

SEROTONIN RECEPTOR AS A TARGET FOR THERAPY

TECHNICAL FIELD

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The present disclosure relates broadly to methods of treating proliferative diseases. In particular, the disclosure relates to a 5-HT_{2A} serotonin receptor inhibitor.

BACKGROUND

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Hepatocellular carcinoma (HCC) is the most common form of liver cancer (more than 800,000 deaths annually worldwide), with the Asia-Pacific region having the largest disease burden. In Singapore, HCC is the fourth most common cancer in men with an age-standardised incidence rate of 17.7 per 100,000 males. Although early-stage HCC may be treated by resection, local ablative therapies, or liver transplant, tumours recur within 5 years in 60-80% of such cases, often progressing to advanced HCC. Advanced HCC (aHCC) is a disease with poor prognosis (median survival < 1 year) and limited treatment options. While combination immunotherapy using anti-PD-L1 + anti-VEGFA monoclonal antibodies has recently made promising breakthrough by surpassing the efficacy of the historical systemic treatment drug sorafenib in the IMbrave150 clinical trial, there is still much room for improvement as two-thirds of aHCC patients were still refractory to any kind of treatment.

Moreover, the molecular pathways targeted across all approved treatments for aHCC are a very narrow range of molecular pathways (including the VEGF, PD-1, and CTLA-4 pathways). Therefore, there is a clinical need to search for and evaluate more potential molecular targets that could be rationally combined with existing approved immunotherapeutic agents. Considering that all three FDA-approved drugs for first line HCC treatment were first approved for use in other non-HCC indications and were then repurposed for HCC following successful clinical trials, there is a particular lack of HCC-specific therapeutics that target the liver immune microenvironment for improved therapeutic efficacy.

Accordingly, there is a need to provide alternative targeted treatment of proliferative disease such as hepatocellular carcinoma.

SUMMARY

In a first aspect, there is provided a serotonin receptor 5-HT_{2A} inhibitor or composition comprising serotonin receptor 5-HT_{2A} inhibitor for use in treating a
 5 proliferative disease in a subject in need thereof.

In a second aspect, there is provided the use of a serotonin receptor 5-HT_{2A} inhibitor in the manufacture of a medicament for treating a proliferative disease in a subject in need thereof.

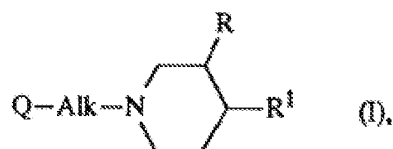
In a third aspect, there is provided a method of treating a proliferative disease in
 10 a subject in need thereof, wherein the method comprises administering an effective amount of a serotonin receptor 5-HT_{2A} inhibitor into the subject.

In some examples, the serotonin receptor 5-HT_{2A} inhibitor ameliorates the suppression of the immune system, optionally ameliorates the suppression of CD8 T cell function.

15 In some examples, the 5-HT_{2A} inhibitor is a selective antagonist of 5-HT_{2A}.

In some examples, the 5-HT_{2A} inhibitor is an agent that targets the serotonin receptor 5-HT_{2A} by disrupting its function in enhancing CD8 T cell activation and/or an agent that targets the serotonin receptor 5-HT_{2A} overall function and therefore disrupts its function in enhancing CD8 T cell activation.

20 In some examples, the 5-HT_{2A} inhibitor is a compound having the formula:

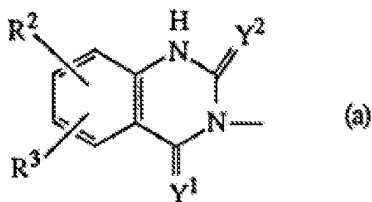


the pharmaceutically acceptable acid-addition salts thereof and the possible stereoisomeric forms thereof; wherein

25 R is hydrogen or C₁₋₆alkyl;

Alk is C₁₋₄alkanediyl;

Q is a radical of formula



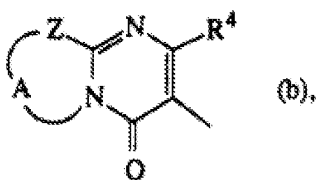
wherein Y¹ and Y² are each independently O or S;

R² is hydrogen, halo, C₁₋₆alkyl or C₁₋₆alkyloxy;

R³ is hydrogen or halo;

5 or

Q is a radical of formula



wherein R⁴ is hydrogen or C₁₋₆alkyl;

Z is -S-, -CH₂- or -CR⁵=CR⁶-; said R⁵ and R⁶ each independently being hydrogen
10 or C₁₋₆alkyl; and A is a bivalent radical -CH₂-CH₂-, -CH₂-CH₂-CH₂- or -CR⁷=CR⁸-, said
R⁷ and R⁸ each independently being hydrogen, halo, amino or C₁₋₆alkyl;

R¹ is a radical of formula



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wherein Ar is phenyl or substituted phenyl, said substituted phenyl bearing an
amino group and/or 1, 2 or 3 halo atoms; and

wherein X is > C=O, > CH-OH, > CH-O-C(O)-R⁹, > CH₂, > C(OC₁₋₆alkyl)₂,



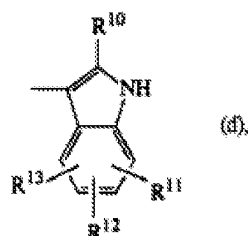
20 > C=N-OH or > C=N-NH₂;

said R⁹ being hydrogen or C₁₋₆ alkyl and

said q being the integer 2 or 3;

or

R¹ is a radical of formula

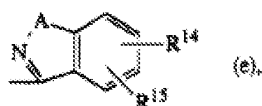


wherein R¹⁰ is hydrogen or C₁₋₆alkyl; R¹¹, R¹² and R¹³ are each independently hydrogen

5 or halo;

or

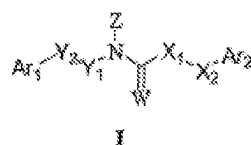
R¹ is a radical of formula



wherein A is O or S; R¹⁴ and R¹⁵ are each independently hydrogen, halo, hydroxy,

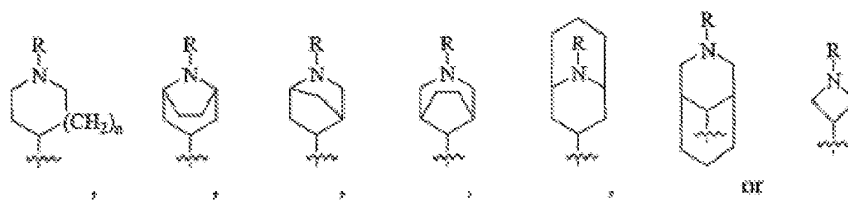
10 C₁₋₆alkoxy or C₁₋₆alkyl.

In some examples, the 5-HT_{2A} inhibitor is a compound having the formula:



wherein

Z is a group selected from



15

R is hydrogen, a cyclic or straight-chained or branched acyclic organyl group, a lower hydroxyalkyl group, a lower aminoalkyl group, or an aralkyl or heteroaralkyl group;

n is 0, 1, or 2;

X₁ is methylene, vinylene, or an NH or N(lower alkyl) group; and

X_2 is methylene, or, when X_1 is methylene or vinylene, X_2 is methylene or a bond; or when X_1 is methylene, X_2 is O, S, NH, or N(lower alkyl) or a bond;

Y_1 is methylene and Y_2 is methylene, vinylene, ethylene, propylene, or a bond; or

Y_1 is a bond and Y_2 is vinylene; or

5 Y_1 is ethylene and Y_2 is O, S, NH, or N(lower alkyl);

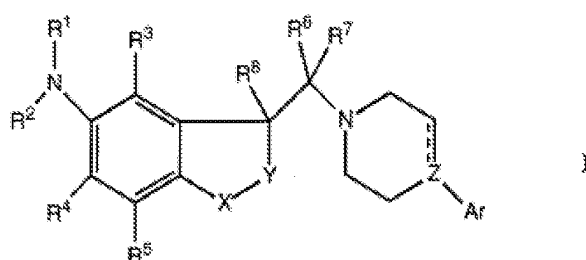
Ar_1 and Ar_2 independently are unsubstituted or substituted aryl or heteroaryl groups;

W is oxygen or sulfur; or

a pharmaceutically acceptable salt, ester, or prodrug thereof.

