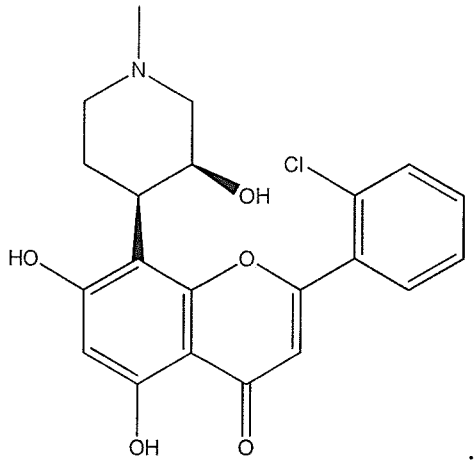


See, for example, U.S. Patent Application Publication No. US2011189175 and US2011189175; PCT International Patent Application Publication Nos. WO 2000044362; WO 2001041747; WO 2001053293; WO 2001053294; WO 2002022133; WO 2007010946, all of which are incorporated by reference herein.

Despite advances in the treatment of HCC, there is a need to provide improved treatment for HCC. Despite advances in the treatment of IHCC, there is a need to provide improved treatment for IHCC.

SUMMARY

In one aspect, the present invention refers to a pharmaceutical formulation comprising Compound 1 or a pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof. In a second aspect, the present invention refers to use of the pharmaceutical formulation as disclosed herein in the manufacture of a medicament for the treatment of hepatocellular carcinoma, wherein said pharmaceutical formulation is to be administered to a patient in need thereof. In a third aspect, the present invention refers to use of the pharmaceutical formulation as disclosed herein in the manufacture of a medicament for the treatment of intrahepatic cholangiocarcinoma, wherein said pharmaceutical formulation is to be administered to a patient in need thereof.

Examples provide a combination therapy, comprising an effective amount of Compound 1 and an effective amount of a CDK4/6 inhibitor. In certain examples the CDK 4/6 inhibitor is palbociclib. In other examples the CDK 4/6 inhibitor is ribociclib. In other examples the CDK 4/6 inhibitor is abemaciclib. In other examples the CDK 4/6 inhibitor is G1T38. In other examples the CDK 4/6 inhibitor is G1T28. Combination therapy provided herein may lead to an enhanced reduction in the viability of HCC cells and may lead to tumor growth inhibition of HCC in patients in need of treatment. Combination therapy provided

herein may also be effective in treatment of hepatic cholangiocarcinoma, including for example intrahepatic cholangiocarcinoma (IHCC).

Examples may provide a method of treating hepatocellular carcinoma in a patient in need thereof, including administering to the patient combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage between 50 mg to 600 mg. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage between 200 mg to 400 mg. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage of 300 mg.

In some examples the CDK 4/6 inhibitor is selected from, for example, 6-acetyl-8-cyclopentyl-5-methyl-2-{[5-(piperazin-1-yl)pyridin-2yl]amino}pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (palbociclib); 7-Cyclopentyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid dimethylamide (ribociclib); and *N*-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-*1H*-benzo[d]imidazol-6-yl)pyrimidin-2-amine (abemaciclib).

In some examples the CDK 4/6 inhibitor is palbociclib. Palbociclib may be administered, for example in a dosage of 75, 100, or 125 mg/day. Typically a dosage is administered orally as a single capsule for 21 consecutive days followed by a 7 day off-treatment period

In some examples the CDK 4/6 inhibitor is ribociclib. Ribociclib may be administered, for example, in a dosage of 200, 400, or 600 mg/day. Typically ribociclib is administered orally as 200 mg capsules or tables, for 21 consecutive days, followed by a 7 day off-treatment period.

In some examples the CDK 4/6 inhibitor is abemaciclib. Abemaciclib may be administered, for example, in a dosage of 200, 300, or 400 mg/day. Typically abemaciclib is administered twice-daily in dosages of 100, 150, or 200 mg/dose. Abemaciclib is typically administered for 21 consecutive days or 28 consecutive days, followed by a 7 day off-

treatment period.

