THERAPEUTIC AGENTS FOR TREATING HEPATOCELLULAR CARCINOMA

PRIORITY

[1] This application claims priority to U.S. Provisional patent application serial number 63/110,148, filed November 5, 2020, which is incorporated herein by reference in its entirety.

BACKGROUND

[2] Liver cancer is a difficult-to-treat disease at least in part because it is not often identified at an early stage. Most common treatments focus on slowing progress of the disease, extending patient survival and improving quality of life. Hepatocellular carcinoma (HCC), also called malignant hepatoma, is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection (hepatitis B or C) or cirrhosis (alcoholism being the most common cause of liver cirrhosis).

[3] HCC is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected (Global Data - HEPATOCELLULAR CARCINOMA – OPPORTUNITY ANALYSIS AND FORECASTS TO 2024, pg. 21). The incidence of HCC is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of HCC. Each year in the United States, approximately 15,000 men and 6,000 women are diagnosed with primary liver cancer.

[4] The presentation of HCC has evolved significantly over the past few decades. Whereas in the past, HCC generally presented at an advanced stage with right-upper-quadrant pain, weight loss, and signs of decompensated liver disease, it is now increasingly recognized at a much earlier stage as a consequence of the routine screening of patients with known cirrhosis, using cross-sectional imaging studies and serum alpha-fetoprotein (AFP) measurements.

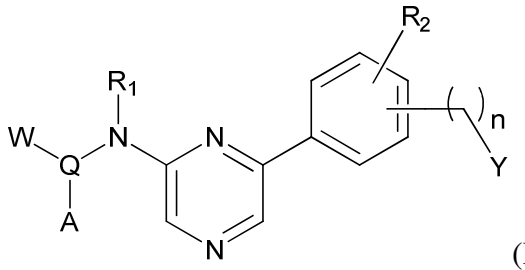
[5] The occurrence of HCC is expected to continue to grow in the coming years. The peak incidence of HCC associated with hepatitis C virus (HCV) infection has not yet occurred. There is also a growing problem with cirrhosis, which develops in the setting of nonalcoholic fatty liver disease (NAFLD), or nonalcoholic steatohepatitis (NASH). NASH typically develops in the setting of obesity, type 2 diabetes, dyslipidemia, and hypertension, and it remains a

significant problem. Thus, developing effective and efficient care for patients with end-stage liver disease and HCC must become a significant focus.

[6] Successful treatments for HCC have been limited (Bruix J, et al. J Hepatol 35:421-430, 2001; Bruix J, et al. Cancer Cell 5:215-219, 2004; Haskell C M. Chapter 46 Liver: Natural History, Diagnosis and Staging in "Cancer Treatment" 5th edition, W. B, Saunders Company, Philadelphia, editors: Haskell C M & Berek J S; Szklaruk J, et al. AJR 180:441-453, 2003). Despite active therapy, survival rates of patients with HCC in the U.S. are among the lowest of all cancer patients (ACS Cancer Facts & Figures (2017)). Thus, there is an urgent need to develop and identify therapeutics that are specific and more effective in the treatment of liver cancers such as HCC.

SUMMARY

[7] In one aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (I):



(I)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

R_1 is H, C_{1-4} alkyl;

Q is a bond, or C_{1-4} alkyl;

A is aryl, heteroaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CH_2F, CHF_2, CF_3, CN, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkylNR_4R_5, O-aryl, O-hetaryl, CO_2R_4, $CONR_4R_5$, nitro, NR_4R_5, C_{1-4} alkylNR_4R_5, NR_6C_{1-4}alkylNR_4R_5, NR_4COR_5, $NR_6CONR_4R_5$, $NR_4SO_2R_5$;

R_4, R_5 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally

substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

R_6 is selected from H, C_{1-4} alkyl;

R_7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl;

R_2 is 0-2 substituents independently selected from halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CH_2F, CHF_2, CF_3, OCF_3, CN, C_{1-4}alkylNR_8R_9, OC_{1-4}alkylNR_8R_9, CO_2R_8, $CONR_8R_9$, NR_8R_9, NR_8COR_9, $NR_{10}CONR_8R_9$, $NR_8SO_2R_9$;

R_8, R_9 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4} alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{11};

R_{10} is selected from H, C_{1-4} alkyl, aryl or hetaryl;

R_{11} is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl;

Y is halogen, OH, $NR_{12}R_{13}$, $NR_{14}COR_{12}$, $NR_{14}CONR_{12}R_{13}$, $N_{14}SO_2R_{13}$;

R_{12} and R_{13} are each independently H, CH_2F, CHF_2, CF_3, CN, C_{1-4} alkyl optionally substituted with OH, OC_{1-4}alkyl or NR15R16, cycloalkyl; cyclohetalkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, or may be joined to form an optionally substituted 3-6 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{14}, R_{15} and R_{16} are each independently selected from H, C_{1-4} alkyl;

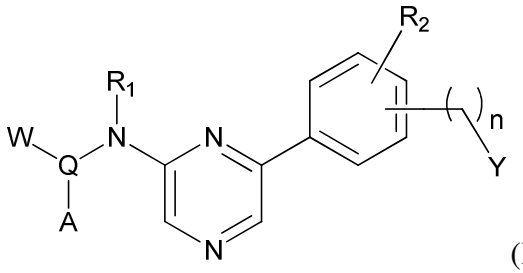
n=0-4;

W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4} alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17}; and

R_{17} is selected from H, C_{1-4} alkyl.

[8] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (II):



(II)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

R_1 is H, C_{1-4} alkyl;

Q is a bond, or C_{1-4} alkyl;

A is aryl, hetaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CH_2F, CHF_2, CF_3, CN, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkylNR_4R_5, O-aryl, O-hetaryl, CO_2R_4, $CONR_4R_5$, nitro, NR_4R_5, C_{1-4} alkylNR_4R_5, NR_6C_{1-4}alkyl$N\ R_4R_5$, NR_4COR_5, $NR_6CON\ R_4R_5$, $NR_4SO_2R_5$;

R_4, R_5 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

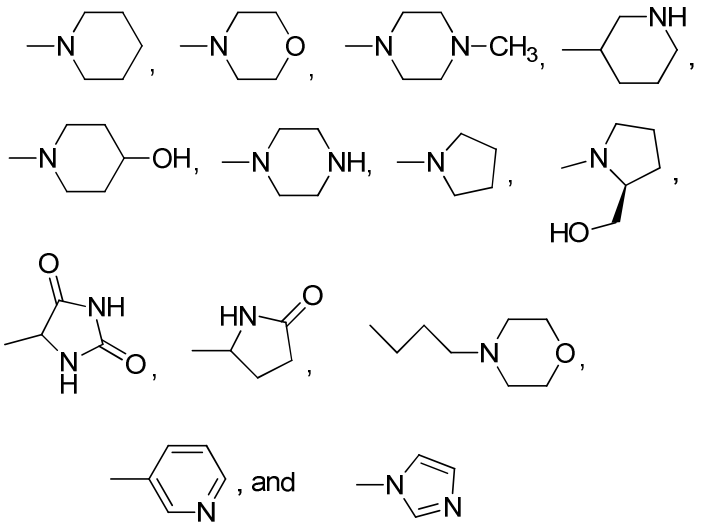
R_6 is selected from H, C_{1-4} alkyl;

R_7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl;

R_2 is 0-2 substituents independently selected from C_{1-4}alkyl and OC_{1-4}alkyl;

Y is CH_2OH, OC_{1-4}alkylOH, OC_{1-4}alkylR_{12}, OC_{1-4}alkyl$NR_{12}NR_{13}$, C(O)R12, CH_2R_{12}, $COOR_{12}$, $CONR_{12}R_{13}$, $OCON\ R_{12}R_{13}$, $CH_2N\ R_{12}R_{13}$, $NHCOR_{12}$, $NHCON\ R_{12}R_{13}$,

R_{12} and R_{13} are each independently H, C_{1-2} alkyl, $(CH_2)_3NEt_2$, $(CH_2)_2NMe_2$, $(CH_2)_5NH_2$, $(CH_2)_2OH$,



n=0-4;

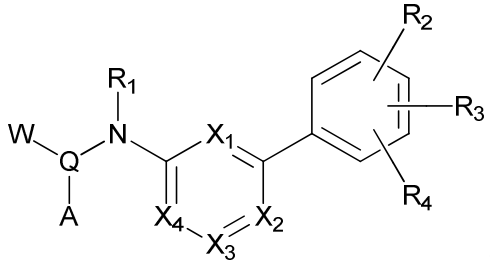
W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4} alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17}

R_{17} is selected from H, C_{1-4} alkyl; and

wherein when Y is CH_2R_{12} then R_{12} is not H, C_{1-2}alkyl.

[9] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (III):



(III)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are selected from the following:

(i) X_1 and X_2 are N and X_3 and X_4 are C independently substituted with Y;

(ii) X_1 and X_4 are N and X_2 and X_3 are C independently substituted with Y;

(iii) X_1 and X_3 are N and X_2 and X_4 are C independently substituted with Y;

(iv) X_2 and X_4 are N and X_1 and X_3 are C independently substituted with Y;

(v) X_1 is N and X_2, X_3, and X_4 are C independently substituted with Y;

(vi) X_3 is N and X_1, X_2, and X_4 are C independently substituted with Y;

(vii) X_4 is N and X_1, X_2, and X_3 are C independently substituted with Y;

(viii) X_2 is N and X_1, X_3, and X_4 are C independently substituted with Y; and

(ix) X_1, X_2 and X_3 are N and X_4 is C substituted with Y;

R_1 is H, C_{1-6}alkyl, C_{1-6}alkylNR_5R_6, C_{1-6}alkylNR_5COR_6, C_{1-6}alkyl$NR_5SO_2R_6$, C_{1-6}alkylCO_2R_5, C_{1-6}alkyl$CONR_5R_6$;

R_5 and R_6 are each independently H, C_{1-4}alkyl, aryl, hetaryl, C_{1-4}alkylaryl, C_{1-4}alkylhetaryl or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

R_7 is selected from H, C_{1-4}alkyl;

R_2 is selected from C_{1-6}alkylOH, OC_{2-6}alkylOH, C_{1-6}alkylNR_8R_9, OC_{2-6}alkyl$N\ R_8R_9$, C_{1-6}alkylNR_8COR_9, OC_{2-6}alkylNR_8COR_9, C_{1-6}alkylhetaryl, OC_{2-6}alkylhetaryl, $OCON\ R_8R_9$, NR_8COOR_9, $NR_{10}CONR_8R_9$, $CONR_8R_9$, NR_8COR_{12};

R_8, R_9 are each independently H, C_{1-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{12} is C_{2-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl;

R_{11}, R_{13} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{14} is selected from H, C_{1-4}alkyl;

R_{10} is H, C_{1-4} alkyl;

R_3 and R_4 are each independently H, halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CF_3, OCF_3;

Q is a bond, or C_{1-4} alkyl;

W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15} and R_{16} are each independently H, C_{1-4}alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4}alkyl cyclohetalkyl, aryl, hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4}alkyl;

A is aryl, hetaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CF_3, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkyl$NR_{18}R_{19}$, Oaryl, Ohetaryl, CO_2R_{18}, $CONR_{18}R_{19}$, $NR_{18}R_{19}$, C_{1-4} alkyl$NR_{18}R_{19}$, $NR_{20}C_{1-4}$alkyl$NR_{18}R_{19}$, $NR_{18}COR_{19}$, $NR_{20}CONR_{18}R_{19}$, NR18SO_2R_{19};

R_{18}, R_{19} are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{21};

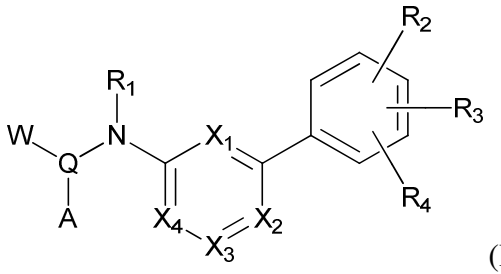
R_{21} is selected from H, C_{1-4}alkyl;

R_{20} is selected from H, C_{1-4}alkyl;

Y is selected from H, C_{1-4}alkyl, OH, $NR_{22}R_{23}$; and

R_{22}, R_{23} are each independently H, C_{1-4}alkyl.

[10] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (IV):



(IV)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are selected from the following:

(i) X_1 and X_2 are N and X_3 and X_4 are C independently substituted with Y;

(ii) X_1 and X_4 are N and X_2 and X_3 are C independently substituted with Y;

(iii) X_1 and X_3 are N and X_2 and X_4 are C independently substituted with Y;

(iv) X_2 and X_4 are N and X_1 and X_3 are C independently substituted with Y;

(v) X_1 is N and X_2, X_3, and X_4 are C independently substituted with Y;

(vi) X_3 is N and X_1, X_2, and X_4 are C independently substituted with Y;

(vii) X_4 is N and X_1, X_2, and X_3 are C independently substituted with Y;

(viii) X_2 is N and X_1, X_3, and X_4 are C independently substituted with Y; and

(ix) X_1, X_2 and X_3 are N and X_4 is C substituted with Y;

R_1 is H, C_{1-6}alkyl, C_{1-6}alkylNR_5R_6, where R_5 and R_6 are each independently H, C_{1-4}alkyl, aryl, hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7; R_7 is selected from H, C_{1-4}alkyl;

R_2 is selected from C_{1-6}alkylOH, OC_{2-6}alkyl OH, C_{1-6}alkylNR_8R_9, OC_{2-6}alkyl NR_8R_9, C_{1-6}alkylNR_8COR_9, OC_{2-6}alkylNR_8COR_9, C_{1-6}alkylhetaryl, OC_{2-6}alkylhetaryl, $OCONR_8R_9$, NR_8COOR_9, $NR_{10}CONR_8R_9$, $CONR_8R_9$, NR_8COR_{12};

R_8, R_9 are each independently H, C_{1-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{12} is C_{2-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl;

R_{11}, R_{13} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14}; R_{14} is selected from H, C_{1-4}alkyl;

R_{10} is H, C_{1-4}alkyl;

R_3 and R_4 are each independently H, halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CF_3, OCF_3;

Q is CH;

W is selected from C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4}alkyl;

A is aryl, hetaryl optionally substituted with 0-2 substituents independently chosen from halogen, C_{1-4}alkyl, CF_3, aryl, hetaryl, OCF_3, OC_{1-4}alkyl; OC_{2-3}alkyl$NR_{18}R_{19}$, Oaryl, Ohetaryl, CO_2R_{18}, $CONR_{18}R_{19}$, $NR_{18}R_{19}$, C_{1-4}alkyl$NR_{18}R_{19}$, $NR_{20}C_{1-4}$alkyl$NR_{18}R_{19}$, $NR_{18}COR_{19}$, $NR_{20}CONR_{18}R_{19}$, $NR_{18}SO_2R_{19}$;

R_{18}, R_{19} are each independently H, C_{1-4}alkyl, C_{1-4}alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4}alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{21};

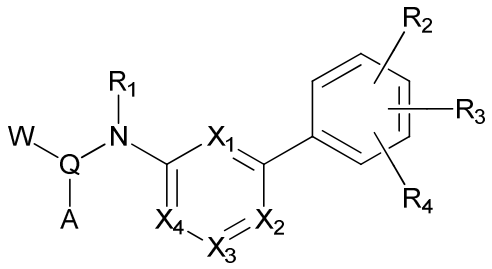
R_{21} is selected from H, C_{1-4}alkyl;

R_{20} is selected from H, C_{1-4}alkyl;

Y is selected from H, C_{1-4}alkyl, $NR_{22}R_{23}$; and

R_{22}, R_{23} are each independently H, C_{1-4}alkyl.