In some examples, the 5-HT_{2A} inhibitor is a compound having the formula:

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wherein one of X and Y is CH₂ and the other one is selected from the group consisting of CH₂, O, and S;

15 the dotted line, emanating from Z, indicates an optional bond; when it does not indicate a bond Z is N, CH or COH; and when it indicates a bond Z is C;

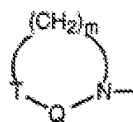
Ar is phenyl, 2-thienyl, 3-thienyl, 2-furanyl, 3-furanyl, 2-pyrimidyl, 1-indolyl, 2-indolyl, 3-indolyl, 1-indol-2-onyl, 3-indol-2-onyl, 2- or 3-benzofuranyl, 2- or 3-benzothiophenyl, 1-naphthyl or 2-naphthyl, each optionally substituted with halogen, lower alkyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, 20 trifluoromethyl, trifluoromethylsulfonyloxy, cycloalkyl, cycloalkyl-lower-alkyl, nitro, amino, lower alkylamino, di-lower alkylamino, acylamino or C₁₋₂ alkylenedioxy;

R^1 is hydrogen, lower alkyl, lower alkenyl, lower alkynyl, cycloalk(en)yl, cycloalk(en)yl-lower alk(en/yn)yl, aryl, aryl-lower alkyl, acyl, thioacyl, lower alkylsulfonyl, trifluoromethylsulfonyl, arylsulfonyl,

25 R^1 is a group R^9VCO- where V is O or S and R^9 is lower alkyl, cycloalkyl, cycloalkyl-lower-alkyl or aryl, or R^1 is a group $R^{10}R^{11}NCO-$ or $R^{10}R^{11}NCS-$ wherein R^{10} and R^{11} are independently hydrogen, lower alkyl, cycloalkyl, cycloalkyl-lower-alkyl or aryl, or R^{10} and R^{11} together with the N-atom to which they are linked, form a pyrrolidinyl, piperidinyl or perhydroazepin group;

R² is hydrogen, lower alkyl, cycloalkyl or cycloalkyl-lower-alkyl;

or R¹ and R² together with the N-atom to which they are linked form a group,



wherein Q is C=O, C=S or CH₂; T is NH, S, O or CH₂; and m is 1-4,

inclusive;

5 R³ - R⁵ are independently hydrogen, halogen, lower alkyl, lower alkylcarbonyl, phenylcarbonyl, halogen substituted phenylcarbonyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, cycloalkyl, cycloalkyl-lower-alkyl or nitro;

 R⁶ and R⁷ are each hydrogen or lower alkyl or they are linked together to
10 constitute a 3 - 7-membered carbocyclic ring;

 R⁸ is hydrogen or lower alkyl;

 any alkyl, cycloalkyl or cycloalkylalkyl group present being optionally substituted with one or two hydroxy groups, which again are optionally esterified with an aliphatic or aromatic carboxylic acid; and any aryl substituent present being optionally substituted
15 with halogen, lower alkyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, trifluoromethylsulfonyloxy, cycloalkyl, cycloalkyl-lower-alkyl or nitro;

 and pharmaceutically acceptable acid addition salts thereof.

 In some examples, the 5-HT_{2A} inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (R)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable
25 salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

 In some examples, the proliferative disease is a tumour and/or a cancer.

 In some examples, the proliferative disease is a liver cancer.

 In some examples, the proliferative disease is a hepatocellular carcinoma.

 In some examples, the serotonin receptor 5-HT_{2A} inhibitor for use as described
30 herein or the use of claim 2 as described herein or the method as described herein further comprising one or more combination therapy selected from the group consisting of an immunotherapy, a chemotherapy, an ablative therapy, and a transplantation.

In some examples, the serotonin receptor 5-HT_{2A} inhibitor for use as described herein or the use as described herein or the method as described herein further comprising an immunotherapy such as, but not limited to, an immune checkpoint blockade (ICB) therapy comprising an administration of monoclonal antibodies, optionally the immunotherapy may include the use of one or more of anti-PD-L1 (such as atezolizumab or nivolumab), anti-VEGFA (such as bevacizumab), anti-CTLA4 (such as ipilimumab), or anti-PD-1 (such as nivolumab) plus anti-CTLA4 (such as ipilimumab), and/or combination thereof.

In some examples, the combination therapy is to be administered to the subject concurrently, sequentially, or separately from the serotonin receptor 5-HT_{2A} inhibitor.

In some examples, the inhibitor is to be administered intravenously, subcutaneously, orally, sublingually, or intraperitoneally.

In a fourth aspect, there is provided a pharmaceutical composition comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy.

In some examples, the serotonin receptor inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

In a fifth aspect, there is provided a combination therapy comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy, wherein the serotonin receptor inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

DEFINITIONS

As used herein, the term "halo" refers to fluoro, chloro, bromo and iodo; the term "C₁₋₆alkyl" refers to straight and branch chained saturated hydrocarbon radicals having from 1 to 6 carbon atoms such as, for example, methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like; and
5 "C₁₋₄alkanediyl" includes bivalent straight or branch chained alkanediyl radicals having from 1 to 4 carbon atoms.

The term "inhibitor", or "antagonist" as it is used herein, refers to a molecule which decreases the amount or the duration of the effect of the biological activity of serotonin, particularly decreasing the effect of engagement of serotonin receptor 5-HT_{2A}. In some
10 examples, inhibitor or antagonist may include compounds, proteins, nucleic acids, carbohydrates, antibodies, or small molecules which decrease the effect of serotonin. For example, an antagonist may be used according to the present disclosure to inhibit the serotonin receptor 5-HT_{2A}.

The term "treating", "treat" and "therapy", and synonyms thereof refer to both
15 therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a medical condition, which includes but is not limited to diseases (such as proliferative disease including tumour and/or cancer), symptoms and disorders. A medical condition also includes a body's response to a disease or disorder, e.g. dysregulated cell proliferation, dysregulated cell metabolism, and/or inflammation.
20 Those in need of such treatment include those already with a medical condition as well as those prone to getting the medical condition or those in whom a medical condition is to be prevented. In some examples, the treatment as described herein delays tumour outgrowth and/or prolongs survival. In some examples, the present disclosure may provide higher survival rate, delayed tumour outgrowth, and/or lower lethality. In some
25 examples, the present disclosure may provide higher survival rate, delayed tumour outgrowth, and/or lower lethality when used alone or in combination with therapies known in the art (for example immune checkpoint blockade (ICB) therapy).

The term "subject" as used herein includes patients and non-patients. The term "patient" refers to individuals suffering or are likely to suffer from a medical condition such
30 as proliferative disease including tumour and/or cancer, while "non-patients" refer to individuals not suffering and are likely to not suffer from the medical condition. "Non-patients" include healthy individuals, non-diseased individuals and/or an individual free from the medical condition. The term "subject" includes humans and animals. Animals may include, but is not limited to, mammals (for example non-human primates, canine,

murine, rabbit, and the like), and the like. "Murine" refers to any mammal from the family Muridae, such as mouse, rat, and the like.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either
5 meaning.

Further, in the description herein, the word "substantially" whenever used is understood to include, but not restricted to, "entirely" or "completely" and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly include
10 elements/components recited after such terms, in addition to other components not explicitly recited. For example, when "comprising" is used, reference to a "one" feature is also intended to be a reference to "at least one" of that feature. Terms such as "consisting", "consist", and the like, may in the appropriate context, be considered as a subset of terms such as "comprising", "comprise", and the like. Therefore, in
15 embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as "consisting", "consist", and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a
20 variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and
25 teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. It is to be appreciated that the
30 individual numerical values within the range also include integers, fractions and decimals. Furthermore, whenever a range has been described, it is also intended that the range covers and teaches values of up to 2 additional decimal places or significant figures (where appropriate) from the shown numerical end points. For example, a description of a range of 1% to 5% is intended to have specifically disclosed the ranges
35 1.00% to 5.00% and also 1.0% to 5.0% and all their intermediate values (such as 1.01%,

1.02% ... 4.98%, 4.99%, 5.00% and 1.1%, 1.2% ... 4.8%, 4.9%, 5.0% etc.,) spanning the ranges. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

5 Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should
10 not be limited to the steps being carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative
15 embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

DESCRIPTION OF EMBODIMENTS

Exemplary, non-limiting embodiments of serotonin receptor 5-HT_{2A} inhibitors and
20 methods of treatments using the inhibitors are disclosed hereinafter.

The 5-HT_{2A} receptor, one of the three members of 5-HT₂ receptor family is a typical 7-transmembrane G-protein receptor, with coupling to the Gq/11 signal transduction protein. 5-HT_{2A} receptor are found postsynaptically to serotonergic neurons. 5-HT_{2A} receptor are found mainly in the frontal cortex or in region that is connected to
25 visual cortex. In the periphery, 5-HT_{2A} receptor can be found eliciting response in vascular smooth muscle and a component of the response of smooth muscle in the gut.

