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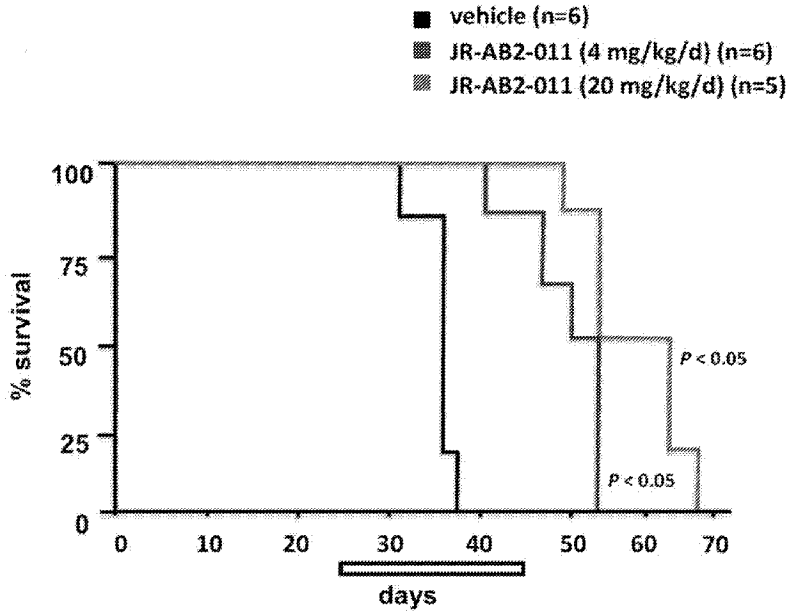

FIG. 6B

(57) Abstract: The present disclosure provides compounds and methods for inhibiting mTORC2, as well as methods for treating cancers such as glioblastoma.

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INHIBITORS OF MTOR-RICTOR INTERACTIONS

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/481,924, filed April 5, 2017, the contents of which are fully incorporated by reference herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

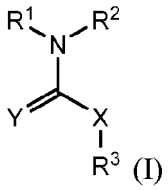
This invention was made with Government support under Grant Number CA168700, awarded by the National Institutes of Health. The Government has certain rights in the invention. This work was supported by the U.S. Department of Veterans Affairs, and the Federal Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Glioblastomas (GBM) are highly malignant and invasive tumors, properties which prevent total surgical resection, and render these neoplasms refractory to chemotherapeutic interventions (1). Prognosis for patients is very poor and most succumb to the disease within a year (2). Patients that do survive typically develop significant long-term toxicities as a result of high-dose chemotherapies ultimately leading to drug resistance (3). Thus, the development of novel therapeutic options based on the biology of these heterogeneous tumors is necessary while circumventing resistance mechanisms. However, current efforts utilizing mTOR inhibitors as a potential targeted therapeutic for the treatment of glioblastoma have failed in the clinic. Accordingly, new mTOR inhibitors are needed.

SUMMARY OF THE INVENTION

In some aspects, the present invention provides compounds having the structure of formula I or a pharmaceutically acceptable salt thereof:



(I)

wherein:

R^1 is aryl, heteroaryl, or heterocyclyl;

R^2 is alkyl, aryl or heteroaryl;

R^3 is alkyl, aryl, or heteroaryl;

X is $C(R^4R^5)$, $N(R^4)$, or O;

Y is S or O; and

R^4 and R^5 are independently selected from H or alkyl.

Exemplary compounds of Formula (I) include the compounds depicted in Table I.

The invention further relates to pharmaceutical compositions of the subject compounds, as well as methods of using these compounds or compositions in the treatment of cancer, such as prostate cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1C. Identification of compounds that inhibit mTORC2 activity in glioblastoma cells. FIG. 1A: JR-AB2-000 inhibits mTORC2 *in vitro* kinase activity. mTORC2 kinase reactions were performed using GST-tagged AKT as a substrate with the indicated concentrations of inhibitor. Reactions were subsequently immunoblotted for phospho-S^{473}-AKT and GST-tagged AKT. **FIG. 1B:** JR-AB2-000 blocks binding of Rictor to mTOR in LN229 cells. Cells were treated with 5 mM Antimycin (control compound), 50 nM JR-AB2-000 or 20 nM rapamycin for 15 min and mTOR immunoprecipitated. Immunoprecipitations were then immunoblotted for the indicated proteins. **FIG. 1C:** mTORC2 signaling is inhibited in GBM lines following 24 h exposure to JR-AB2-000. LN229 or U87 cells were treated with 100 nM of inhibitor as shown and lysates immunoblotted for the indicated proteins. Data shown are representative of experiments repeated two times.

FIGs. 2A-2E. Anti-GBM effects of JR-AB2-000 in cell lines. FIG. 2A: Inhibition of GBM cell line proliferation following culture with JR-AB2-000 (black, 0 nM; blue, 0.5 μM; red, 1 μM; green, 2 μM) for the indicated time points. Data represent mean ±S.D. of three independent experiments. **FIG. 2B:** JR-AB2-000 exposure inhibits anchorage-independent growth. Cells were suspended in soft agar to evaluate anchorage independent growth in the presence of the indicated concentrations of inhibitor and colonies counted after 14 days of growth. Data represent mean +S.D. of three independent experiments. **FIG. 2C:** Migration of U87 and LN229 cells in the presence of the indicated concentration of JR-AB2-000. Cells were seeded into Boyden chambers and allowed to migrate towards BSA (white bars), vitronectin (grey bars) or fibronectin (black bars) (*, $P<0.05$). Data represent mean +S.D. of three independent experiments. **FIG. 2D:** Invasive potential of U87 or LN229 cells in the presence of the indicated concentrations of JR-AB2-000 migrating through matrigel. Data represent mean +S.D. of three independent experiments. **FIG. 2E:** Cell-cycle phase

distributions were determined on the indicated lines treated with JR-AB2-000 as shown. Percent apoptotic cells as determined via annexin V-FITC staining are also shown below each graph. One of three experiments with similar results is shown.

FIGs. 3A-3F. Sensitivity to JR-AB2-000 is dependent on Rictor or SIN1 expression. FIG. 3A: Steady-state Rictor expression levels in GBM lines. Lysates from the indicated lines were immunoblotted for Rictor and actin levels. **FIG. 3B:** Quantification of Rictor expression levels from (A) as determined by densitometric analyses. Relative Rictor expression in U87 cells was arbitrarily assigned a value of 1. Rictor expression in all cell lines is shown as mean +S.D., n=3. **FIG. 3C:** XTT proliferation assays performed on eleven GBM lines with increasing concentration of JR-AB2-000 at 72 h. **FIG. 3D:** Correlation between JR-AB2-000 sensitivity and IC_{50} determined for all GBM cell lines treated with JR-AB2-000 for 72 h and shown as means of 3-5 individual experiments. Relative Rictor expression was obtained from (B) above. **FIG. 3E:** Sensitivity of U87, U87shRictor and U87-Rictor GBM cells to JR-AB2-000 treated with the indicated concentrations at 72 h. Data represent mean ±S.D. of three independent experiments. *, $P < 0.05$ **FIG. 3F:** Sensitivity of wt SIN1 and SIN1-null MEFs to increasing concentrations of JR-AB2-000 at 48 h. Data represent mean ±S.D. of three independent experiments. *, $P < 0.05$

FIG. 4. JR-AB-011 binds to Rictor and prevents Rictor-mTOR association. Surface plasmon resonance analysis of JR-AB-011 binding to immobilized Rictor (top, black), mSIN1 (bottom, light gray) or mTOR (bottom, dark gray) as indicated (left panel). Binding sensorgrams of immobilized mTOR with Rictor over the indicated concentration range (right panel). The Kon, Koff and Kd were calculated by simultaneous nonlinear regression using a 1:1 binding model and BIAevaluation 3.1 software.

FIGs. 5A-5B. JR-AB-011 binds to Rictor and prevents Rictor-mTOR association. FIG. 5A: Competitive binding curves of Rictor-mTOR association in the absence or presence of JR-AB2-011 or JR-AB2-000 as indicated (left panel). Analysis of selectivity of JR-AB2-011 (middle panel) or JR-AB2-000 (right panel) binding to Rictor, Raptor, mLST8 or Deptor as shown. Samples were preincubated with inhibitors and Rictor, Raptor, mLST8 or Deptor proteins as indicated and run over sensor chip containing immobilized mTOR. The IC_{50} values were calculated using the response units at the dissociation phase. **FIG. 5B:** mTOR-Flag coupled beads binding to myc-Rictor in the presence of increasing JR-AB2-011 (top panels) or JR-AB2-000 (bottom panels). myc-Rictor was incubated with inhibitor for 1 h followed by incubation with FLAG agarose beads coupled to mTOR-Flag (mTOR-Flag beads). Binding of

myc-Rictor to mTOR-Flag beads (Rictor-mTOR-Flag beads) was detected by immunoblotting with an anti-myc mAb. The amount of protein bound to FLAG agarose beads was detected with an anti-Flag mAb (loading control). Immunoblots were quantified via densitometric analyses and graphs are shown to the right of the blots. Three independent experiments were performed and one representative result is shown.

FIGs. 6A-6C. Effects of JR-AB2-011 treatment on GBM tumor growth in mice. **FIG. 6A:** Tumor burden of SCID mice implanted with LN229 cells and treated with the indicated schedules of vehicle (top trace), JR-AB2-011 (4 mg/kg/d) (middle trace) and JR-AB2-011 (20 mg/kg/d) (bottom trace) for ten consecutive days and tumor growth assessed every two days following initiation of treatment (start, day 0). *, $P < 0.05$, significantly different from vehicle, JR-AB2-011 (4 mg/kg/d) and JR-AB2-011 (20 mg/kg/d). **FIG. 6B:** Overall survival of subcutaneous LN229 tumors receiving the indicated treatment schedules of vehicle (left trace), JR-AB2-011 (4 mg/kg/d) (middle trace) and JR-AB2-011 (20 mg/kg/d) (right trace). **FIG. 6C:** Apoptotic cells were identified by TUNEL assays of sections prepared from harvested tumors at day 12 following initiation of treatment regimens (left panel). Data are expressed as the number of positive apoptotic bodies divided by high power field (hpf; 10-12 hpf/tumor). Values are means +S.D., *, $P < 0.05$. Phospho-S^{473}-AKT/total AKT protein ratio levels in tumors (middle panel). Values are means ±S.D., *, $P < 0.05$, significantly different from vehicle, JR-AB2-011 (4 mg/kg/d) and JR-AB2-011 (20 mg/kg/d). Phospho-T^{389}-S6K/total S6K protein ratio levels in tumors. Values are means ±S.D., *, $P < 0.05$, significantly different from vehicle, JR-AB2-011 (4 mg/kg/d) and JR-AB2-011 (20 mg/kg/d). Protein levels were quantified by Western analyses of harvested tumors from mice with the corresponding treatments as indicated and described in "Material & Methods".

FIG. 7. TUNEL staining of human neurons treated with increasing concentrations of JR-AB2-000 (left panel) or JR-AB2-011 (right panel) following 48 h exposure. Data shown are mean +S.D., n = 3.

FIGs. 8A-8B. Kinetics and dose-response of JR-AB2-000 mediated-inhibition of mTORC2 signaling in LN229 cells. **FIG. 8A:** LN229 cells were treated with JR-AB2-000 (1 μM) for the indicated time points and lysates immunoblotted for the indicated proteins. **FIG. 8B:** LN229 cells were treated with JR-AB2-000 at the indicated concentrations for 2.5 h and protein lysates immunoblotted for the indicated proteins.

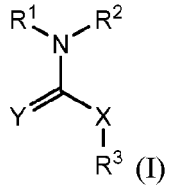
FIG. 9. Treatment of U87 and LN229 GBM cells with JR-AB2-011 (1 μM) for 24 h and lysates immunoblotted for the indicated proteins.

FIG. 10. Peripheral blood RBC and WBC counts in mice (5 mice/group) treated with daily IP injections (10 days) of 0, 4 or 20 mg/kg/d of JR-AB2-011. Data are expressed as percent of control mice that received vehicle only and assigned 100 %.

DETAILED DESCRIPTION

In some aspects, the present invention provides compounds as described herein. The compounds described herein are useful as cancer therapeutics, in particular as inhibitors of the interaction between Rictor and mTORC2, and inhibitors of mTORC2 signaling. In some embodiments, the compounds described herein do not substantially affect mTORC1 signaling (e.g., affect mTORC1 with an IC_{50} at least 10 times or preferably even at least 100 times their IC_{50} for mTORC2).

In some aspects, the present invention provides compounds having the structure of formula I or pharmaceutically acceptable salts thereof:



(I)

wherein:

R^1 is aryl, heteroaryl, or heterocyclyl;

R^2 is alkyl, aryl or heteroaryl;

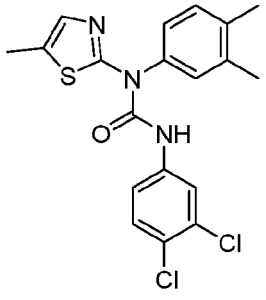
R^3 is alkyl, aryl, or heteroaryl;

X is $C(R^4R^5)$, $N(R^4)$, or O;

Y is S or O;

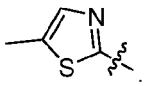
R^4 and R^5 are independently selected from H or alkyl.

In some embodiments, R^1 is not 3-methylisothiazolyl. In some embodiments, R^2 is not 3,4-dimethylphenyl. In some embodiments, R^3 is not 3,4-dichlorophenyl. In some embodiments, X is not N(H). In some embodiments, the compound is not



In some embodiments of formula I, R^1 is 5-membered heteroaryl or heterocyclyl, e.g., thiazolyl, isothiazolyl, oxazolyl, 4,5-dihydrooxazolyl, 4,5-dihydrothiazolyl, benzothiazolyl, benzoxazolyl, pyridyl, or phenyl. In certain preferred embodiments, R^1 is selected from thiazolyl, oxazolyl, 4,5-dihydrooxazolyl, 4,5-dihydrothiazolyl, benzothiazolyl, benzoxazolyl, pyridyl, or phenyl.

In some embodiments of formula I, R^1 is substituted with one or more alkyl moieties. In certain preferred embodiments, R^1 is 3-methylisothiazolyl:



In some embodiments of formula I, R^2 is heteroaryl or heterocyclyl. In other embodiments, R^2 is phenyl.

In some embodiments of formula I, R^2 is substituted with one or more R^6; each R^6 independently selected from $N(R^7R^8)$, alkyl, alkoxy, or halo; wherein R^7 and R^8 are independently selected from alkyl.

In certain preferred embodiments, R^2 is halophenyl, such as 4-bromophenyl or 4-fluorophenyl.

In some embodiments of formula I, R^3 is heteroaryl or heterocyclyl. In other embodiments, R^3 is phenyl.

In some embodiments of formula I, R^3 is optionally substituted with one or more R^9; each R^9 independently selected from $N(R^{10}R^{11})$, alkyl, alkoxy, or halo; wherein R^{10} and R^{11} are independently selected from alkyl.

In certain preferred embodiments, R^3 is halophenyl, such as 3,4-dichlorophenyl.

In some embodiments of formula I, X is $N(R^4)$, preferably N(H).

In some embodiments of formula I, Y is O. In other embodiments, Y is S.

In some aspects, the present invention provides a pharmaceutical composition comprising a compound as described herein and a pharmaceutically acceptable excipient.

In some aspects, the present invention provides the use of a compound or composition as disclosed herein for inhibiting or preventing the formation of mTORC2 in a cell. In some such embodiments, the compound or composition does not substantially inhibit or prevent the formation of mTORC1 in the cell.

In some aspects, the present invention provides the use of a compound or composition as disclosed herein for treating a mammal suffering from cancer, such as glioblastoma.

In some aspects, the present invention provides a method for inhibiting or preventing the formation of mTORC2 in a cell, comprising contacting the cell with a compound or composition as disclosed herein. In some such embodiments, the formation of mTORC1 in the cell is not substantially inhibited or prevented.

In some aspects, the present invention provides a method for treating a mammal suffering from cancer, comprising administering a compound or composition as disclosed herein. In some embodiments, the cancer is glioblastoma.

In some aspects, the present invention provides methods of treating proliferative diseases, such as glioblastoma, methods of inhibiting the interaction between Rictor and mTORC2, and methods of inhibiting mTORC2 signaling.

In certain embodiments, compounds of the invention are prodrugs of the compounds described herein. For example, wherein a hydroxyl in the parent compound is presented as an ester or a carbonate, or a carboxylic acid present in the parent compound is presented as an ester. In certain such embodiments, the prodrug is metabolized to the active parent compound in vivo (e.g., the ester is hydrolyzed to the corresponding hydroxyl or carboxylic acid).

In certain embodiments, compounds of the invention may be racemic. In certain embodiments, compounds of the invention may be enriched in one enantiomer. For example, a compound of the invention may have greater than 30% ee, 40% ee, 50% ee, 60% ee, 70% ee, 80% ee, 90% ee, or even 95% or greater ee. In certain embodiments, compounds of the invention may have more than one stereocenter. In certain such embodiments, compounds of the invention may be enriched in one or more diastereomers. For example, a compound of the invention may have greater than 30% de, 40% de, 50% de, 60% de, 70% de, 80% de, 90% de, or even 95% or greater de.

In certain embodiments, the present invention provides pharmaceutical compositions comprising a compound of Formula I. In certain embodiments, the pharmaceutical compositions further comprise a pharmaceutically acceptable excipient.

In certain embodiments, the pharmaceutical compositions may be for use in treating or preventing a condition or disease as described herein.

In certain embodiments, the present invention relates to methods of treatment with a compound of Formula I. In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one enantiomer or isomer of a compound. An enantiomerically enriched mixture may comprise, for example, at least 60 mol percent of one

enantiomer, or more preferably at least 75, 90, 95, or even 99 mol percent. In certain embodiments, the compound enriched in one enantiomer is substantially free of the other enantiomer, wherein substantially free means that the substance in question makes up less than 10%, or less than 5%, or less than 4%, or less than 3%, or less than 2%, or less than 1% as compared to the amount of the other enantiomer, *e.g.*, in the composition or compound mixture. For example, if a composition or compound mixture contains 98 grams of a first enantiomer and 2 grams of a second enantiomer, it would be said to contain 98 mol percent of the first enantiomer and only 2% of the second enantiomer.

In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one diastereomer of a compound. A diastereomerically enriched mixture may comprise, for example, at least 60 mol percent of one diastereomer, or more preferably at least 75, 90, 95, or even 99 mol percent.

In certain embodiments, the present invention provides a pharmaceutical preparation suitable for use in a human patient, comprising any of the compounds shown above, and one or more pharmaceutically acceptable excipients.

Compounds of any of the above structures may be used in the manufacture of medicaments for the treatment of any diseases or conditions disclosed herein.

Compositions and Modes of Administration

The compounds of this invention may be used in treating the conditions described herein, in the form of the free base, salts (preferably pharmaceutically acceptable salts), solvates, hydrates, prodrugs, isomers, or mixtures thereof. All forms are within the scope of the disclosure. Acid addition salts may be formed and provide a more convenient form for use; in practice, use of the salt form inherently amounts to use of the base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the subject organism in pharmaceutical doses of the salts, so that the beneficial properties inherent in the free base are not vitiated by side effects ascribable to the anions. Although pharmaceutically acceptable salts of the basic compounds are preferred, all acid addition salts are useful as sources of the free base form even if the particular salt per se is desired only as an intermediate product as, for example, when the salt is formed only for the purposes of purification and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures.

Pharmaceutically acceptable salts within the scope of the disclosure include those derived from the following acids; mineral acids such as hydrochloric acid, sulfuric acid, phosphoric acid and sulfamic acid; and organic acids such as acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, quinic acid, and the like.

The compounds of the present invention can be formulated as pharmaceutical compositions and administered to a subject in need of treatment, for example a mammal, such as a human patient, in a variety of forms adapted to the chosen route of administration, for example, orally, nasally, intraperitoneally, or parenterally (e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal or topical routes). Parenteral administration may be by continuous infusion over a selected period of time.

In accordance with the methods of the disclosure, the described compounds may be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compositions containing the compounds of the disclosure can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

A composition comprising a compound of the present disclosure may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

A person skilled in the art would know how to prepare suitable formulations. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (1990 - 18th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

Thus, compounds of the invention may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier; or by inhalation or insufflation. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the compounds may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The compounds may be combined with a fine inert powdered carrier and inhaled by the subject or insufflated. Such compositions and preparations should contain at least 0.1% of compounds of formula Ia or Ib. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of a given unit dosage form. The amount of the compounds in such therapeutically useful compositions is such that an effective dosage level will be obtained.

In certain embodiments of the disclosure, compositions comprising a compound of the present disclosure for oral administration include capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and the like, each containing a predetermined amount of the compound of the present disclosure as an active ingredient.

In solid dosage forms for oral administration (capsules, tablets, troches, pills, dragees, powders, granules, and the like), one or more compositions comprising the compound of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, gum tragacanth, corn starch, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain

silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the compounds may be incorporated into sustained-release preparations and devices. For example, the compounds may be incorporated into time release capsules, time release tablets, and time release pills.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the compound of the present disclosure, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol (ethanol), isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming, and preservative agents.

Suspensions, in addition to the active compounds, salts and/or prodrugs thereof, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol, and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

In certain embodiments, pharmaceutical compositions suitable for parenteral administration may comprise the compound of the present disclosure in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the disclosure include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The compounds may be administered intravenously or intraperitoneally by infusion or injection. Solutions of the compounds or their salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the compounds which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable

compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the compounds may be applied in pure form. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Other solid carriers include nontoxic polymeric nanoparticles or microparticles. Useful liquid carriers include water, alcohols or glycols or water/alcohol/glycol blends, in which the compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508), all of which are hereby incorporated by reference.

Useful dosages of the compounds of formulas I and II can be determined by comparing their in vitro activity, and in vivo activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949, which is hereby incorporated by reference.

For example, the concentration of the compounds in a liquid composition, such as a lotion, can be from about 0.1-25% by weight, or from about 0.5-10% by weight. The concentration in a semi-solid or solid composition such as a gel or a powder can be about 0.1-5% by weight, or about 0.5-2.5% by weight.

The amount of the compounds required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

Effective dosages and routes of administration of agents of the invention are conventional. The exact amount (effective dose) of the agent will vary from subject to subject, depending on, for example, the species, age, weight and general or clinical condition of the subject, the severity or mechanism of any disorder being treated, the particular agent or vehicle used, the method and scheduling of administration, and the like. A therapeutically effective dose can be determined empirically, by conventional procedures known to those of skill in the art. See, e.g., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds., Macmillan Publishing Co., New York. For example, an effective dose can be estimated initially either in cell culture assays or in suitable animal models. The animal model may also be used to determine the appropriate concentration ranges and routes of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutic dose can also be selected by analogy to dosages for comparable therapeutic agents.

The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (e.g., the subject, the disease, the disease state involved, and whether the treatment is prophylactic). Treatment may involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years.

In general, however, a suitable dose will be in the range of from about 0.001 to about 100 mg/kg, e.g., from about 0.01 to about 100 mg/kg of body weight per day, such as above about 0.1 mg per kilogram, or in a range of from about 1 to about 10 mg per kilogram body weight of the recipient per day. For example, a suitable dose may be about 1 mg/kg, 10 mg/kg, or 50 mg/kg of body weight per day.

The compounds of Formula I are conveniently administered in unit dosage form; for example, containing 0.05 to 10000 mg, 0.5 to 10000 mg, 5 to 1000 mg, or about 100 mg of active ingredient per unit dosage form.

The compounds can be administered to achieve peak plasma concentrations of, for example, from about 0.5 to about 75 μM, about 1 to 50 μM, about 2 to about 30 μM, or about 5 to about 25 μM. Exemplary desirable plasma concentrations include at least or no more than 0.25, 0.5, 1, 5, 10, 25, 50, 75, 100 or 200 μM. For example, plasma levels may be from about 1 to 100 micromolar or from about 10 to about 25 micromolar. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the compounds, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the compounds. Desirable blood levels may be maintained by continuous infusion to provide about 0.00005-5 mg per kg body weight per hour, for example at least or no more than 0.00005, 0.0005, 0.005, 0.05, 0.5, or 5 mg/kg/hr. Alternatively, such levels can be obtained by intermittent infusions containing about 0.0002-20 mg per kg body weight, for example, at least or no more than 0.0002, 0.002, 0.02, 0.2, 2, 20, or 50 mg of the compounds per kg of body weight.

The compounds may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator.

The dosage of the compounds and/or compositions of the disclosure can vary depending on many factors such as the pharmacodynamic properties of the compound, the mode of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the frequency of the treatment and the type of concurrent treatment, if any, and the clearance rate of the compound in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. The compounds of the disclosure may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. To calculate the human equivalent dose (HED) from a dosage used in the treatment of age-dependent cognitive impairment in rats, the formula HED (mg/kg) = rat dose (mg/kg) x 0.16 may be employed (see Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002, Center for Biologics Evaluation and Research). For example, using that formula, a dosage of 10 mg/kg in rats is equivalent to 1.6 mg/kg in humans. This conversion is based on a more general

formula HED = animal dose in mg/kg x (animal weight in kg/human weight in kg) 0.33. Similarly, to calculate the HED from a dosage used in the treatment in mouse, the formula HED (mg/kg) = mouse dose (mg/kg) x 0.08 may be employed (see Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers, December 2002, Center for Biologics Evaluation and Research).

Definitions

Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular biology, cell and cancer biology, neurobiology, neurochemistry, virology, immunology, microbiology, pharmacology, genetics and protein and nucleic acid chemistry, described herein, are those well known and commonly used in the art.

The methods and techniques of the present disclosure are generally performed, unless otherwise indicated, according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout this specification. See, e.g. "Principles of Neural Science", McGraw-Hill Medical, New York, N.Y. (2000); Motulsky, "Intuitive Biostatistics", Oxford University Press, Inc. (1995); Lodish et al., "Molecular Cell Biology, 4th ed.", W. H. Freeman & Co., New York (2000); Griffiths et al., "Introduction to Genetic Analysis, 7th ed.", W. H. Freeman & Co., N.Y. (1999); and Gilbert et al., "Developmental Biology, 6th ed.", Sinauer Associates, Inc., Sunderland, MA (2000).