[11] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (V):



or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are selected from the following:

(i) X_1 and X_2 are N and X_3 and X_4 are C independently substituted with Y;

(ii) X_1 and X_4 are N and X_2 and X_3 are C independently substituted with Y;

(iii) X_2 and X_4 are N and X_1 and X_3 are C independently substituted with Y;

(iv) X_1 is N and X_2, X_3, and X_4 are C independently substituted with Y;

(v) X_3 is N and X_1, X_2, and X_4 are C independently substituted with Y;

(vi) X_4 is N and X_1, X_2, and X_3 are C independently substituted with Y;

(vii) X_2 is N and X_1, X_3, and X_4 are C independently substituted with Y; and

(viii) X_1, X_2 and X_3 are N and X_4 is C substituted with Y;

R_1 is H, C_{1-6}alkyl, C_{1-6} alkylNR_5R_6, C_{1-6}alkylNR_5COR_6, C_{1-6}alkyl$NR_5SO_2R_6$, C_{1-6}alkylCO_2R_5, C_{1-6}alkyl$CONR_5R_6$;

R_5 and R_6 are each independently H, C_{1-4}alkyl, aryl, hetaryl, C_{1-4}alkylaryl, C_{1-4}alkylhetaryl or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

R_7 is selected from H, C_{1-4}alkyl;

R_2 is selected from OH, OC_{1-6}alkyl, C_{1-6}alkylOH, OC_{2-6}alkylOH, C_{1-6}alkylNR_8R_9, OC_{2-6}alkylNR_8R_9, C_{1-6}alkylNR_8COR_9, OC_{2-6}alkylNR_8COR_9, C_{1-6}alkylhetaryl, OC_{2-6}alkylhetaryl, $OCONR_8R_9$, NR_8COOR_9, $NR_{10}CONR_8R_9$, $CONR_8R_9$, NR_8COR_{12};

R_8, R_9 are each independently H, C_{1-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{12} is C_{2-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl;

R_{11}, R_{13} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{14} is selected from H, C_{1-4}alkyl;

R_{10} is H, C_{1-4} alkyl;

R_3 and R_4 are each independently H, halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CF_3, OCF_3;

Q is a bond, or C_{1-4}alkyl;

W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4}alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4}alkyl cyclohetalkyl, aryl, hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4}alkyl;

A is aryl, hetaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CF_3, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkyl$NR_{18}R_{19}$, Oaryl, Ohetaryl, CO_2R_{18}, $CONR_{18}R_{19}$, $NR_{18}R_{19}$, C_{1-4} alkyl$NR_{18}R_{19}$, $NR_{20}C_{1-4}$alkyl$NR_{18}R_{19}$, $NR_{18}COR_{19}$, $NR_{20}CONR_{18}R_{19}$, $NR_{18}SO_2R_{19}$;

R_{18}, R_{19} are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{21};

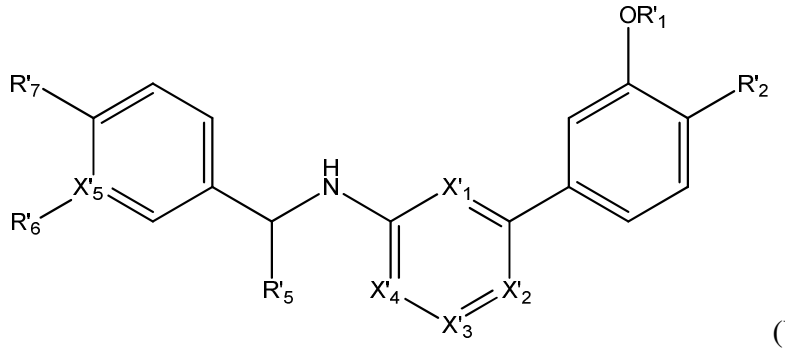
R_{21} is selected from H, C_{1-4} alkyl;

R_{20} is selected from H, C_{1-4} alkyl;

Y is selected from H, C_{1-4}alkyl, OH, $NR_{22}R_{23}$;

R_{22}, R_{23} are each independently H, C_{1-4} alkyl.

[12] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (VI):



(VI)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

R'$_1$ is C_{1-4} alkyl,

R'$_2$ is independently selected from the group consisting of: OH, NHCOR'$_{12}$, and NHCONHR'$_{12}$;

R'$_{12}$ is independently selected from the group consisting of H, C_{1-4} alkyl optionally substituted with OH, OC_{1-4}alkyl or NR'$_{15}$R'$_{16}$;

R'$_{15}$ and R'$_{16}$ are each independently selected from H and C_{1-4} alkyl;

X'$_1$, X'$_2$, X'$_3$, X'$_4$ are selected from the following:

(i) X'$_1$ and X'$_2$ are N and X'$_3$ and X'$_4$ are C independently substituted with Y';

(ii) X'$_1$ and X'$_4$ are N and X'$_2$ and X'$_3$ are C independently substituted with Y';

(iii) X'$_1$ and X'$_3$ are N and X'$_2$ and X'$_4$ are C independently substituted with Y';

(iv) X'$_2$ and X'$_4$ are N and X'$_1$ and X'$_3$ are C independently substituted with Y';

Y' is selected from H, OH, C_{1-4}alkyl, and OC_{1-4}alkyl;

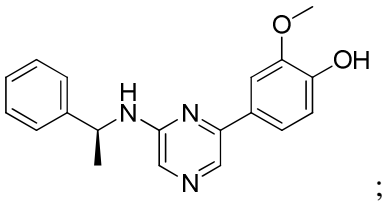
X'$_5$ is selected from N and C, and

when X'$_5$ is C, R'$_6$ is selected from the group H, halogen, C_{1-4} alkyl, OC_{1-4}alkyl, CF_3, and OCF_3:

R'$_5$ is selected from the group C_{1-4} alkyl, OC_{1-4}alkyl, CF_3, and OCF_3; and

R'$_7$ is selected from the group H, halogen, C_{1-4} alkyl, OC_{1-4}alkyl, CF_3, and OCF_3.

[13] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment a compound of the general formula (VI) that is selected from the group consisting of: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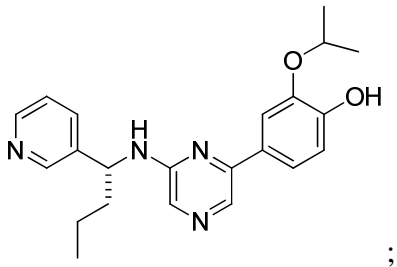
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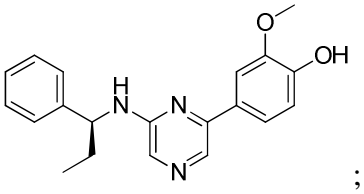
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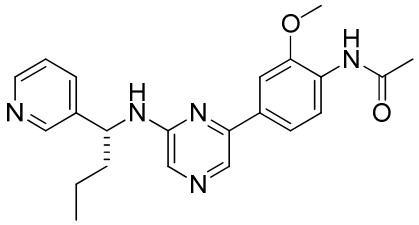
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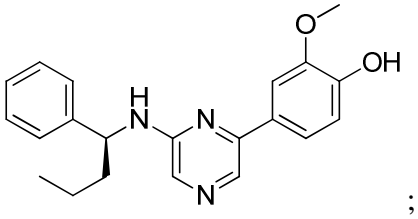
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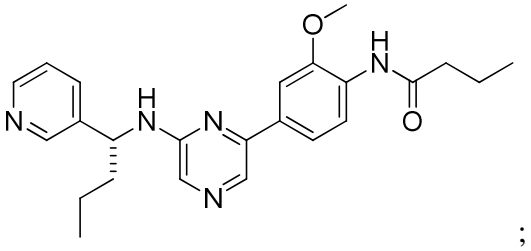
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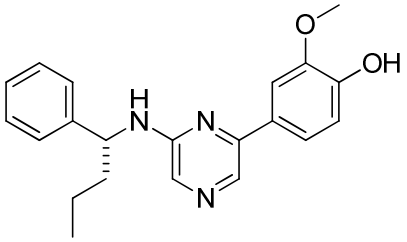
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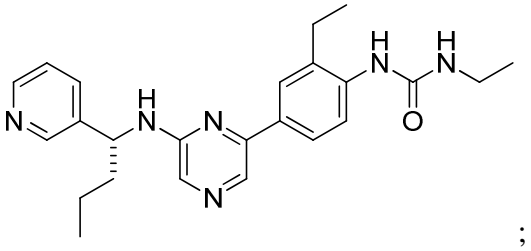
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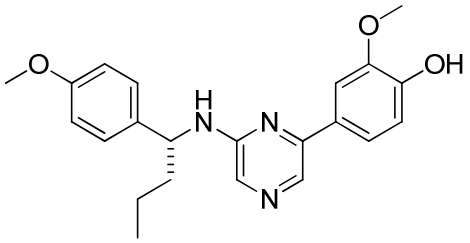
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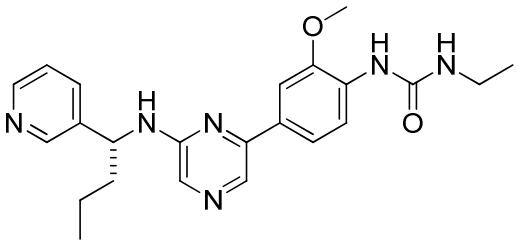
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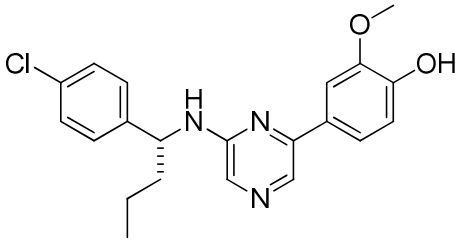
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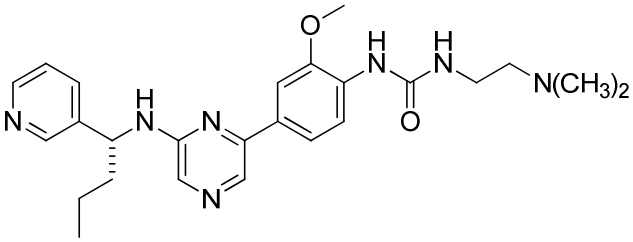
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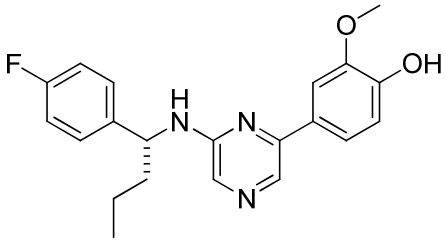
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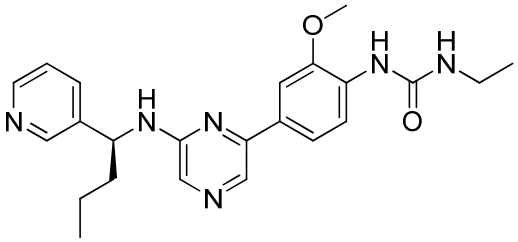
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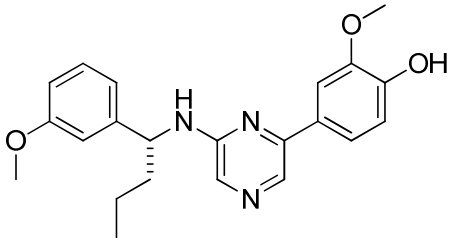
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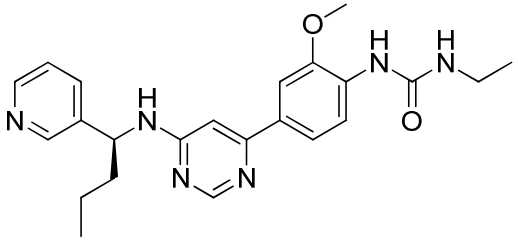
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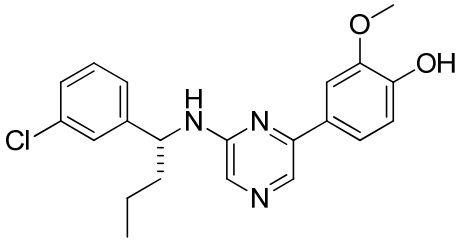
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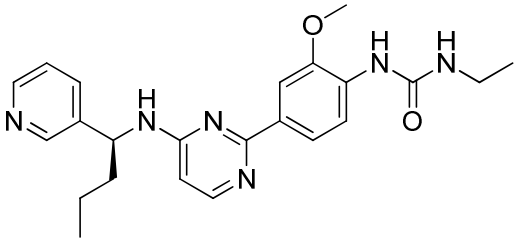
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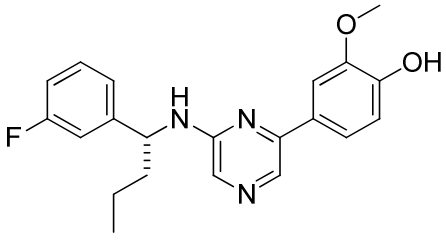

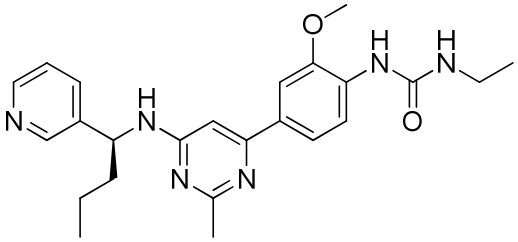
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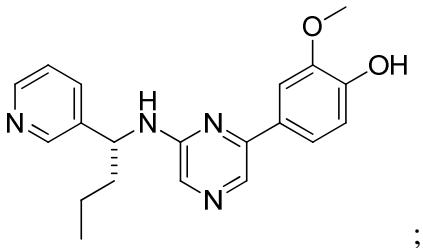
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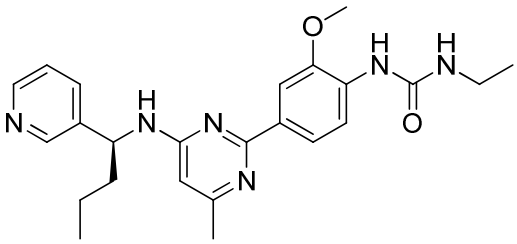
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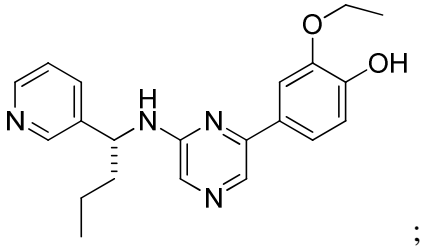
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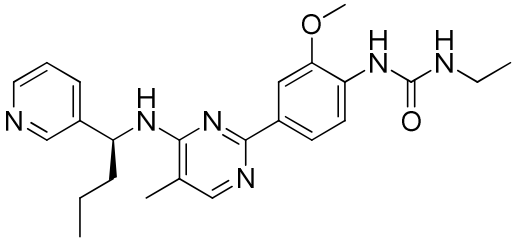
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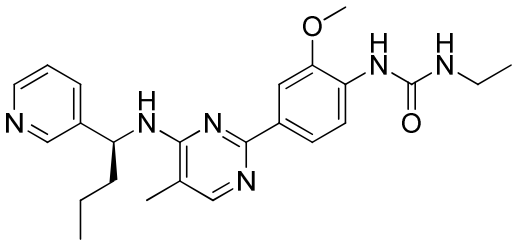
or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or crystal form thereof.