The inventors of the present disclosure have discovered that the serotonin receptor 5-HT_{2A} is a molecule with surprising suppressive effects on liver NPC-activated CD8 T cells. Therefore, the inventors of the present disclosure have found serotonin
30 receptor 5-HT_{2A} to be a surprisingly effective target for an adjuvant therapy together with HCC immunotherapy that has the potential to meet the crucial clinical need for HCC-specific molecular therapies that can be rationally combined with existing approved immunotherapeutics.

The liver immune microenvironment is intrinsically immunosuppressive, as
35 hepatocytes and non-parenchymal cells (NPCs) such as Kupffer cells and sinusoidal

endothelial cells suppress T cell activation under normal resting conditions. Therefore, disrupting these immunosuppressive interactions between liver NPCs and tumour-specific T cells could potentially increase the effectiveness of T cell-targeted immunotherapies in HCC.

5 As such, in one aspect, there is provided a serotonin receptor 5-HT_{2A} inhibitor or composition comprising serotonin receptor 5-HT_{2A} inhibitor for use in treating a proliferative disease in a subject in need thereof.

 In another aspect, there is provided the use of a serotonin receptor 5-HT_{2A} inhibitor in the manufacture of a medicament for treating a proliferative disease in a
10 subject in need thereof.

 In yet another aspect, there is provided a method of treating a proliferative disease in a subject in need thereof, wherein the method comprises administering an effective amount (e.g. therapeutically effective amount) of a serotonin receptor 5-HT_{2A} inhibitor into the subject.

15 In some examples, there is provided methods of modulating the immune system in a subject, wherein the method comprises administering an effective amount of a serotonin receptor 5-HT_{2A} inhibitor into the subject.

 As used herein, the immune system encompasses all types of cells, compounds, composition, and/or protein that is part of the body's self-defense mechanism against
20 foreign objects and/or aberrant cells. In some examples, the immune system includes the innate immune system such as NK cells, neutrophils, eosinophils, basophils, monocytes, and the like. In some examples, the immune system includes the adaptive immune system such as T lymphocytes, B lymphocytes, NK T cells, antigen presenting cells, dendritic cells, and the like. In some examples, the methods as disclosed herein
25 modulates CD8 T cells in a subject.

 In some examples, the methods as disclosed herein removes the suppression on the immune cells by liver cells or liver-resident cells. In some examples, liver-resident cells may include, but is not limited to, liver non-parenchymal cells. In some examples, liver non-parenchymal cells include liver sinusoidal endothelial cells, hepatic stellate
30 cells, and the like. In some examples, liver-resident cells may include, but is not limited to, liver-resident immune cells. In some examples, liver-resident immune cells include liver-resident T cell, liver-resident NK cell, and the like. In some examples, the methods as disclosed herein removes the suppression on T lymphocytes. In some examples, the methods as disclosed herein removes the suppression on CD8 T lymphocytes. In some

examples, the serotonin receptor 5-HT_{2A} inhibitor ameliorates the suppression of the immune system, optionally ameliorates the suppression of CD8 T cell function.

In some examples, the serotonin receptor 5-HT_{2A} inhibitor acts as an adjuvant therapy/immunotherapy for the subject in need thereof.

5 In some examples, the serotonin receptor 5-HT_{2A} inhibitor augment CD8 T cell activation. In some examples, the serotonin receptor 5-HT_{2A} inhibitor augments the secretion of pro-inflammatory cytokine IFN γ in CD8 T cell activation.

 Without wishing to be bound by theory, it is believed that the serotonin receptor 5-HT_{2A} inhibitor (such as ketanserin) disrupts the immunosuppressive interactions
10 between liver cells and tumour-specific immune cells. In some examples, the inventors believe the serotonin receptor 5-HT_{2A} inhibitor (such as ketanserin) surprisingly ameliorates liver non-parenchymal cell (NPC) suppression of CD8 T cell effector function. The inventors found treatment with the serotonin receptor 5-HT_{2A} inhibitor surprisingly leads to significant expansion and/or activation of CD8 T cells effector
15 (function). Thus, in some examples, the serotonin receptor 5-HT_{2A} inhibitor increases the effectiveness of immune cells in immunotherapy. For example, the serotonin receptor 5-HT_{2A} inhibitor increase the effectiveness of T cell-targeted immunotherapy.

 In some examples, functional phenotype of an activated immune cell (such as an activated CD8 T cell) may include, but is not limited to, an upregulation of CD25
20 expression, an increase expression/production of IFN γ and/or TNF α , an increase of CD44 expression, an increase expression/production of granzyme B, an increase expression/production of perforin, an increase cytotoxicity (as demonstrated by in vitro killing of target cells), and the like.

 Also disclosed are uses of serotonin receptor 5-HT_{2A} inhibitors to augment CD8
25 T cell activation, of which one embodiment is the reduction of liver non-parenchymal cell (NPC) suppression of CD8 T cells. In some examples, the specific aspect of CD8 T cell activation that is augmented is secretion of the pro-inflammatory cytokine IFN γ .

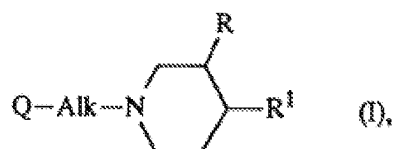
 Without wishing to be bound by theory, targeting the 5-HT_{2A} serotonin receptor to disrupt liver non-parenchymal cell and CD8 T cell function results in enhanced CD8 T
30 cell activation. In some examples, this disruption is mediated by disruption of the gene coding for 5-HT_{2A} (*Htr2a* in mice, *HTR2A* in humans).

 In some examples, the 5-HT_{2A} inhibitor is a selective antagonist of 5-HT_{2A}.

 In some examples, the 5-HT_{2A} inhibitor is an agent that targets the serotonin receptor 5-HT_{2A} by disrupting its function in enhancing CD8 T cell activation and/or an

agent that targets the serotonin receptor 5-HT_{2A} overall function and therefore disrupts its function in suppressing CD8 T cell activation.

In some examples, the 5-HT_{2A} inhibitor is a compound having the formula:



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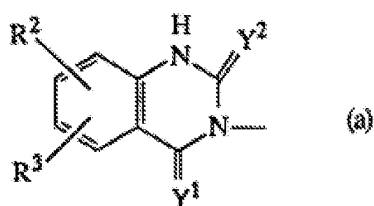
the pharmaceutically acceptable acid-addition salts thereof and the possible stereoisomeric forms thereof; wherein

R is hydrogen or C₁₋₆alkyl;

Alk is C₁₋₄alkanediyl;

10

Q is a radical of formula



wherein Y¹ and Y² are each independently O or S;

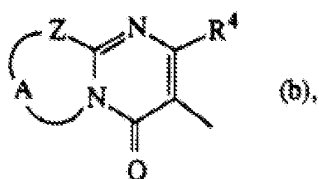
R² is hydrogen, halo, C₁₋₆alkyl or C₁₋₆alkyloxy;

R³ is hydrogen or halo;

15

or

Q is a radical of formula



wherein R⁴ is hydrogen or C₁₋₆alkyl;

Z is -S-, -CH₂- or -CR⁵=CR⁶-; said R⁵ and R⁶ each independently being hydrogen or C₁₋₆alkyl; and A is a bivalent radical -CH₂-CH₂-, -CH₂-CH₂-CH₂- or -CR⁷=CR⁸-, said R⁷ and R⁸ each independently being hydrogen, halo, amino or C₁₋₆alkyl;

5 R¹ is a radical of formula



wherein Ar is phenyl or substituted phenyl, said substituted phenyl bearing an
 10 amino group and/or 1, 2 or 3 halo atoms; and
 wherein X is >C=O , >CH-OH , >CH-O-C(O)-R^9 , >CH_2 , $\text{>C(OC}_{1-6}\text{alkyl)}_2$,



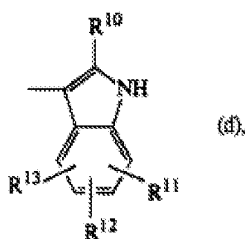
>C=N-OH or >C=N-NH_2 ;

said R⁹ being hydrogen or C₁₋₆ alkyl and

15 said q being the integer 2 or 3;

or

R¹ is a radical of formula



20 wherein R¹⁰ is hydrogen or C₁₋₆alkyl; R¹¹, R¹² and R¹³ are each independently hydrogen or halo;

or

25 R¹ is a radical of formula



wherein A is O or S; R¹⁴ and R¹⁵ are each independently hydrogen, halo, hydroxy, C₁₋₆alkyloxy or C₁₋₆alkyl.

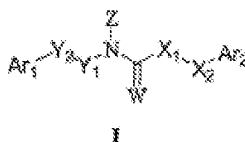
In some examples, compounds of formula (I) according to the present disclosure are those wherein R¹ is a radical of formula (c).