In some examples the CDK 4/6 inhibitor is G1T-38. G1T-38 may be administered, for example, in dosages of 10, 50, or 100 mg/kg. In some examples the CDK 4/6 inhibitor is G1T-28. G1T-28 may be administered, for example, in dosages of between 190 and 200 mg/m^2.

In some examples the CDK 4/6 inhibitor is AT-7519. AT-7519 may be administered, for example, in dosages of 14.4 to 32.4 mg/m^2. AT-7519 may be dosed every three weeks, with drug given on days 1, 4, 8, and 11. In one example the dose is 27 mg/m^2, given at the above frequencies.

In some examples the CDK 4/6 inhibitor is FLX-925. In some examples the CDK 4/6 inhibitor is alvocidib. Alvocidib may be administered, for example, in amounts between 8 and 122 mg/m2. Alvocidib may be administered as a 72 hour infusion. Maximum tolerated dosages of aovocidib have been reported as 40, 50, or 78 mg/m^2.

In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof are administered as separate formulations. Typically the time between administration of each formulation does not exceed 12 hours. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof are administered as a single formulation. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof are administered sequentially with other treatments. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof are administered simultaneously.

In some examples the form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide that is administered is the free base form. In some examples the form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide that is administered is a hydrochloride salt form.

Further examples may provide a pharmaceutical formulation including *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a free base form. In some examples the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a hydrochloride salt form.

Further examples may provide use of a combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-

dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor in the treatment of hepatocellular carcinoma. Further examples may provide use of a combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor in the preparation of a medicament for treatment of hepatocellular carcinoma.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A through FIG. 1C show antitumor effects of Compound 1 and CDK 4/6 inhibitor ribociclib in the JHH7 xenograft model of hepatocellular carcinoma. Both Compound 1 and ribociclib, each as a free base,were given orally (PO) once daily (QD) for 8 days. Data represent the mean ±SEM for tumor volume.

FIG. 1A shows Ribociclib as single agent (****$P \leq 0.0001$ compared to vehicle controls using two way ANOVA followed by Sidak post hoc test).

FIG. 1B shows two dose levels of Ribociclib in combination with 300mg/kg Compound 1 (****$P \leq 0.0001$ compared to 300mg/kg Compound 1 single agent group using two way ANOVA followed by Sidak post hoc test).

FIG. 1C shows two dose levels of Ribociclib in combination with 500mg/kg Compound 1 (****$P \leq 0.0001$ for 500mg/kg Compound 1 single agent group in comparison to the vehicle controls and ****$P \leq 0.0001$ for combinations compared to 500mg/kg Compound 1 single agent group using two way ANOVA followed by Sidak post hoc test).

FIG. 2A through FIG. 2C show antitumor effects of Compound 1 and CDK 4/6 inhibitor palbociclib in the JHH xenograft model of hepatocellular carcinoma. Both Compound 1 and Palbociclib, each as a free base, were given orally (PO) once daily (QD) for 8 days. Data represent the mean ±SEM for Tumor Volume.

FIG. 2A shows palbociclib as single agent (*$P \leq 0.05$ compared to vehicle controls using two way ANOVA followed by Sidak post hoc test).

FIG. 2B shows two dose levels of palbociclib in combination with 300mg/kg Compound 1 (**$P \leq 0.01$ compared to vehicle control and ****$P \leq 0.0001$ compared to

Claims

1. A pharmaceutical formulation comprising *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and a CDK 4/6 inhibitor or a pharmaceutically acceptable salt thereof.

2. The pharmaceutical formulation of claim 1, comprising a free base form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.

3. The pharmaceutical formulation of claim 1, wherein the pharmaceutically acceptable salt of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a hydrochloride salt form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.

4. The pharmaceutical formulation as defined in claim 1 for use in therapy.

5. Use of the pharmaceutical formulation as defined in claim 1 in the manufacture of a medicament for the treatment of hepatocellular carcinoma, wherein said pharmaceutical formulation is to be administered to a patient in need thereof.