[14] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment a compound having the structure:



or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or crystal form thereof.

[15] In some embodiments of the above aspects, the method can treat a liver cancer that is selected from the group consisting of hepatocellular carcinoma (HCC), fibrolamellar HCC, bile duct cancer, angiosarcoma, and secondary liver cancer. In yet further embodiments, the method may treat HCC.

[16] In some embodiments of the above aspects, the subject may be a mammal that has a liver cancer. In some further embodiments, the subject is a human who has liver cancer.

[17] In some embodiments of the above aspects, the methods can further comprise monitoring the subject for a change in a sign and/or a symptom of liver cancer that is responsive to administering the compound.

[18] In some embodiments, the methods of treatment may be administered as a monotherapy. In alternative embodiments, the methods of treatment may be administered as part of a combination therapy and can further comprise one or more additional therapeutic interventions. In some further embodiments, the methods of treatment may further comprise

surgery. In some further embodiments, the methods of treatment may further comprise administration of at least one additional anti-cancer agent.

[19] In some embodiments of the above aspects, the compound can be administered intravenously, subcutaneously, or orally.

[20] In some further aspects, the disclosure provides for the use of the compounds disclosed herein, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or crystal form thereof, for the treatment of a liver cancer.

[21] In another further aspect, the disclosure provides for the use of the compounds disclosed herein, or a pharmaceutically acceptable prodrug, salt, hydrate, solvate, or crystal form thereof, for the preparation of a medicament for the treatment of a liver cancer.

[22] In embodiments of these further aspects, the use can be for the treatment of a liver cancer selected from the group consisting of: hepatocellular carcinoma (HCC), fibrolamellar HCC, bile duct cancer, angiosarcoma, and secondary liver cancer. In yet further embodiments the liver cancer is HCC.

[23] Other features, aspects, embodiments, and advantages provided by the disclosure will be apparent from the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[24] **Figures 1A-1E** depict viability for individual HCC cell lines HepG2 (Figure 1A); Hep3B (Figure 1B); Hep40 (Figure 1C); Huh7 (Figure 1D); and PLC5 (Figure 1E) as a function of candidate compound concentration.

[25] **Figure 2** depicts IC_{50} summary information for candidate compounds in HCC cell lines and primary human hepatocytes.

[26] **Figure 3** depicts the maximum tolerated dose survival curve for TXR-311 in BALB/c mice.

[27] **Figure 4** depicts body weight changes in maximum tolerated dose assays for different concentrations of TXR-311.

[28] **Figure 5** depicts body weight changes in the PDX1 cohort for animals treated with TXR-311 relative to vehicle.

[29] **Figure 6** depicts tumor measurement in the PDX1 cohort for animals treated with TXR-311 relative to vehicle.

[30] **Figure 7** depicts tumor images (IVIS) for animals in the PDX1 cohort that were treated with vehicle

[31] **Figure 8** depicts tumor images (IVIS) for animals in the PDX1 cohort that were treated with TXR-311.

[32] **Figure 9** depicts differences in tumor size on Day 25 for animals in the PDX1 cohort for animals treated with TXR-311 relative to vehicle.

[33] **Figure 10** depicts the difference in tumor volume in the PDX1 cohort for animals treated with TXR-311 relative to vehicle.

[34] **Figure 11** depicts the body weight in the PDX2 cohort for animals treated with TXR-311 or sorafenib relative to vehicle.

[35] **Figure 12** depicts the IVIS-derived tumor measurement in the PDX2 cohort for animals treated with TXR-311 or sorafenib relative to vehicle.

[36] **Figure 13** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 0.

[37] **Figure 14** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 7.

[38] **Figure 15** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 14.

[39] **Figure 16** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 21.

[40] **Figure 17** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 28.

[41] **Figure 18** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 35.

[42] **Figure 19** depicts tumor images (IVIS) for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib at Day 42.

[43] **Figure 20** depicts tumor images (IVIS) summarizing tumor growth for animals in the PDX2 cohort that were treated with vehicle.

[44] **Figure 21** depicts tumor images (IVIS) summarizing tumor growth for animals in the PDX2 cohort that were treated with TXR-311.

[45] **Figure 22** depicts tumor images (IVIS) summarizing tumor growth for animals in the PDX2 cohort that were treated with sorafenib.

[46] **Figure 23** depicts differences in tumor size for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib.

[47] **Figure 24** depicts a graphical summary of the tumor volume data for animals in the PDX2 cohort that were treated with vehicle, TXR-311, or sorafenib.

DETAILED DESCRIPTION

[48] The disclosure provides methods that are directed to the treatment of liver cancer that comprise administration of compounds and compositions that are identified through a process that combines an initial computational-based analysis of approved or investigational drugs using DUMA, a proprietary artificial intelligence platform, and reviewing existing information available for candidate compounds along with performing additional research and development of the identified candidate compounds specifically directed to determine the efficacy of the compounds in the treatment of liver cancer. Using this type of evaluation provides several advantages relative to conventional therapeutic agent discovery, including reduction in the overall cost and time of providing new treatments and therapies for particular diseases and conditions. The initial screening of candidate compounds that have existing human safety and pharmacodynamic data provides a reduced risk of expensive failures late in development due to safety concerns.

[49] Therefore, in a general sense, the disclosure provides methods of treating liver cancer, including hepatocellular carcinoma (HCC), comprising administering to a subject in need of treatment certain 2-phenyl pyrazines that have been previously described as microtubule inhibitors, and that have now, for the first time, been shown to be effective in the treatment of liver cancer.

[50] Microtubule binding agents include a broad range of structurally diverse compounds that can function to stabilize or destabilize the microtubule structure and assembly. Both stabilizing and destabilizing agents are used as clinically relevant anti-cancer drugs including

stabilizing agents such as docetaxel, paclitaxel, and ixabepilone, and destabilizing agents such as the alkaloids, vincristine, vindesine, and vinblastine. Microtubule binding agents that destabilize microtubule structure are usually classified based on the domain to which they bind, either the vinca-domain or the colchicine binding site (or "CBS"). Some agents that bind the vinca-domain (e.g., vincristine, vindesine, and vinblastine) have been developed as therapeutics against specific types of cancers (e.g., breast, ovarian). In contrast, and despite the existence of data that shows some CBS binding agents possess *in vitro* cytotoxicity against certain cancer cell lines, no CBS binding agents have yet been shown to be effective in the treatment of any cancers.

[51] The instant disclosure and the Examples demonstrate, the inventor have identified a class of microtubule binding agents that target the colchicine binding site and are effective in the treatment of liver cancers including HCC in particular. Furthermore, the data demonstrates that the compounds identified and recited in the methods of treatment exert a specific cytotoxic effect against tumorigenic cells of the liver relative to normal hepatocytes.

Definitions

[52] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[53] It should be appreciated that all numerical ranges disclosed herein are intended to include any particular number within that range as well as sub-ranges that fall within the scope of the broader range. For example, a range of 0.01% to 5.0% will be understood to also encompass ranges falling at or above 0.01% and at or below 5.0% (e.g., 3.7%, 1.0%, 0.02% - .04%, 0.02% - 4.5%, 0.05% - 4.08%, or 0.03% - 1.0%, etc). These are just examples of the types of numbers and ranges that would be encompassed.

[54] The term "tissue" refers to an aggregate of multiple cells and/or cell products.

[55] The "gene" refers to a nucleic acid sequence or portion thereof that encodes a protein. Nucleic acid sequences may include, but are not limited to, DNA in any form, such as genomic DNA, cDNA, synthesized DNA. Nucleic acid sequences may also include RNA and RNA transcripts corresponding to DNA.

[56] The terms "peptide," "polypeptide" and "protein" refer interchangeably to a molecule comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide may contain at least three amino acids, and no limitation may be placed on the maximum number of amino acids that can comprise a protein or peptide sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[57] The terms "patient" and "subject" are used interchangeably to refer to animals that may be in need of a therapy that comprises the methods and compositions described herein. Non-limiting examples of animals that may be a patient or subject include animals such as primates (*e.g.,* humans, and non-human primates, such chimpanzees, gorillas), and other mammals (*e.g.,* cats, dogs, agricultural mammals, such as equines, bovines, ovines, porcines, horses, pigs, cows, sheep, laboratory rodents/animal model systems, such as hamsters, mice and rats, and rabbits).

[58] A subject at risk of a condition includes subjects who by virtue of a known characteristic, such as a genetic marker, biomarker, existing disease or condition, or family history, are at a measurably or significantly higher risk ($p \leq 0.05$) relative to a control population of individuals not known to have such genetic marker, biomarker or family history or the like, of developing the condition. Certain risk factors may be associated with occurrence and/or onset of liver cancer, and may be used to identify subject who may be at risk of developing liver cancer, or who may be screened for liver cancer prior to onset of any symptoms (i.e., clinical conditions) associated with liver cancer. Non-limiting examples of risk factors include hepatitis B or hepatitis C infections, cirrhosis of the liver caused by alcoholism, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH).

[59] The term "disease" or "clinical conditions" refer to a physiological state of an organism with an abnormal biological state. Abnormal biological states include, but are not

limited to, an interruption, cessation or disorder of cells, tissues, body functions, systems or organs that may be inherent, inherited, caused by an infection (e.g., sepsis), caused by abnormal cell function, caused by abnormal cell division (e.g., cancer), and the like. As discussed in the aspects and embodiments herein, the methods relate to the treatment of liver cancer.

[60] Liver cancer, as discussed herein, can refer broadly to any abnormal cell growth, neoplasm, or tumor in the liver. In embodiments of the methods disclosed herein, liver cancer relates to a malignant tumor or neoplasm and may include the non-limiting examples of hepatocellular carcinoma (HCC, or hepatoma), fibrolamellar HCC, cholangiocarcinoma (bile duct cancer), angiosarcoma (hemangiocarcinoma), and secondary liver cancer (metastasis to the liver, often (but not required to be) originating from colon or colorectal cancer). In certain embodiments, the liver cancer is HCC. In some embodiments, the liver cancer is a secondary liver cancer.

[61] The phrase "pharmaceutically acceptable" refers to an agent that does not interfere with the effectiveness of the biological activity of an active ingredient, and which may be approved by a regulatory agency of the Federal government or a state government, or is listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly for use in humans. Accordingly, suitable pharmaceutically acceptable carriers include agents that do not interfere with the effectiveness of a pharmaceutical composition.

[62] The phrase "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable, preferably nontoxic, acids and bases, including inorganic and organic acids and bases, including but not limited to, sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydro bromide, hydro iodide, nitrate, sulfate, bisulfite, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, fornate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Pharmaceutically acceptable salts include those formed with free amino groups such as, but not limited to, those derived from hydrochloric, phosphoric, acetic, oxalic, and tartaric acids. Pharmaceutically acceptable salts also include those formed with free carboxyl groups such as, but not limited to, those derived from sodium, potassium, ammonium,

sodium lithium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, and procaine.

[63] The terms "therapy" and "treatment" refer to those medical steps taken to alleviate or alter a disease state, *e.g.,* a course of treatment intended to reduce or eliminate the affects or symptoms of a disease using pharmacological, surgical, dietary and/or other techniques. A therapeutic regimen may include a prescribed dosage of one or more therapeutic agents.

[64] The term "therapeutic agent" refers to any drug, agent, biologically active agent, biological substance, chemical substance or biochemical substance that is capable of being administered in a therapeutically effective amount to a subject. In various aspects and embodiments of the disclosure therapeutic agents can include one or more compounds disclosed herein.

[65] The term "therapeutically effective amount" refers to an amount of a therapeutic agent, compound, formulation, material, or composition, as described herein effective to achieve a particular biological result. Such results may include, but are not limited to, the inhibition of a disease as determined by any means suitable in the art.

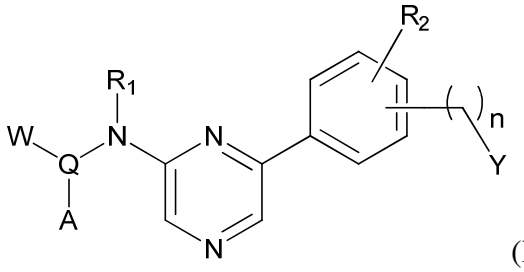
[66] An effective regime refers to a combination of an amount, frequency of administration and route of administration effective to treat a condition. Effective treatment delays onset of, reduces, inhibits deterioration of, or ameliorates at least one sign or symptom of the condition. A regime can be considered effective by demonstrating a statistically significant delay, reduction, inhibition, or amelioration ($p \leq 0.05$) in a sign or symptom comparing a population of treated human subjects (or animal models) having or at risk of the condition being treated relative to a control population of subjects (or animal models) with the condition who are not treated with the agent. The control population can be contemporaneously treated with a placebo or can be a historical control. A treatment can alternatively or additionally be considered effective if it reduces or ameliorates at least one sign or symptom of the condition in a treated subject relative to the same subject before treatment.

[67] Unless otherwise apparent from the context, reference to a particular drug, e.g., by its international non-proprietary name, should be understood as encompassing the drug in the exact form associated with the name as well as minor variations in which the active moiety is supplied as free acid or free base instead of pharmaceutically acceptable salt or vice versa, or is supplied as different pharmaceutically acceptable salt or is supplied as an amide or ester of the active

moiety, such variations being in pharmaceutically acceptable form and with insubstantial, if any, variation in activity.