Chemistry terms used herein, unless otherwise defined herein, are used according to conventional usage in the art, as exemplified by "The McGraw-Hill Dictionary of Chemical Terms", Parker S., Ed., McGraw-Hill, San Francisco, C.A. (1985).

All of the above, and any other publications, patents and published patent applications referred to in this application are specifically incorporated by reference herein. In case of conflict, the present specification, including its specific definitions, will control.

The term "agent" is used herein to denote a chemical compound (such as an organic or inorganic compound, a mixture of chemical compounds), a biological macromolecule (such as a nucleic acid, an antibody, including parts thereof as well as humanized, chimeric and human antibodies and monoclonal antibodies, a protein or portion thereof, e.g., a peptide, a lipid, a carbohydrate), or an extract made from biological materials such as bacteria, plants,

fungi, or animal (particularly mammalian) cells or tissues. Agents include, for example, agents whose structure is known, and those whose structure is not known. The ability of such agents to inhibit AR or promote AR degradation may render them suitable as "therapeutic agents" in the methods and compositions of this disclosure.

A "patient," "subject," or "individual" are used interchangeably and refer to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (e.g., canines, felines, etc.) and rodents (e.g., mice and rats).

"Treating" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

The term "preventing" is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount.

"Administering" or "administration of" a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, e.g., through a skin duct). A compound or agent can also

appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, e.g., patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age and/or the physical condition of the subject and the chemical and biological properties of the compound or agent (e.g., solubility, digestibility, bioavailability, stability and toxicity). In some embodiments, a compound or an agent is administered orally, e.g., to a subject by ingestion. In some embodiments, the orally administered compound or agent is in an extended release or slow release formulation, or administered using a device for such slow or extended release.

As used herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapeutic agents such that the second agent is administered while the previously administered therapeutic agent is still effective in the body (e.g., the two agents are simultaneously effective in the patient, which may include synergistic effects of the two agents). For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic agents.

A "therapeutically effective amount" or a "therapeutically effective dose" of a drug or agent is an amount of a drug or an agent that, when administered to a subject will have the intended therapeutic effect. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. The precise effective amount needed for a subject will depend upon, for example, the subject's size, health and age, and the nature and extent of the condition being treated, such as cancer or MDS. The skilled worker can readily determine the effective amount for a given situation by routine experimentation.

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

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

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

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

The term "alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term "alkyl" refers to saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_{1-30} for straight chains, C_{3-30} for branched chains), and more preferably 20 or fewer.

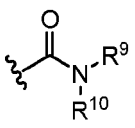
Moreover, the term "alkyl" as used throughout the specification, examples, and claims is intended to include both unsubstituted and substituted alkyl groups, the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc.

The term "C_{x-y}" or "C_x-C_y", when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. C_0alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. A C_{1-6}alkyl group, for example, contains from one to six carbon atoms in the chain.

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

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

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



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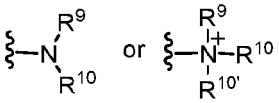
wherein R^9 and R^{10} each independently represent a hydrogen or hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

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



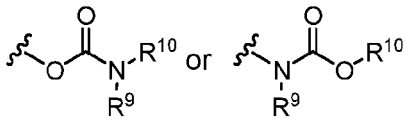
wherein R^9, R^{10}, and $R^{10'}$ each independently represent a hydrogen or a hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

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

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

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

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



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group.

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

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

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

The term "carbonate" is art-recognized and refers to a group $-OCO_2-$.

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

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

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

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

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

The terms "heteroaryl" and "hetaryl" include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heteroaryl" and "hetaryl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

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

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

The terms "heterocyclyl", "heterocycle", and "heterocyclic" refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heterocyclyl" and "heterocyclic" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

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

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

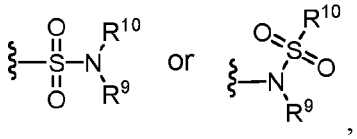
The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the

recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

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

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

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



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl.

The term "sulfoxide" is art-recognized and refers to the group–S(O)-.

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

The term "sulfone" is art-recognized and refers to the group $-S(O)_2-$.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate

organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

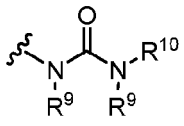
The term "thioalkyl", as used herein, refers to an alkyl group substituted with a thiol group.

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

wherein R^9 represents a hydrocarbyl.

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

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



,

wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl.

The term "modulate" as used herein includes the inhibition or suppression of a function or activity (such as cell proliferation) as well as the enhancement of a function or activity.

The phrase "pharmaceutically acceptable" is art-recognized. In certain embodiments, the term includes compositions, excipients, adjuvants, polymers and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation,

allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

"Pharmaceutically acceptable salt" or "salt" is used herein to refer to an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt of any base compounds represented by Formula I. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable salts, e.g., oxalates, may be used, for example, in the isolation of compounds of Formula I for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

The term "pharmaceutically acceptable basic addition salt" as used herein means any non-toxic organic or inorganic base addition salt of any acid compounds represented by Formula I or any of their intermediates. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium, or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic, or aromatic organic amines such as methylamine, trimethylamine and picoline or ammonia. The selection of the appropriate salt will be known to a person skilled in the art.

Many of the compounds useful in the methods and compositions of this disclosure have at least one stereogenic center in their structure. This stereogenic center may be present in a R or a S configuration, said R and S notation is used in correspondence with the rules described in Pure Appl. Chem. (1976), 45, 11-30. The disclosure contemplates all

stereoisomeric forms such as enantiomeric and diastereoisomeric forms of the compounds, salts, prodrugs or mixtures thereof (including all possible mixtures of stereoisomers). See, e.g., WO 01/062726.

Furthermore, certain compounds which contain alkenyl groups may exist as Z (zusammen) or E (entgegen) isomers. In each instance, the disclosure includes both mixture and separate individual isomers.

Some of the compounds may also exist in tautomeric forms. Such forms, although not explicitly indicated in the formulae described herein, are intended to be included within the scope of the present disclosure.

"Prodrug" or "pharmaceutically acceptable prodrug" refers to a compound that is metabolized, for example hydrolyzed or oxidized, in the host after administration to form the compound of the present disclosure (e.g., compounds of formula I). Typical examples of prodrugs include compounds that have biologically labile or cleavable (protecting) groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, or dephosphorylated to produce the active compound. Examples of prodrugs using ester or phosphoramidate as biologically labile or cleavable (protecting) groups are disclosed in U.S. Patents 6,875,751, 7,585,851, and 7,964,580, the disclosures of which are incorporated herein by reference. The prodrugs of this disclosure are metabolized to produce a compound of Formula I. The present disclosure includes within its scope, prodrugs of the compounds described herein. Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in "Design of Prodrugs" Ed. H. Bundgaard, Elsevier, 1985.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filter, diluent, excipient, solvent or encapsulating material useful for formulating a drug for medicinal or therapeutic use.

The term "Log of solubility", "LogS" or "logS" as used herein is used in the art to quantify the aqueous solubility of a compound. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. A low solubility often

goes along with a poor absorption. LogS value is a unit stripped logarithm (base 10) of the solubility measured in mol/liter.

Discussion

There is increasing evidence to support dysregulation of mTORC2/AKT signaling in the pathogenesis and progression of several cancers (8,36). Thus, activation of mTORC2, downstream of PI-3K or Rac1, or as a result of Rictor amplification or alterations in protein stability may serve as a nodal point in cell signaling highlighting its potential as a drug target. The present disclosure describes an inhibitor of mTORC2 and demonstrates its significant anti-GBM activity. SAR studies identified an improved inhibitor which also demonstrated significant anti-GBM properties *in vitro* and *in vivo*. Experiments addressing the mechanism of action of the inhibitors demonstrate that the inhibitors bind Rictor in such a manner as to preclude subsequent mTOR association. The studies are most consistent with the hypothesis that these inhibitors likely disrupt Rictor-mTOR binding by binding to Rictor on a site(s) either directly involved in mTOR binding or a site which allosterically prevents mTOR association. The improved ability of the SAR optimized analog JR-AB2-011 to block mTORC2 signaling may be due to JR-AB2-011s greater apparent affinity for Rictor. This is supported by SPR experiments in FIG. 5A (left panel), in which the K_i for JR-AB2-011 was found to be 6-7 fold lower relative to JR-AB2-000 in competitive binding assays.

The mechanistic target of rapamycin (mTOR) kinase is the major mediator of phosphatidylinositol 3-kinase (PI-3K) signaling and is an important target for molecular therapeutics in GBM (4,5). mTOR is a common element in two separate multicomponent kinase complexes (6,7). The mTOR-mLST8-Raptor complex (mTORC1) integrates signals regulating cell size and growth whereas the mTOR-mLST8-mSIN1-Rictor complex (mTORC2) regulates cell cycle-dependent cytoskeleton assembly in addition to growth (8). mTORC2 has emerged as a promising target in GBM, as recent data indicate that mTORC2 activity is essential for the transformation and invasive characteristics of these tumors, yet in many normal cells mTORC2 activity appears nonessential (9-11).

Essential to the successful targeting of mTORC2 is an understanding of the mechanisms of its dysregulation. Constitutive activation of upstream signaling pathways contribute to mTORC2 activation, via activation of growth factors PI-3K and TSC1-TSC2 (9). Recent data demonstrates association with the ribosome directly can activate mTORC2 (12). Another mechanism by which cells may regulate mTORC2 activity is by governing the

expression levels of essential regulatory subunits. It has been demonstrated that in gliomas, increased Rictor expression can be correlated with increased phospho-S473-AKT levels (13,14) and this has now been described in a variety of cancer cell types (15-17). Moreover, overexpressing Rictor enhances oncogenic traits of tumor cells (14) and GEMM studies demonstrate that it is indeed a bone fide oncoprotein (18). Frustratingly so, inhibiting components of the EGFR/PI-3K/AKT/mTORC signaling axis in GBM has proved to be cytostatic rather then inducing cell death or apoptosis, even though AKT and mTOR have clearly established anti-apoptotic roles (3). Alternative survival pathways may become activated allowing cells to evade pro-apoptotic signals mediated by EGFR/PI-3K/AKT/mTORC blockade.

The discovery that mTORC2 directly activates AKT led to hypotheses that mTORC2-specific inhibitors may be valuable anti-cancer drugs (9). Such a compound, in addition to blocking AKT phosphorylation, would have the advantage of not disrupting the mTORC1-dependent negative feedback loops, which have now been demonstrated to be a major mode of drug resistance to mTORC1 inhibitors (19,20).

There are currently three classes of compounds which can inhibit mTORC2 activity (ATP-competitive, dual PI-3K/mTOR inhibitors and rapamycin), however none of these inhibitors are specific. All suffer from the potential limitation of blocking mTORC1 and inactivating negative feedback loops resulting in the activation of AKT, even in the context of mTORC2 inhibition (9). Additionally, inhibition of mTORC1 also activates autophagy which may promote glioma cell survival (21,22).

Mammalian TORC2 is ~1.3 MDa and contains two copies of each of six subunits: mTOR, mLST8, Rictor, mSIN1, Deptor and PRR5 or its paralog PRR5L (37). Besides mTOR, Rictor is the largest subunit of TORC2. Disruption or RNAi-mediated depletion of Rictor results in the disassembly of TORC2 suggesting that Rictor performs a critical scaffolding function (38). In binding studies both the parent and JR-AB2-011 inhibitors bound to Rictor and prevented the association of mTOR, consistent with the notion that these compounds are able to inhibit assembly of mTORC2. It is unknown if JR-AB2-011 can also result in the disassembly of preformed mTORC2. While unlikely, it cannot be ruled out that JR-AB2-011 affects PRR5 function such that mTORC2 activity is impaired. Future mechanistic studies will be required to address these questions.

The inhibitors described herein are specific for mTORC2 in that mTORC1 was apparently unaffected by exposure, as one of the most well established markers of mTORC1

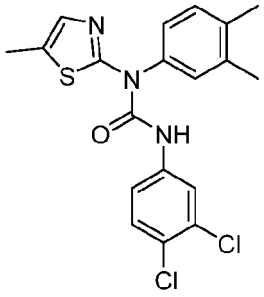
activity, phospho-T^{389}-S6K, was monitored as a readout. Additionally, phospho-S^{312} and total IRS1 levels were unchanged suggesting that at least the S6K/IRS1/PI-3K feedback loop remained intact in the presence of JR-AB2-000 (Fig. 8A). MAPK activation is also downstream of this feedback loop and ERK activity was unresponsive to JR-AB2-000 (Fig. 8B).

An important determinant of sensitivity to JR-AB2-000 is relative Rictor or SIN1 expression. This is most likely a result of increased mTORC2 formation, as increased expression of these subunits would be expected to promote the nucleation of signaling competent mTORC2 kinases. These inhibitors bind Rictor, and it is possible that post-translational modifications of Rictor, such as phosphorylation or acetylation may additionally be critical determinants of sensitivity for these compounds. Indeed, recent experiments show that glucose-dependent acetylation of Rictor promotes resistance to EGFR, PI-3K or AKT targeted therapies in GBM (39). It is conceivable that such modifications to Rictor may affect the affinity of JR-AB2-011 for its putative binding site. Another important consideration is the possibility that JR-AB2-000 or JR-AB2-011 bound Rictor may exert effects on several mTORC2-independent signaling cascades regulating cell proliferation and motility (15,40-42).

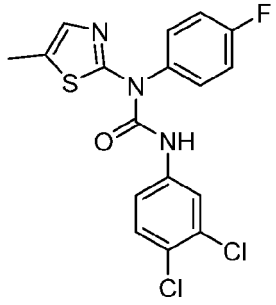
Recent studies have described the development of strategies to specifically inhibit TORC2 acutely in yeast utilizing reverse chemical genetics (37). To investigate the selective pharmacology of TORC2 blockade, Kliegman *et al.* engineered an allele of TOR2 which accepted an orthogonal kinase inhibitor that did not inhibit TOR1 (43). These studies implicated known regulatory relationships between TORC2 and sphingolipid biosynthesis and also identified novel regulation of the pentose phosphate pathway by TORC2. Rispal *et al.*, characterized the downstream effectors of TORC2 by engineering TORC1 to be resistant to the ATP-competitive TOR inhibitor NVP-BHS345 and subsequent treatment with the compound specifically blocked TORC2 (44). Phosphoproteomic analyses elucidated regulatory effects of TORC2 on actin polarization and endocytosis, via the phospholipid flippase kinases Fpk1 and Fpk2 and identified a broad spectrum of potential TORC2 effectors. The combined use of JR-AB2-011 and phosphoproteomics in mammalian cells may permit a similarly comprehensive analysis of mTORC2 substrates.

In summary, inhibitors of mTORC2 activity have been identified which target the regulatory subunit Rictor. The compounds and compositions are useful in inhibiting the interaction of the regulatory subunit Rictor with mTOR and in blunting mTORC2 signaling

while leaving mTORC1 signaling unaffected. These inhibitors have broad anti-GBM effects *in vitro* and in xenograft experiments, blocking growth, motility and invasive characteristics of GBM cell lines. Sensitivity to the inhibitor is correlated with elevated Rictor or mSIN1 expression. SPR and mTOR-bead pull-down experiments suggest that JR-AB2-000 and JR-AB2-011 specifically bind to Rictor and block association with mTOR. These results demonstrate that mTORC2 is a compelling target in GBM and support the use of these compounds in further dissection of mTORC2-mediated signaling processes.



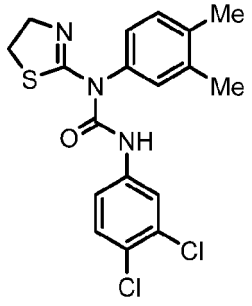
JR-AB2-000



JR-AB2-011

In addition to these compounds, a number of additional compounds have been prepared and tested, as listed in Table 1:

Table 1



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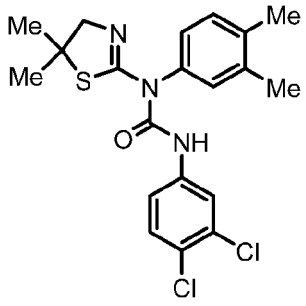

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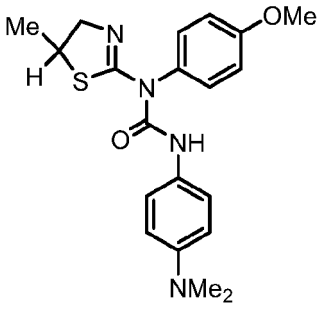

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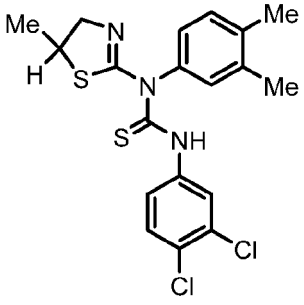

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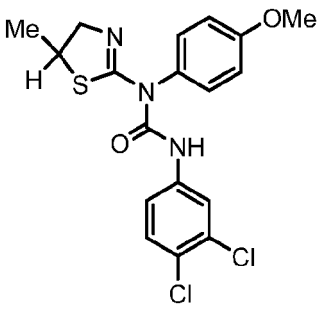

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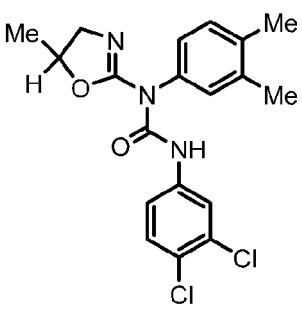

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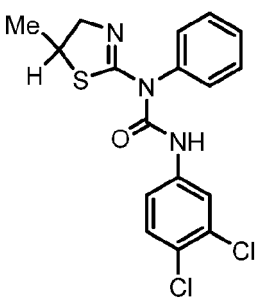

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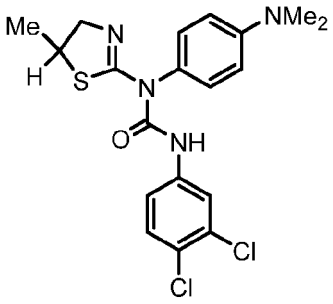

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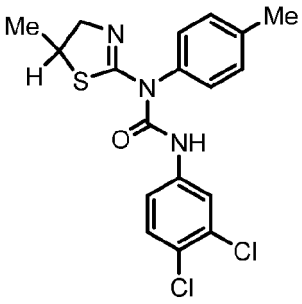

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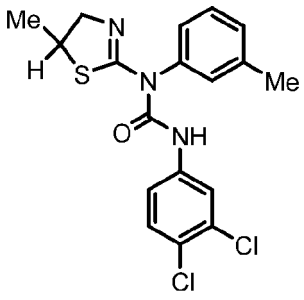

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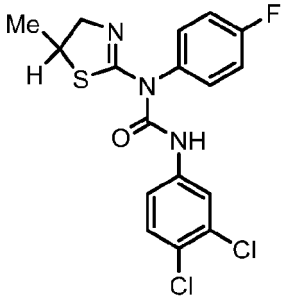

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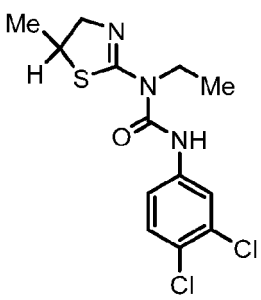

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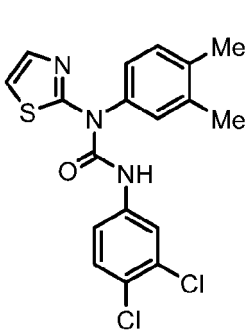

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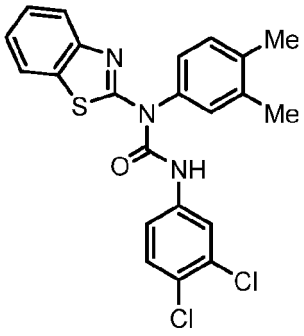

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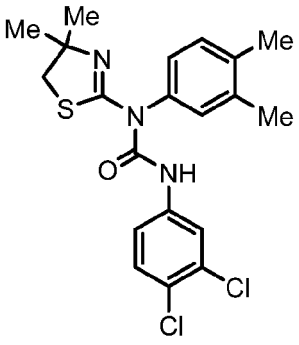

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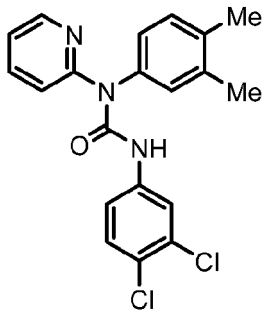

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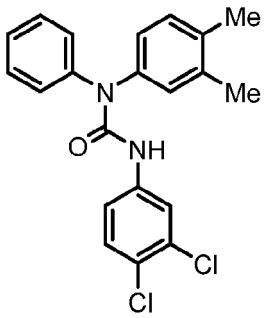

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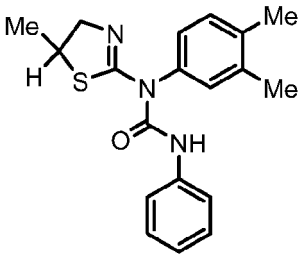

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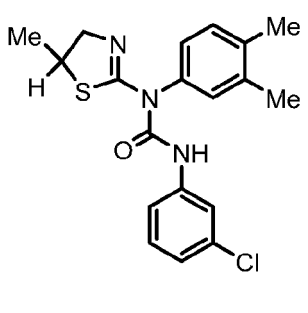

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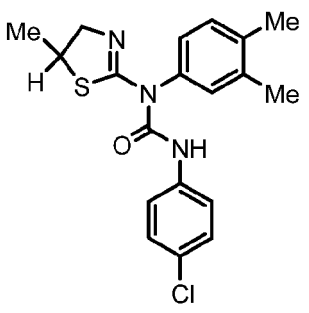

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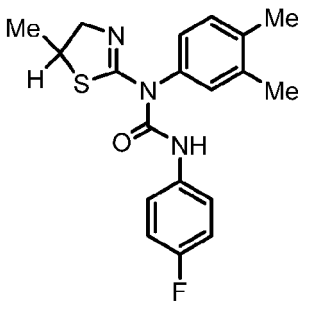

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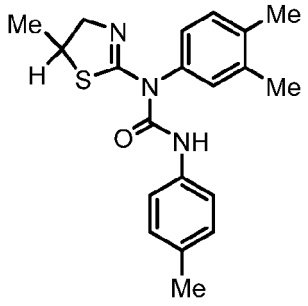

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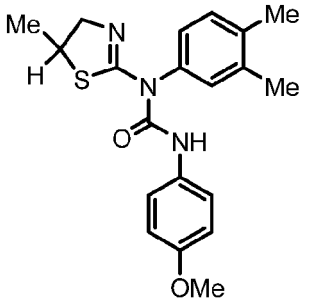

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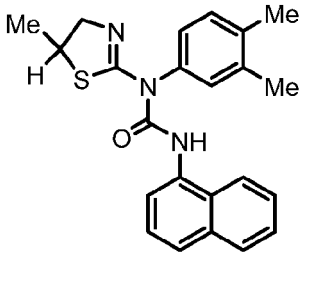

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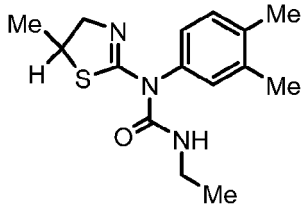

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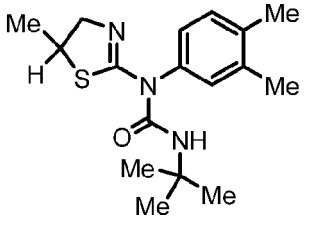

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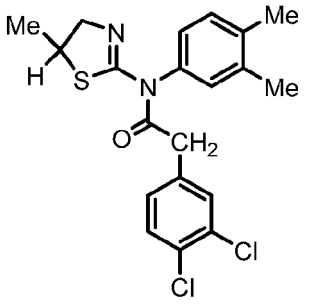

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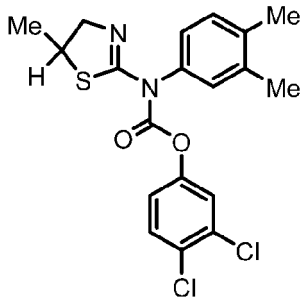

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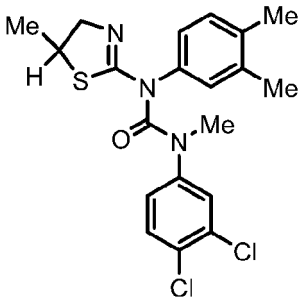
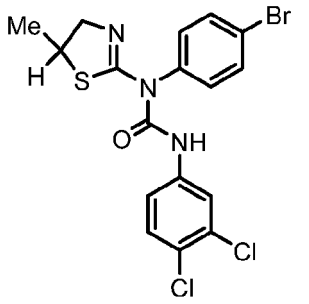


JR-AB2-029



JR-AB2-030

EXAMPLES

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of

certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1: General Methods

Cell lines, constructs and transfections- The SF763 line was obtained from the UCSF Neurosur-gery Tissue Bank and all other lines were from ATCC (Manassas, VA). Normal mature human neurons were obtained from ScienCell (Carlsbad, CA). Wild-type and SIN1-null MEFs were gen-erously provided by Dr. Bing Su (Yale University). Lines were obtained from 2001-2012 and rou-tinely tested to confirm the absence of mycoplasma. Cell authentication by ATCC is done by STR profiling. The myc-Rictor (corrected) and Deptor constructs were gifts from Dr. David Sabatini (Whitehead Institute/MIT) and the Flag-mTOR and mSIN1-HA constructs were gifts from Dr. Jie Chen (University of Illinois). DNA transfections were performed using Effectene transfection rea-gent according to the manufacturer (Qiagen).