In some examples, compounds of formula (I) according to the present disclosure are those compounds of formula (I) wherein Q is a radical of formula (a) wherein Y¹ and Y² are both oxygen atoms and R² and R³ are both hydrogen; or wherein Q is a radical of formula (b), wherein R⁴ is methyl, Z is -CR⁵=CR⁶-wherein R⁵ and R⁶ are independently hydrogen or methyl, A is -CR⁷=CR⁸- wherein R⁷ and R⁸ are independently hydrogen or methyl; and/or wherein in the radical (c)X is > C=O and Ar is halo substituted phenyl.

In some examples, compound according to the present disclosure are selected from the group consisting of 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1H,3H)-quinazolinedione which compound is generically designated as ketanserin, and 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,7-dimethyl-4H-pyrido-[1,2-a]-pyrimidin-4-one, and the pharmaceutically acceptable acid-addition salts thereof.

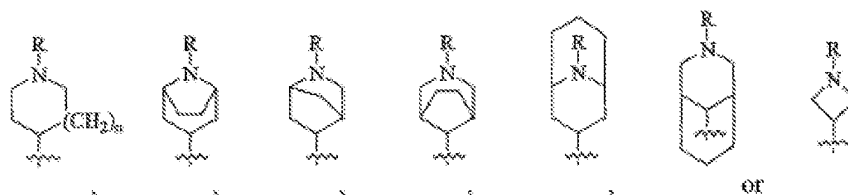
The compounds of formula (I) can be used as such or in their acid-addition salt form. The latter can conveniently be obtained by treating the base-form with appropriate acids, such as, for example, inorganic acids, such as hydrohalic acid, e.g. hydrochloric, hydrobromic and the like, and sulfuric acid, nitric acid, phosphoric acid and the like; or organic acids, such as, for example, acetic, propanoic, hydroxyacetic, 2-hydroxypropanoic, 2-oxopropanoic, ethanedioic, propanedioic, butanedioic, (Z)-2-butenedioic, (E)-2-butenedioic, 2-hydroxybutanedioic, 2,3-dihydroxybutanedioic, 2-hydroxy-1,2,3-propanetricarboxylic, methanesulfonic, ethanesulfonic, benzenesulfonic, 4-methylbenzenesulfonic, cyclohexanesulfamic, 2-hydroxybenzoic, 4-amino-2-hydroxybenzoic and the like acids.

In some examples, the 5-HT_{2A} inhibitor is a compound having the formula:



wherein

Z is a group selected from



R is hydrogen, a cyclic or straight-chained or branched acyclic organyl group, a lower hydroxyalkyl group, a lower aminoalkyl group, or an aralkyl or heteroaralkyl group;

5 n is 0, 1, or 2;

X₁ is methylene, vinylene, or an NH or N(lower alkyl) group; and

X₂ is methylene, or, when X₁ is methylene or vinylene, X₂ is methylene or a bond; or when X₁ is methylene, X₂ is O, S, NH, or N(lower alkyl) or a bond;

Y₁ is methylene and Y₂ is methylene, vinylene, ethylene, propylene, or a bond; or

10 Y₁ is a bond and Y₂ is vinylene; or

Y₁ is ethylene and Y₂ is O, S, NH, or N(lower alkyl);

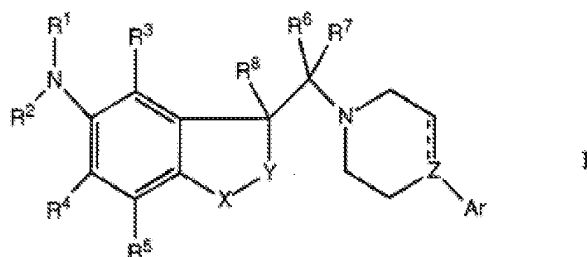
Ar₁ and Ar₂ independently are unsubstituted or substituted aryl or heteroaryl groups;

W is oxygen or sulfur; or

a pharmaceutically acceptable salt, ester, or prodrug thereof.

15 Also provided are pharmaceutical compositions comprising an effective amount of a compound of formula (I) or pharmaceutically acceptable salts, esters, or prodrugs thereof.

In some examples, the 5-HT_{2A} inhibitor is a compound having the formula:



20

wherein one of X and Y is CH₂ and the other one is selected from the group consisting of CH₂, O, and S;

the dotted line, emanating from Z, indicates an optional bond; when it does not indicate a bond Z is N, CH or COH; and when it indicates a bond Z is C;

25 Ar is phenyl, 2-thienyl, 3-thienyl, 2-furanyl, 3-furanyl, 2-pyrimidyl, 1-indolyl, 2-indolyl, 3-indolyl, 1-indol-2-onyl, 3-indol-2-onyl, 2- or 3-benzofuranyl, 2- or 3-

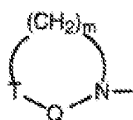
benzothiophenyl, 1-naphthyl or 2-naphthyl, each optionally substituted with halogen, lower alkyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, trifluoromethylsulfonyloxy, cycloalkyl, cycloalkyl-lower-alkyl, nitro, amino, lower alkylamino, di-lower alkylamino, acylamino or C₁₋₂ alkylenedioxy;

5 R^1 is hydrogen, lower alkyl, lower alkenyl, lower alkynyl, cycloalk(en)yl, cycloalk(en)yl-lower alk(en/yn)yl, aryl, aryl-lower alkyl, acyl, thioacyl, lower alkylsulfonyl, trifluoromethylsulfonyl, arylsulfonyl,

R^1 is a group R^9VCO- where V is O or S and R^9 is lower alkyl, cycloalkyl, cycloalkyl-lower-alkyl or aryl, or R^1 is a group $R^{10}R^{11}NCO-$ or $R^{10}R^{11}NCS-$ wherein R^{10} and R^{11} are independently hydrogen, lower alkyl, cycloalkyl, cycloalkyl-lower-alkyl or aryl, or R^{10} and R^{11} together with the N-atom to which they are linked, form a pyrrolidinyl, piperidinyl or perhydroazepin group;

R^2 is hydrogen, lower alkyl, cycloalkyl or cycloalkyl-lower-alkyl;

or R^1 and R^2 together with the N-atom to which they are linked form a group,



15 Q is C=O, C=S or CH₂; T is NH, S, O or CH₂; and m is 1-4, inclusive;

$R^3 - R^5$ are independently hydrogen, halogen, lower alkyl, lower alkylcarbonyl, phenylcarbonyl, halogen substituted phenylcarbonyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, cycloalkyl, cycloalkyl-lower-alkyl or nitro;

R^6 and R^7 are each hydrogen or lower alkyl or they are linked together to constitute a 3 - 7-membered carbocyclic ring;

R^8 is hydrogen or lower alkyl;

any alkyl, cycloalkyl or cycloalkylalkyl group present being optionally substituted with one or two hydroxy groups, which again are optionally esterified with an aliphatic or aromatic carboxylic acid; and any aryl substituent present being optionally substituted with halogen, lower alkyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, trifluoromethylsulfonyloxy, cycloalkyl, cycloalkyl-lower-alkyl or nitro;

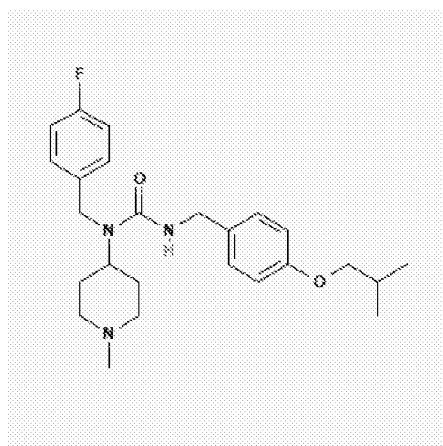
30 and pharmaceutically acceptable acid addition salts thereof.