Methods and Therapeutic agents

[68] In one aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (I):



(I)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

R_1 is H, or C_{1-4} alkyl;

Q is a bond, or C_{1-4} alkyl;

A is aryl, heteroaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CH_2F, CHF_2, CF_3, CN, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkylNR_4R_5, Oaryl, Ohetaryl, CO_2R_4, $CONR_4R_5$, nitro, NR_4R_5, C_{1-4} alkylNR_4R_5, NR_6C_{1-4}alkylNR_4R_5, NR_4COR_5, $NR_6CONR_4R_5$, $NR_4SO_2R_5$;

R_4, R_5 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

R_6 is selected from H, C_{1-4} alkyl;

R_7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl;

R_2 is 0-2 substituents independently selected from halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CH_2F, CHF_2, CF_3, OCF_3, CN, C_{1-4}alkylNR_8R_9, OC_{1-4}alkylNR_8R_9, CO_2R_8, $CONR_8R_9$, NR_8R_9, NR_8COR_9, $NR_{10}CONR_8R_9$, $NR_8SO_2R_9$;

R_8, R_9 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4} alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{11};

R_{10} is selected from H, C_{1-4} alkyl, aryl or hetaryl;

R_{11} is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl;

Y is halogen, OH, $NR_{12}R_{13}$, $NR_{14}COR_{12}$, $NR_{14}CONR_{12}R_{13}$, $N_{14}SO_2R_{13}$;

R_{12} and R_{13} are each independently H, CH_2F, CHF_2, CF_3, CN, C_{1-4} alkyl optionally substituted with OH, OC_{1-4}alkyl or NR15R16, cycloalkyl; cyclohetalkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, or may be joined to form an optionally substituted 3-6 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{14}, R_{15} and R_{16} are each independently selected from H, C_{1-4} alkyl;

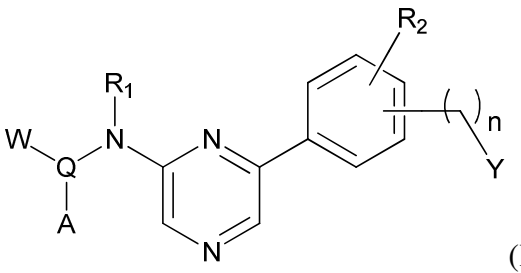
n=0-4;

W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4} alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4} alkyl.

[69] In another aspect disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (II):



(II)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

R_1 is H, C_{1-4} alkyl;

Q is a bond, or C_{1-4} alkyl;

A is aryl, hetaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CH_2F, CHF_2, CF_3, CN, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkylNR_4R_5, Oaryl, Ohetaryl, CO_2R_4, $CONR_4R_5$, nitro, NR_4R_5, C_{1-4} alkyl$N\ R_4R_5$, NR_6C_{1-4}alkyl$N\ R_4R_5$, NR_4COR_5, $NR_6CON\ R_4R_5$, $NR_4SO_2R_5$;

R_4, R_5 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

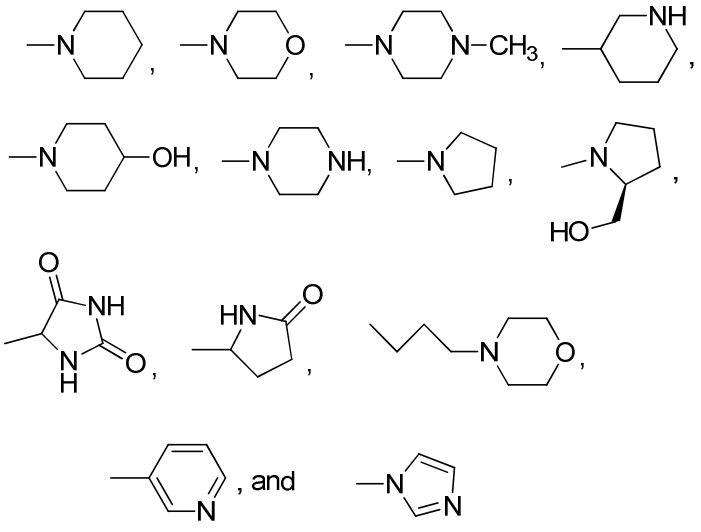
R_6 is selected from H, C_{1-4} alkyl;

R_7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl;

R_2 is 0-2 substituents independently selected from C_{1-4}alkyl and OC_{1-4}alkyl;

Y is CH_2OH, OC_{1-4}alkylOH, OC_{1-4}alkylR_{12}, OC_{1-4}alkyl$NR_{12}NR_{13}$, C(O)R12, CH_2R_{12}, $COOR_{12}$, $CONR_{12}R_{13}$, OCON $R_{12}R_{13}$, $CH_2N\ R_{12}R_{13}$, $NHCOR_{12}$, NHCON $R_{12}R_{13}$,

R_{12} and R_{13} are each independently H, C_{1-2} alkyl, $(CH_2)_3NEt_2$, $(CH_2)_2NMe_2$, $(CH_2)_5NH_2$, $(CH_2)_2OH$,



n=0-4;

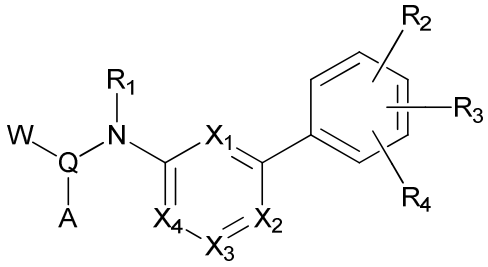
W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4} alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17}

R_{17} is selected from H, C_{1-4} alkyl; and

wherein when Y is CH_2R_{12} then R_{12} is not H, C_{1-2}alkyl.

[70] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (III):



(III)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are selected from the following:

(i) X_1 and X_2 are N and X_3 and X_4 are C independently substituted with Y;

(ii) X_1 and X_4 are N and X_2 and X_3 are C independently substituted with Y;

(iii) X_1 and X_3 are N and X_2 and X_4 are C independently substituted with Y;

(iv) X_2 and X_4 are N and X_1 and X_3 are C independently substituted with Y;

(v) X_1 is N and X_2, X_3, and X_4 are C independently substituted with Y;

(vi) X_3 is N and X_1, X_2, and X_4 are C independently substituted with Y;

(vii) X_4 is N and X_1, X_2, and X_3 are C independently substituted with Y;

(viii) X_2 is N and X_1, X_3, and X_4 are C independently substituted with Y; and

(ix) X_1, X_2 and X_3 are N and X_4 is C substituted with Y;

R_1 is H, C_{1-6}alkyl, C_{1-6}alkylNR_5R_6, C_{1-6}alkylNR_5COR_6, C_{1-6}alkyl$NR_5SO_2R_6$, C_{1-6}alkylCO_2R_5, C_{1-6}alkyl$CONR_5R_6$;

R_5 and R_6 are each independently H, C_{1-4}alkyl, aryl, hetaryl, C_{1-4}alkylaryl, C_{1-4}alkylhetaryl or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

R_7 is selected from H, C_{1-4}alkyl;

R_2 is selected from C_{1-6}alkylOH, OC_{2-6}alkylOH, C_{1-6}alkylNR_8R_9, OC_{2-6}alkyl$N\ R_8R_9$, C_{1-6}alkylNR_8COR_9, OC_{2-6}alkylNR_8COR_9, C_{1-6}alkylhetaryl, OC_{2-6}alkylhetaryl, $OCON\ R_8R_9$, NR_8COOR_9, $NR_{10}CONR_8R_9$, $CONR_8R_9$, NR_8COR_{12};

R_8, R_9 are each independently H, C_{1-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{12} is C_{2-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl;

R_{11}, R_{13} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{14} is selected from H, C_{1-4}alkyl;

R_{10} is H, C_{1-4} alkyl;

R_3 and R_4 are each independently H, halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CF_3, OCF_3;

Q is a bond, or C_{1-4} alkyl;

W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15} and R_{16} are each independently H, C_{1-4}alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4}alkyl cyclohetalkyl, aryl, hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4}alkyl;

A is aryl, hetaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CF_3, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkyl$NR_{18}R_{19}$, Oaryl, Ohetaryl, CO_2R_{18}, $CONR_{18}R_{19}$, $NR_{18}R_{19}$, C_{1-4} alkyl$NR_{18}R_{19}$, $NR_{20}C_{1-4}$alkyl$NR_{18}R_{19}$, $NR_{18}COR_{19}$, $NR_{20}CONR_{18}R_{19}$, $NR18SO_2R_{19}$;

R_{18}, R_{19} are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{21};

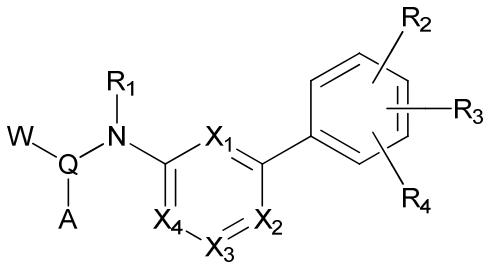
R_{21} is selected from H, C_{1-4}alkyl;

R_{20} is selected from H, C_{1-4}alkyl;

Y is selected from H, C_{1-4}alkyl, OH, $NR_{22}R_{23}$; and

R_{22}, R_{23} are each independently H, C_{1-4}alkyl.

[71] In another aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (IV):



(IV)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are selected from the following:

(i) X_1 and X_2 are N and X_3 and X_4 are C independently substituted with Y;

(ii) X_1 and X_4 are N and X_2 and X_3 are C independently substituted with Y;

(iii) X_1 and X_3 are N and X_2 and X_4 are C independently substituted with Y;

(iv) X_2 and X_4 are N and X_1 and X_3 are C independently substituted with Y;

(v) X_1 is N and X_2, X_3, and X_4 are C independently substituted with Y;

(vi) X_3 is N and X_1, X_2, and X_4 are C independently substituted with Y;

(vii) X_4 is N and X_1, X_2, and X_3 are C independently substituted with Y;

(viii) X_2 is N and X_1, X_3, and X_4 are C independently substituted with Y; and

(ix) X_1, X_2 and X_3 are N and X_4 is C substituted with Y;

R_1 is H, C_{1-6}alkyl, C_{1-6}alkylNR_5R_6, where R_5 and R_6 are each independently H, C_{1-4}alkyl, aryl, hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7; R_7 is selected from H, C_{1-4}alkyl;

R_2 is selected from C_{1-6}alkylOH, OC_{2-6}alkyl OH, C_{1-6}alkylNR_8R_9, OC_{2-6}alkyl NR_8R_9, C_{1-6}alkylNR_8COR_9, OC_{2-6}alkylNR_8COR_9, C_{1-6}alkylhetaryl, OC_{2-6}alkylhetaryl, $OCONR_8R_9$, NR_8COOR_9, $NR_{10}CONR_8R_9$, $CONR_8R_9$, NR_8COR_{12};

R_8, R_9 are each independently H, C_{1-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{12} is C_{2-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl;

R_{11}, R_{13} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14}; R_{14} is selected from H, C_{1-4}alkyl;

R_{10} is H, C_{1-4}alkyl;

R_3 and R_4 are each independently H, halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CF_3, OCF_3;

Q is CH;

W is selected from C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4}alkyl;

A is aryl, hetaryl optionally substituted with 0-2 substituents independently chosen from halogen, C_{1-4}alkyl, CF_3, aryl, hetaryl, OCF_3, OC_{1-4}alkyl; OC_{2-3}alkyl$NR_{18}R_{19}$, Oaryl, Ohetaryl, CO_2R_{18}, $CONR_{18}R_{19}$, $NR_{18}R_{19}$, C_{1-4}alkyl$NR_{18}R_{19}$, $NR_{20}C_{1-4}$alkyl$NR_{18}R_{19}$, $NR_{18}COR_{19}$, $NR_{20}CONR_{18}R_{19}$, $NR_{18}SO_2R_{19}$;

R_{18}, R_{19} are each independently H, C_{1-4}alkyl, C_{1-4}alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4}alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{21};

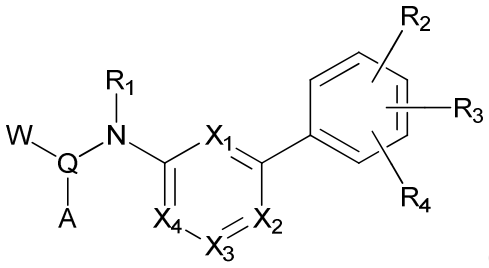
R_{21} is selected from H, C_{1-4}alkyl;

R_{20} is selected from H, C_{1-4}alkyl;

Y is selected from H, C_{1-4}alkyl, $NR_{22}R_{23}$;

R_{22}, R_{23} are each independently H, C_{1-4}alkyl.