Recombinant Proteins, Antibodies, Reagents and JR-AB2-000 Structure-activity relationship (SAR) Analog preparation- Proteins were expressed and purified from HEK293 cells using anti-myc, an-ti-HA or anti-Flag Sepharose column chromatography as previously described (23). Recombinant Raptor was from Abcam (Cambridge, MA) and recombinant mLST8 was obtained from Abnova (Walnut, CA). Antibodies were from the following sources. mTOR (#2972, Cell Signaling Technologies, CST), phospho-S^{312}-IRS1 (#ab66154, Abcam), total IRS1 (#ab131487, Abcam), phospho-$T^{202/Y204}$-ERK (#4370, CST), total ERK (#9102, CST), phospho-S^{657}-PKCα (#SAB-4504096, Sigma), total PKCα (#sc-208, Santa Cruz Biotechnology), phospho-T^{389}-S6K (#9205, CST), total S6K (#9202, CST), phospho-T^{346}-NDRG1 (#3217, CST), total NDRG1 (#ab124689, Abcam), phospho-S^{473}-AKT (#9271, CST), total AKT (#9272, CST), Rictor (#A300-459A, Bethyl Laboratories), actin (#ab3280, Abcam), α-GST (#2622, CST), α-myc (#2276, CST), α-Flag (#F3165, Sigma). Antimycin A was from Sig-ma and rapamycin was obtained from LC Laboratories (Woburn, MA). CID613034 (JR-AB2-000) was obtained from the Developmental Therapeutics Program repository at the NCI. Additionally, large-scale amounts of analog JR-AB2-011 (ID# STK377726) was obtained from Vitas-M Ltd., (Champaign, IL).

Protein expression, co-immunoprecipitation and in vitro kinase analyses- Western blot analyses were performed as previously described (14). Briefly, cells were lysed in RIPA (lysis) buffer containing protease inhibitor cocktail and phosSTOP phosphatase inhibitor

cocktail (Roche) and extracts resolved by SDS-PAGE. Proteins were transferred to PVDF membranes and incubated with the indicated antibodies. Antigen-antibody complexes were detected using appropriate horseradish peroxidase-conjugated secondary antibodies (GE Healthcare) and enhanced chemiluminescence (Amersham ECL Prime). Co-immunoprecipitations were performed as previously described (26). mTORC2 in vitro kinase assays were performed as described utilizing GST-tagged AKT as a substrate (14).

Surface plasmon resonance and mTOR-Flag binding assays- SPR experiments were carried out on a Biacore 2000 optical biosensor (BioCore AB, Piscataway NJ) using immobilized recombinant Rictor, mSIN1 or mTOR as described (27). Binding was observed as the change in response units (RU) as analyte was injected at a flow rate of 10 pl/min at 25°C. For SPR competitive solution binding experiments, on an mTOR immobilized CM5 chip, pre-incubated rictor, raptor, mLST8 or Deptor with inhibitor (30 min) reaction mixtures were injected over the surfaces of the chip. Re-sponse units were measured in the dissociation phase and specific binding was calculated by subtracting the control surface signal from the surfaces with immobilized mTOR. For mTOR-Flag binding assays, purified myc-Rictor was pre-treated with increasing concentrations of JR-AB2-000 or JR-AB2-011 for 1h at 4°C and subsequently added to mTOR-Flag beads and incubated overnight at 4°C. Incubated beads were washed five times and immunoblotted using an anti-myc or anti-Flag antibody as indicated.

Cell Proliferation, Cell-cycle distribution and TUNEL assays- Cells were plated into 96-well plates and after culturing for various time points, cell numbers were measured by 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) assay (Roche) as described by the manufacturer. Viability of human neurons was assessed by trypan blue- exclusion. Cell-cycle analysis was done by propidium iodide staining of cells and flow cytometry as previously described (28). Cells were stained using a FITC-conjugated annexin V (Annexin V-FITC Early Apoptosis Detection kit, Cell Signaling) to monitor apoptosis. TUNEL staining of tumor sections was performed using the TACSXL DAB In Situ Apoptosis Detection kit (Trevigen) according to the manufacturer's instructions (28).

Xenograft Studies- All animal experiments were performed under an approved Institutional Animal Care and Use Committee protocol and conformed to the guidelines established by the Association for the Assessment and Accreditation of Laboratory Animal Care. Xenografts of LN229 cells were performed in female C.B.-17-scid (Taconic) mice as previously described (29). Tumors were har-vested at autopsy for Western blot analysis.

Sections of paraffin-embedded tumors on slides were processed for immunohistochemistry as previously described (30).

Statistical analysis- Statistical analyses were performed with Student's t test and ANOVA models using Systat 13 (Systat Software, Chicago, IL). P values of less then 0.05 were considered significant.

Example 2: JR-AB2-000 Inhibition of mTORC2 in vitro kinase activity

As shown in FIG. 1A, JR-AB2-000, in a concentration-dependent manner, inhibited mTORC2 *in vitro* kinase activity. JR-AB2-000 also blocked the association of Rictor with mTOR in immunoprecipitates of mTOR from U87 cells treated with the inhibitor (Fig. 1B). The ability of the compound to block rictor-mTOR association was specific as raptor-mTOR binding was unaffected. In U87 and LN229 GBM lines, JR-AB2-000 markedly inhibited phosphorylation of known mTORC2 substrates such as, phospho-S^{473}-AKT, phospho-T^{346}-NDRG1 and phospho-S^{657}-PKCα, while having no discernible affect on the levels of phospho-T^{389}-S6K, a mTORC1 specific phospho-site. Moreover, in time-course and dose-response experiments (Figs. 8A, 8B), JR-AB2-000 inhibited mTORC2 signaling in a concentration-dependent manner with marked inhibition of activity within 2 hours of treatment in GBM cells, while having no significant affects on mTORC1 activity. These data suggest that JR-AB2-000 is a potent mTORC2-specific inhibitor.

Example 3: Anti-GBM effects of JR-AB2-000 in vivo

To determine the response of GBM cell lines to JR-AB2-000, XTT assays were performed in U87 and LN229 cells at various concentrations of inhibitor over the course of 96 hours. As shown in Fig. 2A, JR-AB2-000 treatment resulted in dose-dependent cytotoxicity in both lines. Treatment with the compound also had marked affects on GBM motility and invasive characteristics consistent with the blockade of mTORC2 activity, which is known to regulate these properties (14). To initially determine whether JR-AB2-000 affected the ability of GBM cells to form colonies in soft agar, U87 and LN229 cells were treated with the inhibitor in clonogenic growth assays. As shown in Fig. 2B, clonogenic growth of both U87 and LN229 were markedly inhibited by the compound. To determine if JR-AB2-000 treatment affected cell migration, the ability of JR-AB2-000 treated cells to traverse a vitronectin-coated or fibronectin-coated Boyden chamber was assessed as compared to chambers coated with BSA as a control. In a dose-dependent fashion JR-AB2-000 significantly inhibited the numbers of cells that migrated towards vitronectin or

fibronectin-coated surfaces as compared to control BSA-coated surfaces (Fig. 2C). JR-AB2-000 was also tested for whether it affected the ability of U87 or LN229 cells to invade Matrigel. As shown in Fig. 2D, the compound significantly blocked migratory ability with increasing concentration. Finally, the cell-cycle phase distributions of U87 and LN229 cells treated with the inhibitor were examined. As shown in Fig. 2E, JR-AB2-000 increased the percentage of cells in G1/G0 with a concomitant reduction in S-phase and unchanged G2/M numbers. JR-AB2-000 treatment also induced significant apoptosis in these cells relative to untreated controls. Taken together, these data demonstrate significant inhibitory affects of JR-AB2-000 on GBM cell growth, migration and invasive properties.

Example 5: Evaluation of whether JR-AB2-000 Sensitivity Depends on Rictor Levels

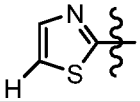
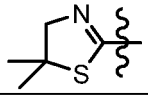
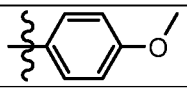
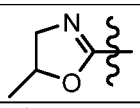
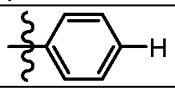
The relative expression of Rictor has been demonstrated to regulate the formation of mTORC2 and its activity (13,14). To determine whether Rictor expression can alter the sensitivity of GBM cells to JR-AB2-000 Rictor levels were determined in several cell lines and their relative expression was correlated with drug sensitivity. As shown in Fig. 3A, Rictor expression was varied in these lines and expression levels were quantified via densitometry and normalized to levels observed in U87 cells. In Fig. 3B, the relative Rictor expression in the lines tested was high in most cases except for H4, U373 and U138 which displayed low Rictor levels and LN229 and M059J in which expression was at intermediate levels. These cell lines were subsequently tested in XTT assays to determine the cellular responses to JR-AB2-000 over a wide range of concentrations (Fig. 3C). A significant inverse correlation was observed between the IC_{50} for JR-AB2-000 and relative Rictor expression in the cell lines tested (Fig. 3D) demonstrating that lines which harbored elevated levels of Rictor were the most sensitive to the drug, while those with low Rictor expression were relatively resistant. The effects of JR-AB2-000 were also tested on U87 cells in which Rictor was knocked-down via stable overexpression of an shRNA targeting Rictor or cells that stably overexpressed Rictor via an expression construct (Fig. 3E). U87 cells stably overexpressing Rictor were very sensitive to JR-AB2-000 while $U87_{shRictor}$ cells were markedly resistant. Similarly, the sensitivity of wt and mSIN1-null MEFs to JR-AB2-000 was tested. These cells also displayed differential sensitivities to the compound, as wildtype MEFs were significantly more sensitive as compared to mSIN-null MEFs at concentrations over 0.25 μM.

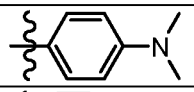
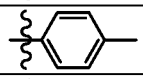
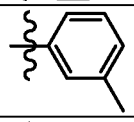
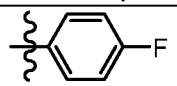
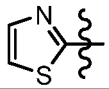
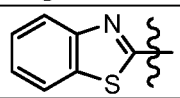
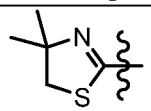
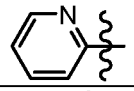
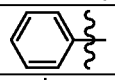
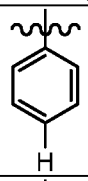
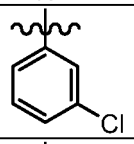
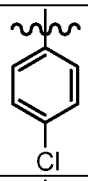
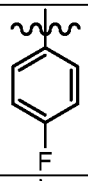
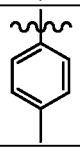
Example 6: Structure-Activity Relationship Investigations

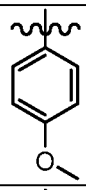
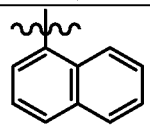
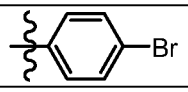
A series of thirty analogs were synthesized based on the structure of JR-AB2-000 to explore the structure-activity relationship (SAR) of the inhibitor. Modifications were made to all functional groups of the molecule as shown in Fig. 4, and these analogs' effects on mTORC2 signaling and anti-GBM responses were evaluated. These data are summarized in Table 2.

In table 2, the fold decrease mTORC2 activity was determined by densitometric quantification of immunoblots probed for phospho-S473-AKT abundance in LN229 cells treated with 1 μM analog for 24 h as compared to values obtained with parent compound (JR-AB2-000) under identical conditions. The "Structure" column in Table 2 depicts the structural modifications to JR-AB2-000 that were made, with reference to Scheme A. No significant alterations in p-T389-S6K levels were observed for any of the analogs (1 μM, 24 h) as compared to untreated controls (not shown). For each analog the IC50 was determined via XTT proliferation assays as in Figs. 3C and 3D. Data represent mean ±S.D. of three independent experiments. Percent apoptosis was determined for LN229 cells treated with analog (1μM) at 48 h via annexin V-FITC staining. One of three experiments with similar results is shown.

Table 2: Summary of anti-GBM effects of JR-AB2-000 Analogs

Compound	Structure	Chemical Formula	mTORC2 activity (fold decrease)	IC50 (μM)	% Apoptosis (1 μM)
JR-AB2-000	Scheme A	$C_{19}H_{19}C_{12}N_3OS$	1.00	1.76±0.51	18.2
JR-AB2-001		$C_{18}H_{17}Cl_2N_3OS$	0.95	1.30±0.11	19.4
JR-AB2-002		$C_{20}H_{21}Cl_2N_3OS$	1.10	1.62±0.37	12.6
JR-AB2-003	Scheme A	$C_{20}H_{24}N_4O_2S$	0.94	2.46±0.49	15.7
JR-AB2-004	Scheme A	$C_{19}H_{19}Cl_2N_3S_2$	1.05	1.51±0.82	11.0
JR-AB2-005		$C_{18}H_{17}Cl_2N_3O_2S$	1.19	5.77±0.63	13.8
JR-AB2-006		$C_{19}H_{19}Cl_2N_3O_2$	1.05	1.05±0.36	11.5
JR-AB2-007		$C_{17}H_{15}Cl_2N_3OS$	1.10	1.48±0.95	10.2

JR-AB2-008		$C_{19}H_{20}Cl_2N_4OS$	1.36	1.86±0.21	12.7
JR-AB2-009		$C_{18}H_{17}Cl_2N_3OS$	1.05	1.93±0.54	13.9
JR-AB2-010		$C_{18}H_{17}Cl_2N_3OS$	1.17	1.37±0.68	17.4
JR-AB2-011		$C_{17}H_{14}Cl_2FN_3OS$	3.94	0.31±0.17	36.3
JR-AB2-012		$C_{13}H_{15}Cl_2N_3OS$	1.18	0.96±0.14	16.4
JR-AB2-013		$C_{18}H_{15}Cl_2N_3OS$	1.12	2.15±0.10	20.2
JR-AB2-014		$C_{22}H_{17}Cl_2N_3OS$	1.24	1.39±0.37	19.7
JR-AB2-015		$C_{20}H_{21}Cl_2N_3OS$	1.03	1.19±0.63	10.6
JR-AB2-016		$C_{20}H_{17}Cl_2N_3O$	1.29	1.50±0.24	11.5
JR-AB2-017		$C_{21}H_{18}Cl_2N_2O$	1.05	1.72±0.93	16.1
JR-AB2-018		$C_{19}H_{21}N_3OS$	1.67	1.14±0.59	12.2
JR-AB2-019		$C_{19}H_{20}ClN_3OS$	1.93	1.28±0.83	15.7
JR-AB2-020		$C_{19}H_{20}ClN_3OS$	1.58	2.11±0.38	17.8
JR-AB2-021		$C_{19}H_{20}FN_3OS$	1.59	1.83±0.15	10.2
JR-AB2-022		$C_{20}H_{23}N_3OS$	1.13	1.42±0.32	15.9

JR-AB2-023		$C_{20}N_{23}N_3O_2S$	1.24	0.98±0.24	16.3
JR-AB2-024		$C_{23}H_{23}N_3OS$	1.65	1.26±0.27	26.0
JR-AB2-025		$C_{15}H_{21}N_3OS$	1.37	1.39±0.48	17.5
JR-AB2-026		$C_{17}H_{25}N_3OS$	1.18	2.82±0.75	11.3
JR-AB2-027	Scheme A	$C_{20}H_{20}Cl_2N_2OS$	1.41	1.86±0.39	12.2
JR-AB2-028	Scheme A	$C_{19}H_{18}Cl_2N_2O_2S$	1.30	2.10±0.29	14.6
JR-AB2-029	Scheme A	$C_{20}H_{21}Cl_2N_3OS$	1.72	1.14±0.38	11.7
JR-AB2-030		$C_{17}H_{14}BrCl_2OS$	2.69	0.57±0.20	20.5

Analogs with modifications to the left-side of the molecule (R^1 in Formula (I)), showed no significant improvements in mTORC2 signaling blockade, as determined by the degree of p-S^{473}-AKT inhibition relative to values obtained with the parent inhibitor were observed. Additionally, no alterations in IC_{50} or apoptosis values were observed with these analogs, suggesting that this region of the molecule was not critical for inhibition of mTORC2. Within the analogs containing modifications to the right-side of the molecule (R^2 in Formula (I)), JR-AB2-005 displayed a significant increase in IC_{50}, however p-S-^{473}-AKT levels and the degree of apoptosis in cells following exposure were unaffected. JR-AB2-011 markedly reduced mTORC2 signaling (see also Fig. 9) and IC_{50} while enhancing apoptotic levels in GBM cells compared to the parent compound. Similarly, JR-AB2-030 also blocked mTORC2 signaling and lowered the IC_{50} value while increasing apoptosis levels, albeit to a lesser degree than analog JR-AB2-011. Additional analogs were synthesized shown in Scheme A (modifications to R^3 in Formula (I) and JR-AB2-027 thru -029). None of these significantly altered mTORC2 signal blockade or anti-GBM properties as compared to the parent inhibitor.

The *in vitro* cytotoxicities of analogs JR-AB2-011 and JR-AB2-030 relative to JR-AB2-000 in human neurons were also determined. JR-AB2-011 displayed the least toxicity to normal neurons with no significant cytotoxic effects for concentrations up to 10 mM and was chosen for further study.

Example 7: JR-AB2-000 and JR-AB2-011 Inhibition of Rictor-mTOR Association

To begin to investigate the mechanism of action of JR-AB2-000 and its analog JR-AB2-011 surface plasmon resonance (SPR) analyses were performed of JR-AB2-000 binding to either immobilized Rictor, mTOR or mSIN1. As shown in Fig. 4 (left-panel), JR-AB2-000 selectively bound to Rictor and reached equilibrium rapidly. The K_D was determined from steady-state binding associations and was calculated at 1 μM. Contrastingly, JR-AB2-000 was unable to bind mTOR or mSIN1, another regulatory subunit of mTORC2. mTOR binding to immobilized Rictor was not observed, however in the reverse configuration immobilized mTOR bound Rictor with a calculated Kd of 257 nM (Fig. 4, right panel). In competitive SPR assays in which immobilized mTOR was allowed to bind analyte containing preincubated Rictor bound to either JR-AB2-000 or JR-AB2-011, JR-AB2-011 showed increased potency and a 6-7 fold improved binding affinity to Rictor and (JR-AB2-011; IC50 = 0.36 μmol/L; Ki = 0.19 μmol/L) in comparison with the parent compound (JR-AB2-000; IC50 = 1.64 μmol/L; Ki = 1.46 μmol/L) (Fig 5A, left panel). To further confirm specificity, each of the inhibitors was preincubated with either Raptor (mTORC1 subunit), mLST8 or Deptor (both mTORC1 & 2 subunits) and these reaction mixtures were passed over immobilized mTOR. Neither JR-AB2-011 or JR-AB2-000 significantly affected the binding of Raptor, mLST8 or Deptor to immobilized mTOR (Fig 5A, middle & right panels). Additionally, the ability of JR-AB2-011 or JR-AB2-000 to inhibit mTOR-Rictor association was determined by a pull-down assay utilizing Flag-mTOR and myc-Rictor. As shown in Fig. 5B, both inhibitors blocked the ability of bead bound Flag-mTOR to bind myc-Rictor. JR-AB2-011 appeared to more effectively block mTOR-Rictor association at the highest concentration of inhibitor relative to the parent compound in this assay. Taken together, these results support the notion that the analog JR-AB2-011 and its parent bind Rictor and specifically block mTOR association. Additionally, these data suggest that JR-AB2-011 more effectively inhibits mTOR binding relative to the parent compound.

Example 8: JR-AB2-011 Therapy in GBM Xenografts

To determine the *in vivo* affects of JR-AB2-011 xenograft studies were conducted utilizing LN229 cells in mice. Mice were subcutaneously implanted with tumor cells and when tumors were palpable (~200 mm^3), mice were randomized into treatment groups receiving vehicle, JR-AB2-011 (4 mg/kg/d) and JR-AB2-011 (20 mg/kg/d). As shown in Fig. 6A, mice receiving JR-AB2-011 at either dosing regimen displayed marked inhibition of

tumor growth rate (JR-AB2-011 at 4 mg/kg/d; 74% inhibition at end of dosing period; tumor growth delay 10.0 days; JR-AB2-011 at 20 mg/kg/d; 80% inhibition at end of dosing period; tumor growth delay 12.0 days) as compared to mice receiving vehicle alone. Consistent with the effects on xenograft growth, overall survival of mice at either JR-AB2-011 dosing regimens were significantly extended relative to vehicle treated mice. Notably, mice tolerated either of these dosing regimens without obvious short or longterm toxicity or weight loss. Blood cell counts were not affected by JR-AB2-011 (Fig. 10). The induction of apoptotic cell death was monitored by TUNEL staining of sections from harvested tumors and JR-AB2-011 at both dosing regimens markedly enhanced apoptosis, supporting the increases in apoptotic death observed *in vitro* with the parent compound (Fig 6C, (left panel), see also Fig. 2E). The ratio of phospho-S^{473}-AKT to total AKT was significantly reduced in tumors at both doses of JR-AB2-011, while the ratio of phospho-T^{389}-S6K to total S6K was not significantly altered (Fig. 6C, center and right panels).

Example 9: General Methods

Antibodies: Antibodies were from the following sources. mTOR (#2972, Cell Signaling Technologies, CST), phospho-S^{312}-IRS1 (#ab66154, Abcam), total IRS1 (#ab131487, Abcam), phospho-$T^{202/Y204}$-ERK (#4370, CST), total ERK (#9102, CST), phospho-S^{657}-PKCα (#SAB-4504096, Sigma), total PKCα (#sc-208, Santa Cruz Biotechnology), phospho-T^{389}-S6K (#9205, CST), total S6K (#9202, CST), phospho-T^{346}-NDRG1 (#3217, CST), total NDRG1 (#ab124689, Abcam), phospho-S^{473}-AKT (#9271, CST), total AKT (#9272, CST), Rictor (#A300-459A, Bethyl Laboratories), actin (#ab3280, Abcam), α-GST (#2622, CST), α-myc (#2276, CST), α-Flag (#F3165, Sigma)

Yeast methods: Standard techniques were used for yeast manipulations (1). Yeast reagents used are derivatives of the MATCHMAKER Gal4 Two-Hybrid System 3 (Clontech). The drug sensitive screening strain AR109D (*MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ, avo3::KanMX, tor2::KanMX pRS316::Ypk2^{D239A}-HA, pdr1:CUP1pro-HXT9, pdr3:CUP1pro-HXT11)* was derived from AR109 (2). Expression of *Ypk2^{D239A}* (constitutively active Ypk2 (D239A) allele) was well tolerated in this strain and conferred viability in the absence of *Avo3* and *Tor2* (3).

Chemistry:

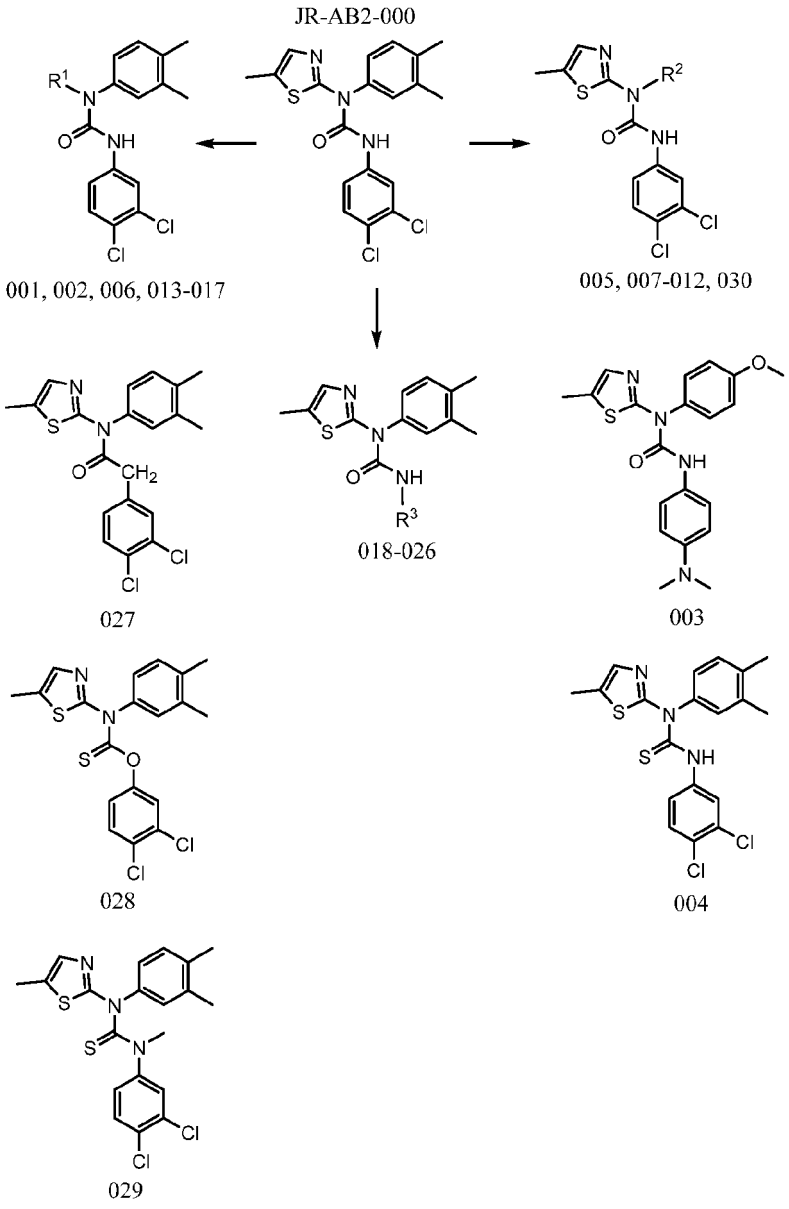
All reactions were carried out under open-air conditions unless otherwise specified. Dichloromethane (DCM) and triethylamine (TEA) were distilled from calcium hydride under

an argon atmosphere. Chemicals were purchased from Sigma-Aldrich, Alfa aesar and TCI in ≥95% purity, all other solvents or reagents were purified according to literature procedures if necessary. ^{1}H-NMR spectra were recorded on Bruker spectrometers at 400 or 500 MHz and are reported relative to deuterated solvent signals ($CHCl_3$ δ 7.26; DMSO δ 2.48 ppm). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Splitting patterns are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doubles; dt, doublet of triplets; t, triplet; td, triplet of doublets; q, quartet; sext, sextet; m, multiplet; and br, broad. ^{13}C NMR spectra were recorded on Bruker Spectrometers at 125 MHz and are reported relative to deuterated solvent signals ($CHCl_3$ δ 77.0; DMSO δ 40.0 ppm). Data for ^{13}C spectra are reported in terms of chemical shift. The chemical shifts are reported in parts per million (ppm, δ). Melting points were obtained using Buchi B-545 melting point apparatus and are uncorrected. The reactions were monitored with a silica gel TLC plate under UV light (254 and 365 nm) followed by visualization with a ninhydrin or phosphomolybdic acid staining solution. Column chromatography was performed on silica gel 60, 230-400 mesh. DART-HRMS spectra were collected on a Thermo Exactive Plus MSD (Thermo Scientific) equipped with an ID-CUBE ion source and a Vapur Interface (IonSense). Both the source and MSD were controlled by Excalibur, version 3.0. The purity of the compounds was assayed by high field proton and carbon NMR and was ≥95%.