In some examples, the 5-HT_{2A} inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione (ketanserin tartrate), 1-

[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

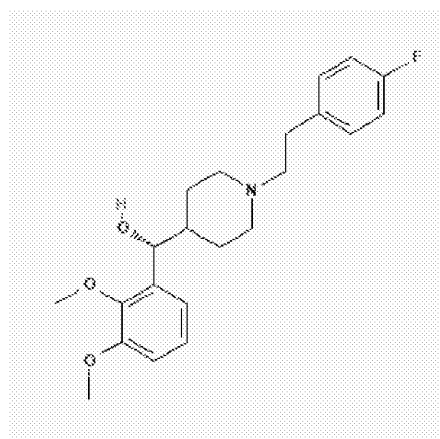
5 As used herein, the term Pimavanserin refers to a member of the class of ureas in which three of the four hydrogens are replaced by 4-fluorobenzyl, 1-methylpiperidin-4-yl, and 4-(isopropoxy)benzyl groups. Pimavanserin has CAS no. 706779-91-1 or 706782-28-7, or also known as 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea, or the following formula:

10

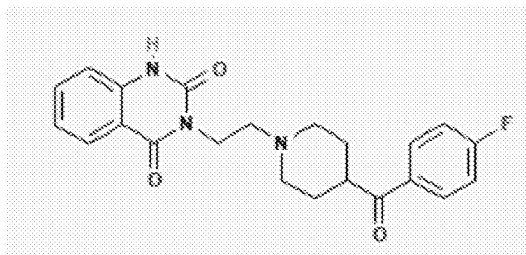


As used herein, the term Volinanserin refers to a selective 5-HT_{2A} receptor antagonist. Volinanserin has CAS no. 139290-65-6, or also known as: (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol, or the following formula:

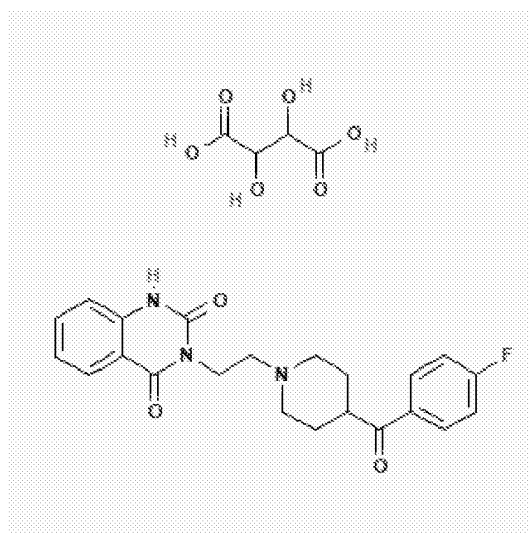
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As used herein, the term ketanserin refers to a quinazoline derivative that acts as a selective 5-HT_{2A} serotonin antagonist. Ketanserin has CAS no. 74050-98-9 or 83846-83-7, or also known as: [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione], or the following formula:



In some examples, the ketanserin as described herein may be ketanserin tartrate, having CAS: 83846-83-7, or also known as 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1H-quinazoline-2,4-dione, or the following formula:



In some examples, the specific inhibitors of 5-HT_{2A} which may be based on the molecular structure of ketanserin may show efficacy in augmenting CD8 T cell activation.

15 In some examples, the inhibitor of 5-HT_{2A} is ketanserin.

Pharmaceutically acceptable salts that may be mentioned include acid addition salts and base addition salts. Such salts may be formed by conventional means, for example by reaction of a free acid or a free base form of a compound of formula I with one or more equivalents of an appropriate acid or base, optionally in a solvent, or in a

medium in which the salt is insoluble, followed by removal of said solvent, or said medium, using standard techniques (e.g. in vacuo, by freeze-drying or by filtration). Salts may also be prepared by exchanging a counter-ion of a serotonergic compound in the form of a salt with another counter-ion, for example using a suitable ion exchange resin.

5 Examples of pharmaceutically acceptable salts include acid addition salts derived from mineral acids and organic acids, and salts derived from metals such as sodium, magnesium, or preferably, potassium and calcium.

 Examples of acid addition salts include acid addition salts formed with acetic, 2,2-dichloroacetic, adipic, alginic, aryl sulphonic acids (e.g. benzenesulphonic, naphthalene-2-sulphonic, naphthalene-1,5-disulphonic and p-toluenesulphonic), ascorbic (e.g. L-ascorbic), L-aspartic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulphonic, (+)-(1S)-camphor-10-sulphonic, capric, caproic, caprylic, cinnamic, citric, cyclamic, dodecylsulphuric, ethane-1,2-disulphonic, ethanesulphonic, 2-hydroxyethanesulphonic, formic, fumaric, galactaric, gentisic, glucoheptonic, gluconic
10 (e.g. D-gluconic), glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α -oxoglutaric, glycolic, hippuric, hydrobromic, hydrochloric, hydriodic, isethionic, lactic (e.g. (+)-L-lactic and ($\pm$)-DL-lactic), lactobionic, maleic, malic (e.g. (-)-L-malic), malonic, ($\pm$)-DL-mandelic, metaphosphoric, methanesulphonic, 1-hydroxy-2-naphthoic, nicotinic, nitric, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, L-pyroglutamic,
15 salicylic, 4-amino-salicylic, sebacic, stearic, succinic, sulphuric, tannic, tartaric (e.g. (+)-L-tartaric), thiocyanic, undecylenic and valeric acids.

 Particular examples of salts are salts derived from mineral acids such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids; from organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic,
20 gluconic, succinic, arylsulphonic acids; and from metals such as sodium, magnesium, or preferably, potassium and calcium.

 As mentioned above, also encompassed are any solvates of the compounds and their salts. Preferred solvates are solvates formed by the incorporation into the solid state structure (e.g. crystal structure) of the compounds of the invention of molecules of a non-toxic pharmaceutically acceptable solvent (referred to below as the solvating solvent).
30 Examples of such solvents include water, alcohols (such as ethanol, isopropanol and butanol) and dimethylsulphoxide. Solvates can be prepared by recrystallizing the compounds of the invention with a solvent or mixture of solvents containing the solvating solvent. Whether or not a solvate has been formed in any given instance can be
35 determined by subjecting crystals of the compound to analysis using well known and

standard techniques such as thermogravimetric analysis (TGE), differential scanning calorimetry (DSC) and X-ray crystallography.

The solvates can be stoichiometric or non-stoichiometric solvates. Particularly preferred solvates are hydrates, and examples of hydrates include hemihydrates, monohydrates and dihydrates.

Compounds of the present disclosure will generally be administered as a pharmaceutical formulation in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, which may be selected with due regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutically acceptable carriers may be chemically inert to the active compounds and may have no detrimental side effects or toxicity under the conditions of use. Suitable pharmaceutical formulations are known in the art. For parenteral administration, a parenterally acceptable aqueous solution may be employed, which is pyrogen free and has requisite pH, isotonicity, and stability. Suitable solutions will be well known to the skilled person, with numerous methods being described in the literature.

Otherwise, the preparation of suitable formulations may be achieved routinely by the skilled person using routine techniques and/or in accordance with standard and/or accepted pharmaceutical practice.

The amount of a compound in any pharmaceutical formulation used in accordance with the present disclosure will depend on various factors, such as the severity of the condition to be treated, the particular patient/subject to be treated, as well as the compound(s) which is/are employed. In any event, the amount of a compound in the formulation may be determined routinely by the skilled person.

In some examples, the proliferative disease is a tumour and/or a cancer.

In some examples, the tumour/cancer is selected from the group comprising: liver cancer (such as hepatocellular carcinoma), bone cancer, pancreatic cancer (such as pancreatic ductal adenocarcinoma (PDAC), pancreatic adenocarcinoma, and the like), skin cancer (such as melanoma, including but not limited to, cutaneous or intraocular malignant melanoma), cancer of the head or neck, breast cancer, lung cancer, renal cancer, uterine cancer, bowel cancer, ovarian cancer, colorectal cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, non-Hodgkin's lymphoma, cancer of the oesophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland,

sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumours of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumour angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, environmentally induced cancers including those induced by asbestos, hematologic malignancies including, for example, multiple myeloma, B-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, non-Hodgkin's lymphomas, acute myeloid lymphoma, chronic myelogenous leukaemia, chronic lymphoid leukaemia, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, acute lymphoblastic leukaemia, mycosis fungoides, anaplastic large cell lymphoma, T-cell lymphoma, and precursor T-lymphoblastic lymphoma, and any combinations of cancers thereof.

In some examples, the proliferative disease is a liver cancer.

In some examples, the proliferative disease is a hepatocellular carcinoma.

In some examples, the present disclosure further comprising an additional/combination therapy, including, but is not limited to an immunotherapy (such as an immune checkpoint blockade/ICB therapy/adoptive immune cell transfer therapy such as CAR-T cell therapy), a chemotherapy, an ablative therapy (local ablative therapy) such as a radiotherapy or a surgery (e.g., resection), a transplantation, and the like.

In some examples, the present disclosure further comprising an immunotherapy such as, but not limited to, an immune checkpoint blockade (ICB) therapy comprising an administration of monoclonal antibodies, optionally the immunotherapy may include the use of one or more of anti-PD-L1 (such as atezolizumab or nivolumab), anti-VEGFA (such as bevacizumab), anti-CTLA4 (such as ipilimumab), or anti-PD-1 (such as nivolumab) plus anti-CTLA4 (such as ipilimumab), and/or combination thereof.