[72] In another aspect, the disclosure provides a method of treating HCC comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (V):



(V)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are selected from the following:

(i) X_1 and X_2 are N and X_3 and X_4 are C independently substituted with Y;

(ii) X_1 and X_4 are N and X_2 and X_3 are C independently substituted with Y;

(iii) X_2 and X_4 are N and X_1 and X_3 are C independently substituted with Y;

(iv) X_1 is N and X_2, X_3, and X_4 are C independently substituted with Y;

(v) X_3 is N and X_1, X_2, and X_4 are C independently substituted with Y;

(vi) X_4 is N and X_1, X_2, and X_3 are C independently substituted with Y;

(vii) X_2 is N and X_1, X_3, and X_4 are C independently substituted with Y; and

(viii) X_1, X_2 and X_3 are N and X_4 is C substituted with Y;

R_1 is H, $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$ alkylNR_5R_6, $C_{1\text{-}6}$alkylNR_5COR_6, $C_{1\text{-}6}$alkyl$NR_5SO_2R_6$, $C_{1\text{-}6}$alkylCO_2R_5, $C_{1\text{-}6}$alkyl$CONR_5R_6$;

R_5 and R_6 are each independently H, $C_{1\text{-}4}$alkyl, aryl, hetaryl, $C_{1\text{-}4}$alkylaryl, $C_{1\text{-}4}$alkylhetaryl or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_7;

R_7 is selected from H, $C_{1\text{-}4}$alkyl;

R_2 is selected from OH, $OC_{1\text{-}6}$alkyl, $C_{1\text{-}6}$alkylOH, $OC_{2\text{-}6}$alkylOH, $C_{1\text{-}6}$alkylNR_8R_9, $OC_{2\text{-}6}$alkylNR_8R_9, $C_{1\text{-}6}$alkylNR_8COR_9, $OC_{2\text{-}6}$alkylNR_8COR_9, $C_{1\text{-}6}$alkylhetaryl, $OC_{2\text{-}6}$alkylhetaryl, $OCONR_8R_9$, NR_8COOR_9, $NR_{10}CONR_8R_9$, $CONR_8R_9$, NR_8COR_{12};

R_8, R_9 are each independently H, C_{1-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{14};

R_{12} is C_{2-4}alkyl, C_{1-4}alkyl$NR_{11}R_{13}$, hetaryl, cyclohetalkyl;

R_{11}, R_{13} are each independently H, C_{1-4}alkyl, or may be joined to form an optionally substituted 3-8membered ring optionally containing an atom selected from O, S, NR_{14};

R_{14} is selected from H, C_{1-4}alkyl;

R_{10} is H, C_{1-4} alkyl;

R_3 and R_4 are each independently H, halogen, C_{1-4}alkyl, OH, OC_{1-4}alkyl, CF_3, OCF_3;

Q is a bond, or C_{1-4}alkyl;

W is selected from H, C_{1-4}alkyl, C_{2-6}alkenyl; where C_{1-4}alkyl or C_{2-6}alkenyl may be optionally substituted with C_{1-4}alkyl, OH, OC_{1-4}alkyl, $NR_{15}R_{16}$;

R_{15}, and R_{16} are each independently H, C_{1-4}alkyl, C_{1-4}alkyl cycloalkyl, C_{1-4}alkyl cyclohetalkyl, aryl, hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{17};

R_{17} is selected from H, C_{1-4}alkyl;

A is aryl, hetaryl optionally substituted with 0-3 substituents independently chosen from halogen, C_{1-4} alkyl, CF_3, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkyl$NR_{18}R_{19}$, Oaryl, Ohetaryl, CO_2R_{18}, $CONR_{18}R_{19}$, $NR_{18}R_{19}$, C_{1-4} alkyl$NR_{18}R_{19}$, $NR_{20}C_{1-4}$alkyl$NR_{18}R_{19}$, $NR_{18}COR_{19}$, $NR_{20}CONR_{18}R_{19}$, $NR_{18}SO_2R_{19}$;

R_{18}, R_{19} are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR_{21};

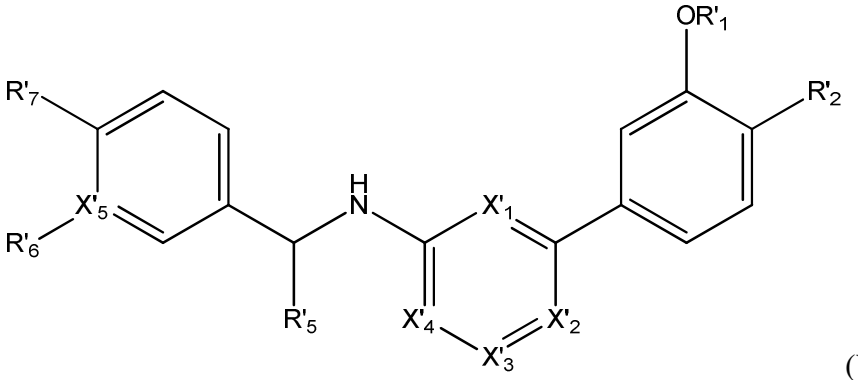
R_{21} is selected from H, C_{1-4} alkyl;

R_{20} is selected from H, C_{1-4} alkyl;

Y is selected from H, C_{1-4}alkyl, OH, $NR_{22}R_{23}$;

R_{22}, R_{23} are each independently H, C_{1-4} alkyl.

[73] In yet a further aspect, the disclosure provides a method of treating liver cancer comprising administering to a subject in need of treatment an amount of at least one compound of the general formula (VI):



(VI)

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

R'$_1$ is C$_{1-4}$ alkyl,

R'$_2$ is independently selected from the group consisting of: OH, NHCOR'$_{12}$, and NHCONHR'$_{12}$;

R'$_{12}$ is independently selected from the group consisting of H, C$_{1-4}$ alkyl optionally substituted with OH, OC$_{1-4}$alkyl or NR'$_{15}$R'$_{16}$;

R'$_{15}$ and R'$_{16}$ are each independently selected from H and C$_{1-4}$ alkyl;

X'$_1$, X'$_2$, X'$_3$, X'$_4$ are selected from the following:

(i) X'$_1$ and X'$_2$ are N and X'$_3$ and X'$_4$ are C independently substituted with Y';

(ii) X'$_1$ and X'$_4$ are N and X'$_2$ and X'$_3$ are C independently substituted with Y';

(iii) X'$_1$ and X'$_3$ are N and X'$_2$ and X'$_4$ are C independently substituted with Y';

(iv) X'$_2$ and X'$_4$ are N and X'$_1$ and X'$_3$ are C independently substituted with Y';

Y' is selected from H, OH, C$_{1-4}$alkyl, and OC$_{1-4}$alkyl;

X'$_5$ is selected from N and C, and

when X'$_5$ is C, R'$_6$ is selected from the group H, halogen, C$_{1-4}$ alkyl, OC$_{1-4}$alkyl, CF$_3$, and OCF$_3$:

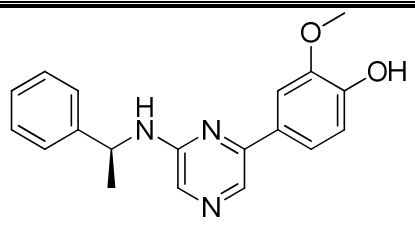
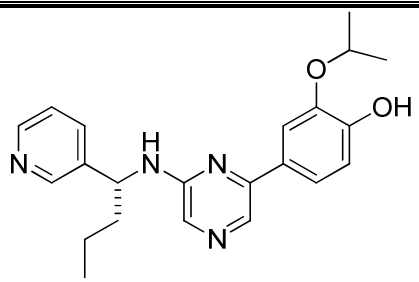
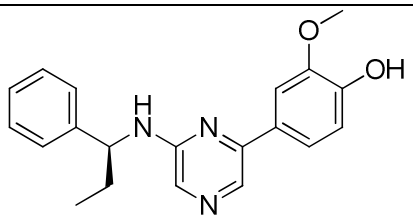
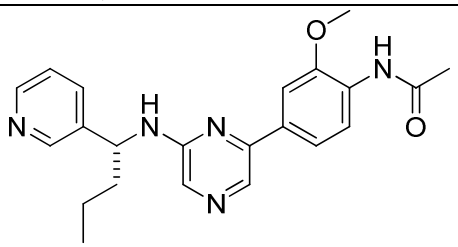
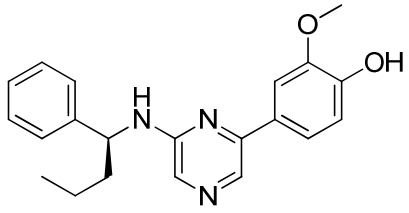
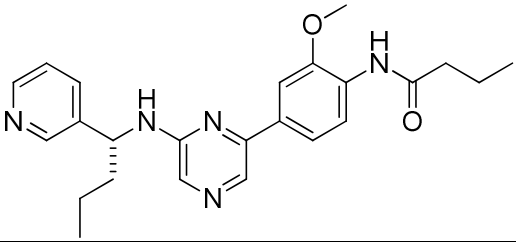
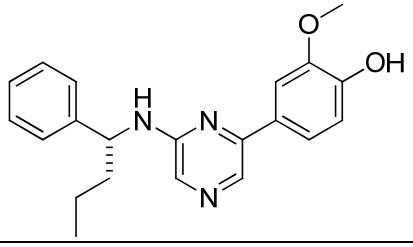
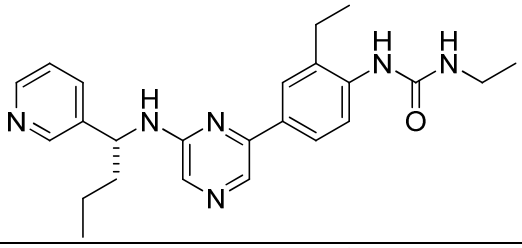
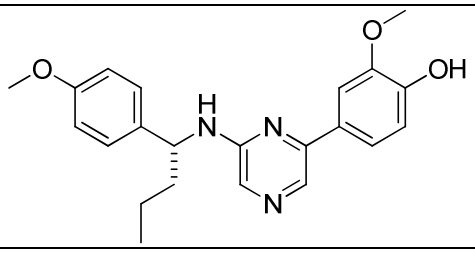
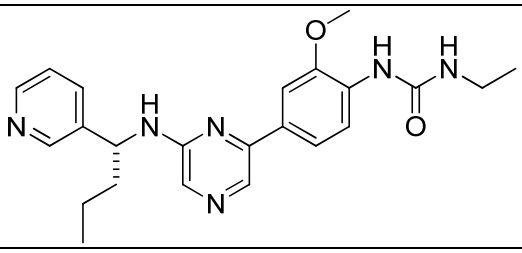
R'$_5$ is selected from the group C$_{1-4}$ alkyl, OC$_{1-4}$alkyl, CF$_3$, and OCF$_3$;

R'$_7$ is selected from the group H, halogen, C$_{1-4}$ alkyl, OC$_{1-4}$alkyl, CF$_3$, and OCF$_3$.

[74] In the above described aspects it should be noted that: C$_{1-4}$alkyl means an unsubstituted or optionally substituted straight or branched alkyl chain; Aryl means unsubstituted or optionally substituted phenyl or naphthyl; Hetaryl means an unsubstituted or optionally substituted 5- or 6-membered heteroaromatic ring containing one or more heteroatoms selected

from O, N, S; Cycloalkyl means a 3-8 membered saturated ring; and Cyclohetalkyl means an optionally substituted 3-8 membered saturated ring containing 1-3 heteroatoms selected from O, S, NR_{24}, where R_{24} is H, C_{1-4}alkyl, aryl, hetaryl.

[75] In some embodiments, the method comprises administering to a subject in need of treatment an amount of at least one compound of the general formula (VI) selected from the group consisting of:

CMP No.	Structure	CMP No.	Structure
1		13	
2		14	
3		15	
4		16	
5		17	

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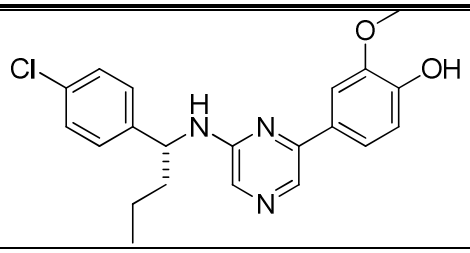
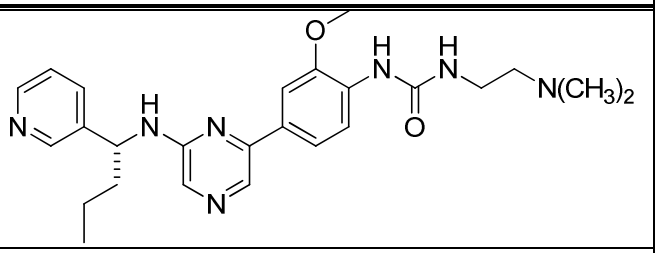
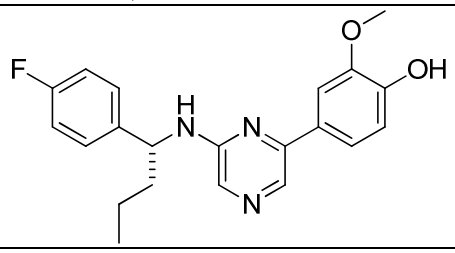
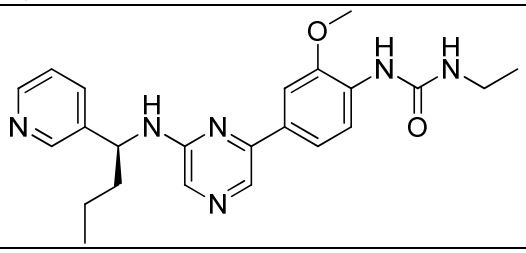
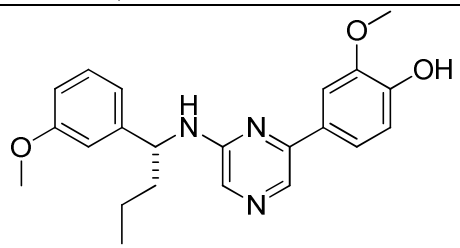
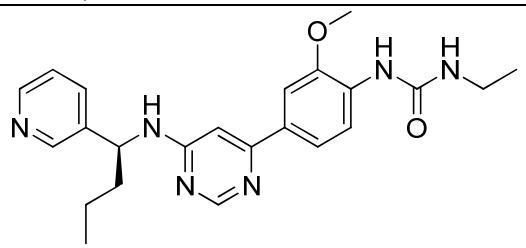
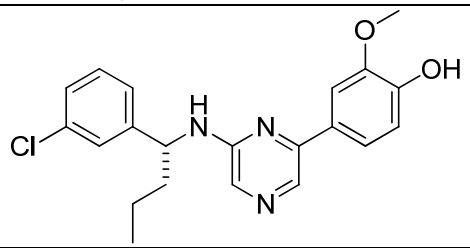
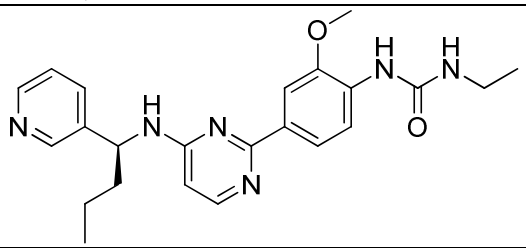
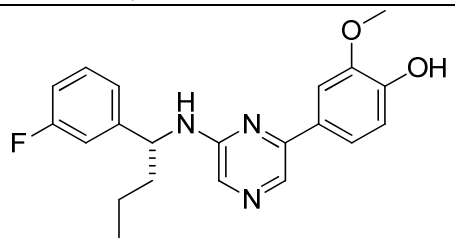
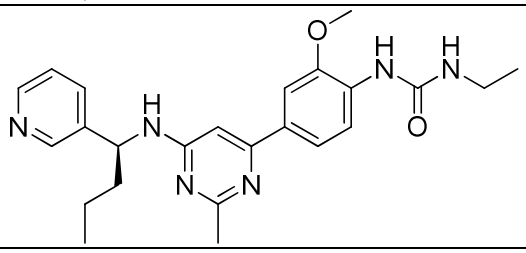
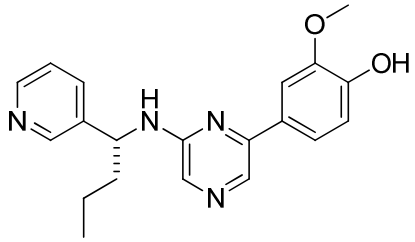
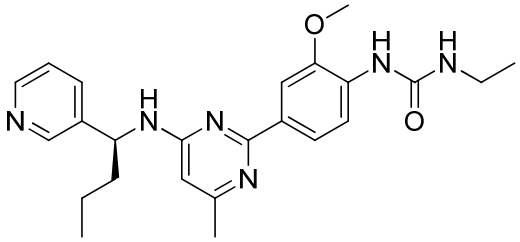
6		18	
7		19	
8		20	
9		21	
10		22	
11		23	

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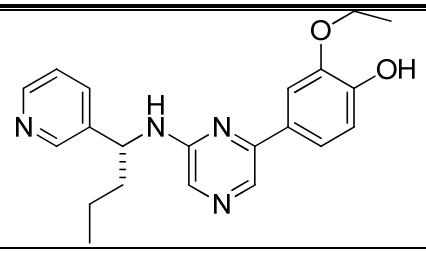
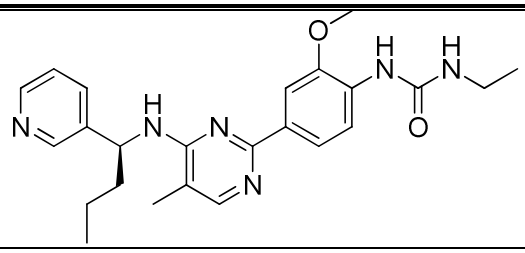
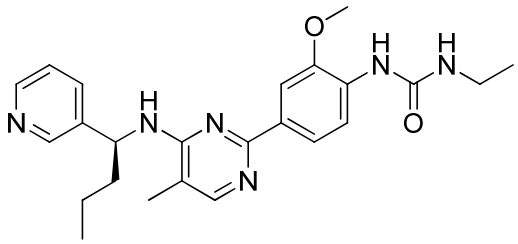
12		24	

[76] In some further embodiments, the methods comprise administering a compound of the structure:



(TXR-311, or compound 24).