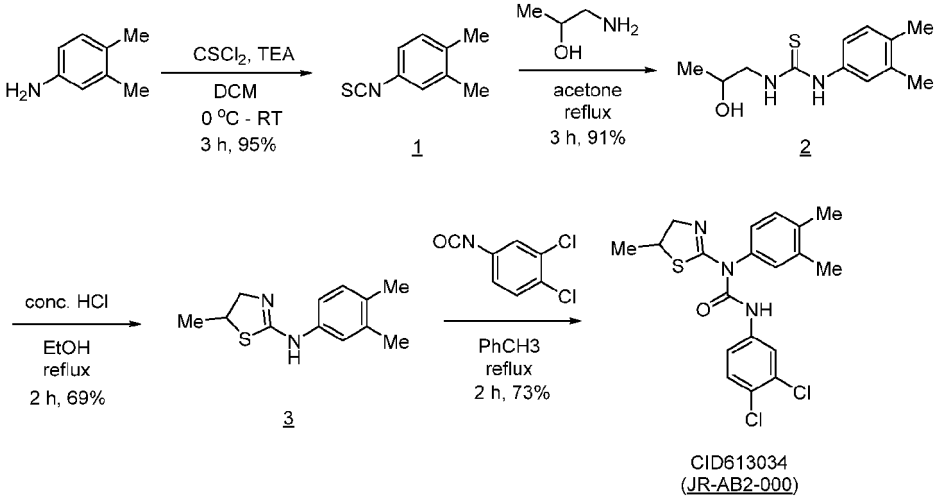
Example 10: Synthetic procedures and Characterization data.

Thirty analogs of JR-AB2-000 (CID613034) were synthesized with the indicated group modification shown and further detailed in Scheme A. Analog identification numbers are shown with the structures corresponding to analogs where a particular functional group is modified.

Scheme A


JR-AB2-000
001, 002, 006, 013-017
005, 007-012, 030
018-026
027
003
028
004
029

Scheme 1. Synthesis of Compound CID613034 (JR-AB2-000)



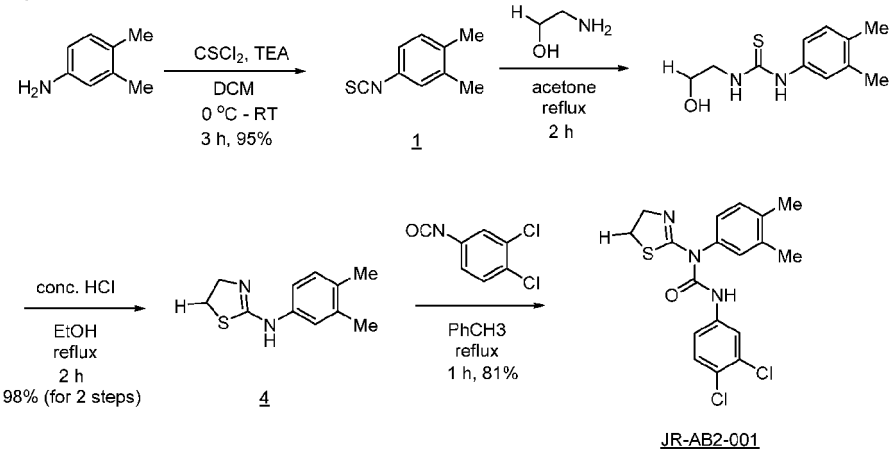
3,4-Dimethylphenylisothiocyanate, 1. To a dichloromethane (100 mL) solution of 3,4-dimethylaniline (1212 mg, 10.0 mmol, 1.0 eq) was added thiophosgene (1.0 mL, 13.0 mmol, 1.3 eq) and triethylamine (3.49 mL, 25.0 mmol, 2.5 eq) at 0 °C. The mixture was warmed to room temperature and stirred for 3 h. After the reaction was completed, the mixture was diluted with dichloromethane (200 mL) and washed with water (100 mL X 2). The combined organic layer was dehydrated with brine and $MgSO_4$, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 50:1, v/v) to afford the desired product **1** (1.548 g, 95%) as pale yellow oil: Rf = 0.6 (hexane/ethyl acetate, 50:1, v/v); ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.21 (s, 1H), 7.18 (d, J = 8.0 Hz, 1H), 7.13 (dd, J = 8.0, 2.0 Hz, 1H), 2.20 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (DMSO-d6, 125 MHz) δ 138.8, 137.3, 133.0, 131.1, 127.7, 127.1, 123.5, 19.6, 19.5.

1-(3,4-Dimethylphenyl)-3-(2-hydroxypropyl)thiourea, 2. (4) To an acetone (20 mL) solution of 3,4-dimethylphenylisothiocyanate (**1**, 460 mg, 2.87 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.29 mL, 3.73 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 3 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product **2** (810 mg, 91%) as pale yellow oil: Rf = 0.25 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.68 (br, 1H), 7.16 (d, J = 7.5 Hz, 1H), 6.97 (s, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.44 (br, 1H), 4.04-4.01 (m, 1H), 3.91-3.88 (m, 1H), 3.44 (ddd, J = 13.5, 5.0, 8.0 Hz, 1H), 2.25 (s, 6H), 1.19 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.3, 138.9, 136.4, 133.4. 131.2, 126.5, 122.7, 67.3, 52.1, 21.2, 19.9, 19.4.

***N*-(3,4-Dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine, 3.** (4) To an ethanol (10 mL) solution of 1-(3,4-dimethylphenyl)-3-(2-hydroxypropyl)thiourea (**2**, 810 mg, 3.4 mmol, 1.0 eq) was added conc. HCl (10 mL) at room temperature, and the mixture was refluxed for 2 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product **3** (516 mg, 68%) as pale brown solid: Rf = 0.2 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.03 (d, *J* = 8.0 Hz, 1H), 6.90 (s, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 3.89-3.84 (m, 2H), 3.45-3.44 (m, 1H), 2.22 (s, 3H), 2.20 (s, 3H), 1.42 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.5, 144.6, 137.2, 131.7, 130.0, 122.5, 118.5, 57.8, 43.7, 20.4, 19.9, 19.2.

3-(3,4-Dichlorophenyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-000. A toluene (10 mL) solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 510 mg, 2.3 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (433 mg, 2.3 mmol, 1.0 eq) was refluxed for 2 h under argon gas atmosphere. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-000** (690 mg, 73%) as white powder: Rf = 0.2 (hexane/ethyl acetate, 5:1, v/v); mp 158-159 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.2 (s, 1H), 7.73 (s, 1H), 7.33 (d, *J* = 1.0 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.74 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.44 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.95 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.70 (sext, *J* = 6.5 Hz, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 1.45 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.3, 150.9, 146.6, 137.9, 137.7, 133.5, 132.7, 130.4, 130.3, 126.6, 122.7, 121.4, 119.1, 118.5, 55.5, 36.9, 20.0, 19.9, 19.3; DART-HRMS found 408.0689 $[M+H]^+$, calcd for $C_{19}H_{20}Cl_2N_3OS$ 408.0704.

Scheme 2. Synthesis of JR-AB2-001



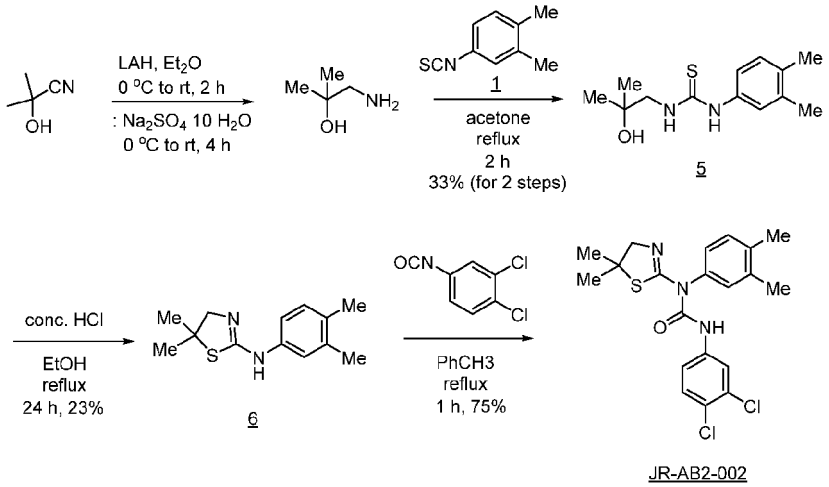
JR-AB2-001

***N*-(3,4-Dimethylphenyl)-4,5-dihydrothiazol-2-amine, 4.** (4) To an acetone (20 mL) solution of 3,4-dimethylphenylisothiocyanate (**1**, 326 mg, 2.0 mmol, 1.0 eq) was added aminoethanol (0.19 mL, 2.6 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 2 h, and then concentrated in vacuo. The crude mixture was dissolved in ethanol (10 mL) and conc. HCl (10 mL) at room temperature, and the mixture was refluxed for 2 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product **4** (404 mg, 98%) as pale yellow solid: Rf = 0.15 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.03 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 6.91 (d, J = 7.5 Hz, 1H), 3.85 (t, J = 7.0 Hz, 2H), 3.30 (t, J = 7.0 Hz, 2H), 2.23 (s, 3H), 2.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.5, 144.2, 137.2, 131.5, 130.1, 122.2, 118.1, 32.4, 29.7, 19.9, 19.2.

3-(3,4-Dichlorophenyl)-1-(4,5-dihydrothiazol-2-yl)-1-(3,4-dimethylphenyl)urea, JR-AB2-001. A toluene (10 mL) solution of *N*-(3,4-dimethylphenyl)-4,5-dihydrothiazol-2-amine (**4**, 206 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 1 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-001** (280 mg, 71%) as white powder: Rf = 0.5 (hexane/ethyl acetate, 3:1, v/v); mp 166-168 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.2 (s, 1H), 7.73 (t, J = 1.0 Hz, 1H), 7.32 (d, J = 1.0 Hz, 2H), 7.13 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 6.75 (dd, J = 8.0, 2.0 Hz, 1H), 4.36 (t, J = 7.0 Hz, 2H), 3.18 (t, J = 7.0 Hz, 2H), 2.28 (s, 3H), 2.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.3, 150.8, 146.6, 137.8, 137.7, 133.5, 132.7, 130.39, 130.38, 126.6,

122.6, 121.4, 119.1, 118.4, 48.8, 25.7, 19.9, 19.3; DART-HRMS found 394.0560 $[M+H]^+$, calcd for $C_{18}H_{18}Cl_2N_3OS$ 394.0548.

Scheme 3. Synthesis of JR-AB2-002



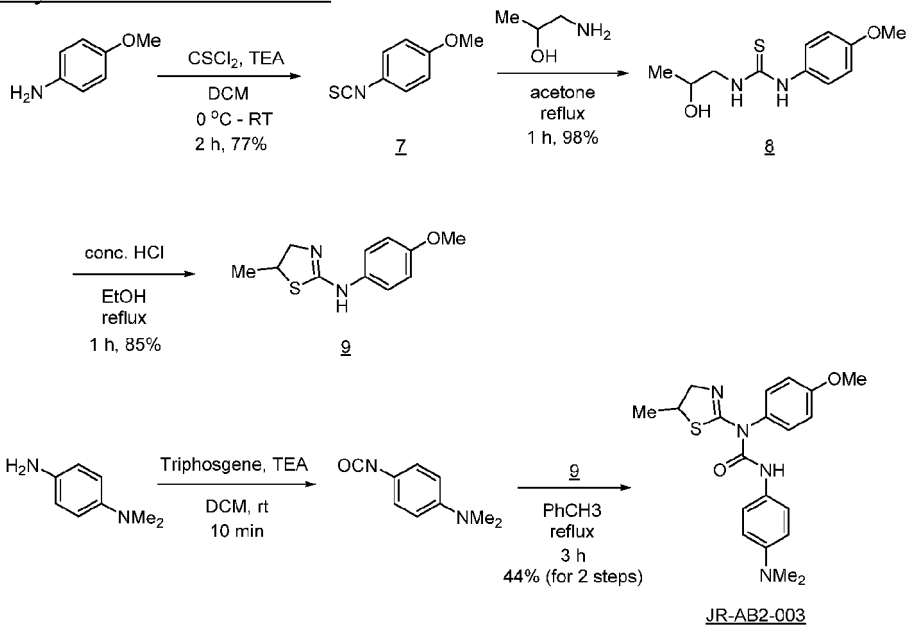
1-(3,4-Dimethylphenyl)-3-(2-hydroxy-2-methylpropyl)thiourea, 5. (4,5) To a diethyl ether (30 mL) suspension of lithium aluminum hydride (380 mg, 10.0 mmol, 2.0 eq) was added acetone cyanohydrin (0.46 mL, 5.0 mmol, 1.0 eq) in diethyl ether (20 mL) dropwise at 0 °C. The mixture was stirred for 2 h at room temperature. To the reaction mixture was added excess amount of sodium sulfate hydrate at 0 °C with vigorous stirring, and the mixture was stirred for 4 h at room temperature. The resulting mixture was filtered through celite and the residue was washed with diethyl ether. The ether washings were all combined and dehydrated with MgSO4 and then concentrated in vacuo. The crude mixture was dissolved in acetone (20 mL) and added 3,4-dimethylphenyl isothiocyanate (1, 571 mg, 3.5 mmol, 0.7 eq), then the mixture was refluxed for 2 h and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1 → 3:1, v/v) to afford the desired product 5 (287 mg, 33% based on 1) as pale yellow solid: Rf = 0.15 (hexane/ethyl acetate, 3:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 (br, 1H), 7.16 (d, J = 5.5 Hz, 1H), 6.98 (s, 1H), 6.97 (d, J = 6.0 Hz, 1H), 6.45 (s, 1H), 3.68 (s, 2H), 2.25 (s, 6H), 2.00 (br, 1H), 1.23 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.4, 138.9, 136.4, 133.4, 131.2, 126.5, 122.7, 71.4, 55.5, 27.6, 19.9, 19.4.

***N*-(3,4-Dimethylphenyl)-5,5-dimethyl-4,5-dihydrothiazol-2-amine, 6.** (4) To an acetone (10 mL) solution of 1-(3,4-dimethylphenyl)-3-(2-hydroxy-2-methylpropyl)thiourea (5, 252 mg, 1.0 mmol, 1.0 eq) was added conc. HCl (5 mL) and the mixture was refluxed for 24 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was

dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1 → 2:1, v/v) to afford the desired product **6** (53 mg, 23%) as white powder: Rf = 0.15 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.03 (d, J = 8.0 Hz, 1H), 6.88 (s, 1H), 6.84 (d, J = 8.0 Hz, 1H), 3.51 (s, 2H), 2.22 (s, 3H), 2.20 (s, 3H), 1.54 (s, 6H).

3-(3,4-Dichlorophenyl)-1-(5,5-dimethyl-4,5-dihydrothiazol-2-yl)-1-(3,4-dimethylphenyl)urea, JR-AB2-002. A toluene (2 mL) solution of *N*-(3,4-dimethylphenyl)-5,5-dimethyl-4,5-dihydrothiazol-2-amine (**6**, 43 mg, 0.18 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (35 mg, 0018 mmol, 1.0 eq) was refluxed for 1 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-002** (57 mg, 75%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 3:1, v/v); mp 161-162 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.3 (s, 1H), 7.74 (t, J = 1.5 Hz, 1H), 7.33 (d, J = 1.5 Hz, 2H), 7.12 (d, J = 8.0 Hz, 1H), 6.78 (s, 1H), 6.73 (dd, J = 8.0, 2.0 Hz, 1H), 4.09 (s, 2H), 2.28 (s, 3H), 2.26 (s, 3H), 1.54 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.4, 151.1, 146.4, 137.9, 137.7, 133.5, 132.7, 130.4, 130.3, 126.5, 122.7, 121.4, 119.1, 118.5, 61.1, 47.5, 28.3, 20.0, 19.3; DART-HRMS found 422.0837 $[M+H]^+$, calcd for $C_{20}H_{22}Cl_2N_3OS$ 422.0861.

Scheme 4. Synthesis of JR-AB2-003



4-Methoxyphenyl isothiocyanate, 7. To a dichloromethane (100 mL) solution of p-anisidine (1232 mg, 10.0 mmol, 1.0 eq) was added thiophosgene (1.0 mL, 13.0 mmol, 1.3 eq)

and triethylamine (3.49 mL, 25.0 mmol, 2.5 eq) at 0 °C. The mixture was warmed to room temperature and stirred for 2 h. After the reaction was completed, the mixture was diluted with dichloromethane (200 mL) and washed with water (100 mL X 2). The combined organic layer was dehydrated with brine and $MgSO_4$, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **7** (1.274 g, 77%) as yellow oil: Rf = 0.8 (hexane/ethyl acetate, 5:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.17 (ddd, *J* = 9.0, 3.0, 2.5 Hz, 2H), 6.85 (ddd, *J* = 9.5, 3.0, 2.5 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.7, 134.0, 127.1, 123.7, 114.9, 55.7.

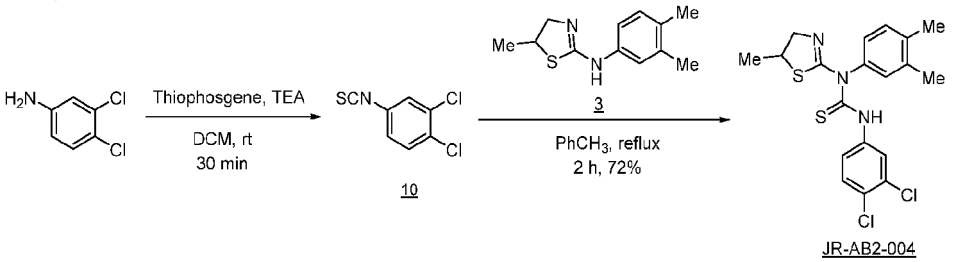
1-(2-Hydroxypropyl)-3-(4-methoxyphenyl)thiourea, 8. (4) To an acetone (20 mL) solution of 4-methoxyphenyl isothiocyanate (**7**, 374 mg, 2.26 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.23 mL, 2.94 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 1 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1 → 1:1, v/v) to afford the desired product **8** (532 mg, 98%) as pale brown caramel: Rf = 0.1 (hexane/ethyl acetate, 3:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.83 (br, 1H), 7.15 (d, *J* = 8.0 Hz, 2H), 6.92 (d, *J* = 8.5 Hz, 2H), 6.32 (br, 1H), 4.02-3.99 (m, 1H), 3.87-3.85 (m, 1H), 3.42 (ddd, *J* = 14.0, 5.0, 3.0 Hz, 1H), 2.31 (br, 1H), 1.18 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.7, 159.1, 128.5, 127.7, 115.4, 67.3, 55.7, 52.1, 21.2.

***N*-(4-Methoxyphenyl)-5-methyl-4,5-dihydrothiazol-2-amine, 9.** (4) To an ethanol (10 mL) solution of 1-(2-hydroxypropyl)-3-(4-methoxyphenyl)thiourea (**8**, 580 mg, 2.41 mmol, 1.0 eq) was added conc. HCl (10 mL) at room temperature, and the mixture was refluxed for 1 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product **9** (457 mg, 85%) as pale yellow solid: Rf = 0.15 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.02 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 3.88-3.80 (m, 2H), 3.78 (s, 3H), 3.39 (dd, *J* = 10.0, 5.5 Hz, 1H), 1.42 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.6, 155.9, 141.5, 122.6, 114.3, 57.2, 55.6, 43.6, 20.5.

3-(4-(Dimethylamino)phenyl)-1-(4-methoxyphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-003. (6) To a dichloromethane solution (3 mL) of triphosgene (149 mg, 0.5 mmol, 1.0 eq) was added 4-*N*,*N*-dimethylamino-aniline (68 mg, 0.5

mmol, 1.0 eq) in dichloromethane (2 mL) and triethylamine (0.15 mL. 1.1 mmol, 2.2 eq) dropwise. After 30 min stirring at room temperature, dichloromethane was removed in vacuo. To the toluene (3 mL) solution of the crude mixture was added *N*-(4-methoxyphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**9**, 111 mg, 0.5 mmol, 1.0 eq) in toluene (2 mL) and the mixture was refluxed for 3 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was diluted with ethyl acetate (50 mL) and washed with aq. $NaHCO_3$ solution (20 mL). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 3:1, v/v) to afford the desired product **JR-AB2-003** (84 mg, 44% for 2 steps) as light yellow solid: Rf = 0.3 (hexane/ethyl acetate, 3:1, v/v); mp 143-145 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.7 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 2H), 6.94 (d, *J* = 7.5 Hz, 2H), 6.89 (d, *J* = 7.5 Hz, 2H), 6.71 (d, *J* = 8.0 Hz, 2H), 4.45 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.96 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.81 (s, 3H), 3.68 (sext, *J* = 6.5 Hz, 1H), 2.91 (s, 6H), 1.44 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.0, 156.8, 151.3, 147.7, 142.7, 128.1, 122.5, 121.8, 114.3, 113.3, 55.7, 55.5, 41.1, 36.9, 20.0; DART-HRMS found 385.1697 $[M+H]^+$, calcd for $C_{20}H_{25}N_4O_2S$ 385.1698.

Scheme 5. Synthesis of JR-AB2-004

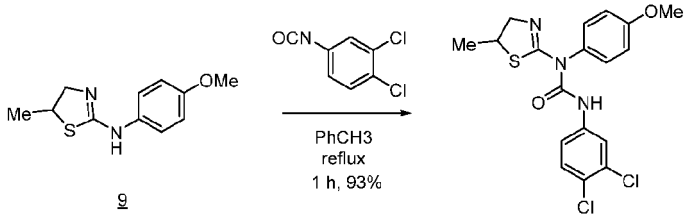


3,4-Dichlorophenyl isothiocyanate, 10. To a dichloromethane (20 mL) solution of 3,4-dichloroaniline (324 mg, 2.0 mmol, 1.0 eq) was added thiophosgene (0.2 mL, 2.6 mmol, 1.3 eq) and triethylamine (0.7 mL, 5.0 mmol, 2.5 eq) at 0 °C. The mixture was warmed to room temperature and stirred for 30 min. After the reaction was completed, the mixture was diluted with ethyl acetate (100 mL) and washed with water (50 mL X 2). The combined organic layer was dehydrated with brine and $MgSO_4$, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 50:1, v/v) to afford the desired product **10** (323 mg, 79%) as pale yellow oil: Rf = 0.85 (hexane/ethyl acetate, 5:1, v/v).

3-(3,4-Dichlorophenyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)thiourea, JR-AB2-004. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-

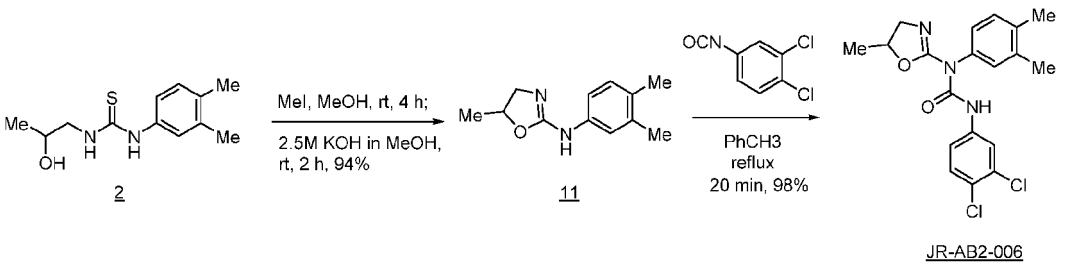
dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and phenyl 3,4-dichlorophenyl isothiocyanate (**10**, 204 mg, 1.0 mmol, 1.0 eq) was refluxed for 2 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 20:1, v/v) to afford the desired product **JR-AB2-004** (72 mg, 72%) as white powder: Rf = 0.4 (hexane/ethyl acetate, 10:1, v/v); mp 124-126 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 14.3 (s, 1H), 7.79 (d, *J* = 2.0 Hz, 1H), 7.45 (dd, *J* = 9.0, 3.0 Hz, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 7.13 (d, *J* = 9.0 Hz, 1H), 6.78 (s, 1H), 6.73 (dd, *J* = 7.5, 2.0 Hz, 1H), 5.01 (dd, *J* = 12.0, 6.5 Hz, 1H), 4.39 (dd, *J* = 12.0, 6.5 Hz, 1H), 3.69 (sext, *J* = 6.5 Hz, 1H), 2.27 (s, 3H), 2.26 (s, 3H), 1.46 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.0, 159.1, 145.7, 138.3, 137.8, 134.0, 132.4, 130.4, 130.2, 129.6, 126.3, 124.1, 122.5, 118.3, 61.5, 35.8, 19.9, 19.7, 19.3; DART-HRMS found 424.0258 $[M+H]^+$, calcd for $C_{19}H_{20}Cl_2N_3S_2$ 424.0476.

Scheme 6. Synthesis of JR-AB2-005



3-(3,4-Dichlorophenyl)-1-(4-methoxyphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-005. A toluene (5 mL) solution of *N*-(4-methoxyphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**9**, 67 mg, 0.3 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (56.4 mg, 0.3 mmol, 1.0 eq) was refluxed for 1 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-005** (114 mg, 93%) as white powder: Rf = 0.5 (hexane/ethyl acetate, 2:1, v/v); mp 129-131 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.3 (s, 1H), 7.75 (s, 1H), 7.33 (s, 2H), 6.95 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.0 Hz, 2H), 4.44 (dd, *J* = 11.0, 7.0 Hz, 1H), 3.95 (dd, *J* = 11.0, 7.0 Hz, 1H), 3.82 (s, 3H), 3.71 (sext, *J* = 6.0 Hz, 1H), 1.46 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.5, 157.1, 150.9, 142.0, 137.9, 132.7, 130.4, 126.6, 122.5, 121.4, 119.2, 114.5, 55.54, 55.53, 36.9, 20.0; DART-HRMS found 410.0464 $[M+H]^+$, calcd for $C_{18}H_{18}Cl_2N_3O_2S$ 410.0497.