6. Use of the pharmaceutical formulation as defined in claim 1 in the manufacture of a medicament for the treatment of intrahepatic cholangiocarcinoma, wherein said pharmaceutical formulation is to be administered to a patient in need thereof.

7. The use of claim 5 or claim 6, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is to be administered in a daily dosage selected from the group consisting of between 50 mg to 600 mg/day, between 200 mg to 400 mg/day, between 50 mg/day and 3000 mg/day, 150 mg/day, 300 mg/day, 600 mg/day, 1000 mg/day, 1500 mg/day, and 2000 mg/day.

8. The use of any one of claims 5 to 7, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is to be administered as a single dosage or in multiple dosages.

9. The use of any one of claims 5 to 8, wherein the CDK 4/6 inhibitor is selected from the group consisting of 6-acetyl-8-cyclopentyl-5-methyl-2-{[5-(piperazin-1-yl)pyridin-2yl]amino}pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (palbociclib) and pharmaceutically acceptable

salts thereof; *N*-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-*1H*-benzo[d]imidazol-6-yl)pyrimidin-2-amine (abemaciclib) and pharmaceutically acceptable salts thereof; and 7-cyclopentyl-2-(5-piperazin-1-yl-pyridin-2-ylamino)-7H-pyrrolo[2,3-d]pyrimidine-6-carboxylic acid dimethylamide (ribociclib); G1T-38; 2'-((5-(4-methylpiperazin-1-yl)pyridin-2-yl)amino)-7',8'-dihydro-6'H-spiro[cyclohexane-1,9'-pyrazino[1',2':1,5]pyrrolo[2,3-d]pyrimidin]-6'-one (G1T-28); N-(4-piperidinyl)-4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3 carboxamide (AT-7519); 2-Hydroxy-1-[2-[[9-(trans-4-methylcyclohexyl)-9*H*-pyrido[4',3':4,5]pyrrolo[2,3-d]pyrimidin-2-yl]amino]-7,8-dihydro-1,6-naphthyridin-6(5*H*)-yl]ethanone (FLX-925); 2-(2-chlorophenyl)-5,7-dihydroxy-8-((3*S*,4*R*)-3-hydroxy-1-methylpiperidin-4-yl)-4*H*-chromen-4-one (alvocidib) and pharmaceutically acceptable salts thereof.

10. The use of any one of claims 5 to 9, wherein the CDK 4/6 inhibitor is palbociclib.

11. The use of claim 10, wherein the palbociclib is to be administered in a daily dosage selected from the group consisting of 75 mg/day, 100 mg/day, and 125 mg/day.

12. The use of any one of claims 5 to 9, wherein the CDK 4/6 inhibitor is ribociclib.

13. The use of claim 12, wherein the ribociclib is to be administered in a daily dosage selecting from the group consisting of 200 mg/day, 400 mg/day, and 600 mg/day.

14. The use of any one of claims 5 to 9, wherein the CDK 4/6 inhibitor is abemaciclib.

15. The use of claim 14, wherein the abemaciclib is to be administered in a daily dosage selected from the group consisting of 200 mg/day, 300 mg/day, and 400 mg/day.

16. The use of any one of claims 5 to 15, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or pharmaceutically acceptable salt thereof are to be administered as separate formulations.

17. The use of any one of claims 5 to 15, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or pharmaceutically acceptable salt thereof are to be administered as a single formulation.

18. The use of any one of claims 5 to 15, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-

yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or pharmaceutically acceptable salt thereof are to be administered sequentially.

19. The use of any one of claims 5 to 15, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the CDK 4/6 inhibitor or pharmaceutically acceptable salt thereof are to be administered simultaneously.

20. The use of any one of claims 5 to 19, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is the free base form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.

21. The use of any one of claims 5 to 19, wherein the pharmaceutically acceptable salt of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a hydrochloride salt form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.