[77] Compound 24, or (*S*)-1-ethyl-3-(2-methoxy-4-(5-methyl-4-((1-(pyridin-3-yl)butyl)amino)pyrimidin-2-yl)phenyl)urea, is also identified as CYT997 (Lexibulin), a microtubule targeting agent reported previously in *Bioorg Med Chem Lett* **19:**4639–4642, 2009; *Mol Cancer Ther* **8:**3036–3045, 2009.

[78] In the some of the above aspects and embodiments discussed above, the methods treat a subject who has HCC.

Use of the Compounds for Treatment of Liver Cancer

[79] In some aspects and embodiments the disclosure provides for the use of the compounds disclosed herein for the treatment of liver cancer. Thus, in one aspect, the disclosure relates to the use of compounds of general formula (I) in the treatment of liver cancer. In another aspect, the disclosure relates to the use of compounds of general formula (II) in the treatment of liver cancer. In another aspect, the disclosure relates to the use of compounds of general formula (III) in the treatment of liver cancer. In yet another aspect, the disclosure relates to the use of compounds of general formula (IV) in the treatment of liver cancer. In another aspect, the disclosure relates to the use of compounds of general formula (V) in the treatment of liver cancer. In another aspect, the disclosure relates to the use of compounds of general formula (VI)

in the treatment of liver cancer. In some embodiments of the aspects relating to the use of the compounds for treating liver cancer, the compound may be selected from any of compounds (1)-(24). In further embodiments, the disclosure relates to the use of compound 24 for the treatment of liver cancer. In certain of the above aspects and embodiments relating to the use of compounds, the use relates to the treatment of HCC. In further embodiments, the disclosure provides for the use of compound 24 in the treatment of HCC.

Patients and Subjects Amenable to Treatment

[80] In certain embodiments, the methods disclosed above relate to the treatment of a mammalian subject and preferably relate to methods for treating a human patient who has liver cancer. No single system is available that could be called the "standard" for classifying a liver cancer such as, for example, HCC. Like with any cancer, the goals of a tumor staging system in HCC are to estimate a patient's prognosis, which allows for appropriate therapy to be selected. The identification of that appropriate therapy, in turn, requires a staging paradigm that standardizes the platform for researchers to exchange data regarding treatments and outcomes. Currently, seven HCC staging systems with respect to their development and limitations are commonly utilized. These staging systems include TNM, Okuda staging, BCLC staging classification, Child-Pugh score, Japan integrated staging, Chinese University Prognostic Index (CUPI), and the French scoring system (GRETCH). (*See, e.g.*, Subramaniam et al., Chin Clin Oncol (2013) 2(4):33.)

[81] The Okuda system is a prognostic score introduced in 1985 and incorporates both tumor features as well as the degree of underlying cirrhosis. Using a cohort of 850 patients with an unequivocal diagnosis of HCC between 1975-1983, Okuda and colleagues devised a staging system based on four factors representing advanced disease. This includes tumor occupying greater or less than 50% of the liver, the presence or absence of ascites, and serum albumin and bilirubin levels (Table 1). In the original cohort, median survival was 11.5 months for Stage I, 3.0 months for Stage II and 0.9 months for Stage III. (*See, e.g.*, Subramaniam et al., Chin Clin Oncol (2013) 2(4):33.)

[82] The Child-Pugh score is a scoring system to measure the severity of chronic liver disease inclusive of cirrhosis. The intention is to provide a system with which clinicians can

objectively communicate about liver function. (*See, e.g.*, Subramaniam et al., Chin Clin Oncol (2013) 2(4):33.)

Table 1. Okuda staging

Factors representing advanced disease	
- Tumor size >50% of liver - Ascites - Albumin <3 g/dL - Bilirubin >3 mg/dL	
Stage I	No factors present
Stage II	1-2 factors
Stage III	3-4 factors

[83] Among the above seven staging systems, BCLC is used by many practitioners to guide clinical decision-making, because of its widespread presence in contemporary HCC research. The BCLC classification was first published in 1999 and is considered the standard HCC system by the American Association of for the Study of Liver Disease (AASLD) and European Association for the Study of the Liver. (*See, e.g.*, Subramaniam et al., Chin Clin Oncol (2013) 2(4):33.)

[84] Derived from a single institution experience, BCLC takes into account size and extent of the primary tumor, liver function and physiological factors and incorporates the Okuda stage and Child-Pugh score (Table 2). There is a corresponding treatment schedule for each stage (Table 3), ranging from curative therapies such as resection or transplant for early stage patients to best supportive care for end-stage patients. (*See, e.g.*, Subramaniam et al., Chin Clin Oncol (2013) 2(4):33.)

Table 2. Barcelona Clinic Liver Cancer (BCLC) staging classification

Stage	**PST**	**Tumor status**		**Liver function studies**
		Tumor stage	**Okuda Stage**	
Stage A: early HCC				
A1	0	Single	I	No portal hypertension and normal bilirubin
A2	0	Single	I	Portal hypertension and normal bilirubin
A3	0	Single	I	Portal hypertension and abnormal bilirubin
A4	0	3 tumors <3cm	I-II	Child-Pugh A-B

Stage B: intermediate HCC	0	Large multinodular	I-II	Child-Pugh A-B
Stage C: advanced HCC	1-2*	Vascular invasion or extrahepatic spread	I-II	Child-Pugh A-B
Stage D: end-stage HCC	3-4†	Any	III	Child-Pugh C
PST, Performance Status Test; Stage A and B, all criteria should be fulfilled; *, Stage C, at least one criteria: PST1-2 or vascular invsion/extrahepatic spread; †, Stage D, at least one criteria: PST3-4 or Okuda Stage III/Child-Pugh C.				

Table 3. Treatment schedule proposed for hepatocellular carcinoma (HCC) cirrhotic patients according to the BCLC classification system

Stage	**Treatment intention**	**First/second choice**
Stage A: early HCC		
A1	Radical	Surgical resection
A2		Surgical resection → OLT/percutaneous treatment
A3		OLT/percutaneous treatment
A4		OLT/percutaneous treatment
Stage B: intermediate HCC	Palliative*	
Stage C: advanced HCC	Palliative*	
Stage D: end-stage HCC	Symptomatic	Supportive treatment
*In the setting of phase II investigations or randomized control trials.		

[85] Signs and symptoms of HCC may include:

- Pain in the upper right part of your belly
- A lump or feeling of heaviness in your upper belly
- Bloating or swelling in your belly
- Loss of appetite and feelings of fullness
- Weight loss
- Weakness or deep fatigue

- Nausea and vomiting
- Yellow skin and eyes
- Pale, chalky bowel movements and dark urine
- Fever

[86] Although the mainstay of therapy for HCC is surgical resection, the majority of patients are not eligible because of tumor extent or underlying liver dysfunction. Several other treatment modalities are available, including:1) liver transplantation; 2) radiofrequency ablation (RFA) and microwave ablation; and 3) percutaneous ethanol or acetic acid ablation.

[87] The most commonly offered therapy is transcatheter arterial chemoembolization (TACE). TACE is performed by an interventional radiologist who selectively cannulates the feeding artery to the tumor and delivers high local doses of chemotherapy, including doxorubicin, cisplatin, or mitomycin C. To prevent systemic toxicity, the feeding artery is occluded with gel foam or coils to prevent flow. Because most hepatocellular carcinomas derive 80-85% of their blood flow from the hepatic artery, the therapy can be well targeted, leaving the normal parenchyma, which is primarily supplied by portal blood, minimally affected. A reduction in tumor burden can be achieved in 16-61% of treated patients.

[88] Subjects amenable to treatment include subjects having a diagnosis of probable or possible hepatocellular carcinoma, or who do not yet meet such criteria but have sufficient sign(s), symptom(s), or genetic risk factor(s) of hepatocellular carcinoma as to place them at a statistically significant risk of developing hepatocellular carcinoma relative to the general population.

Treatment

[89] In subjects already exhibiting a sign(s) and/or symptom(s) of liver cancer such as HCC, for example, administration of the compounds, compositions, and formulations disclosed herein can reverse, halt, or delay progression of disease (e.g., HCC) and/or reduce the severity of the clinical sign(s) or symptom(s) associated with liver cancer such as HCC.

[90] Some subjects are asymptomatic, but have one or more risk factors or may be at an early stage of the condition(s). In such subjects, administration of the compounds disclosed herein can inhibit or delay onset or progression of a condition to later stage and/or reduce the severity of the condition, once present.

Formulations and Administration of Therapeutic Agents

[91] The methods may include administration of the compounds at a dosage level that is appropriate based on a number of factors, including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the subject undergoing therapy. Dosages may generally be in a range of about 0.01 to about 500 mg per kg patient body weight per day which can be administered in single or multiple doses. In embodiments, the dosage level can range from about 0.1 to about 250 mg/kg per day; or from about 0.5 to about 100 mg/kg per day; or from about 0.1 to about 50 mg/kg per day. One of ordinary skill will appreciate that within these ranges the dosage may be about 0.05 to 0.5, about 0.5 to 5, or about 5 to 50 mg/kg per day.

[92] The methods can include any route of administration. For example, when administered orally, the compounds or compositions can be provided in the form of tablets containing 1.0 to 1000 milligrams of the active ingredient, (e.g., 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0, 75.0, 100.0, 150.0, 200.0, 250.0, 300.0, 400.0, 500.0, 600.0, 750.0, 800.0, 900.0, and 1000.0 milligrams of the active ingredient) for the symptomatic adjustment of the dosage to the patient to be treated. The compounds may be administered on a regimen of 1 to 4 times per day (e.g., including twice or three times per day).

[93] The compounds used in the methods herein may be administered by any suitable route, for example, orally, such as in the form of tablets, capsules, granules, suspensions, or powders; sublingually; buccally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intracisternal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The compounds may, for example, be administered in a form suitable for immediate release or extended release as is generally known in the art.

Combination Therapies

[94] As discussed above, the methods disclosed herein can be used as a monotherapy for treatment or prophylaxis of liver cancer (e.g., HCC). In some embodiments of the above aspects,

the disclosed methods of treatment can be used in combination with one or more additional therapeutic interventions. Non-limiting examples include radiation, surgery, immunotherapy, and other anti-cancer (i.e., anti-neoplastic) agents. In some embodiments, the methods further comprise administration of the compounds disclosed above in combination with an additional anti-cancer agent that may be selected from the non-limiting examples of alkylating agents, antimetabolites, natural antineoplastic agents, hormonal antineoplastic agents, angiogenesis inhibitors, differentiating reagents, RNA inhibitors, antibodies or immunotherapeutic agents, gene therapy agents, small molecule enzymatic inhibitors, biological response modifiers, and anti-metastatic agents. In non-limiting embodiments, a combination therapy can include sorafenib, which represents the current standard of care.

Synthesis of Compounds

[95] The compounds described herein can be obtained through commercial sources or prepared using synthetic methods known in the art. Non-limiting examples for compound synthesis are disclosed in U.S. Patent 7,981,900 which refers to a 2-step synthetic process starting from a dihaloheterocycle.

[96] Briefly, the first step is a nucleophilic aromatic substitution to generate a monoamino-monohalo intermediate. The nucleophilic aromatic substitution can be carried out by addition of a primary or secondary amine to the di-halogenated heterocycle in an organic solvent (e.g., alcohols, aliphatic and cyclic ethers, ethanol, DMF, toluene, or xylene). The reaction is typically performed at elevated temperature in the presence of excess amine or a non-nucleophilic base such as triethylamine or diisopropylethylamine, or an inorganic base such as potassium carbonate or sodium carbonate.

[97] Alternatively, the amino substituent may be introduced through a transition metal catalyzed amination reaction, using catalysts such as $Pd(OAc)_2/P(t\text{-}Bu)_3$, $Pd_2(dba)_3$/BINAP and $Pd(OAc)_2$/BINAP. These reactions are typically carried out in solvents such as toluene or dioxane, in the presence of bases such as cesium carbonate or sodium or potassium tert-butoxide at temperatures ranging from room temperature to reflux.

[98] The amines employed in the first step of the synthesis of these compounds are obtained commercially or are prepared using methods well known to those skilled in the art (e.g., α-alkylbenzylamines prepared through reduction of oximes using reductants known in the art).

The α-alkylbenzylamines may also be prepared using known methods, such as for example reductive amination of ketones (e.g., the Leuckart-Wallach reaction) or from α-alkylbenzyl alcohols. Further, methods for chiral reduction are known in the art.

[99] The second step of the synthesis involves cross-coupling of the monoamino-monohalo intermediate with a functionalized coupling reagent, typically using palladium. Typical coupling reagents can include those used in Suzuki coupling (boronic acids or esters, see e.g., Miyaura, N. and Suzuki, *Chem. Rev.* 1995, 952457); Stille coupling (tin hydrides, see e.g., Stifle, J. K., *Angew. Chem., Int. Ed. Engl.,* 1986, 25, 508), Kumada coupling (Grignard reagents, see e.g., Kumada, M.; Tamao, K.; Sumitani, K. *Org. Synth.* 1988, Coll. Vol. 6, 407.), or Negishi coupling (organo-zinc species, see e.g., Negishi, E.; *J. Organomet. Chem.* 2002, 653, 34) each incorporated by reference. For example, Suzuki coupling may be performed in an organic solvent in the presence of an inorganic base at elevated temperatures, and using palladium catalyst known in the art.