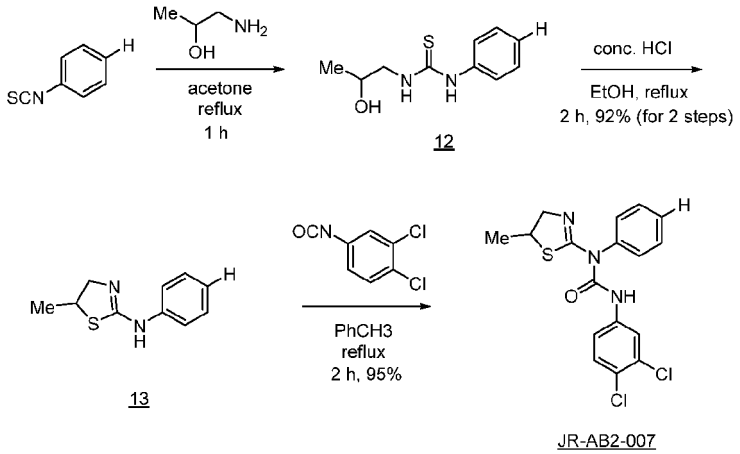
Scheme 7. Synthesis of JR-AB2-006



***N*-(3,4-Dimethylphenyl)-5-methyl-4,5-dihydrooxazol-2-amine, 11.** (4) To a methanol (10 mL) solution of 1-(3,4-dimethylphenyl)-3-(2-hydroxypropyl)thiourea (**2**, 238 mg, 1.0 mmol, 1.0 eq) was iodomethane (0.31 mL, 5.0 mmol, 5.0 eq), and the mixture was stirred for 4 h. After the completion of reaction, the mixture was concentrated in vacuo to remove the excess reagent and solvent. To the crude mixture was added 2.5M KOH in methanol (10 mL, 25.0 mmol, 25.0 eq) and the mixture was stirred for 2 h at room temperature. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and washed with water (30 mL X2). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The desired product **11** (192 mg, 94%) was adducted as white solid without further purification: Rf = 0.4 (dichloromethane/methanol, 10:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.06 (s, 1H), 7.02 (s, 2H), 4.73 (sext, *J* = 6.0 Hzm 1H), 4.40 (br, 1H), 3.91 (dd, *J* = 11.0, 8.0 Hz, 1H), 3.39 (dd, *J* = 11.0, 7.5 Hz, 1H), 2.22 (s, 3H), 2.19 (s, 3H), 1.41 (d, *J* = 6.0 Hz, 3H).

3-(3,4-Dichlorophenyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrooxazol-2-yl)urea, JR-AB2-006. A toluene (10 mL) solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrooxazol-2-amine (**11**, 102 mg, 0.5 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (94 mg, 0.5 mmol, 1.0 eq) was refluxed for 20 min. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-006** (192 mg, 98%) as white powder: Rf = 0.4 (hexane/ethyl acetate, 3:1, v/v); mp 183-184 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.0 (s, 1H), 7.77 (s, 1H), 7.35 (s, 2H), 7.09 (d, *J* = 8.0 Hz, 1H), 6.93 (s, 1H), 6.90 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.79 (sext, *J* = 6.5 Hz, 1H), 4.25 (dd, *J* = 10.5, 8.0 Hz, 1H), 3.67 (dd, *J* = 10.5, 7.5 Hz, 1H), 2.27 (s, 3H), 2.24 (s, 3H), 1.51 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 150.0, 149.4, 141.6, 137.8, 137.1, 132.7, 132.5, 130.5, 130.0, 126.5, 124.5, 121.1, 120.4, 118.8, 73.5, 49.4, 20.1, 19.9, 19.2; DART-HRMS found 392.0919 $[M+H]^+$, calcd for $C_{19}H_{20}Cl_2N_3O_2$ 392.0933.

Scheme 8. Synthesis of JR-AB2-007



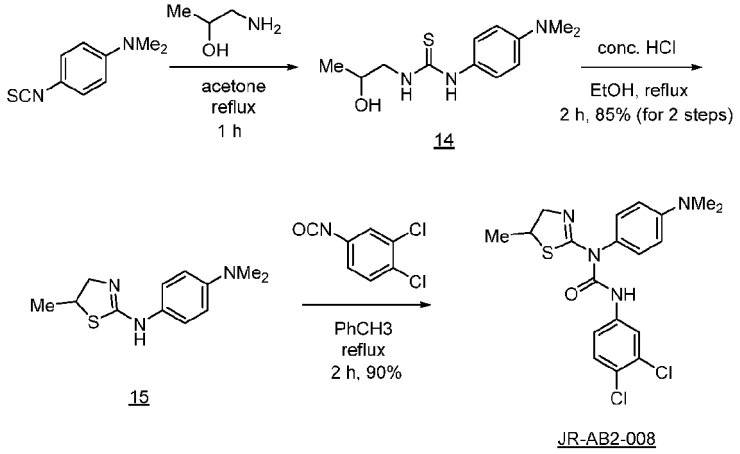
1-(2-Hydroxypropyl)-3-phenylthiourea, 12. (4) To an acetone (20 mL) solution of phenyl isothiocyanate (0.597 mL, 5.0 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.502 mL, 6.5 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 1 h, and then concentrated in vacuo. The crude mixture was directly used for the synthesis of compound 13: Rf = 0.2 (hexane/ethyl acetate, 1:1, v/v).

5-Methyl-*N*-phenyl-4,5-dihydrothiazol-2-amine, 13. (4) To an ethanol (10 mL) solution of 1-(2-hydroxypropyl)-3-phenylthiourea (**12**, 5.0 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 2 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1 → 0:1, v/v) to afford the desired product **13** (881 mg, 92% for 2 steps) as white solid: Rf = 0.15 (hexane/ethyl acetate, 1:1, v/v).

3-(3,4-Dichlorophenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)-1-phenylurea, JR-AB2-007. A toluene (20 mL) solution of 5-methyl-*N*-phenyl-4,5-dihydrothiazol-2-amine (**13**, 385 mg, 2.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (376 mg, 2.0 mmol, 1.0 eq) was refluxed for 2 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:0 → 10:1, v/v) to afford the desired product **JR-AB2-007** (720 mg, 95%) as white powder: Rf = 0.8 (hexane/ethyl acetate, 1:1, v/v); mp 126-128 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.2 (s, 1H), 7.74 (s, 1H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.33 (s, 2H), 7.19 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 7.5 Hz, 2H), 4.45 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.97 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.72 (sext, *J* = 6.5 Hz, 1H), 1.46 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.8,

150.8, 148.8, 137.8, 132.7, 130.4, 129.3, 126.7, 125.1, 121.50, 121.48, 119.2, 55.6, 37.0, 20.0; DART-HRMS found 380.0361 $[M+H]^+$, calcd for $C_{17}H_{16}Cl_2N_3OS$ 380.0391.

Scheme 9. Synthesis of Compound JR-AB2-008



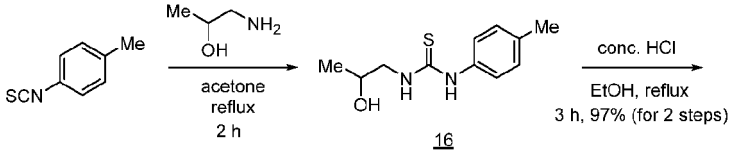
1-(4-(Dimethylamino)phenyl)-3-(2-hydroxypropyl)thiourea, 14. (4) To an acetone (20 mL) solution of 4-Dimethylaminophenyl isothiocyanate (535 mg, 3.0 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.301 mL, 3.9 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 1 h, and then concentrated in vacuo. The crude mixture was directly used for the synthesis of compound 15: Rf = 0.2 (hexane/ethyl acetate, 1:1, v/v).

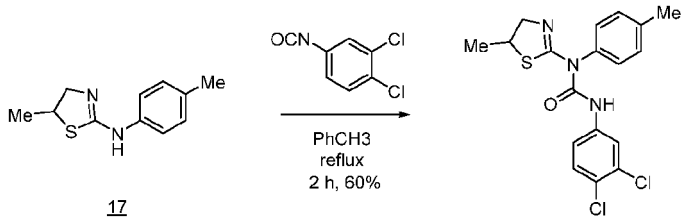
***N*1,*N*1-Dimethyl-*N*4-(5-methyl-4,5-dihydrothiazol-2-yl)benzene-1,4-diamine, 15.** (4) To an ethanol (10 mL) solution of 1-(4-(dimethylamino)phenyl)-3-(2-hydroxypropyl)thiourea (**14**, 3.0 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 2 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1 → ethyl acetate/methanol, 10:1, v/v) to afford the desired product **15** (600 mg, 85% for 2 steps) as brown solid: Rf = 0.2 (hexane/ethyl acetate, 1:1, v/v); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.03 (d, J = 8.0 Hz, 2H), 6.69 (d, J = 8.0 Hz, 2H), 3.90-3.86 (m, 2H), 3.47-3.45 (m, 1H), 2.91 (s, 6H), 1.43 (d, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.2, 147.9, 135.6, 123.0, 113.3, 47.9, 43.9, 41.0, 20.5.

3-(3,4-Dichlorophenyl)-1-(4-(dimethylamino)phenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-008. A toluene (20 mL) solution of *N*1,*N*1-dimethyl-*N*4-(5-methyl-4,5-dihydrothiazol-2-yl)benzene-1,4-diamine (**15**, 471 mg, 2.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (376 mg, 2.0 mmol, 1.0 eq) was refluxed for 2 h. After the

completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:0 → 10:1, v/v) to afford the desired product **JR-AB2-008** (761 mg, 90%) as white powder: Rf = 0.6 (hexane/ethyl acetate, 1:1, v/v); mp 135-137 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.4 (s, 1H), 7.75 (s, 1H), 7.33 (s, 2H), 6.93 (dd, *J* = 9.0, 2.0 Hz, 2H), 6.75 (dd, *J* = 9.0, 2.0 Hz, 2H), 4.42 (ddd, *J* = 11.0, 6.5, 2.0 Hz, 1H), 4.94 (ddd, *J* = 11.0, 6.5, 2.0 Hz, 1H), 3.69 (sext, *J* = 6.5 Hz, 1H), 2.96 (s, 6H), 1.45 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.4, 151.1, 148.4, 138.5, 138.0, 132.6, 130.4, 126.4, 122.2, 121.4, 119.1, 113.1, 55.4, 40.9, 36.8, 20.0; DART-HRMS found 423.0786 $[M+H]^+$, calcd for $C_{19}H_{21}Cl_2N_4OS$ 423.0813.

Scheme 10. Synthesis of JR-AB2-009





JR-AB2-009

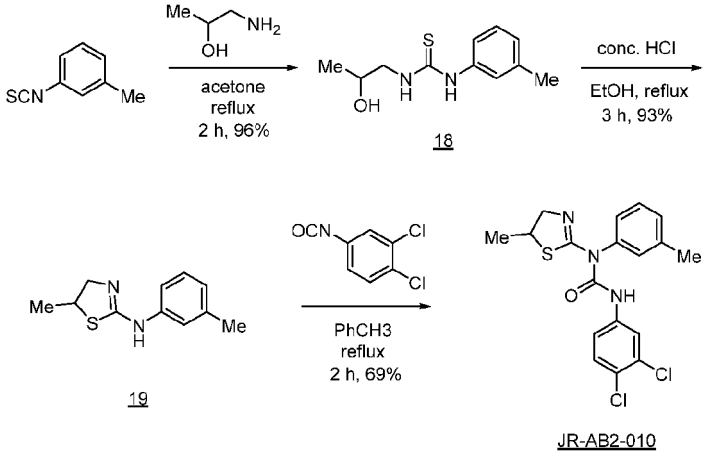
1-(2-Hydroxypropyl)-3-(*p*-tolyl)thiourea, 16. (4) To an acetone (20 mL) solution of 4-Methylphenyl isothiocyanate (448 mg, 3.0 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.301 mL, 3.9 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 2 h, and then concentrated in vacuo. The crude mixture was directly used for the synthesis of compound 17: Rf = 0.2 (hexane/ethyl acetate, 1:1, v/v).

5-Methyl-N-(*p*-tolyl)-4,5-dihydrothiazol-2-amine, 17. (4) To an ethanol (10 mL) solution of 1-(2-hydroxypropyl)-3-(*p*-tolyl)thiourea (**16**, 3.0 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 3 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product 17 (600 mg, 97% for 2 steps) as brown solid: Rf = 0.2 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.08 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 2H), 5.10 (br, 1H),

3.87-3.80 (m, 2H), 3.39 (dd, J = 10.0, 6.0 Hz, 1H), 2.30 (s, 3H), 1.42 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.5, 145.5, 132.6, 129.5, 121.2, 57.0, 43.4, 20.9, 20.4.

3-(3,4-Dichlorophenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)-1-(*p*-tolyl)urea, JR-AB2-009. A toluene (10 mL) solution of 5-methyl-N-(*p*-tolyl)-4,5-dihydrothiazol-2-amine (**17**, 206 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 2 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate to afford the desired product **JR-AB2-009** (236 mg, 60%) as white powder: Rf = 0.6 (hexane/ethyl acetate, 3:1, v/v); mp 160-162 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.2 (s, 1H), 7.74 (s, 1H), 7.33 (s, 2H), 7.18 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 4.44 (dd, J = 11.5, 6.5 Hz, 1H), 3.96 (dd, J = 11.5, 6.5 Hz, 1H), 3.71 (sext, J = 6.5 Hz, 1H), 2.36 (s, 3H), 1.45 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.5, 150.9, 146.2, 137.8, 134.8, 132.7, 130.4, 129.9, 126.6, 121.4, 121.3, 119.1, 55.6, 36.9, 21.0, 20.0; DART-HRMS found 394.0531 $[M+H]^+$, calcd for $C_{18}H_{18}Cl_2N_3OS$ 394.0548.

Scheme 11. Synthesis of Compound JR-AB2-010



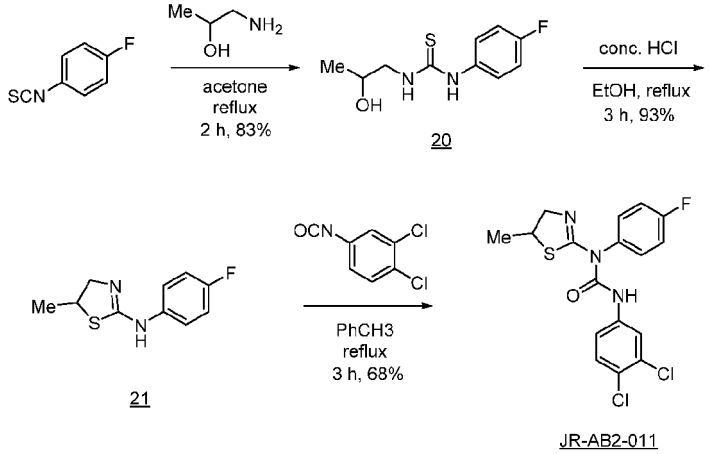
1-(2-Hydroxypropyl)-3-(*m*-tolyl)thiourea, 18. (4) To an acetone (20 mL) solution of 3-methylphenyl isothiocyanate (448 mg, 3.0 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.301 mL, 3.9 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 2 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 3:1 → 1:1, v/v) to afford the desired product **18** (646 mg, 96%) as pale yellow solid: Rf = 0.3 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73 (br, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.10 (d, J = 7.5 Hz, 1H), 7.02 (s, 2H), 6.51 (br, 1H), 4.08-4.00 (m, 1H), 3.98-3.85 (m, 1H), 3.45 (ddd, J = 13.5, 7.5, 5.0

Hz, 1H), 2.36 (s, 3H), 1.21 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.1, 140.5, 135.9, 130.0, 128.2, 125.7, 122.1, 67.2, 52.1, 21.4, 21.2.

5-Methyl-*N*-(*m*-tolyl)-4,5-dihydrothiazol-2-amine, 19. (4) To an ethanol (10 mL) solution of 1-(2-hydroxypropyl)-3-(*m*-tolyl)thiourea (**18**, 630mg, 2.81 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 3 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product **19** (538 mg, 93%) as brown solid: Rf = 0.3 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.16 (t, *J* = 7.0 Hz, 1H), 6.93 (s, 1H), 6.91 (d, *J* = 6.5 Hz, 1H), 6.85 (d, *J* = 6.5 Hz, 1H), 3.89-3.82 (m, 2H), 3.44-3.41 (m, 1H), 2.31 (s, 3H), 1.42 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.1, 147.5, 138.8, 128.8, 124.0, 121.8, 118.1, 57.6, 43.7, 21.5, 20.4.

3-(3,4-Dichlorophenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)-1-(*m*-tolyl)urea, JR-AB2-010. A toluene (10 mL) solution of 5-methyl-*N*-(*m*-tolyl)-4,5-dihydrothiazol-2-amine (**19**, 206 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 2 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **JR-AB2-010** (273 mg, 69%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 3:1, v/v); mp 111-113 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.2 (s, 1H), 7.74 (s, 1H), 7.33 (s, 2H), 7.26 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 7.5 Hz, 1H), 6.82 (s, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 4.45 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.96 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.71 (sext, *J* = 6.5 Hz, 1H), 2.38 (s, 3H), 1.46 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.5, 150.8, 148.7, 139.3, 137.8, 132.7, 130.4, 129.1, 126.6, 125.9, 122.2, 121.5, 119.2, 118.3, 55.6, 36.9, 21.5, 20.0; DART-HRMS found 394.0526 $[M+H]^+$, calcd for $C_{18}H_{18}Cl_2N_3OS$ 394.0548.

Scheme 12. Synthesis of JR-AB2-011

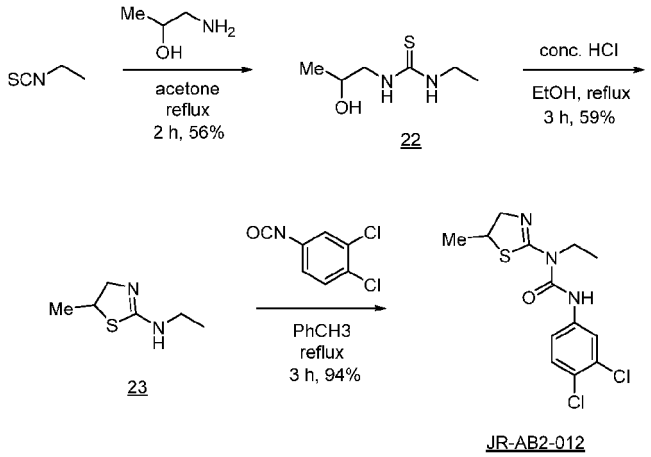


1-(4-Fluorophenyl)-3-(2-hydroxypropyl)thiourea, 20. (4) To an acetone (20 mL) solution of 4-fluorophenyl isothiocyanate (460 mg, 3.0 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.301 mL, 3.9 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 2 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 3:1 → 1:1, v/v) to afford the desired product **20** (566 mg, 83%) as pale yellow solid: Rf = 0.3 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.67 (br, 1H), 7.25-7.22 (m, 2H), 7.12 (t, *J* = 8.0 Hz, 2H), 6.34 (br, 1H), 4.08-4.00 (m, 1H), 3.99-3.82 (m, 1H), 3.42 (ddd, *J* = 13.5, 8.0, 5.0 Hz, 1H), 1.95 (br, 1H), 1.21 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.6. 161.5 (d, *J* = 248.8 Hz, 1C), 131.9, 127.8, 117.2 (d, *J* = 21 Hz, 2C), 67.1, 52.1, 21.2.

***N*-(4-Fluorophenyl)-5-methyl-4,5-dihydrothiazol-2-amine, 21.** (4) To an ethanol (10 mL) solution of 1-(4-fluorophenyl)-3-(2-hydroxypropyl)thiourea (**20**, 550 mg, 2.41 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 3 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 1:1, v/v) to afford the desired product **21** (472 mg, 93%) as pale brown solid: Rf = 0.3 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.04-6.99 (m, 2H), 6.96 (t, J = 9.0 Hz, 2H), 3.86 (sext, *J* = 6.5 Hz, 1H), 3.78 (dd, *J* = 10.0, 6.5 Hz, 1H), 3.36 (dd, *J* = 10.0. 6.5 Hz, 1H), 1.43 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.0, 159.1 (d, *J* = 240.0 Hz, 1C), 145.0, 122.7 (d, *J* = 7.5 Hz, 2C), 115.5 (d, *J* = 22.5 Hz, 2C), 55.8, 43.2, 20.3.

3-(3,4-Dichlorophenyl)-1-(4-fluorophenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-011. A toluene (10 mL) solution of *N*-(4-fluorophenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**21**, 210 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 3 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **JR-AB2-011** (272 mg, 68%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 3:1, v/v); mp 126-128 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.1 (s, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.32 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.06 (td, *J* = 8.5, 2.0 Hz, 2H), 6.95 (ddd, *J* = 9.0, 5.0, 2.0, 2H), 4.45 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.97 (dd, *J* = 11.5, 6.5 Hz, 1H)m 3.72 (sext, *J* = 6.5 Hz, 1H), 1.46 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.4, 158.7 (d, *J* = 243.8 Hz, 1C), 150.7, 144.9, 137.7, 132.7, 130.4, 126.8, 122.9 (d, *J* = 8.8 Hz, 2C), 121.5, 119.2, 116.0 (d, *J* = 22.5 Hz, 2C), 55.7, 37.0, 20.0; DART-HRMS found 398.0266 $[M+H]^+$, calcd for $C_{17}H_{14}Cl_2FN_3OS$ 398.0297.

Scheme 13. Synthesis of JR-AB2-012



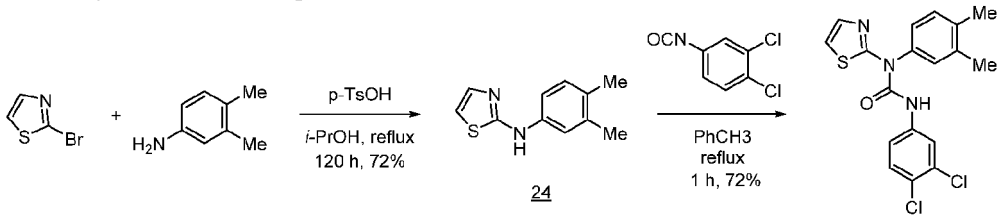
1-Ethyl-3-(2-hydroxypropyl)thiourea, 22. (4) To an acetone (20 mL) solution of ethyl isothiocyanate (0.26 mL, 3.0 mmol, 1.0 eq) was added *DL*-1-amino-2-propanol (0.301 mL, 3.9 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 2 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1 → 1:1, v/v) to afford the desired product **22** (274 mg, 56%) as white powder: Rf = 0.15 (hexane/ethyl acetate, 1:1, v/v).

***N*-Ethyl-5-methyl-4,5-dihydrothiazol-2-amine, 23.** (4) To an ethanol (10 mL) solution of 1-ethyl-3-(2-hydroxypropyl)thiourea (**22**, 250mg, 1.54 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 3 h. After the

completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The desired product **23** (130 mg, 59%) was afforded without further purification as yellow solid: Rf = 0.05 (hexane/ethyl acetate, 0:1, v/v); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.01 (dd, J = 13.0, 7.0 Hz, 1H), 3.92 (sext, J = 7.0 Hz, 1H), 3.65 (dd, J = 13.0, 5.0 Hz, 1H), 3.30 (q, J = 7.5 Hz, 2H), 1.37 (d, J = 6.5 Hz, 3H), 1.18 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.8, 67.5, 47.8, 39.6, 21.3, 15.1.

3-(3,4-Dichlorophenyl)-1-ethyl-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-012. A toluene (10 mL) solution of *N*-ethyl-5-methyl-4,5-dihydrothiazol-2-amine (**23**, 101 mg, 0.7 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (132 mg, 0.7 mmol, 1.0 eq) was refluxed for 3 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **JR-AB2-012** (218 mg, 94%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 3:1, v/v); mp 114-115 °C; ^1H NMR ($CDCl_3$, 500 MHz) δ 12.6 (s, 1H), 7.71 (d, J = 2.5 Hz, 1H), 7.33 (d, J = 9.0 Hz, 1H), 7.28 (dd, J = 9.0, 2.5 Hz, 1H), 4.32 (dd, J = 11.5, 6.5 Hz, 1H), 3.85 (dd, J = 11.5, 6.5 Hz, 1H), 3.72 (sext, J = 6.5 Hz, 1H), 3.29 (d, J = 7.5 Hz, 2H), 1.47 (d, J = 7.0 Hz, 3H), 1.31 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.4, 151.4, 138.3, 132.6, 130.4, 126.2, 121.2, 118.9, 54.9, 49.4, 37.1, 20.3, 16.1; DART-HRMS found 332.0367 $[M+H]^+$, calcd for $C_{13}H_{16}Cl_2N_3OS$ 332.0391.

Scheme 14. Synthesis of Compound JR-AB2-013

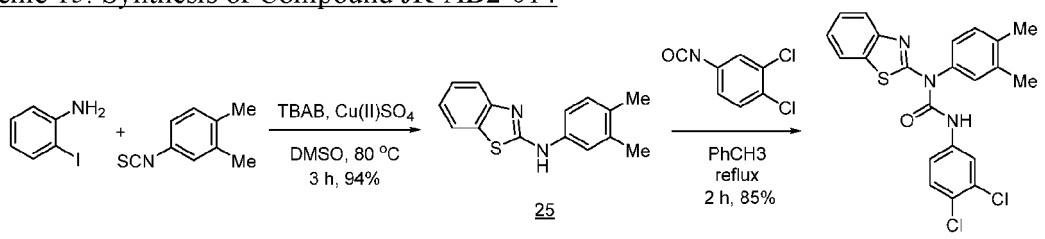


***N*-(3,4-Dimethylphenyl)thiazol-2-amine, 24.** (7) To an isopropanol (25 mL) solution of 3,4-dimethylaniline (606mg, 5.0 mmol, 1.0 eq) and p-toluenesulfonic acid hydrate (476 mg, 2.5 mmol, 0.5 eq) was added 2-bromothiazole (0.68 mL. 7.5 mmol, 1.5 eq) at room temperature, and the mixture was refluxed for 120 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1 → 5:1, v/v) to afford the desired product **24** (738 mg, 72%) as pale yellow solid: Rf =

0.2 (hexane/ethyl acetate, 5:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (d, J = 3.5 Hz, 1H), 7.12-7.09 (m, 3H), 6.58 (d, J = 3.5 Hz, 1H), 2.27 (s, 3H), 2.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.6, 138.7, 138.4, 137.9, 131.7, 130.5, 120.2, 116.1, 107.1, 20.0, 19.1.

