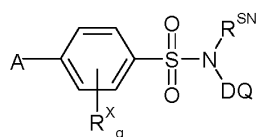
23. The combination according to any one of claims 1 to 3 or 20 to 22 or the composition for the use according to any one of claims 4 to 22, wherein the MCIM binding site comprises one or more amino acid residues from NDUSF2 and/or NDUSF7.

24. The combination or composition for use according claim 23, wherein the MCIM compound interacts with one or more amino acid residues in NDUSF2 selected from His92, Gly85, Tyr141, His88, Leu95, Asp193 and Phe458.

25. The combination or composition for use according to claim 24, wherein the MCIM compound interacts with at least one amino acid residues in NDUSF2 selected from Tyr141, His92 and Asp139.

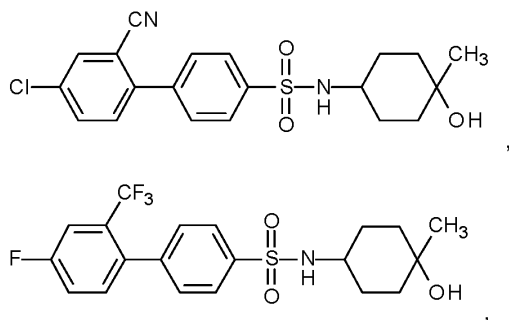
26. The combination according to any one of claims 1 to 3 or 20 to 25 or the composition for use according to any one of claims 4 to 25, wherein a 3D conformation of the MCIM compound as annotated by *Molecular Operating Environment (MOE) 2022* unified annotation scheme comprises 4 or more pharmacophore features which conform to the pharmacophore model represented in Figure 16, and Tables 8-A, 8-B and 8-C.

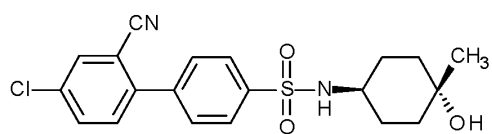
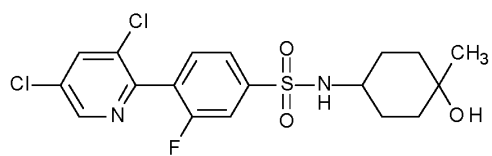
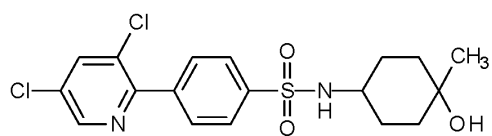
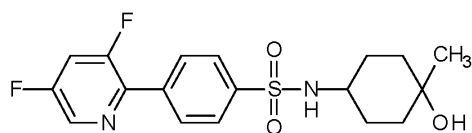
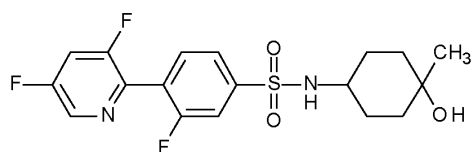
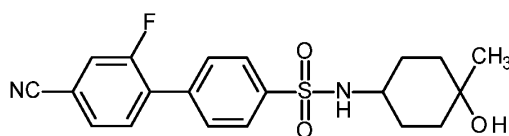
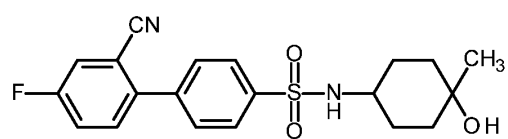
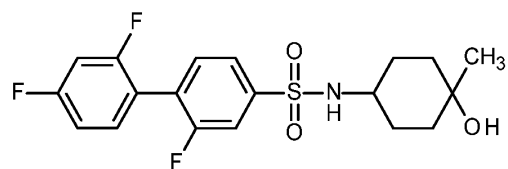
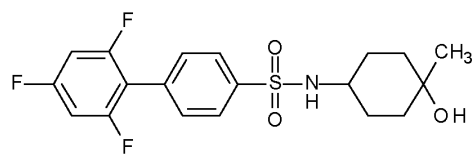
27. The combination according to any one of claims 1 to 3 or 20 to 26 or the composition for use according to any of claims 4 to 26, wherein the MCIM compound is an MCIM compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:

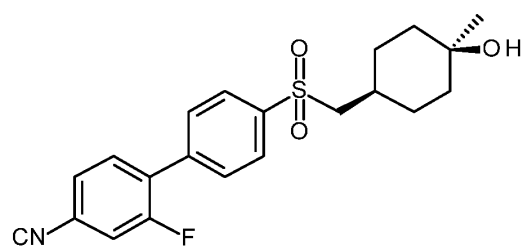
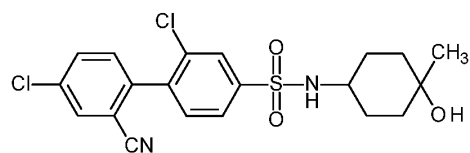
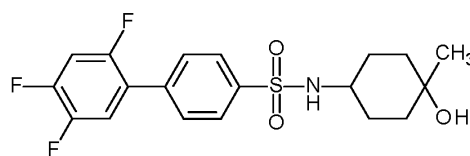
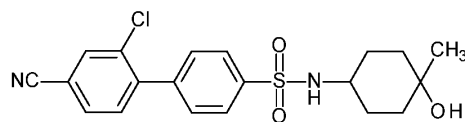
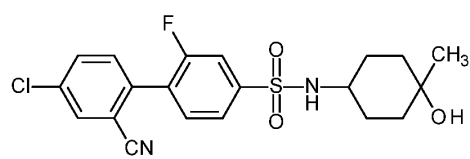
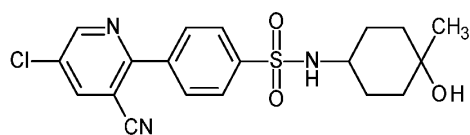
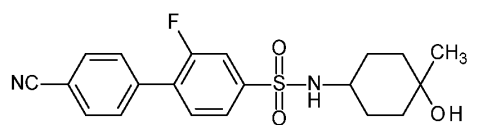


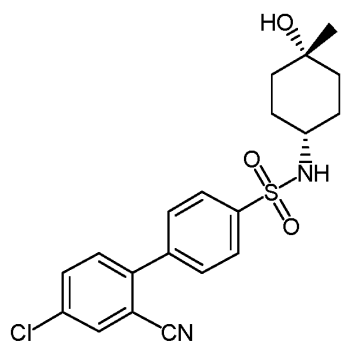
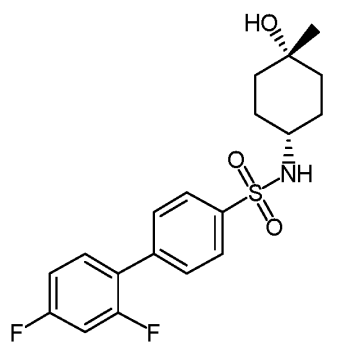
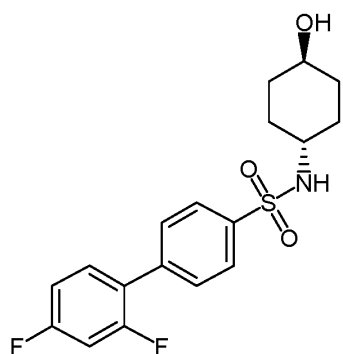
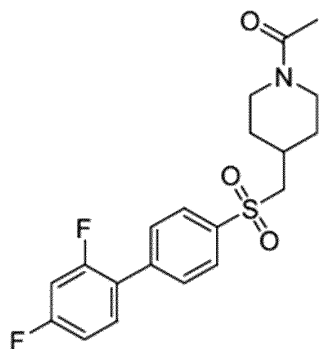
as defined in claim 1 of WO2010/032009.