Also disclosed are methods of treating HCC comprising administering therapeutically effective amount of serotonin receptor 5-HT_{2A} inhibitors as disclosed herein with immune checkpoint blockade (ICB) therapy. In some examples, immune checkpoint blockade therapy can take the form of a combination of anti-PD-L1 and anti-VEGFA mAbs.

In some examples, the methods as disclosed herein provide higher survival rate, delayed tumour outgrowth and lower lethality as compared to using ICB therapy alone. In one such embodiment, this ICB therapy can take the form of a combination of anti-PD-L1 and anti-VEGFA mAbs.

In some examples, the combination therapy is to be administered to the subject concurrently, sequentially, or separately from the serotonin receptor 5-HT_{2A} inhibitor.

In some examples, the inhibitor is to be administered intravenously, subcutaneously, orally, sublingually, or intraperitoneally.

5 Also disclosed are compositions comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy.

In another aspect, there is provided a pharmaceutical composition comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy.

10 In some examples, the serotonin receptor inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical
15 acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

Also disclosed are combination therapies comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy.

20 In yet another aspect, there is provided a combination therapy comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy, wherein the serotonin receptor inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable
25 salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

In some examples, a patient or subject who received the treatment/therapy may exhibit a decrease in the size or speed of tumour growth following administration of the
30 inhibitor and/or combination therapy as described herein. In some examples, the decrease can be observed at week 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some examples, the decrease may be about 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more. For example, at about week 12 following weekly administration of the inhibitor and/or combination therapy as described herein the
35 patient may exhibit a decrease in tumour growth (size and/or rate of grown) by about

10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

Also disclosed is a combination therapy as described herein.

Also disclosed is therapy / a composition / a method as described herein.

5

BRIEF DESCRIPTION OF FIGURES

Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following discussions and if applicable, in conjunction with the figures. It should be appreciated that other modifications to the serotonin inhibitors may be made without deviating from the scope of the invention. Example embodiments are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new exemplary embodiments. The example embodiments should not be construed as limiting the scope of the disclosure.

15 **Fig. 1** shows *in vitro* screening of candidate small molecules that enhance liver NPC-activated CD8 T cell effector function. Ovalbumin-specific OT-I CD8 T cells were co-cultured in the presence of an equal number of liver NPCs that were pulsed with ovalbumin peptide in 96-well plates. Individual compounds of the Prestwick chemical library of FDA-approved drugs were diluted into separate wells of the co-cultures to a final concentration of 10 μ M in individual wells. As a negative control, an equal volume of DMSO (to a final concentration of 0.5% volume/volume) was added to the co-cultures (black symbols). As a positive control, OT-I CD8 T cells were activated with plate-bound α CD3 ϵ (3 μ g/mL) + soluble α CD28 antibody (1 μ g/mL). The functional phenotype of the activated CD8 T cells was assayed using high-throughput multi-parameter flow cytometry 4 days post-activation. The perturbations of each drug on each parameter were evaluated by the direction and extent of perturbation from the mean of the negative controls for each parameter and scored by the total number of positive perturbations. (A) Distribution of scores of total positive perturbations across all 640 drugs screened. (B-F) Distribution of extent and direction of perturbations (normalised by standard deviations from means of negative controls) for 5 functional parameters assayed. **Left panel** – negative controls, **Center panel**– 50 preliminary hits, **Right panel**– positive controls. Each drug was tested in a single well and evaluated against 80 negative and 80 positive control wells.

35 **Fig. 2** shows the effects of increasing concentrations of ketanserin on markers of CD8 T cell effector function during CD8 T cell activation by liver NPCs. Ketanserin was

added at the indicated concentrations to liver NPC-CD8 T cell co-cultures as in Fig. 1. On day 4 post-activation, CD8 T cells were assayed for effector function by flow cytometry for the indicated parameters. Statistical analysis using Kruskal-Wallis test with Dunn's multiple comparisons test, * $p < 0.05$, ** $p < 0.01$. Data are representative of 2 independent biological replicates.

Fig. 3 shows the effects of genetic and/or pharmacological ablation of 5-HT_{2A} activity on CD8 T cells activated by liver NPCs. OT-I Cas9^{+/+} CD8 T cells were transduced with lentiviral vectors carrying gRNA sequences targeting either *Htr2a* or *LacZ* for CRISPR gene editing. (A) Immunoblot of 5-HT_{2A} to evaluate gene editing efficiency of each candidate gRNA. Red arrows (gRNA sequence #5) indicate the sequence used to generate CRISPR-knockouts of *Htr2a* in subsequent experiments. (B) *Htr2a*-KO or control gRNA (*LacZ*)-transduced OT-I cells were co-cultured with ovalbumin-pulsed liver NPCs as in Fig. 1 in the presence of 5 μ M ketanserin or an equivalent concentration of DMSO vehicle. On day 4 post-activation, CD8 T cells were assayed for effector function by flow cytometry for the indicated parameters. Statistical analysis using 2-way ANOVA with Tukey's multiple comparisons test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. 4 shows 5-HT_{2A} is upregulated in CD8 T cell following activation in mice and in humans. Polyclonal CD8 T cells isolated from wildtype mice and healthy human PBMCs were activated *in vitro* with plate-bound α -CD3 and soluble α -CD28 antibodies for four days. (A) Immunoblot analysis for 5-HT_{2A} and GAPDH in whole cell lysates prepared from mouse CD8 T cells sampled at indicated time points. Each lane was loaded with lysate equivalent to 5×10^5 cells. (B) Quantification of 5-HT_{2A} protein levels relative to GAPDH loading control for samples in (A). (C) Immunoblot analysis for 5-HT_{2A} and Histone H3 in whole cell lysates prepared from human CD8 T cells sampled at indicated time points. Each lane was loaded with lysate equivalent to 5×10^5 cells. (D) Quantification of 5-HT_{2A} protein levels relative to Histone H3 loading control for samples in (C). (E) Dynamics of 5-HT_{2A} protein expression relative to loading controls during activation of CD8 T cells isolated from mice and two healthy human donors.

Fig. 5 shows 5-HT_{2A} inhibition using ketanserin during T cell activation augments the cytotoxic effector phenotype in both mice and human CD8 T cells. Polyclonal CD8 T cells isolated from (A) wildtype mice and (B) healthy human PBMCs were activated *in vitro* with plate-bound α -CD3 and soluble α -CD28 antibodies for four days. On day 4 post-activation, CD8 T cells were assayed for effector function by flow cytometry for the

indicated parameters. Statistical analysis using Welch's two-tailed t-test, *p < 0.05, **p < 0.01.

Fig. 6 shows the effects of targeting 5-HT_{2A} with various approaches in a spontaneously induced mouse model of HCC. HCC was induced in immunocompetent mice by hydrodynamic injection of plasmids expressing oncogenes and a firefly luciferase reporter. **(a)** Survival of HCC tumour-bearing mice treated with 2.5 mg/kg of ketanserin (red symbols) or an equivalent volume of 2.5% (v/v) DMSO vehicle (blue symbols) by intraperitoneal injection twice a week starting from day 25 post-tumour induction. Data is pooled from two independent repeats. Statistical using Mantel-Cox logrank test. **(b)** Survival of mice induced with *Htr2a*-knockout (+*Htr2a* gRNA) or *Htr2a*-wildtype (+*LacZ* gRNA) HCC tumours. Data is pooled from two independent repeats. Statistical analysis using Mantel-Cox logrank test. **(c)** Log2-fold change in tumour burden of HCC-bearing mice that were adoptively transferred with either *Htr2a*-KO or -WT tumour-specific CD8 T cells. Statistical analysis using Mann-Whitney test.

15

EXPERIMENTAL DATA

The inventors of the present disclosure identified ketanserin, a selective antagonist of a serotonin receptor, as a lead hit in an *in vitro* screen of a Prestwick library of US FDA-approved drugs for small molecules that could ameliorate liver non-parenchymal cell (NPC) suppression of CD8 T cell effector function (Fig. 1). Ketanserin scored highly in the number of positively perturbed parameters of CD8 T cell function, with a score of 7 out of a maximum possible of 9 (Fig.1A). Ketanserin treatment of liver NPC-activated CD8 T cells increased their survival and proliferation (Fig.1B), and also resulted in increased expression of the activation marker CD25 (Fig.1C) and increased expression of the effector cytokines IFN γ and TNF α (Fig.1D and 1E), but not of the cytotoxic effector molecule granzyme B (Fig.1F).