3-(3,4-Dichlorophenyl)-1-(3,4-dimethylphenyl)-1-(thiazol-2-yl)urea, JR-AB2-013. A toluene (20 mL) solution of *N*-(3,4-dimethylphenyl)thiazol-2-amine (**24**, 480 mg, 2.35 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (442 mg, 2.35 mmol, 1.0 eq) was refluxed for 1 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) and the resulting white caramel was crystallized using dichloromethane and hexane to afford the desired product **JR-AB2-013** (663 mg, 72%) as white powder: Rf = 0.6 (hexane/ethyl acetate, 5:1, v/v); mp 122-123 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.5 (br, 1H), 7.83 (d, J = 2.0 Hz, 1H), 7.42 (d, J = 3.5 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.16 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 3.5 Hz, 1H), 2.34 (s, 3H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.4, 151.3, 139.1, 138.8, 138.3, 138.0, 137.4, 132.7, 131.5, 130.4, 130.0, 126.6, 126.4, 121.5, 119.1, 113.1, 20.0, 19.7; DART-HRMS found 392.0376 $[M+H]^+$, calcd for $C_{18}H_{16}Cl_2N_3OS$ 392.0391.

Scheme 15. Synthesis of Compound JR-AB2-014



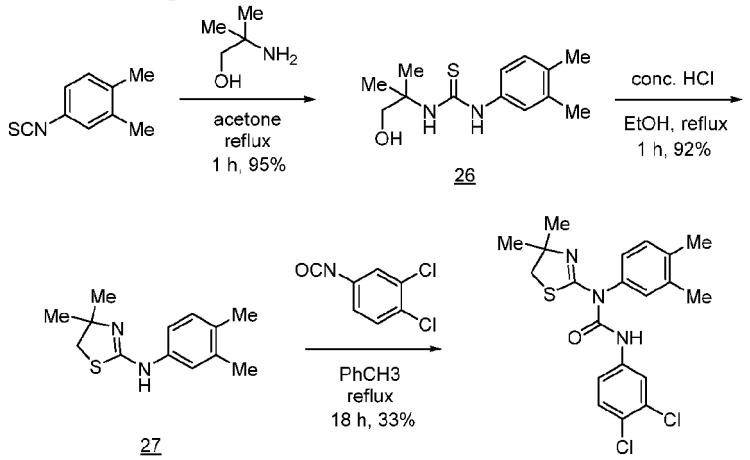
JR-AB2-014

***N*-(3,4-Dimethylphenyl)benzo[*d*]thiazol-2-amine, 25.** (8) To a dimethyl sulfoxide (4 mL) solution of 2-iodoaniline (438 mg, 2.0 mmol, 1.0 eq), 3,4-dimethylphenyl isothiocyanate (359 mg, 2.2 mmol, 1.1 eq), copper(II) sulfate (3.2 mg, 0.02 mmol, 0.01 eq) and tetrabutylamminium bromide (7.9 mg, 2.2 mmol, 1.1 eq) was stirred for 3 h at 80 °C. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and washed with water. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **25** (479 mg, 94%) as yellow solid: Rf = 0.2 (hexane/ethyl acetate, 5:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.61 (dd, J = 8.0, 0.5 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.32 (td, J = 8.0, 1.0 Hz, 1H), 7.23-7.20 (m, 2H), 7.15 (d, J = 8.5 Hz, 1H), 7.14 (td, J = 8.0, 1.0 Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H); ^{13}C NMR

($CDCl_3$, 125 MHz) δ 165.0, 151.6, 138.0, 137.4, 133.2, 130.6, 130.1, 126.1, 122.3, 122.1, 120.8, 119.4, 118.1, 20.0, 19.3.

1-(Benzo[*d*]thiazol-2-yl)-3-(3,4-dichlorophenyl)-1-(3,4-dimethylphenyl)urea, JR-AB2-014. A toluene (10 mL) solution of *N*-(3,4-dimethylphenyl)benzo[*d*]thiazol-2-amine (**25**, 254 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 2 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **JR-AB2-014** (375 mg, 85%) as white powder: Rf = 0.8 (hexane/ethyl acetate, 5:1, v/v); mp 175-177 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.3 (s, 1H), 7.91 (d, *J* = 2.5 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.47 (td, *J* = 9.0, 2.5 Hz, 1H), 7.44 (dd, *J* = 9.0, 1.0 Hz,1H), 7.40 (d, *J* = 9.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.26 (td, *J* = 7.5, 1.0 Hz, 1H), 7.20 (d, *J* = 1.5 Hz, 1H), 7.16 (dd, *J* = 8.0, 1.5 Hz,1H), 2.36 (s, 3H), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.5, 151.1, 150.1, 139.04 138.99, 138.0, 137.2, 132.8, 131.5, 131.3, 130.5, 130.2, 126.8, 126.62, 126.59, 124.0, 121.6, 120.8, 120.7, 119.2, 20.0, 19.7; DART-HRMS found 442.0516 $[M+H]^+$, calcd for $C_{22}H_{18}Cl_2N_3OS$ 442.0548.

Scheme 16. Synthesis of Compound JR-AB2-015



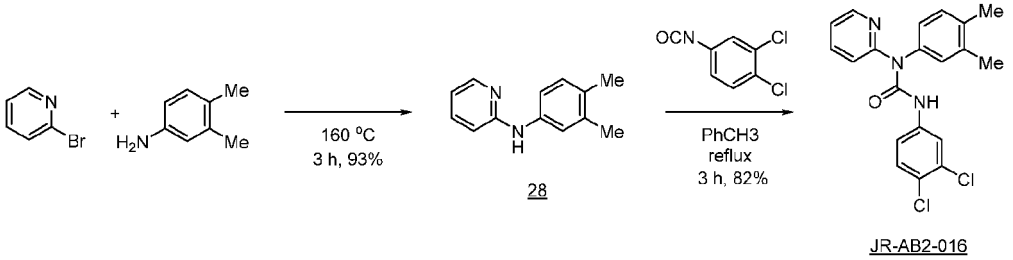
1-(3,4-Dimethylphenyl)-3-(1-hydroxy-2-methylpropan-2-yl)thiourea, 26. (4) To an acetone (20 mL) solution of 3,4-dimethylphenyl isothiocyanate (490 mg, 3.0 mmol, 1.0 eq) was added 2-amino-2-methyl-1-propanol (0.372 mL, 3.9 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 1 h, and then concentrated in vacuo. The residue was crystallized using ethyl acetate and hexane to afford the desired product **26** (720 mg, 95%) as white powder: Rf = 0.4 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz)

δ 7.91 (br, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 6.98 (s, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.11 (br, 1H), 3.83 (s, 2H), 3.17 (br, 1H), 2.25 (s, 6H), 1.39 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.6, 138.6, 135.8, 134.3, 130.9, 126.1, 122.3, 69.3, 57.7, 24.8, 19.9, 19.4.

***N*-(3,4-Dimethylphenyl)-4,4-dimethyl-4,5-dihydrothiazol-2-amine, 27.** (4) To an ethanol (10 mL) solution of 1-(3,4-dimethylphenyl)-3-(1-hydroxy-2-methylpropan-2-yl)thiourea (**26**, 600mg, 2.52 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 1 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The desired product **27** (545 mg, 92%) was afforded without further purification as white powder: Rf = 0.3 (hexane/ethyl acetate, 2:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.09 (d, *J* = 8.0 Hz, 1H), 6.96 (d, *J* = 2.0 Hz, 1H), 6.93 (dd, *J* = 8.0, 2.0 Hz, 1H), 3.17 (s, 2H), 2.233 (s, 3H), 2.228 (s, 3H), 1.51 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.6, 139.4, 137.8, 134.5, 130.3, 123.9, 119.8, 64.5, 42.9, 27.4, 19.8, 19.3.

3-(3,4-Dichlorophenyl)-1-(4,4-dimethyl-4,5-dihydrothiazol-2-yl)-1-(3,4-dimethylphenyl)urea, JR-AB2-015. A toluene (10 mL) solution of *N*-(3,4-dimethylphenyl)-4,4-dimethyl-4,5-dihydrothiazol-2-amine (**27**, 234 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 18 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **JR-AB2-015** (140 mg, 33%) as white powder: Rf = 0.8 (hexane/ethyl acetate, 2:1, v/v); mp 143-144 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.6 (s, 1H), 7.73 (d, *J* = 2.5 Hz, 1H), 7.30 (d, *J* = 9.0 Hz, 1H), 7.27 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.79 (s, 1H), 6.74 (dd, *J* = 7.5, 2.0 Hz, 1H), 2.98 (s, 2H), 2.28 (s, 3H), 2.27 (s, 3H), 1.78 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.2, 151.1, 146.4, 138.1, 137.7, 133.4, 132.6, 130.34, 130.26, 126.4, 122.8, 121.7, 119.4, 118.6, 67.4, 40.3, 26.3, 19.9, 19.3; DART-HRMS found 422.0841 $[M+H]^+$, calcd for $C_{20}H_{22}Cl_2N_3OS$ 422.0861.

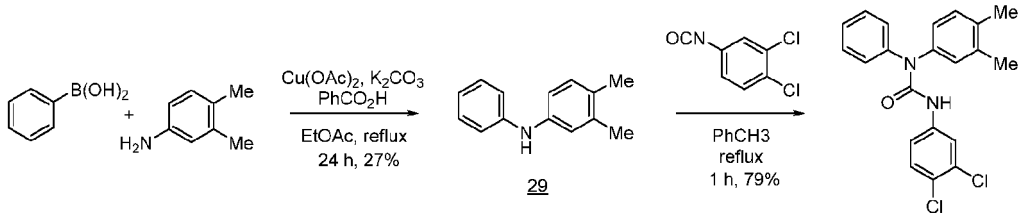
Scheme 17. Synthesis of Compound JR-AB2-016



***N*-(3,4-Dimethylphenyl)pyridin-2-amine, 28.** (9) A mixture of 2-bromopyridine (4.77 mL, 50.0 mmol, 10.0 eq) and 3,4-dimethylaniline (606 mg, 5.0 mmol, 1.0 eq) was refluxed for 3 h at 160 °C. After the completion of reaction, the mixture was cooled down to room temperature, and directly purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **28** (920 mg, 93%) as white powder: Rf = 0.15 (hexane/ethyl acetate, 5:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.17 (dd, *J* = 5.0, 1.0 Hz, 1H), 7.45 (ddd, *J* = 9.0, 7.5, 1.5 Hz, 1H), 7.07 (q, *J* = 8.0 Hz, 2H), 7.04 (dd, *J* = 7.0, 2.5 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 6.68 (ddd, *J* = 7.0, 5.0, 1.0 Hz, 1H), 6.48 (br, 1H), 2.25 (s, 3H), 2.24 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.6, 148.5, 138.0, 137.62, 137.59, 131.6, 130.3, 122.7, 118.7, 114.6, 107.6, 20.0, 19.1.

3-(3,4-Dichlorophenyl)-1-(3,4-dimethylphenyl)-1-(pyridin-2-yl)urea, JR-AB2-016. A toluene (20 mL) solution of *N*-(3,4-dimethylphenyl)pyridin-2-amine (**28**, 595 mg, 3.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (564 mg, 3.0 mmol, 1.0 eq) was refluxed for 3 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-016** (950 mg, 82%) as white powder: Rf = 0.5 (hexane/ethyl acetate, 5:1, v/v); mp 133-134 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 13.2 (s, 1H), 8.35 (ddd, *J* = 5.0, 2.0, 0.5, 1H), 7.90 (d, *J* = 2.5 Hz, 1H), 7.50 (ddd, *J* = 9.0, 7.5, 2.0 Hz, 1H), 7.44 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 1.5 Hz, 1H), 7.03 (dd, *J* = 7.5, 2.5 Hz, 1H), 6.98 (ddd, *J* = 8.0, 5.0, 0.5 Hz, 1H), 6.42 (d, *J* = 8.5 Hz, 1H), 2.32 (s, 3H), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.1, 153.5, 145.2, 138.81, 138.76, 138.5, 137.2, 137.1, 132.5, 131.3, 130.5, 130.2, 126.8, 125.9, 121.5, 119.2, 117.5, 114.0, 20.0, 19.6; DART-HRMS found 386.0801 $[M+H]^+$, calcd for $C_{20}H_{18}Cl_2N_3O$ 386.0827.

Scheme 18. Synthesis of Compound JR-AB2-017

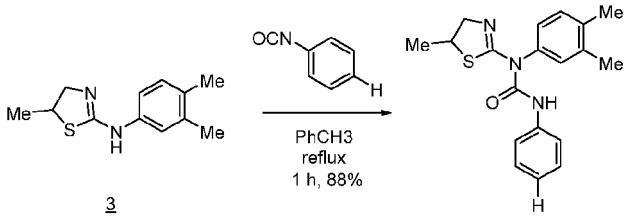


3,4-Dimethyl-N-phenylaniline, 29. (10) An ethyl acetate (25 mL) suspension of 3,4-dimethylaniline (606 mg, 5.0 mmol, 1.0 eq), phenylboronic acid (1829 mg, 15.0 mmol, 3.0 eq), copper(II) acetate (182 mg, 1.0 mmol, 0.2 eq), potassium carbonate (676 mg, 5.0 mmol, 1.0 eq) and benzoic acid (305 mg, 2.5 mmol, 0.5 eq) was refluxed for 24 h, and then the

mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 100:1, v/v) to afford the desired product **29** (270 mg, 27%) as yellow solid: Rf = 0.7 (hexane/ethyl acetate, 5:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.23 (td, J = 7.5, 1.5 Hz, 2H), 7.04 (d, J = 8.0 Hz, 1H), 7.01 (dd, J = 7.5, 1.0 Hz, 1H), 6.90-6.85 (d, 3H), 5.57 (br, 1H), 2.23 (s, 3H), 2.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.0, 140.6, 137.6, 130.3, 129.7, 129.3, 120.3, 120.2, 116.9, 116.3, 20.0, 19.0.

3-(3,4-dichlorophenyl)-1-(3,4-dimethylphenyl)-1-phenylurea, JR-AB2-017. A toluene (10 mL) solution of 3,4-dimethyl-N-phenylaniline (**29**, 197 mg, 1.0 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (188 mg, 1.0 mmol, 1.0 eq) was refluxed for 1 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) and the following product was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-017** (305 mg, 79%) as white powder: Rf = 0.4 (hexane/ethyl acetate, 10:1, v/v); mp 118-119 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.56 (d, J = 2.5 Hz, 1H), 7.38-7.22 (m, 6H), 7.19-7.16 (m, 2H), 7.11 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.47 (br, 1H), 2.28 (s, 3H), 2.26 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.2, 142.2, 139.2, 138.7, 138.2, 136.5, 132.6, 131.1, 130.3, 129.4, 129.0, 126.9, 126.4, 126.1, 125.3, 120.8, 118.5, 19.9, 19.4; DART-HRMS found 385.0857 $[M+H]^+$, calcd for $C_{21}H_{19}Cl_2N_2O$ 385.0874.

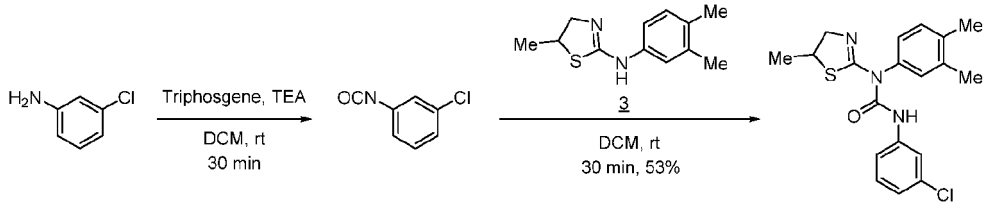
Scheme 19. Synthesis of JR-AB2-018



1-(3,4-Dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)-3-phenylurea, JR-AB2-018. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and phenyl isocyanate (0.109 mL, 1.0 mmol, 1.0 eq) was refluxed for 1 h under argon gas atmosphere. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-018** (280 mg, 88%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 2:1, v/v); mp 142-143 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.1 (s, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.12 (d, J = 8.0 Hz, 1H), 7.06 (t, J = 7.5 Hz, 1H), 6.81 (s, 1H), 6.75 (dd, J = 8.0, 2.0 Hz, 1H), 4.46 (dd, J = 11.5, 6.5 Hz, 1H), 3.97 (dd, J =

11.5, 7.0 Hz, 1H), 3.69 (sext, J = 6.5 Hz, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 1.45 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9, 151.1, 146.9, 138.2, 137.6, 133.2, 130.3, 128.9, 123.6, 122.7, 120.0, 118.5, 55.6, 36.8, 20.0, 19.9, 19.3; DART-HRMS found 340.1475 $[M+H]^+$, calcd for $C_{19}H_{22}N_3OS$ 340.1484.

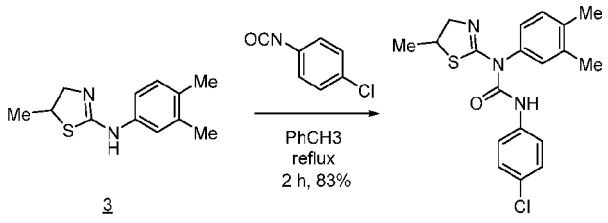
Scheme 20. Synthesis of JR-AB2-019



JR-AB2-019

3-(3-Chlorophenyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-019. (11) To a dichloromethane solution (5 mL) of triphosgene (110 mg, 0.37 mmol, 0.37 eq) was added 3-chloroaniline (0.106 mL, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) and triethylamine (0.14 mL. 1.1 mmol, 1.1 eq) dropwise. After 30 min stirring at room temperature, then *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) was added and the mixture was stirred 30 min at room temperature. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was diluted with ethyl acetate (100 mL) and washed with aq. $NaHCO_3$ solution (30 mL). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-019** (200 mg, 53%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 2:1, v/v); mp 148-149 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.2 (s, 1H), 7.62 (t, J = 2.0 Hz, 1H), 7.35 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 7.20 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.03 (ddd, J = 8.0, 2.0, 1.0 Jz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.75 (dd, J = 8.0, 2.5 Hz, 1H), 4.45 (dd, J = 11.5, 6.5 Hz, 1H), 3.96 (dd, J = 11.5, 6.5 Hz, 1H), 3.70 (sext, J = 6.5 Hz, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 1.45 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.1, 151.0, 146.7, 139.5, 137.7, 134.6, 133.4, 130.3, 129.9, 123.6, 122.7, 119.9, 118.5, 117.9, 55.6, 36.9, 20.0, 19.9, 19.3; DART-HRMS found 374.1068 $[M+H]^+$, calcd for $C_{19}H_{21}ClN_3OS$ 374.1094.

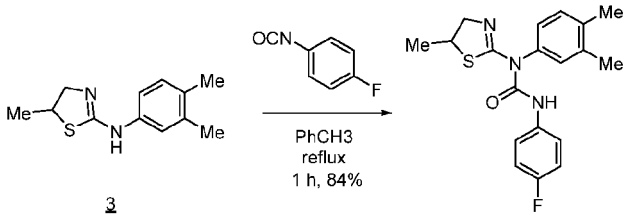
Scheme 21. Synthesis of JR-AB2-020



3 JR-AB2-020

3-(4-Chlorophenyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-020. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and 4-chlorophenyl isocyanate (153 mg, 1.0 mmol, 1.0 eq) was refluxed for 2 h under argon gas atmosphere. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-020** (310 mg, 83%) as white powder: Rf = 0.8 (hexane/ethyl acetate, 2:1, v/v); mp 140-142 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.1 (s, 1H), 7.46 (dt, *J* = 9.0, 2.0 Hz, 2H), 7.26-7.24 (m, 2H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.74 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.44 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.96 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.69 (sext, *J* = 6.5 Hz, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 1.45 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.1, 151.0, 146.7, 137.6, 136.9, 133.3, 130.3, 128.9, 128.5, 122.7, 121.1, 118.5, 55.6, 36.9, 20.0, 19.9, 19.3; DART-HRMS found 374.1081 $[M+H]^+$, calcd for $C_{19}H_{21}ClN_3OS$ 374.1094.

Scheme 22. Synthesis of JR-AB2-021

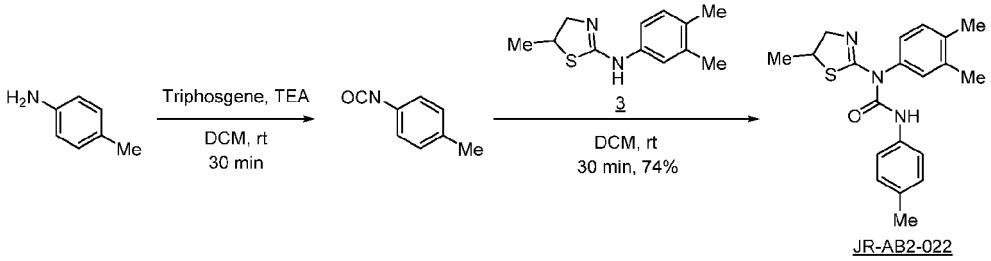


3 JR-AB2-021

1-(3,4-Dimethylphenyl)-3-(4-fluorophenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-021. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and 4-fluorophenyl isocyanate (0.114 mL, 1.0 mmol, 1.0 eq) was refluxed for 1 h under argon gas atmosphere. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-021** (300 mg, 84%) as white powder: Rf = 0.7 (hexane/ethyl acetate, 2:1, v/v); mp 135-136 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.0 (s, 1H), 7.47-7.45 (m, 2H), 7.12 (d, *J* = 7.5 Hz, 1H), 7.01-6.97 (m, 2H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.74 (dd, *J* = 7.5, 2.0 Hz, 1H), 4.45 (dd, *J* = 11.5, 6.5,

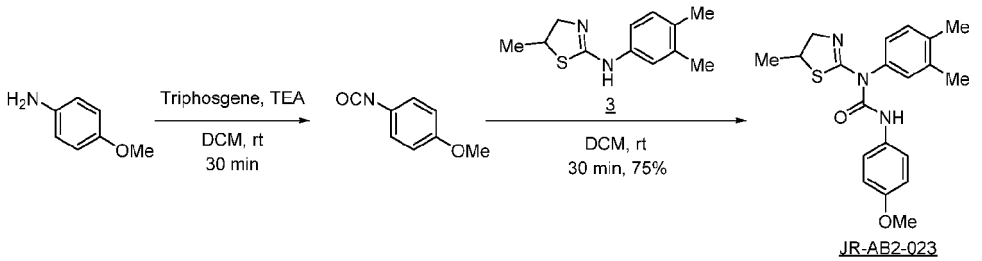
1H), 3.96 (dd, J = 11.5, 7.0 Hz, 1H), 3.69 (sext, J = 6.5 Hz, 1H), 2.27 (s, 3H), 2.26 (s, 3H), 1.45 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1 (d, J = 241.3 Hz, 1C), 158.1, 151.2, 146.8, 137.6, 134.2 (d, J = 2.5 Hz, 1C), 133.3, 130.3, 122.7, 121.6 (d, J = 7.5 Hz, 2C), 118.5, 115.5 (d, J = 22.5 Hz, 2C), 55.6, 36.9, 20.0, 19.9, 19.3; DART-HRMS found 358.1378 $[M+H]^+$, calcd for $C_{19}H_{21}FN_3OS$ 358.1389.

Scheme 23. Synthesis of JR-AB2-022



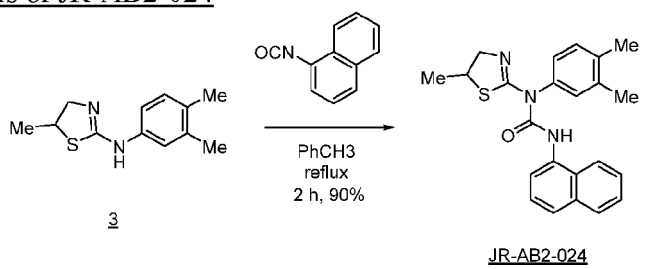
1-(3,4-Dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)-3-(*p*-tolyl)urea, JR-AB2-022. (11) To a dichloromethane solution (5 mL) of triphosgene (110 mg, 0.37 mmol, 0.37 eq) was added p-toluidine (107 mg, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) and triethylamine (0.14 mL. 1.1 mmol, 1.1 eq) dropwise. After 30 min stirring at room temperature, then *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) was added and the mixture was stirred 30 min at room temperature. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was diluted with ethyl acetate (100 mL) and washed with aq. $NaHCO_3$ solution (30 mL). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-022** (260 mg, 74%) as white powder: Rf = 0.75 (hexane/ethyl acetate, 2:1, v/v); mp 129-130 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.9 (s, 1H), 7.38 (dt, J = 7.5, 2.0 Hz, 2H), 7.12 (d, J = 7.5 Hz, 1H), 7.10 (d, J = 9.0 Hz, 2H), 6.80 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 7.5, 2.0 Hz, 1H), 4.45 (dd, J = 11.5, 6.5 Hz, 1H), 3.96 (dd, J = 11.5, 6.5 Hz, 1H), 3.68 (sext, J = 6.5 Hz, 1H), 2.30 (s, 3H), 2.27 (s, 3H), 2.26 (s, 3H), 1.44 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9, 151.1, 147.0, 137.6, 135.6, 133.1, 130.3, 129.4, 123.0, 122.7, 120.0, 118.5, 55.6, 36.8, 20.9, 20.0, 19.9, 19.3; DART-HRMS found 354.1617 $[M+H]^+$, calcd for $C_{20}H_{24}N_3OS$ 354.1640.