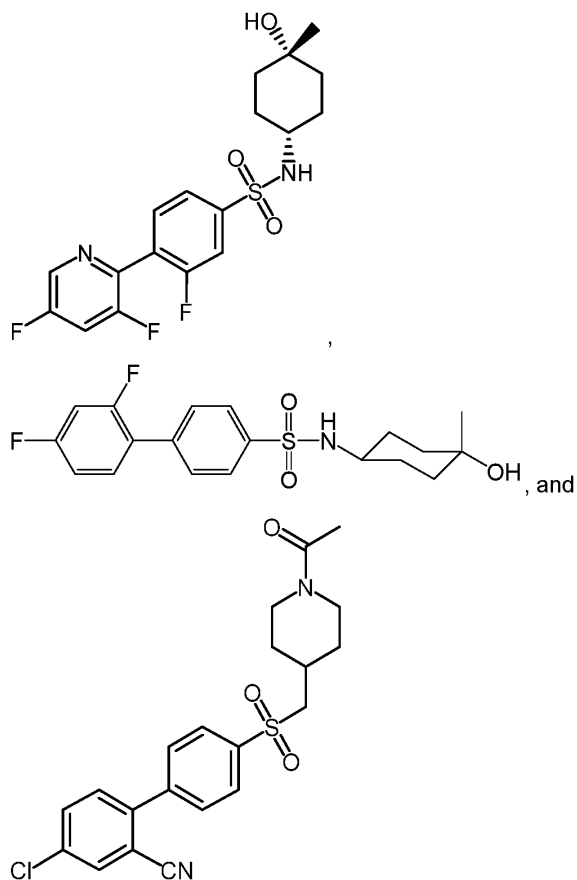
28. The combination or composition for use according to claim 27, wherein the MCIM compound is an MCIM compound selected from compounds of the following formulae, or a pharmaceutically acceptable salt, hydrate, or solvate thereof:



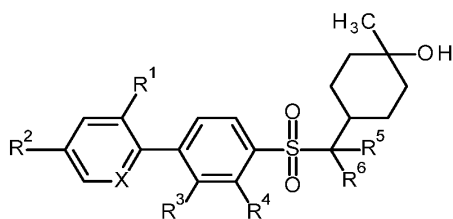






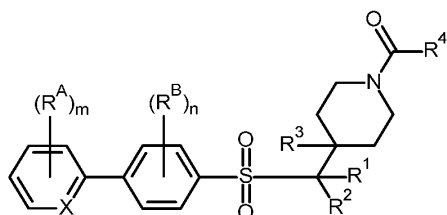


29. The combination according to any one of claims 1 to 3 or 20 to 28 or the composition for use according to any of claims 4 to 28, wherein the MCIM compound is an MCIM compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:



as defined in claim 1 of WO2020/035560 A1.

30. The combination according to any one of claims 1 to 3 or 20 to 29 or the composition for use according to any of claims 4 to 29, wherein the MCIM compound is an MCIM compound, or a pharmaceutically acceptable salt, hydrate, or solvate thereof, of the following formula:



as defined in claim 1 of WO2020/212581 A1.

31. The combination according to any one of claims 1 to 3 or 20 to 30 or the composition for use according to any one of claims 4 to 30, wherein the anti-fibrotic compound inhibits platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), transforming growth factor receptor beta receptor (TGF- β R), colony stimulating factor 1 receptor (CSF1R), Lck, Lyn, Src, TGF β 1, tumor necrosis factor (TNF), phosphodiesterase 4B (PDE4B), Death Receptor 3 (DR3), or any combination of the foregoing.

32. The combination according to any one of claims 1 to 3 or 20 to 31 or the composition for use according to any one of claims 4 to 31, wherein the anti-fibrotic compound comprises nintedanib, pirfenidone, tulisokibart, nerandomilast, or a combination thereof.

33. The combination according to any one of claims 1 to 3 or 20 to 32 or the composition for use according to any one of claims 4 to 32, wherein the MCIM compound and the anti-fibrotic compound are uniformly dispersed in a solution, suspension, semi-solid or solid mixture within a single dosage form and one or more pharmaceutically acceptable excipients.

34. The combination according to any one of claims 1 to 3 or 20 to 32 or the composition for use according to any one of claims 4 to 32, wherein the MCIM compound and the anti-fibrotic compound are heterogeneously dispersed and separated from each other in different regions within a single dosage form.