The inventors subsequently validated this hit molecule by incubating CD8 T cells activated *in vitro* with liver NPCs with increasing doses of ketanserin tartrate and found that ketanserin treatment showed dose-dependent effects on live cell numbers, CD25 expression, and secretion of IFN γ and TNF α (Fig. 2). As further validation, the inventors generated CD8 T cells that were edited with CRISPR-Cas9 to disrupt the *Htr2a* locus (coding for 5-HT_{2A}, Fig. 3A), and activated these *Htr2a*-KO cells with liver NPCs in the presence or absence of ketanserin. The inventors found that genetic disruption of *Htr2a* recapitulated the phenotype of increased proliferation, CD25 expression, and increased

35

IFN γ secretion that the inventors previously found with ketanserin-mediated inhibition of 5-HT_{2A} (Fig. 3B).

To determine whether the 5-HT_{2A} serotonin receptor was expressed by human CD8 T cells, the inventors profiled the dynamics of 5-HT_{2A} expression during the
5 activation of mouse and human CD8 T cells by immunoblot analysis (Fig. 4). The inventors found that expression of 5-HT_{2A} consistently increased following *in vitro* activation of both mouse (consistent with the data of Leon-Ponte *et al.* (Blood, 2007)) and human CD8 T cells (a novel finding not previously described in the literature). These data indicate that the molecular target of the tool compound ketanserin is indeed present
10 in activated CD8 T cells and thus can be inhibited by specific action of ketanserin.

The inventors further compared the effects of inhibiting 5-HT_{2A} activity using ketanserin in mouse and human CD8 T cells during activation (Fig. 5). In both mouse and human CD8 T cells, ketanserin treatment significantly increased the percentage of granzyme B⁺ cells post-*in vitro* activation. In human CD8 T cells, there was a trend
15 towards increased cell numbers and IFN γ expression although these were not significant, in contrast to mouse CD8 T cells.

The inventors evaluated the therapeutic efficacy of ketanserin administration in our custom MITCH model of HCC (SIGN/TDF/121). The inventors previously described that the MITCH model of HCC only partially responds to combination monoclonal
20 antibody blockade of PD-L1 and VEGFR2, which mimics the resistance to treatment observed when HCC patients were treated with a combination of atezolizumab (anti-PD-L1) + bevacizumab (anti-VEGFA) approved as first line HCC therapy in the clinic. Ketanserin-treated mice survived significantly longer than mice in the control cohort, with a general delay of tumour outgrowth and lethality (Fig. 6A). The protective effect of
25 ketanserin is like to be mediated by its action on cells other than HCC tumour cells, as mice with *Htr2a*-deficient HCC tumours showed no significant difference in survival or spontaneous tumour rejection relative to mice with *Htr2a*-wildtype HCC tumours (Fig. 6B). Furthermore, adoptive transfer therapy of HCC tumour-bearing mice with *Htr2a*-KO tumour-specific CD8 T cells also reduced tumour burden more strongly as compared to
30 mice treated with equal numbers of *Htr2a*-wildtype CD8 T cells (Fig. 6C).

These data therefore provide multiple lines of evidence that disrupting the function of the serotonin receptor 5-HT_{2A} could potentially be an efficacious adjuvant for T cell-targeted immunotherapy of HCC.

APPLICATIONS

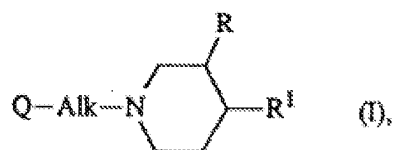
Embodiments of inhibitors of serotonin receptor 5-HT_{2A} disclosed herein
5 provide an alternative adjuvant immunotherapy for treating proliferative disease.

Advantageously, specifically targeting of 5-HT_{2A} serotonin receptor provide
specific activation of tumour-specific CD8 T cells by way of amelioration of
suppression of CD8 T cell by liver non-parenchymal cells.

It will be appreciated by a person skilled in the art that other variations and/or
10 modifications may be made to the embodiments disclosed herein without departing
from the spirit or scope of the disclosure as broadly described. For example, in the
description herein, features of different exemplary embodiments may be mixed,
combined, interchanged, incorporated, adopted, modified, included etc. or the like
across different exemplary embodiments. The present embodiments are, therefore,
15 to be considered in all respects to be illustrative and not restrictive.

CLAIMS

1. A serotonin receptor 5-HT_{2A} inhibitor or composition comprising serotonin
receptor 5-HT_{2A} inhibitor for use in treating a proliferative disease in a subject in need
5 thereof.
2. Use of a serotonin receptor 5-HT_{2A} inhibitor in the manufacture of a medicament
for treating a proliferative disease in a subject in need thereof.
- 10 3. A method of treating a proliferative disease in a subject in need thereof, wherein
the method comprises administering an effective amount of a serotonin receptor 5-HT_{2A}
inhibitor into the subject.
- 15 4. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or the use of claim 2
or the method of claim 3, wherein the serotonin receptor 5-HT_{2A} inhibitor ameliorates the
suppression of the immune system, optionally ameliorates the suppression of CD8 T cell
function.
- 20 5. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or 4 or the use of claim
2 or 4 or the method of claim 3 or 4, wherein the 5-HT_{2A} inhibitor is a selective antagonist
of 5-HT_{2A}.
- 25 6. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4
to 5 or the use of claim 2 or any one of claims 4 to 5 or the method of claim 3 or any one
of claims 4 to 5, wherein the 5-HT_{2A} inhibitor is an agent that targets the serotonin
receptor 5-HT_{2A} by disrupting its function in enhancing CD8 T cell activation and/or an
agent that targets the serotonin receptor 5-HT_{2A} overall function and therefore disrupts
its function in enhancing CD8 T cell activation.
- 30 7. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4
to 6 or the use of claim 2 or any one of claims 4 to 6 or the method of claim 3 or any one
of claims 4 to 6, wherein the 5-HT_{2A} inhibitor is a compound having the formula:

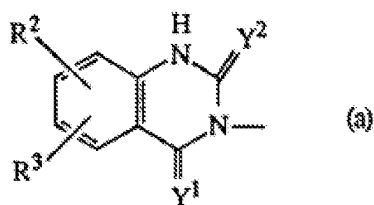


the pharmaceutically acceptable acid-addition salts thereof and the possible stereoisomeric forms thereof; wherein

R is hydrogen or C₁₋₆alkyl;

5 Alk is C₁₋₄alkanediyl;

Q is a radical of formula



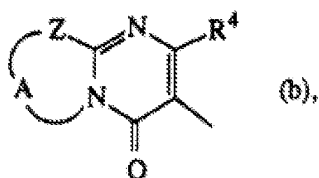
wherein Y¹ and Y² are each independently O or S;

R² is hydrogen, halo, C₁₋₆alkyl or C₁₋₆alkyloxy;

10 R³ is hydrogen or halo;

or

Q is a radical of formula



wherein R⁴ is hydrogen or C₁₋₆alkyl;

15 Z is -S-, -CH₂- or -CR⁵=CR⁶-; said R⁵ and R⁶ each independently being hydrogen or C₁₋₆alkyl; and A is a bivalent radical -CH₂-CH₂-, -CH₂-CH₂-CH₂- or -CR⁷=CR⁸-, said R⁷ and R⁸ each independently being hydrogen, halo, amino or C₁₋₆alkyl;

R¹ is a radical of formula

20



wherein Ar is phenyl or substituted phenyl, said substituted phenyl bearing an amino group and/or 1, 2 or 3 halo atoms; and

wherein X is >C=O , >CH-OH , >CH-O-C(O)-R^9 , >CH_2 , $\text{>C(OC}_{1-6}\text{alkyl)}_2$,



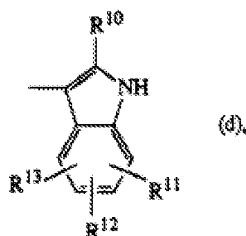
5 >C=N-OH or >C=N-NH_2 ;

said R^9 being hydrogen or C_{1-6} alkyl and

said q being the integer 2 or 3;

or

R^1 is a radical of formula

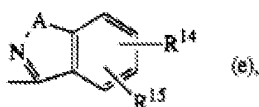


10

wherein R^{10} is hydrogen or C_{1-6} alkyl; R^{11} , R^{12} and R^{13} are each independently hydrogen or halo;

or

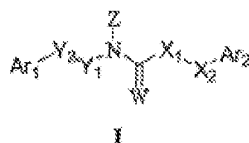
R^1 is a radical of formula



15

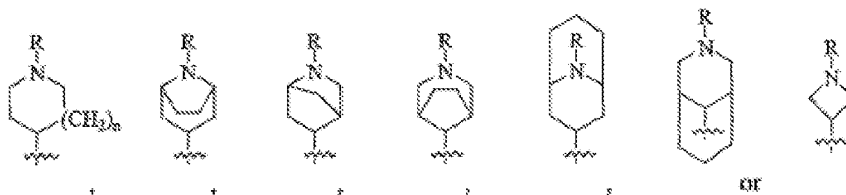
wherein A is O or S; R^{14} and R^{15} are each independently hydrogen, halo, hydroxy, C_{1-6} alkyloxy or C_{1-6} alkyl.

8. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4
20 to 7 or the use of claim 2 or any one of claims 4 to 7 or the method of claim 3 or any one of claims 4 to 7, wherein the 5-HT_{2A} inhibitor is a compound having the formula:



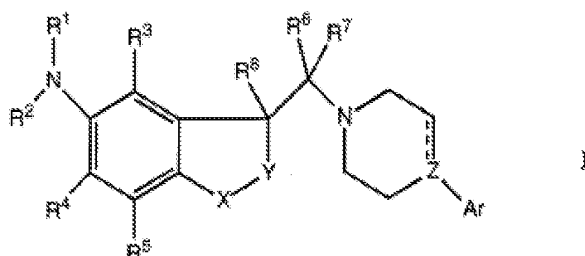
Wherein

Z is a group selected from



- 5 R is hydrogen, a cyclic or straight-chained or branched acyclic organyl group, a lower hydroxyalkyl group, a lower aminoalkyl group, or an aralkyl or heteroaralkyl group;
 n is 0, 1, or 2;
 X₁ is methylene, vinylene, or an NH or N(lower alkyl) group; and
 X₂ is methylene, or, when X₁ is methylene or vinylene, X₂ is methylene or a bond; or
 10 when X₁ is methylene, X₂ is O, S, NH, or N(lower alkyl) or a bond;
 Y₁ is methylene and Y₂ is methylene, vinylene, ethylene, propylene, or a bond; or
 Y₁ is a bond and Y₂ is vinylene; or
 Y₁ is ethylene and Y₂ is O, S, NH, or N(lower alkyl);
 Ar₁ and Ar₂ independently are unsubstituted or substituted aryl or heteroaryl groups;
 15 W is oxygen or sulfur; or
 a pharmaceutically acceptable salt, ester, or prodrug thereof.

9. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 8 or the use of claim 2 or any one of claims 4 to 8, or the method of claim 3 or any one
 20 of claims 4 to 8, wherein the 5-HT_{2A} inhibitor is a compound having the formula:



wherein one of X and Y is CH₂ and the other one is selected from the group consisting of CH₂, O, and S;

the dotted line, emanating from Z, indicates an optional bond; when it does not indicate a bond Z is N, CH or COH; and when it indicates a bond Z is C;

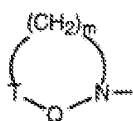
5 Ar is phenyl, 2-thienyl, 3-thienyl, 2-furanyl, 3-furanyl, 2-pyrimidyl, 1-indolyl, 2-indolyl, 3-indolyl, 1-indol-2-onyl, 3-indol-2-onyl, 2- or 3-benzofuranyl, 2- or 3-benzothiophenyl, 1-naphthyl or 2-naphthyl, each optionally substituted with halogen, lower alkyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, trifluoromethylsulfonyloxy, cycloalkyl, cycloalkyl-lower-alkyl, nitro, amino,
10 lower alkylamino, di-lower alkylamino, acylamino or C₁₋₂ alkylenedioxy;

R¹ is hydrogen, lower alkyl, lower alkenyl, lower alkynyl, cycloalk(en)yl, cycloalk(en)yl-lower alk(en/yn)yl, aryl, aryl-lower alkyl, acyl, thioacyl, lower alkylsulfonyl, trifluoromethylsulfonyl, arylsulfonyl,

15 R¹ is a group R⁹VCO- where V is O or S and R⁹ is lower alkyl, cycloalkyl, cycloalkyl-lower-alkyl or aryl, or R¹ is a group R¹⁰R¹¹NCO- or R¹⁰R¹¹NCS- wherein R¹⁰ and R¹¹ are independently hydrogen, lower alkyl, cycloalkyl, cycloalkyl-lower-alkyl or aryl, or R¹⁰ and R¹¹ together with the N-atom to which they are linked, form a pyrrolidinyl, piperidinyl or perhydroazepin group;

R² is hydrogen, lower alkyl, cycloalkyl or cycloalkyl-lower-alkyl;

20 or R¹ and R² together with the N-atom to which they are linked form a group,



wherein Q is C=O, C=S or CH₂; T is NH, S, O or CH₂; and m is 1-4, inclusive;

25 R³ - R⁵ are independently hydrogen, halogen, lower alkyl, lower alkylcarbonyl, phenylcarbonyl, halogen substituted phenylcarbonyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl, cyano, trifluoromethyl, cycloalkyl, cycloalkyl-lower-alkyl or nitro;

R⁶ and R⁷ are each hydrogen or lower alkyl or they are linked together to constitute a 3 - 7-membered carbocyclic ring;

R⁸ is hydrogen or lower alkyl;

30 any alkyl, cycloalkyl or cycloalkylalkyl group present being optionally substituted with one or two hydroxy groups, which again are optionally esterified with an aliphatic or aromatic carboxylic acid; and any aryl substituent present being optionally substituted with halogen, lower alkyl, lower alkoxy, lower alkylthio, hydroxy, lower alkylsulfonyl,

cyano, trifluoromethyl, trifluoromethylsulfonyloxy, cycloalkyl, cycloalkyl-lower-alkyl or nitro;

and pharmaceutically acceptable acid addition salts thereof.

- 5 10. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 9 or the use of claim 2 or any one of claims 4 to 9 or the method of claim 3 or any one of claims 4 to 9, wherein the 5-HT_{2A} inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-
10 [(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.
- 15 11. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 10 or the use of claim 2 or any one of claims 4 to 10 or the method of claim 3 or any one of claims 4 to 10, wherein the proliferative disease is a tumour and/or a cancer.
- 20 12. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 11 or the use of claim 2 or any one of claims 4 to 11 or the method of claim 3 or any one of claims 4 to 11, wherein the proliferative disease is a liver cancer.
- 25 13. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 12 or the use of claim 2 or any one of claims 4 to 12 or the method of claim 3 or any one of claims 4 to 12, wherein the proliferative disease is a hepatocellular carcinoma.
- 30 14. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 13 or the use of claim 2 or any one of claims 4 to 13 or the method of claim 3 or any one of claims 4 to 13 further comprising one or more combination therapy selected from the group consisting of an immunotherapy, a chemotherapy, an ablative therapy, and a transplantation.
- 35 15. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 14 or the use of any claim 2 or any one of claims 4 to 14 or the method of claim 3 or any one of claims 4 to 14 further comprising an immunotherapy such as, but not limited

to, an immune checkpoint blockade (ICB) therapy comprising an administration of monoclonal antibodies, optionally the immunotherapy may include the use of one or more of anti-PD-L1 (such as atezolizumab or nivolumab), anti-VEGFA (such as bevacizumab), anti-CTLA4 (such as ipilimumab), or anti-PD-1 (such as nivolumab) plus
5 anti-CTLA4 (such as ipilimumab), and/or combination thereof.

16. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 15 or the use of any claim 2 or any one of claims 4 to 15 or the method of claim 3 or any one of claims 4 to 15 wherein the combination therapy is to be administered to the
10 subject concurrently, sequentially, or separately from the serotonin receptor 5-HT_{2A} inhibitor.

17. The serotonin receptor 5-HT_{2A} inhibitor for use of claim 1 or any one of claims 4 to 16 or the use of claim 2 or any one of claims 4 to 16 or the method of claim 3 or any
15 one of claims 4 to 16, wherein the inhibitor is to be administered intravenously, subcutaneously, orally, sublingually, or intraperitoneally.

18. A pharmaceutical composition comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy.

20

19. The pharmaceutical composition of claim 17, wherein the serotonin receptor inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-[[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-
25 (2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.

20. A combination therapy comprising a serotonin receptor 5-HT_{2A} inhibitor and an immune checkpoint blockade/ICB therapy, wherein the serotonin receptor inhibitor is [3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione] (ketanserin), 2,3-dihydroxybutanedioic acid;3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-1*H*-quinazoline-2,4-dione (ketanserin tartrate), 1-[(4-fluorophenyl)methyl]-1-(1-methylpiperidin-4-yl)-3-
35 [[4-(2-methylpropoxy)phenyl]methyl]urea (Pimavanserin), (*R*)-(2,3-dimethoxyphenyl)-[1-

[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (Volinanserin) or pharmaceutical acceptable salt or solvate thereof, or pharmaceutically functional derivatives of such compounds.