Scheme 24. Synthesis of JR-AB2-023



1-(3,4-Dimethylphenyl)-3-(4-methoxyphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-023. (11) To a dichloromethane solution (5 mL) of triphosgene (110 mg, 0.37 mmol, 0.37 eq) was added *p*-anisidine (123 mg, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) and triethylamine (0.14 mL. 1.1 mmol, 1.1 eq) dropwise. After 30 min stirring at room temperature, then *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) was added and the mixture was stirred 30 min at room temperature. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was diluted with ethyl acetate (100 mL) and washed with aq. $NaHCO_3$ solution (30 mL). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-023** (275 mg, 75%) as white powder: Rf = 0.6 (hexane/ethyl acetate, 2:1, v/v); mp 120-122 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 11.9 (s, 1H), 7.41 (dt, *J* = 9.0, 2.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 1H), 6.84 (dt, *J* = 9.0, 2.0 Hz, 2H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.74 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.45 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.96 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.78 (s, 3H), 3.68 (sext, *J* = 6.5 Hz, 1H), 2.27 (s, 3H), 2.25 (s, 3H), 1.44 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.9, 156.0, 151.3, 147.0, 137.6, 133.1, 131.3, 150.3, 122.7, 121.7, 118.5, 114.1, 55.6, 55.5, 36.9, 20.0 19.9, 19.3; DART-HRMS found 370.1565 $[M+H]^+$, calcd for $C_{20}H_{24}N_3O_2S$ 370.1589.

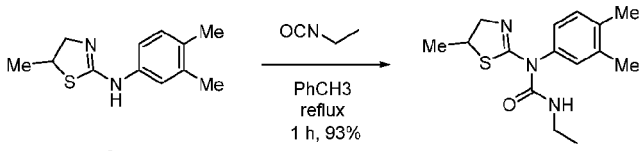
Scheme 25. Synthesis of JR-AB2-024



1-(3,4-Dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)-3-(naphthalen-1-yl)urea, JR-AB2-024. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and 1-naphthyl isocyanate (0.144 mL, 1.0 mmol, 1.0 eq) was refluxed for 2 h under argon gas atmosphere. After the completion of

reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetate and hexane to afford the desired product **JR-AB2-024** (350 mg, 90%) as white powder: Rf = 0.6 (hexane/ethyl acetate, 2:1, v/v); mp 137-139 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.6 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.48 (t, J = 8.0 Hz, 1H), 7.46-7.40 (m, 2H), 7.15 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 6.85 (dd, J = 8.0, 2.0 Hz, 1H), 4.55 (ddd, J = 11.5, 6.5, 2.0 Hz, 1H), 4.04 (ddd, J = 11.5, 6.5, 2.0 Hz, 1H), 3.75 (sext, J = 6.5 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 1.48 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.3, 151.5, 146.8, 137.6, 134.1, 133.7, 133.3, 130.4, 128.6, 126.3, 126.2, 126.0, 125.7, 124.1, 122.8, 121.3, 118.6, 117.8, 55.8, 37.0, 20.0, 19.99, 19.3; DART-HRMS found 390.1604 $[M+H]^+$, calcd for $C_{23}H_{24}N_3OS$ 390.1640.

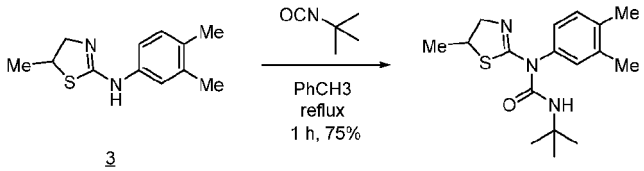
Scheme 26. Synthesis of JR-AB2-025



3 JR-AB2-025

1-(3,4-Dimethylphenyl)-3-ethyl-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-025. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and ethyl isocyanate (0.079 mL, 1.0 mmol, 1.0 eq) was refluxed for 1 h under argon gas atmosphere. After the completion of reaction, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-025** (270 mg, 93%) as white solid: Rf = 0.7 (hexane/ethyl acetate, 2:1, v/v); mp 47-49 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.53 (s, 1H), 7.08 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 2.0 Hz, 1H), 6.68 (dd, J = 7.5, 2.0 Hz, 1H), 4.37 (dd, J = 11.5, 6.5 Hz, 1H), 3.87 (dd, J = 11.5, 7.0 Hz, 1H), 3.62 (sext, J = 6.5 Hz, 1H), 3.35 (qd, J = 7.5, 5.5 Hz, 2H), 2.25 (s, 3H), 2.24 (s, 3H), 1.40 (d, J = 7.0 Hz, 3H), 1.18 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.6, 153.8, 147.6, 137.4, 132.8, 130.2, 122.7, 118.5, 55.7, 36.9, 35.0, 19.93, 19.91, 19.3, 15.1; DART-HRMS found 292.1476 $[M+H]^+$, calcd for $C_{15}H_{22}N_3OS$ 292.1484.

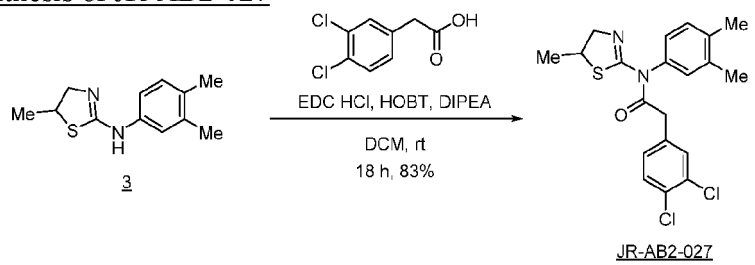
Scheme 27. Synthesis of JR-AB2-026



3 JR-AB2-026

3-(tert-butyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-026. A toluene solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq) and tert-butyl isocyanate (0.114 mL, 1.0 mmol, 1.0 eq) was refluxed for 1 h under argon gas atmosphere. After the completion of reaction, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-026** (240 mg, 75%) as yellow solid: Rf = 0.5 (hexane/ethyl acetate, 2:1, v/v); mp 75-77 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.69 (s, 1H), 7.08 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 2.0 Hz, 1H), 6.69 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.35 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.84 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.60 (sext, *J* = 6.5 Hz, 1H), 2.25 (s, 3H), 2.23 (s, 3H), 1.39 (d, *J* = 6.5 Hz, 3H), 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.5, 152.4, 147.5, 137.4, 132.7, 130.1, 122.7, 118.5, 55.4, 50.7, 36.6, 29.0, 19.94, 19.92, 19.2; DART-HRMS found 320.1790 $[M+H]^+$, calcd for $C_{17}H_{26}N_3OS$ 320.1797.

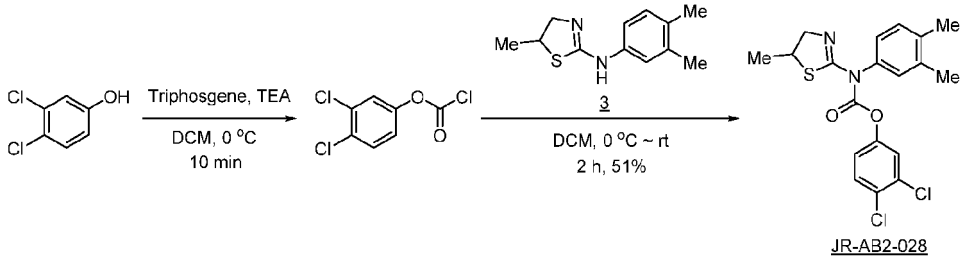
Scheme 28. Synthesis of JR-AB2-027



2-(3,4-Dichlorophenyl)-N-(3,4-dimethylphenyl)-N-(5-methyl-4,5-dihydrothiazol-2-yl)acetamide, JR-AB2-027. To a dichloromethane solution of *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 220 mg, 1.0 mmol, 1.0 eq), 3,4-dichlorophenylacetic acid (226 mg, 1.1 mmol, 1.1 eq), EDC HCl (211 mg, 1.1 mmol, 1.1 eq) and HOBT hydrate <14% (175 mg, 1.1 mmol, 1.1 eq) was added diisopropylethylamine (0.37 mL, 2.1 mmol, 2.1 eq) at room temperature. The mixture was stirred for 18 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was diluted with ethyl acetate (100 mL) and washed with water (30 mL). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1, v/v) to afford the desired product **JR-AB2-027** (340 mg, 83%) as pale yellow solid: Rf = 0.85 (hexane/ethyl acetate, 2:1, v/v); mp 68-70 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42 (d, *J* = 2.0 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.14 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.66 (s, 1H), 6.63 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.41 (d, *J* = 15.5 Hz, 1H), 4.39 (d, *J* = 15.5 Hz, 1H), 4.34 (dd, *J* = 11.5, 6.5 Hz,

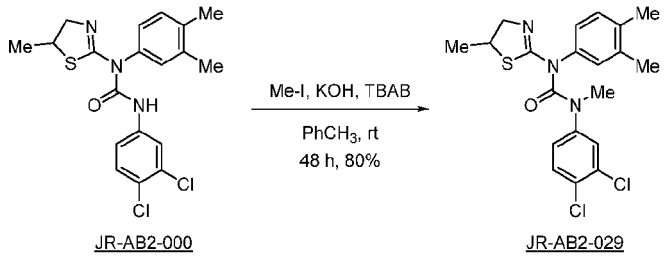
1H), 3.78 (dd, J = 11.5, 7.0 Hz, 1H), 3.63 (sext, J = 6.5 Hz, 1H), 2.26 (s, 3H), 2.24 (s, 3H), 1.37 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5, 154.5, 147.7, 137.6, 135.2, 132.9, 132.2, 131.8, 130.9, 130.3, 130.2, 129.2, 121.9, 117.6, 55.2, 42.6, 37.3, 19.95, 19.94, 19.3; DART-HRMS found 407.0723 $[M+H]^+$, calcd for $C_{20}H_{21}Cl_2N_2OS$ 407.0752.

Scheme 29. Synthesis of JR-AB2-028



3,4-Dichlorophenyl (3,4-dimethylphenyl)(5-methyl-4,5-dihydrothiazol-2-yl)carbamate, JR-AB2-028. (11) To a dichloromethane solution (5 mL) of triphosgene (119 mg, 0.4 mmol, 0.4 eq) was added 3,4-dichlorophenol (163 mg, 1.0 mmol, 1.0 eq) in dichloromethane (3 mL) and triethylamine (0.28 mL. 2.2 mmol, 2.2 eq) dropwise at 0 °C. After 10 min stirring at room temperature, then *N*-(3,4-dimethylphenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**3**, 198 mg, 0.9 mmol, 0.9 eq) in dichloromethane (3 mL) was added and the mixture was stirred 2 h at 0 °C. The mixture was concentrated in vacuo, and the residue was diluted with ethyl acetate (100 mL) and washed with aq. $NaHCO_3$ solution (30 mL). The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 10:1, v/v) to afford the desired product **JR-AB2-028** (208 mg, 51%) as paly yellow oil: Rf = 0.6 (hexane/ethyl acetate, 5:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44 (d, J = 9.0 Hz, 1H), 7.39 (d, J = 3.0 Hz, 1H), 7.12 (dd, J = 9.0, 3.0 Hz, 1H), 7.07 (d, J = 7.5 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.69 (dd, J = 8.0, 2.0 Hz, 1H), 4.38 (dd, J = 11.0, 6.0 Hz, 1H), 3.84 (dd, J = 11.0, 6.5 Hz, 1H), 3.74 (sext, J = 6.5 Hz, 1H), 2.24 (s, 3H), 2.23 (s, 3H), 1.45 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.9, 153.3, 149.2, 148.5, 137.3, 132.9, 132.7, 130.6, 130.1, 124.0, 121.9, 121.3, 117.6, 115.3, 56.4, 37.7, 19.9, 19.6, 19.3; DART-HRMS found 409.1515 $[M+H]^+$, calcd for $C_{19}H_{19}Cl_2N_2O_2S$ 409.0544.

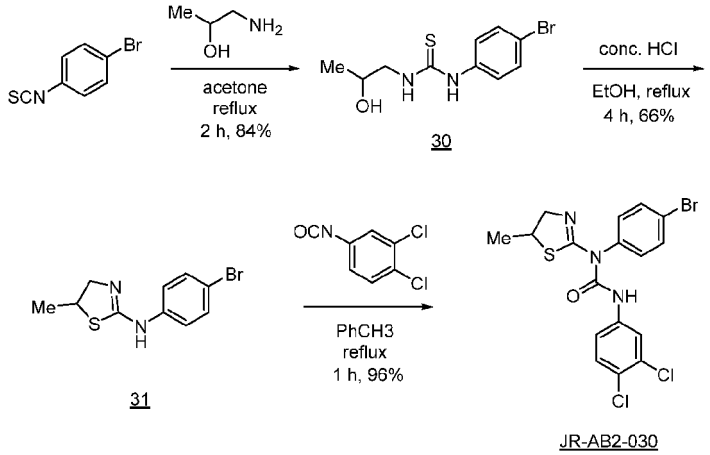
Scheme 30. Synthesis of JR-AB2-029



JR-AB2-000 JR-AB2-029

1-(3,4-Dichlorophenyl)-3-(3,4-dimethylphenyl)-1-methyl-3-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-029. To a toluene solution of 3-(3,4-dichlorophenyl)-1-(3,4-dimethylphenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea (**JR-AB2-000**, 102 mg, 0.25 mmol, 1.0 eq), potassium hydroxide (70 mg, 1.25 mmol, 5.0 eq) and tetrabutylammonium bromide (8 mg, 0.025 mmol, 0.1 eq) was added iodomethane (0.078 mL, 1.25 mmol, 5.0 eq) at room temperature. The mixture was stirred for 48 h. After the completion of reaction, the solid was filtered and washed with ethyl acetate. The filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the desired product **JR-AB2-029** (85 mg, 80%) as white powder: Rf = 0.3 (hexane/ethyl acetate, 2:1, v/v); mp 112-113 °C; ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.63 (d, J = 2.5 Hz, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.31 (dd, J = 8.5, 2.5 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.09 (dd, J = 7.5, 2.5 Hz, 1H), 5.95 (s, 1H), 4.01-3.94 (m, 1H), 3.68 (sext, J = 6.5 Hz, 1H), 3.58-3.53 (m, 1H), 3.29 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 1.29 (d, J = 6.5 Hz, 3H); ^{13}C NMR (DMSO-d6, 125 MHz) δ 154.6, 154.0, 148.6, 144.5, 136.9, 131.5, 131.2, 130.5, 130.1, 129.4, 128.7, 125.8, 122.2, 118.0, 56.2, 38.0, 21.5, 20.0, 19.2, 14.0; DART-HRMS found 422.0828 $[M+H]^+$, calcd for $C_{20}H_{22}Cl_2N_3OS$ 422.0861.

Scheme 31. Synthesis of Compound JR-AB2-030



30

31 JR-AB2-030

1-(4-Bromophenyl)-3-(2-hydroxypropyl)thiourea, 30. (4) To an acetone (20 mL) solution of 4-bromophenyl isothiocyanate (428 mg, 2.0 mmol, 1.0 eq) was added *DL*-1-

amino-2-propanol (0.2 mL, 2.6 mmol, 1.3 eq) dropwise at room temperature. The mixture was refluxed for 2 h, and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 5:1 → 1:1, v/v) to afford the desired product **30** (485 mg, 84%) as brown oil: Rf = 0.1 (hexane/ethyl acetate, 5:1, v/v).

***N*-(4-Bromophenyl)-5-methyl-4,5-dihydrothiazol-2-amine, 31.** (4) To an ethanol (10 mL) solution of 1-(4-bromophenyl)-3-(2-hydroxypropyl)thiourea (**30**, 290 mg, 1.0 mmol, 1.0 eq) was added conc. HCl (5 mL) at room temperature, and the mixture was refluxed for 4 h. After the completion of reaction, the mixture was diluted with ethyl acetate (100 mL) and quenched with aq. $NaHCO_3$ to make HCl salt to free form. The organic layer was dehydrated with brine and $MgSO_4$ and then concentrated in vacuo. The residue was purified by flash column chromatography over silica gel (hexane/ethyl acetate, 2:1, v/v) to afford the desired product **31** (180 mg, 66%) as white powder: Rf = 0.5 (hexane/ethyl acetate, 1:1, v/v); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 (d, *J* = 8.5 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 3.89 (sext, *J* = 6.5 Hz, 1H), 3.81 (dd, *J* = 10.5, 6.5 Hz, 1H), 3.38 (dd, *J* = 10.0, 6.5 Hz, 1H), 1.44 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.1, 147.4, 132.0, 123.2, 116.1, 55.7, 43.2, 20.3.

1-(4-Bromophenyl)-3-(3,4-dichlorophenyl)-1-(5-methyl-4,5-dihydrothiazol-2-yl)urea, JR-AB2-030. A toluene (10 mL) solution of *N*-(4-bromophenyl)-5-methyl-4,5-dihydrothiazol-2-amine (**31**, 135 mg, 0.5 mmol, 1.0 eq) and 3,4-dichlorophenyl isocyanate (94 mg, 0.5 mmol, 1.0 eq) was refluxed for 1 h. After the completion of reaction, the mixture was concentrated in vacuo, and the residue was crystallized using ethyl acetated and hexane to afford the desired product **JR-AB2-030** (220 mg, 96%) as white powder: Rf = 0.8 (hexane/ethyl acetate, 2:1, v/v); mp 155-157 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.0 (s, 1H), 7.72 (d, *J* = 2.0 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 9.0 Hz, 1H), 7.30 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 4.45 (dd, *J* = 11.5, 6.5 Hz, 1H), 3.98 (dd, *J* = 11.5, 7.0 Hz, 1H), 3.73 (sext, *J* = 6.5 Hz, 1H), 1.47 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.3, 150.6, 147.7, 137.6, 132.7, 132.4, 130.4, 126.8, 123.3, 121.5, 119.2, 118.3, 55.7, 37.1, 20.1; DART-HRMS found 457.9460 $[M+H]^+$, calcd for $C_{17}H_{14}BrCl_2N_3OS$ 457.9496.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims. Those skilled in the art will also recognize that all combinations of embodiments described herein are within the scope of the invention.

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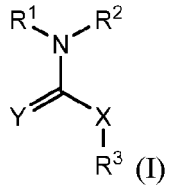
CLAIMS

What is claimed is:

1. A compound having the structure of formula I or a pharmaceutically acceptable salt thereof:



(I)

wherein:

R^1 is aryl, heteroaryl, or heterocyclyl;

R^2 is alkyl, aryl or heteroaryl;

R^3 is alkyl, aryl, or heteroaryl;

X is $C(R^4R^5)$, $N(R^4)$, or O;

Y is S or O;

R^4 and R^5 are independently selected from H or alkyl.

2. The compound of any one of the preceding claims, provided that:

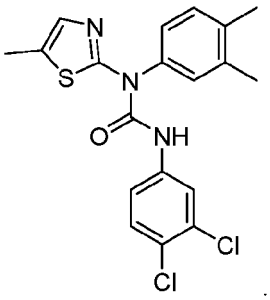
R^1 is not 3-methylisothiazolyl;

R^2 is not 3,4-dimethylphenyl;

R^3 is not 3,4-dichlorophenyl; or

X is not N(H).

3. The compound of any one of the preceding claims, wherein the compound is not



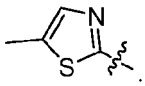
4. The compound of any one of the preceding claims, wherein R^1 is 5-membered heteroaryl or heterocyclyl.

5. The compound of any one of the preceding claims, wherein R^1 is selected from thiazolyl, isothiazolyl, oxazolyl, 4,5-dihydrooxazolyl, 4,5-dihydrothiazolyl, benzothiazolyl, benzoxazolyl, pyridyl, or phenyl.

6. The compound of any one of the preceding claims, wherein R^1 is selected from thiazolyl, oxazolyl, 4,5-dihydrooxazolyl, 4,5-dihydrothiazolyl, benzothiazolyl, benzoxazolyl, pyridyl, or phenyl.

7. The compound of any one of the preceding claims, wherein R^1 is substituted with one or more alkyl.

8. The compound of any one of the preceding claims, wherein R^1 is 3-methylisothiazolyl:



9. The compound of any one of the preceding claims, wherein R^2 is heteroaryl or heterocyclyl.

10. The compound of any one of claims 1-8, wherein R^2 is phenyl.

11. The compound of any one of the preceding claims, wherein R^2 is substituted with one or more R^6; each R^6 is independently selected from $N(R^7R^8)$, alkyl, alkoxy, or halo; and R^7 and R^8 are independently selected from alkyl.

12. The compound of claim 11, wherein R^2 is halophenyl.

13. The compound of claim 12, wherein R^2 is 4-bromophenyl or 4-fluorophenyl.

14. The compound of any one of the preceding claims, wherein R^3 is heteroaryl or heterocyclyl.

15. The compound of any one of the claims 1-13, wherein R^3 is phenyl.

16. The compound of any one of the preceding claims, wherein R^3 is optionally substituted with one or more R^9; each R^9 is independently selected from $N(R^{10}R^{11})$, alkyl, alkoxy, or halo; and R^{10} and R^{11} are independently selected from alkyl.

17. The compound of claim 16, wherein R^3 is halophenyl.

18. The compound of claim 8, wherein R^3 is 3,4-dichlorophenyl.

19. The compound of any one of the preceding claims, wherein X is $N(R^4)$.

20. The compound of any one of the preceding claims, wherein X is N(H).

21. The compound of any one of the preceding claims, wherein Y is O.

22. The compound of any one of claims 1-20, wherein Y is S.

23. A pharmaceutical composition comprising the compound of any one of preceding claims and a pharmaceutically acceptable excipient.

24. Use of a compound or composition of any one of claims 1-23 for inhibiting or preventing the formation of mTORC2 in a cell.

25. The use of claim 24, wherein the compound or composition does not substantially inhibit or prevent the formation of mTORC1 in the cell.

26. Use of a compound or composition of any one of claims 1-23 for treating a mammal suffering from cancer.

27. The use of claim 26, wherein the cancer is glioblastoma.

28. A method of inhibiting or preventing the formation of mTORC2 in a cell, comprising contacting the cell with a compound or composition of any one of claims 1-23.

29. The method of inhibiting or preventing the formation of mTORC2 in a cell of claim 28, wherein the formation of mTORC1 in the cell is not substantially inhibited or prevented.

30. A method of treating a mammal suffering from cancer, comprising administering a compound or composition of any one of claims 1-23.