[100] Derivative compounds may be prepared from the compounds formed by above reactions using techniques known in the art. The synthetic steps above can be modified as to start with the palladium mediated cross-coupling reaction to furnish a mono-halo heterocyclic species. Amine displacement of the halo substituent can then be performed as described above.

[101] The following Examples provide an illustration of some of the aspects and embodiments described above, and are not intended to limit the scope of the claimed invention.

EXAMPLES

Example 1*: Identifying candidate compounds using in vitro cytotoxicity assays in human hepatocellular carcinoma cells and primary human hepatocytes*

[102] A set of structurally diverse candidate compounds were identified for investigation based on existing available information using a process that combines an initial computational-based analysis and review of existing information available for candidate compounds with additional research and development of the identified candidate compounds specifically directed to determine the efficacy of the compounds in the treatment of liver cancer. To screen the candidate compounds, five human HCC cell lines were used in a cell proliferation assay study: HepG2, Hep3B, Hep40, Huh7 and PLC/PRF/5 (PLC5). All cell lines were obtained from either the American Type Culture Collection (ATCC; Manassas, VA) or provided as a gift. Cells were

maintained in Dulbecco's Modified Eagle's Medium, 10% fetal calf serum and penicillin/streptomycin. Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2.

[103] A stock solution (10 mM in DMSO, 100%) was prepared for each screened candidate compound. Cells were seeded at 5000 cells per well in 96-well plates and allowed to adhere overnight. Compounds were then added at the desired final concentrations, and incubated for an additional 72 hours before cell proliferation was assessed.

[104] Liver cytotoxicity was assessed using three primary human hepatocytes: HU1767, HU8216, and HU8264 (purchased from ThermoFisher Scientific (Waltham, MA)) for candidate compounds identified from the HCC cell line assay, the compounds identified as TXR-311 (also identified as Compound 24 herein) and TXR-312. Cryopreserved hepatocytes were thawed using cryopreserved hepatocytes recovery medium. These cells were plated in 96-well plates and maintained in Williams' Medium E and supplemented with hepatocyte maintenance supplement hPack (serum-free). Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2.

[105] For the hepatocyte toxicity assay, a stock solution of (10 mM in 100% DMSO) was prepared for the selected candidate compounds. Cells were seeded at 30,000 cells per well in 96-well plates and allowed to adhere overnight. Compounds were then added at the desired final concentrations, and incubated for an additional 72 hours before cell proliferation was assessed.

[106] Two methods were used for the calculation of IC_{50}: cell proliferation and cell viability assays. Both assays were performed using the respective commercial kits and in accordance with manufacturer's protocol (CellTiter 96® AQueous One Solution Cell Proliferation kit and CellTiter-Glo® Luminescent Cell Viability Assay; (Promega; Madison, Wisconsin). The IC_{50} was calculated based on an estimate of the anti-proliferative and cytotoxic effects observed in the cell proliferation and cell viability assays, respectively.

[107] Results.

[108] <u>HCC toxicity</u>. Two of the screened candidate compounds in the HCC cytotoxicity assay were selected for evaluation in the hepatocyte toxicity assays based on calculated IC_{50} values (0.044 ± 0.019 μM; and 5.5 ± 1.53 μM). Data for the two selected compounds, TXR-311 and TXR-312, were obtained in triplicate for each cell line at each concentration. The mean IC_{50} for each selected compound and for each cell line was calculated and presented in Tables 4 and 5. The mean IC_{50} for each selected compound was calculated and presented in Table 6 and plotted in Figures 1A-1E.

Table 4. Cytotoxicity of TXR-311 in HCC Cell lines, normalized data

Cell Line	TXR-311 Concentration (µM) (for each cell line experiments at each concentration performed in triplicate)							IC50 (µM)
Hep2G	0	0.001	0.01	0.1	1	10	100	**0.048**
Hep3B	0	0.001	0.01	0.1	1	10	100	**0.077**
Hep40	0	0.001	0.01	0.1	1	10	100	**0.002**
Huh7	0	0.001	0.01	0.1	1	10	100	**0.002**
PLC-5	0	0.001	0.01	0.1	1	10	100	**0.091**
							Mean IC50 (µM)	**0.044**

Table 5. Cytotoxicity of TXR-312 in HCC Cell lines, normalized data

Cell Line	TXR-312 Concentration (µM) (for each cell line experiments at each concentration performed in triplicate)							IC50 (µM)
Hep2G	0	0.001	0.01	0.1	1	10	100	**3.603**
Hep3B	0	0.001	0.01	0.1	1	10	100	**8.604**
Hep40	0	0.001	0.01	0.1	1	10	100	**7.716**
Huh7	0	0.001	0.01	0.1	1	10	100	**0.411**
PLC-5	0	0.001	0.01	0.1	1	10	100	**7.143**
							Mean IC50 (µM)	**5.495**

Table 6. Summary of IC_{50} of TXR-311 and TXR-312 in HCC Cell Lines

Drug	IC50 (µM) in HCC Cell Line					IC50 (µM)	
	Hep2G	Hep3B	Hep40	Huh7	PLC-5	Mean	StdDev
TXR-311	0.048	0.077	0.002	0.002	0.091	0.044	0.019
TXR-312	3.603	8.604	7.716	0.411	7.143	5.495	1.529

Table 7. Summary of IC_{50} of TXR-311 and TXR-312 in Primary Hepatocytes

Drug	HU1767		HU8216		HU8264		IC50 (µM)	
	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2	Mean	StdDev
TXR-311	33.35	8.16	14.59	22.45	47.14	16.00	23.62	1.14
TXR-312	0.82	0.70	1.70	1.54	1.29	0.79	5.85	0.17

[109] Hepatocyte toxicity. The cytotoxicity of each selected compound was tested in primary human hepatocytes. The results are summarized in Table 7 and plotted in Figure 2. One of the selected compounds, TXR-311, had a significantly higher IC_{50} (~540 fold) observed in hepatocytes compared to the IC_{50} in HCC cell lines (23.62 ± 5.9 μM compared to 0.044 ± 0.018 μM, respectively), indicating that its activity has a high selectivity for HCC tumor cells with very low toxicity to normal liver cells. There is no previously existing data that would have predicted these findings, and in view of this data, the TXR-311 compound was chosen for further characterization in *in vivo* studies.

Example 2: *Maximum tolerated dose (MTD) study in BALB/c mice*

[110] Twenty female/male BALB/c mice of approximately 8-10 weeks of age and weighing 26-29 grams each at the time of dosing were used in a dose range-finding study. Animals were acclimated for 5 days prior to dose administration. The animals were group-housed in sterilized plastic "shoebox" cages with microisolator caps in a single room dedicated to rodents. LabDiet® 5001 Rodent Diet (Purina Mills, Inc.; St. Louis, MO) or other approved diets were provided ad libitum throughout the acclimation and treatment phases. Fresh tap water was provided ad libitum to the animals via water bottles. Twelve hours of light and twelve hours of dark was provided in the animal rooms.

[111] The study (summarized in Table 8) was conducted to provide data relating to a maximum tolerated level of the compound TXR-311 in BALB/c mice following 42 days of tri-weekly dosing. The study included three groups with up to five female or male mice in each group. Starting on Day 0 and continuing to Day 41, animals in Groups 2-4 were dosed tri-weekly (Q3W) via oral gavage (PO) with the compound TXR-311 at 20, 40 and 60 mg/kg, respectively (Table 1). Measurements were recorded throughout the in-life phase, including: clinical observations, at least once daily (QD); body weight, tri-weekly. Animals were sacrificed on day 42, one day after administration of the final dose and a final body weight recorded.

Table 8. Summary of MTD Study Design

Group	Number of Animals	Treatment (Days 0- 41) (PO, Q3W)		In-Life Procedures (Days 0-41)	Terminal Procedures (Day 42)
		Test Article	Dose (mg/kg)		
1	5	Vehicle	N/A	• Clinical observation: QD • Body weight: Pre-study, then Q3W prior to dosing	• Body weight • Sacrifice
2		TXR-311	20		
3			40		
4			60		

[112] The survival curve of the dose range-finding study is presented in Figure 3. A total of 7 unscheduled mortalities were observed, all of which occurred in groups 3 and 4 (Table 9). In group 4 treated with 60 mg/kg of TXR-311, animals exhibited severe diarrhea and signs of dehydration by Day 5. Three animals in this group were found dead on Day 6 and 2 animals were found dead on Day 7. In group 3 treated with 40 mg/kg of TXR-311, animals exhibited signs of dehydration on Day 14. In this group one mouse was found dead on Day 16 and a second mouse was found dead on Day 20. On Day 21, due to the observed toxicity, the animals were removed from study. In group 2 treated with 20 mg/kg of TXR-311, there were no unscheduled mortalities during the in-life period.

[113] Body weight data are provided in Table 10 and plotted in Figure 4. Animals treated with 60 mg/kg of TXR-311 died prior to Day 7, the first body weight measurement, thus no data are presented. Starting on Day 7 animals treated with 40 mg/kg of TXR-311 exhibited a decrease in body weight. By Day 14, these animals showed a 16% decrease in body weight when compared to their pre-dose weight. Animals treated with 20 mg/kg of TXR-311 and vehicle exhibited a gain in body weight. These results indicate that treating mice three times weekly with 20 mg/kg of TXR-311 is safe and well tolerated.

Table 9. Summary of Unscheduled Mortalities

Group	Treatment	Day of Mortality	No. of Dead Animals	Clinical Signs

1	Vehicle	None	NA	NA
2	20mg/kg	None	NA	NA
3	40mg/kg	16	1	Dehydration
		20	1	
4	60mg/kg	6	4	Dehydration and diarrhea
		7	1	

Table 1. MTD study - Summary of Body Weight

Group	Animal No.	Study Day					
		0	7	14	21	28	35
1 Vehicle	1	26.3	26	26.2	26.4	26.7	27.3
	2	28.6	28.7	29	30.1	30.5	30.8
	3	28	28	28.4	28.2	29.1	29.2
	4	28.5	28.3	28	28.4	28	28.1
	5	29.3	28.9	28.9	29.7	29.8	30.1
	Mean	28.1	28.0	28.1	28.6	28.8	29.1
	SEM	0.5	0.5	0.5	0.7	0.7	0.6
2 TXR-311, 20mg/kg	1	25.4	25.9	26.2	26.4	27.4	27.7
	2	28.7	26.1	26.3	25.8	27	29.5
	3	26.5	27.4	27.4	27.1	27.3	28
	4	27.5	28.9	28.1	28.2	28.8	30
	5	28.3	29	29.7	28.9	30.1	31.3
	Mean	27.3	27.5	27.5	27.3	28.1	29.3
	SEM	0.6	0.7	0.6	0.6	0.6	0.7
3 TXR-311 40mg/kg	1	23.5	24.1	22.8			
	2	27.1	26.5	20.3			
	3	28.7	27.6	23.1			
	4	27.4	26.3	23.9			
	5	26.2	25.5	22.6			
	Mean	26.6	26.0	22.5			
	SEM	0.9	0.6	0.6			
4 TXR-311 60mg/kg	1	27.5					
	2	26.5					
	3	26.4					
	4	26.5					
	5	25.5					

Group	Animal No.	Study Day					
		0	7	14	21	28	35
	Mean	26.5					
	SEM	0.3					

Example 3: *Tumor inhibition activity in NSG mice with HCC patient-derived tumor xenografts*

[114] Female/male NSG mice (NOD *scid* gamma, NOD-*scid* IL2Rg^{null}, NOD-*scid* IL2Rgamma^{null}) of approximately 8-10 weeks of age and weighing 27-33 g each at the time of dosing were obtained to establish two patient-derived xenograft (PDX) orthotopic tumor models. The animals were group housed ($n \leq 5$) in sterilized plastic "shoebox" cages with microisolator caps in a single room dedicated to immunocompromised rodents. Sterile LabDiet® 5062 PicoVac® Rodent Diet 20 (Purina Mills, Inc.; St. Louis, MO) or other approved sterilized diets was provided ad libitum throughout the acclimation and in-life phases. Autoclave-sterilized tap water from the Palo Alto Municipal Water Supply was provided ad libitum to the animals via water bottles or a modified rack-watering system. Twelve hours of light and twelve hours of dark was provided in the animal rooms.

[115] Two studies were performed with different patient-derived xenograft tumors: PDX1 and PDX2. PDX1 and PDX2 were established from HCC patients who underwent surgical resection for their HCC tumors. PDX1 was derived from a patient with non-viral HCC with cirrhosis and metastasis to intra-abdominal lymph nodes. The tumor was diagnosed as a moderately-differentiated HCC, stage 1. PDX2 was derived from a patient with HBV-associated HCC, with cirrhosis and vascular invasion. The tumor was diagnosed as being poorly-differentiated HCC, stage 2. Both patients gave informed consent to have their tissue specimens used for research studies. In mice, PDX1 typically has a faster growth rate (about double) compared to PDX2. There are no known genetic mutations for each PDX tumor.

[116] The PDX1 study was performed in 18 tumor-bearing NSG mice, consisting of two groups of nine mice per group. The PDX2 study was performed in 20 tumor-bearing NSG mice, consisting of two groups of seven mice per group and one group of six mice. Both PDX1 and PDX2 cells were labeled with the luciferase reporter gene using a lenti-virus expression vector. Approximately one month before the projected start of each of the PDX studies, two mice from each PDX cohort were implanted with tumor cells. Implantation was performed via

subcutaneous (SC) injection into the cephalad dorsum area with 0.1 mL (~5 x 10^6 cells) per mouse. The tumors cells were administered as a single-cell suspension of each of the PDX cells suspended in 50% Matrigel (BD Biosciences; Bedford, MA) in phosphate-buffered saline (PBS). Following implantation, mice were returned to their cages and tumors were allowed to develop, for up to 28-42 days, depending on the growth rate of the tumors. Tumor growth was monitored once weekly using Xenogen IVIS *in vivo* imaging system. Luciferase images were acquired following intraperitoneal injection (IP) of D-luciferin substrate.

[117] Donor animals were sacrificed once tumors had reached the desired diameter of ~100 mm. The tumor was then removed and dissected into ~2 mm^3 fragments and surgically implanted into the left lobe of the liver in NSG mice. Starting on Day 0 (7 days after tumor implantation) and for a total of up to 42 consecutive days. The in-life study performed as two cohorts as shown in Table 11. In the first cohort, animals implanted with PDX1 were dosed tri-weekly (Q3W) with TXR-311 or vehicle. In the second cohort, animals implanted with PDX2 were dosed tri-weekly (Q3W) with TXR-311 or vehicle and 5 times a week with sorafenib. All mice were dosed via oral gavage (PO) at 5 mL/kg with vehicle, TXR-311, (both on M/W/F each week) or sorafenib (on M/T/W/Th/F each week) as specified in Table 11. Throughout the in-life phase, the following measurements were recorded: tumor growth once weekly using Xenogen IVIS *in vivo* imaging system; body weight, once weekly (Q1W). Luciferase images were acquired following intraperitoneal injection (IP) of D-luciferin substrate. Sedated animals were placed in the imaging chamber and the image was taken 10 mins after IP injection of D-luciferin substrate.

[118] Animals in the PDX1 study were sacrificed on Day 25 due to excessive tumor growth and significant body weight loss (>18%) observed in both vehicle and drug-treated (TXR-311) groups. No unscheduled mortality was observed in the PDX1 or PDX2 studies. Animals in the PDX2 study were sacrificed on Day 42. During necropsy of both cohorts, the liver was removed, and the xenograft tumor was excised, measured and placed in formalin for future analysis.

[119] The study design is summarized in Table 11. The PDX1 study consisted of two groups of nine tumor-harboring mice each, and the PDX2 study consisted of 2 groups of seven and one group of 6 tumor-harboring mice.

Table 11. Summary of Study Design for PDX1 and PDX2

Cohort	Group	No. of Animals	PDX	Treatment (Days 0-41)*		In-Life Procedures (Days 0-41)	Terminal Procedures (Day 42)
				Treatment (PO)	Dose (mg/kg)		
1	1	9	PDX1	10% DMSO in saline	N/A	• Clinical observation: QD • Tumor measurement: Q1W • Body weight: Pre-study, then Q1W and at sacrifice	• Body weight • Necropsy, removal of liver and measurement of xenograft
	2	9		TXR-311 in 10% DMSO	20		
2	1	7	PDX2	10% DMSO in saline	N/A		
	2	7		TXR-311 in 10% DMSO	20		
	3	6		Sorafenib in 10% DMSO	50		

* Animals of Cohort 1 were sacrificed on Day 25

[120] Results

[121] PDX1 study: Tumor size was monitored weekly using IVIS imaging. The luciferase counts for each animal during the in-life period (Days 0-25) are presented in Table 13 and plotted in Figure 6; IVIS images are presented in Figures 7-8. By the end of in-life period (Day 25) the tumors of TXR-311-treated animals were 3-fold smaller compared to vehicle-treated animals. This inhibition in tumor growth was statistically significant when compared to vehicle-treated animals ($p \leq 0.001$, 2-way ANOVA).

[122] Body weight data are provided in Table 12 and plotted in Figure 5. Animals bearing the PDX1 tumor which were treated with vehicle or 20 mg/kg of TXR-311 exhibited a decrease in body weight during the in-life period. By Day 21 animals treated with vehicle showed a 26% body weight loss and animals treated with TXR-311 showed a 18% body weight loss when compared to the average body weight at Day 0. Statistical analysis (2-way ANOVA) indicated that the difference in body weight change between treatment groups was not significant ($p > 0.05$). Due to the severe decrease in body weight along with large tumor dimensions, all mice were sacrificed on Day 25.

Table 12. PDX1 - Body Weight

Treatment	Animal No.	Body Weight (g) on the Indicated Study Day			
		0	7	14	21
TXR-311	1	29.5	29	25.9	22.5
	2	28.3	26.8	25.8	26.4
	3	30.5	27.8	25.8	25.6
	4	28.6	26.5	25.8	25.8
	5	33.5	30.4	28.6	25
	6	28.8	26.5	25.7	24.3
	7	27.2	23.7	21.3	20.5
	8	30.2	28.5	27.7	26.5
	9	28.3	26.3	24.1	22.3
	Mean	29.4	27.3	25.6	24.3
	SEM	0.6	0.6	0.7	0.7
Vehicle	1	29.1	25.6	22.8	22.6
	2	27.1	23.8	20.3	19.5
	3	31.3	29.1	27.3	24.2
	4	31.4	27.3	20.7	19.6

Treatment	Animal No.	Body Weight (g) on the Indicated Study Day			
		0	7	14	21
	5	30.3	26	23	23.2
	6	28.9	26.6	23.8	22.4
	7	31.6	29.7	27.9	21.5
	8	31.1	26.3	26.2	23.8
	9	29.3	25.7	22.3	20.9
	Mean	30.0	26.7	23.8	22.0
	SEM	0.5	0.6	0.9	0.6

Table 13. PDX1 - Tumor Growth and Luciferase Counts

Treatment	Animal No.	Luciferase Counts on the Indicated Study Day			
		0	7	14	21
TXR-311	1	28000	170000	1200000	4590000
	2	7710	64700	21300	39300
	3	8080	237000	458000	940000
	4	10300	146000	413000	306000
	5	10700	10500	155000	357000
	6	10200	306000	307000	858000
	7	14900	78800	1320000	3910000
	8	19800	348000	111000	991000
	9	18700	59000	1690000	1840000
	Mean	14265.6	157777.8	630588.9	1536811.1
	SEM	2253.2	39276.0	203138.6	544035.6
Vehicle	1	27100	511000	1380000	2900000
	2	41700	123000	1100000	4780000
	3	28100	426000	2110000	4420000
	4	17000	128000	1330000	7690000
	5	17800	233000	987000	4870000
	6	15000	108000	2040000	3590000
	7	6770	171000	659000	3980000
	8	5380	63200	505000	5730000
	9	25200	266000	1230000	4970000

	Mean	20450.0	225466.7	1260111.1	4770000.0
	SEM	3800.1	50899.9	181905.6	458469.7

[123] PDX2 study: Tumor size was monitored weekly using IVIS. The luciferase counts for each animal during the in-life period (Days 0-42) is presented in Table 15 and plotted in Figure 12; IVIS images are presented in Figures 13-22. By the end of in-life period (Day 42) animals treated with TXR-311 or sorafenib had tumors that were 4 times smaller compared to vehicle-treated animals. This inhibition in tumor growth was statistically significant when compared to vehicle treated animals ($p \leq 0.001$, 2-way ANOVA). A significant inhibition in tumor growth ($p \leq 0.05$) was also observed on Day 35 in animals treated with TXR-311 but not in animals treated with sorafenib, the standard of care for HCC.

[124] Body weight data are provided in Table 14 and plotted in Figure 11. Animals bearing PDX2 tumors that were treated with vehicle, 20 mg/kg TXR-311 or 50 mg/kg sorafenib showed a small increase (2-5%) in body weight. These results indicate that the severe body weight loss observed in the PDX1 study was the result of tumor growth and not due to TXR-311 toxicity.

Table 14. PDX2 - Body Weight

Treatment	**Body Weight (g) on the Indicated study Day**					
	0	7	14	21	28	35
Vehicle	23.4	23.7	23.6	24.6	25	24.7
	23.1	23.4	24.3	24.8	24.6	25.5
	25.8	25.7	26.7	26.1	25.3	27.1
	25	24.3	24.1	26.4	25.7	26.2
	24.2	23.8	23.6	25	24.8	25.2
	24	21.8	22.3	22	22	23.4
	23.5	23.9	24.7	25	25.2	25.9
Mean	24.1	23.8	24.2	24.8	24.7	25.4
SEM	0.4	0.4	0.5	0.5	0.5	0.4
TXR-311	24.9	25	25.8	24.7	25.4	24.7
	25.4	25.6	24.3	25.2	26.4	25.6
	24.5	25.7	26.7	25.8	25.7	26.5
	23.4	24.2	24.1	24.5	26	25.6
	24.7	25.5	23.6	23.6	26.1	25.3
	25	23.7	22.3	24.7	25.4	24.1
	24.2	25	24.7	24.7	25.1	24.9

Treatment	Body Weight (g) on the Indicated study Day					
	0	7	14	21	28	35
Mean	24.6	25.0	24.5	24.7	25.7	25.2
SEM	0.2	0.3	0.5	0.3	0.2	0.3
Sorafenib	24.8	24.4	25.3	25.6	26.5	26.4
	23.2	23.1	23.4	25.1	24.9	24.4
	24.8	23.9	24	25.2	24.8	24.2
	25.9	24.7	24.5	26.1	26	26.5
	23.6	23.1	23.2	23.5	23.2	23.6
	23.5	23.6	23.8	24.4	23.5	24.4
Mean	24.3	23.8	24.0	25.0	24.8	24.9
SEM	0.4	0.3	0.3	0.4	0.5	0.5

Table 15. PDX2 - Tumor Growth and Luciferase Counts

Treatment	Luciferase Counts on the Indicated Study Day						
	0	**7**	**14**	**21**	**28**	**35**	**42**
Vehicle	25200	40600	47900	138000	187000	2250000	14300000
	30900	38700	218000	2390000	2760000	14600000	23300000
	13100	20200	37100	78500	158000	516000	4310000
	11400	24000	34100	26700	138000	381000	269000
	14800	21800	67000	152000	328000	2970000	8750000
	21300	53000	473000	426000	802000	838000	2040000
	36800	82000	1070000	1150000	6970000	21300000	34900000
Mean	21928.6	40042.9	278157.1	623028.6	1620428.6	6122142.9	12552714.3
SEM	3626.5	8330.0	145067.8	329066.0	959262.4	3160329.4	4785315.1
TXR-311	16200	9290	77700	346000	957000	1650000	3010000
	26400*	131000*	142000*	581000*	3610000*	4060000*	15300000*
	7900	23000	26700	220000	362000	326000	386000
	4690	9500	61700	37200	14000	32100	59400
	11800	24400	27400	66900	45500	166000	733000
	59500	139000	1130000	635000	122000	1310000	2030000
	15200	25700	87300	133000	168000	541000	96200
Mean	20241.4	51698.6	221828.6	288442.9	754071.4	1155014.3	3087800.0
SEM	7048.0	21676.3	152091.6	91294.3	491450.7	534774.9	2077249.2
Sorafenib	7690	45800	56000	42000	56700	102000	139000
	4560	1970	5630	33200	12200	113000	113000
	6800	2840	81400	80300	399000	310000	544000
	6600	5430	4600	10900	24600	24000	81900
	27800	66400	215000	301000	849000	3160000	5030000
	67500	107000	870000	1150000	3040000	11600000	6550000
Mean	20158.3	38240.0	205438.3	269566.7	730250.0	2551500.0	2076316.7
SEM	10100.6	17534.0	136590.7	181341.0	480485.2	1876206.6	1192638.4

* This animal was treated as an outlier as noted below.

Tumor Volume. Animals in the PDX1 and PDX2 studies were sacrificed on Days 25 and 42 respectively. During necropsy, the liver was removed, the xenograft was excised and the tumor diameters (small (S) and large (L)) were measured. Tumor volume was calculated using the following formula: $V= \pi/6 * L*S^2$. Tumor volume is presented in Tables 16 and 17 and plotted in Figures 10 and 24, tumor images are presented in Figures 9 and 23.

Table 16. Tumor Measurements and Volume

	Large Diameter (mm)	Small Diameter (mm)	Tumor Volume (mm3)
TXR-311	16.61	13.9	1680.29
	6.84	5.62	113.11
	9.62	8.59	371.66
	11.31	8.94	473.29
	12.96	11.75	936.84
	9.53	9.26	427.86
	16.14	13.98	1651.60
	11.26	9.87	574.33
	16.71	10.76	1012.95
	Mean		804.66
	SEM		186.91
Vehicle	15.59	13.51	1489.85
	18.23	13.18	1658.07
	18.46	16.82	2734.45
	21.61	13.49	2059.04
	16.25	13.61	1576.00
	19.66	16.99	2971.37
	15.39	10.44	878.26
	16.91	12.97	1489.39
	17.45	14.9	2028.40
	Mean		1876.09
	SEM		218.11

Table 17. Tumor Measurements and Volume

	Large Diameter (mm)	Small Diameter (mm)	Tumor Volume (mm^3)
TXR-311	14.17	11.55	989.74
	(21.14)*	(16.76)*	(3109.13)*
	8.29	7.08	217.57
	5.63	4.58	61.83
	8.06	8.05	273.47

	10.54	8.79	426.39
	7.35	6.67	171.21
		Mean	807.81
		SEM	409.61
Vehicle	19.51	13.93	1982.19
	25.22	19.18	4857.67
	15.45	11.27	1027.45
	10.16	9.78	508.81
	15.99	12.67	1343.96
	17.03	10.24	934.98
	24.19	19.25	4693.35
		Mean	2192.63
		SEM	688.25
Sorafenib	10.45	9.56	500.06
	7.02	5.26	101.69
	11.65	11.12	754.26
	6.21	5.12	85.23
	17.25	15.01	2034.87
	21.02	16.56	3018.14
		Mean	1082.38
		SEM	449.04

*Identified as outlier and removed from the inferential statistics analysis

[125] At termination of the in-life phase of the PDX1 study, animals treated with TXR-311 exhibit a mean tumor volume of 805 ± 187 mm^3 while animals treated with vehicle show a larger mean tumor volume of 1876 ± 218 mm^3. Statistical analysis indicated that this decrease was statistically significant ($p \leq 0.01$, 2-way ANOVA).

[126] At termination of the in-life phase of the PDX2 study, animals treated with TXR-311 had the lowest tumor volume when compared to vehicle- and sorafenib-treated animals: 750 ± 410 mm^3 vs 2193 ± 688 mm^3, and 1082 ± 485 mm^3, respectively. Statistical analysis (non-parametric, one-way ANOVA) following a single outlier identification and removal indicated that these differences were statistically significant at $p \leq 0.05$ only when comparing TXR-311 to vehicle-treated animals. Statistical analysis without the removal of the outlier indicated that these differences did not reach a statistical significance of $p < 0.05$ ($p = 0.06$). Five out of seven mice treated with TXR-311 had significantly reduced tumor volume compared to vehicle-treated animals.

[127] Treating tumor-bearing mice with 20 mg/kg of TXR-311 significantly inhibited the growth of the two orthotopic HCC xenografts when compared to vehicle-treated animals. Measuring the tumor volume at the time of necropsy showed a significant decrease in PDX1 but

not in PDX2 xenograft when compared to vehicle-treated animals. Based on luciferase counts of in-life xenografts, TXR-311 reduced PDX1 and PDX2 tumor volume by 3- and 4-fold, respectively at the end of the treatment period.

[128] In summary, preclinical cytotoxicity studies and patient derived xenograft animal model studies of TXR-311 suggest that certain CBS microtubule destabilizing agents constitute a class of drug candidates for the treatment of liver cancers such as HCC and possesses specificity for tumorigenic cells relative to normal hepatocytes.

[129] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. Any feature, step, element, embodiment, or aspect of the disclosure can be used in combination with any other unless specifically indicated otherwise. Although the disclosure provides description, illustration, and examples for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.