31. The method of claim 30, wherein the cancer is glioblastoma.

DRAWINGS

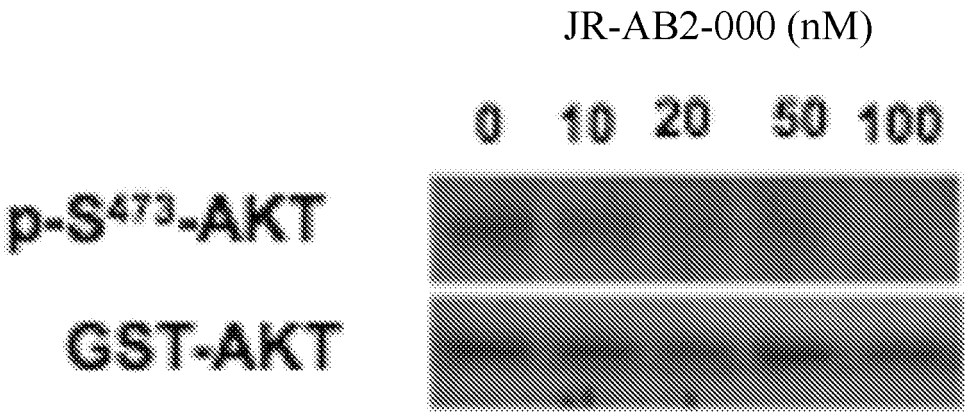


FIG. 1A

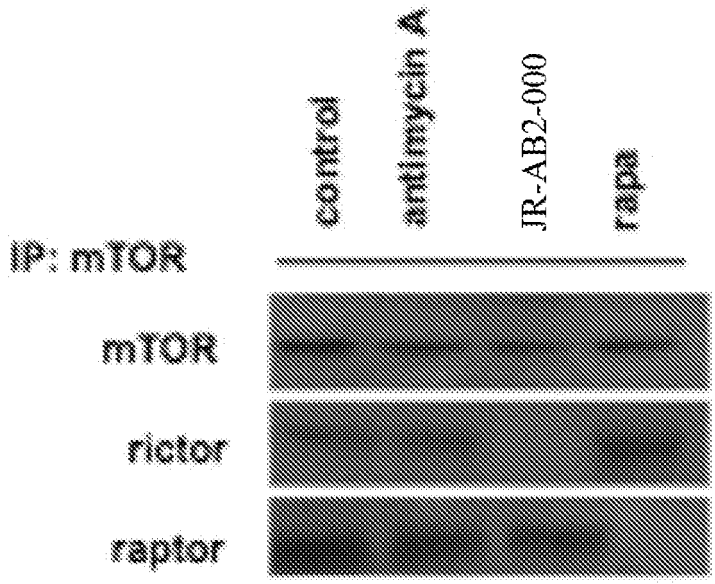


FIG. 1B

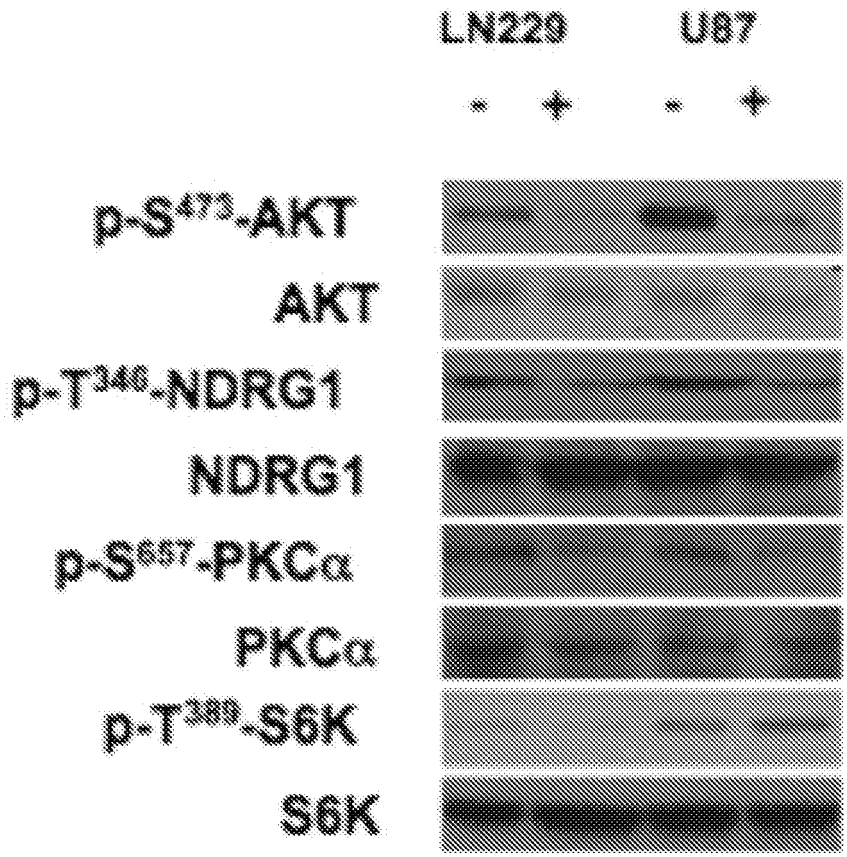

LN229
U87
-
+
-
+
p-S473-AKT
AKT
p-T346-NDRG1
NDRG1
p-S657-PKCα
PKCα
p-T389-S6K
S6K

FIG. 1C

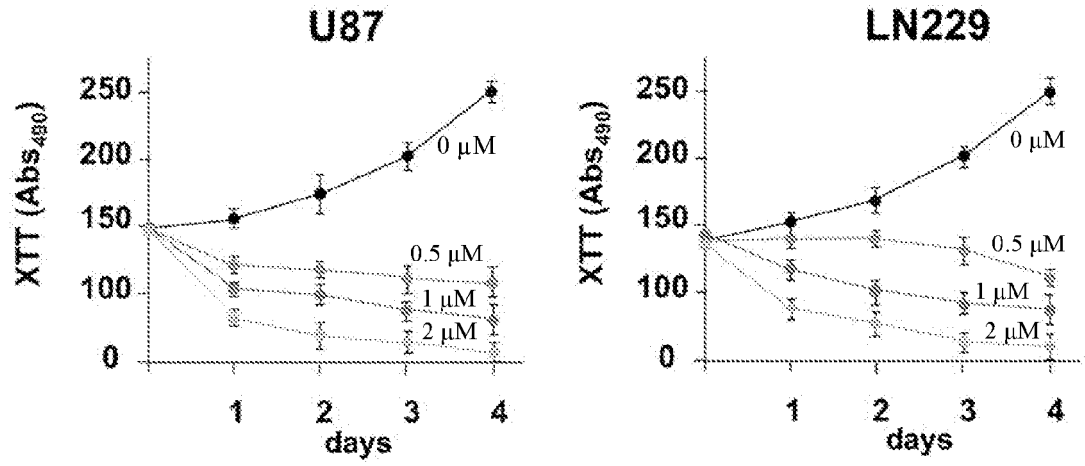


FIG. 2A

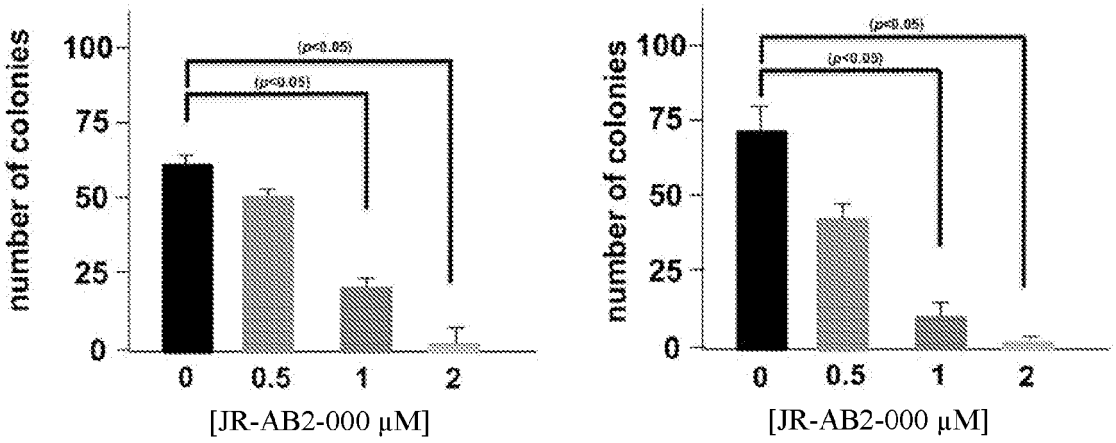


FIG. 2B

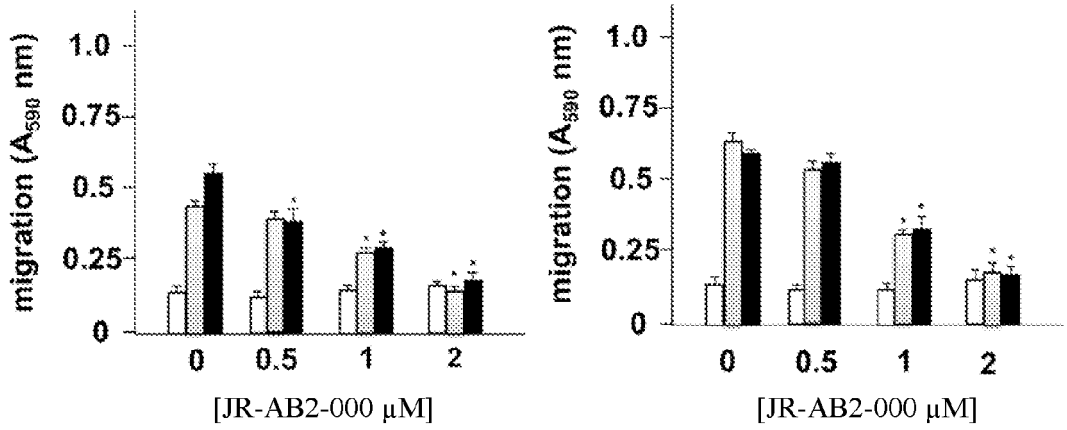


FIG. 2C

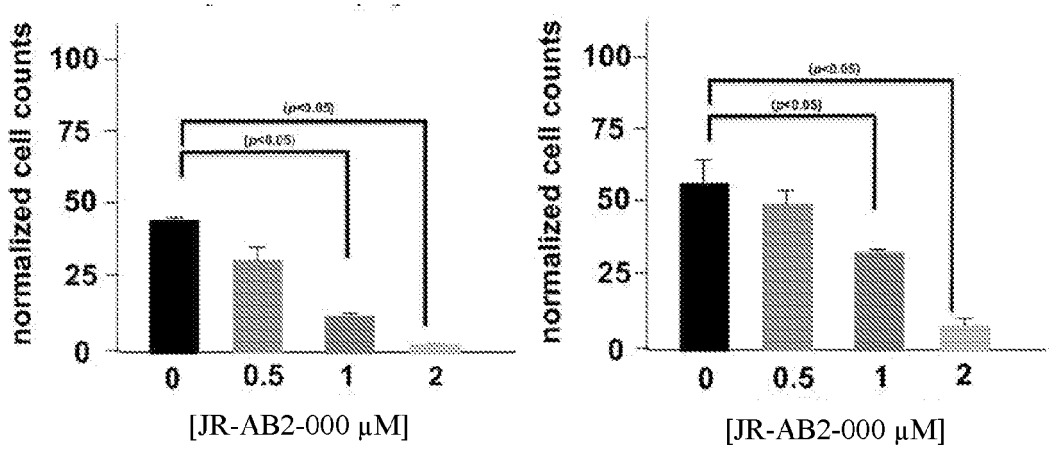


FIG. 2D

S

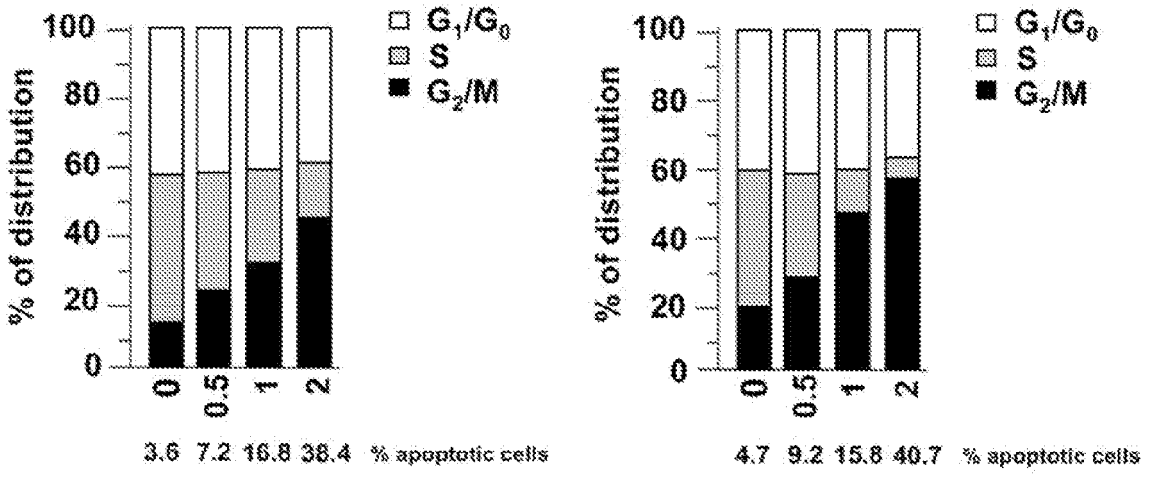


FIG. 2E

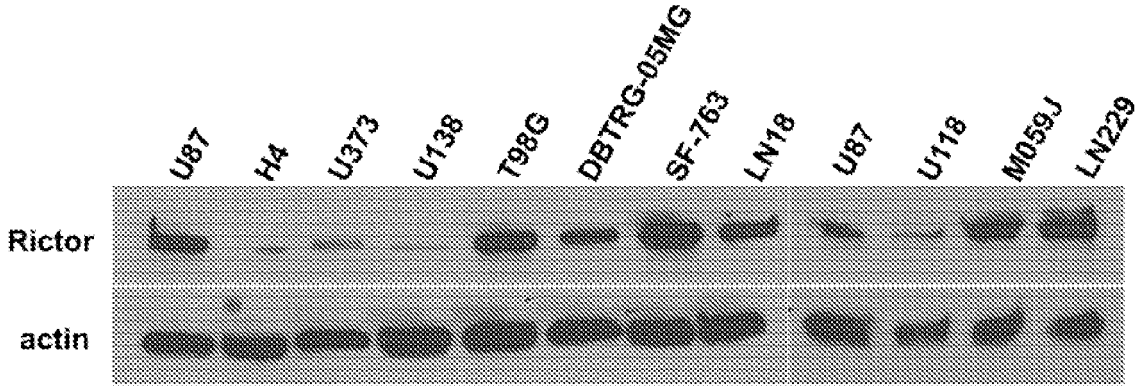

U87
H4
U373
U138
T98G
DBTRG-05MG
SF-763
LN18
U87
U118
M059J
LN229
Rictor
actin

FIG. 3A

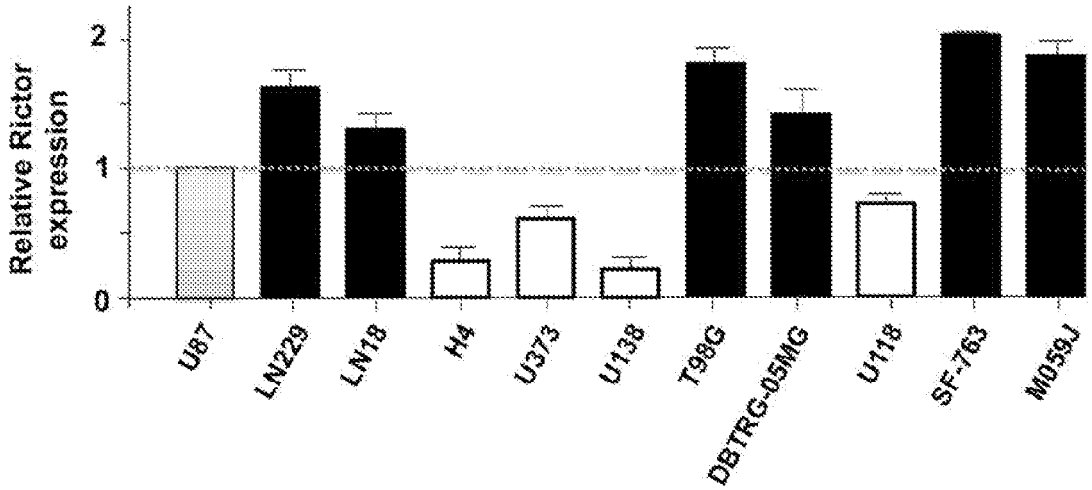

Relative Rictor expression
0
1
2
U87
LN229
LN18
H4
U373
U138
T98G
DBTRG-05MG
U118
SF-763
M059J

FIG. 3B

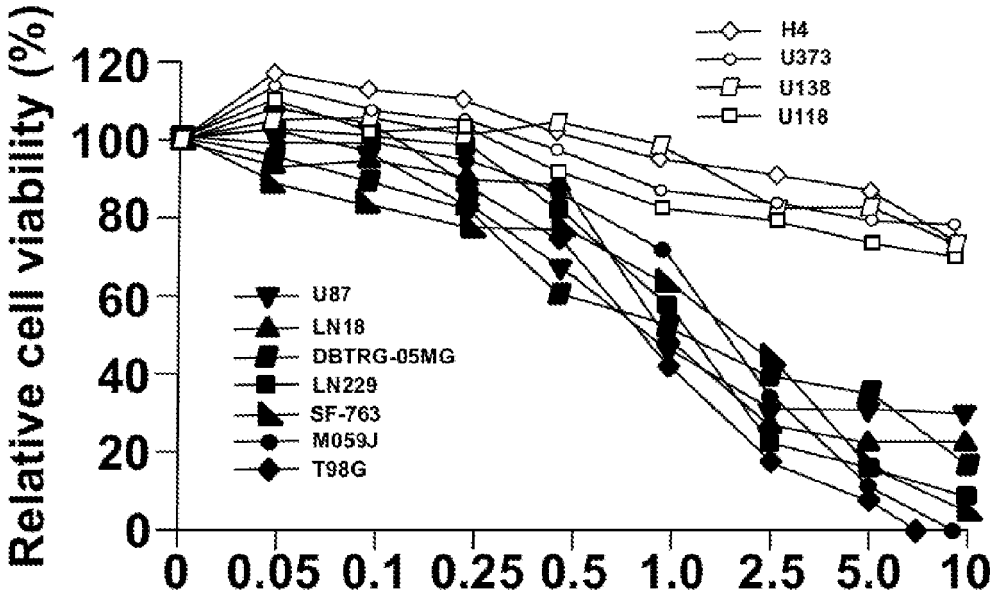

Relative cell viability (%)
120
100
80
60
40
20
0
0 0.05 0.1 0.25 0.5 1.0 2.5 5.0 10
[JR-AB2-000 μM]
H4
U373
U138
U118
U87
LN18
DBTRG-05MG
LN229
SF-763
M059J
T98G

FIG. 3C

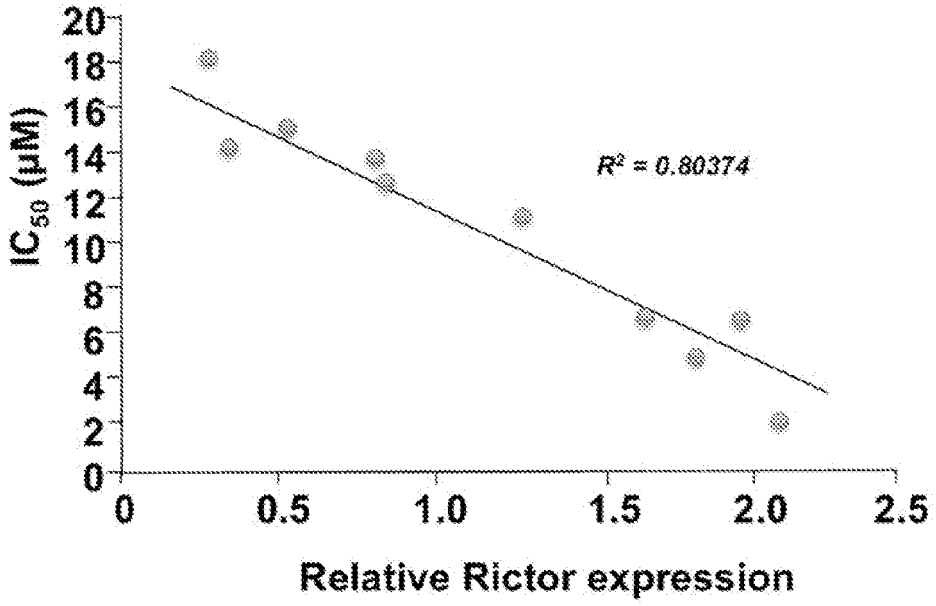

IC50 (μM)
20
18
16
14
12
10
8
6
4
2
0
0 0.5 1.0 1.5 2.0 2.5
Relative Rictor expression
R² = 0.80374

FIG. 3D

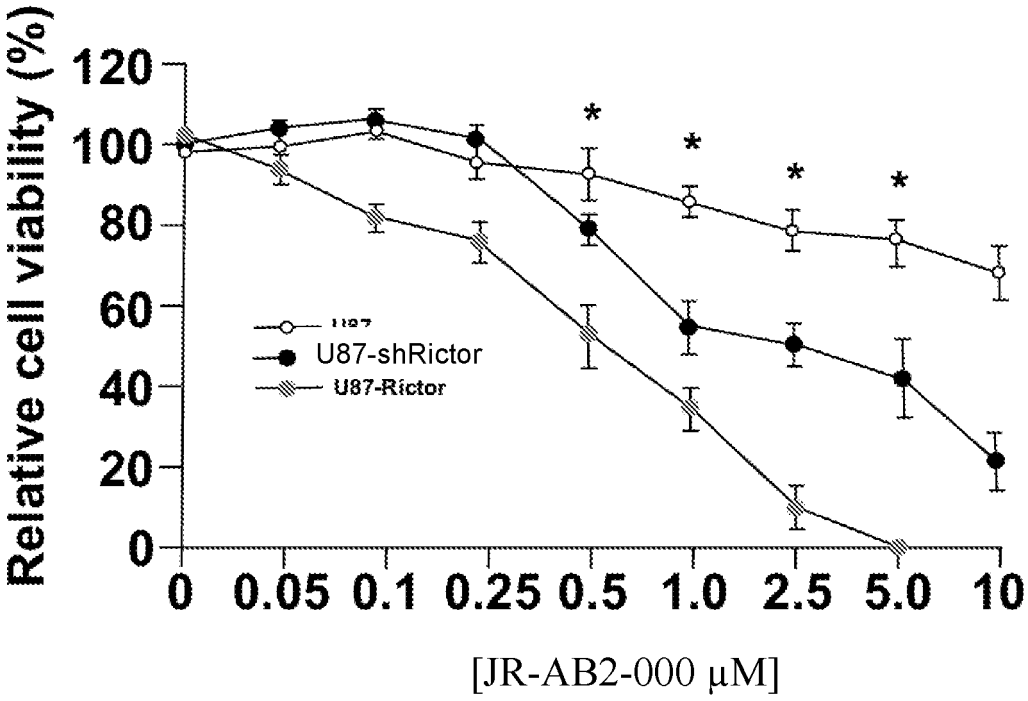

Relative cell viability (%)
120
100
80
60
40
20
0
U87-shRictor
U87-Rictor
*
*
*
*
0 0.05 0.1 0.25 0.5 1.0 2.5 5.0 10
[JR-AB2-000 μM]

FIG. 3E

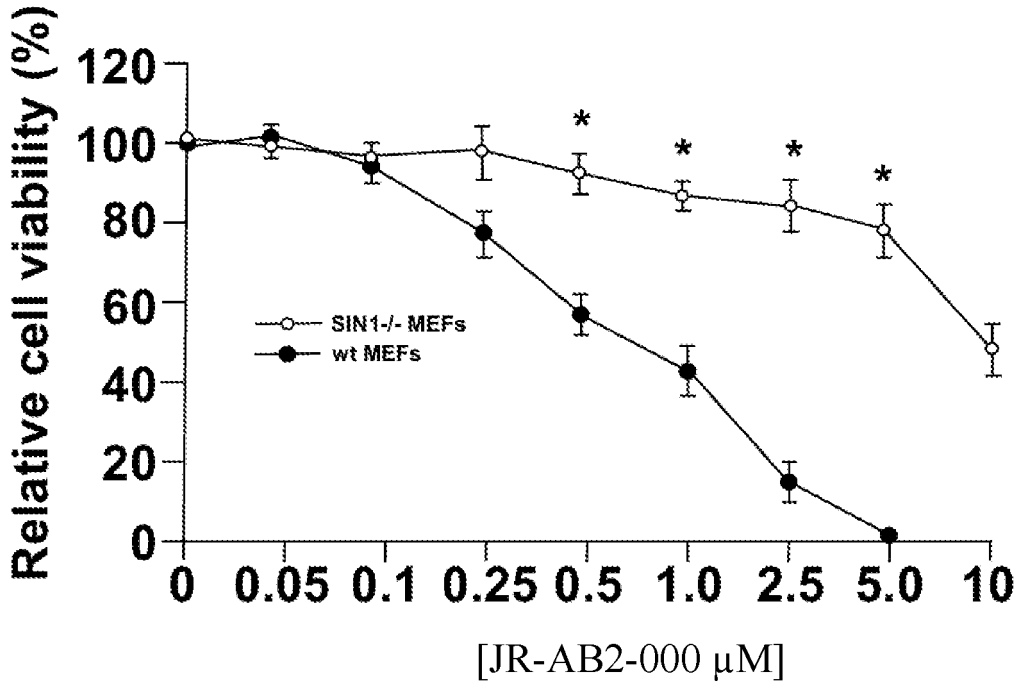

Relative cell viability (%)
120
100
80
60
40
20
0
SIN1-/- MEFs
wt MEFs
*
*
*
*
0 0.05 0.1 0.25 0.5 1.0 2.5 5.0 10
[JR-AB2-000 μM]

FIG. 3F

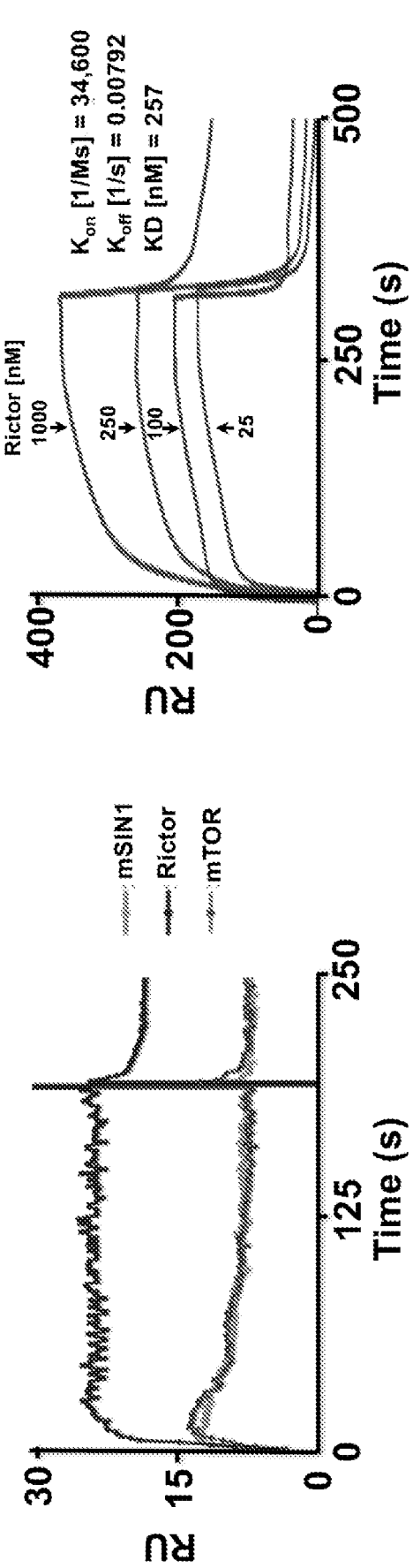

Rictor [nM]
1000
250
100
25
K_{on} [1/Ms] = 34,600
K_{off} [1/s] = 0.00792
KD [nM] = 257
RU
Time (s)
mSIN1
Rictor
mTOR

FIG. 4

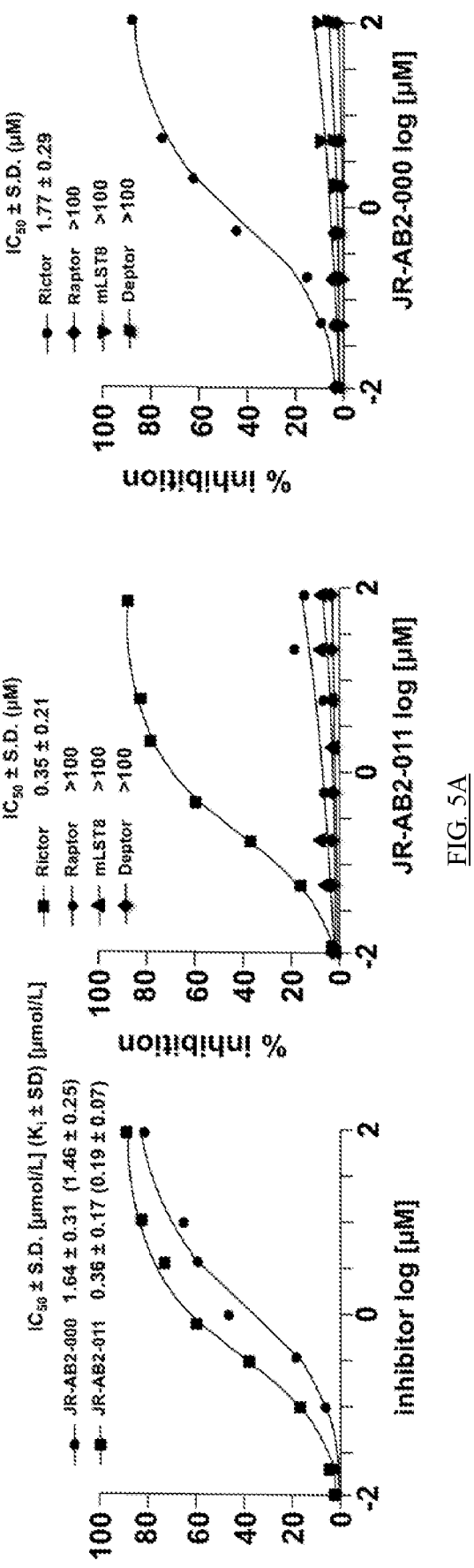

IC_{50} ± S.D. [µmol/L] (K_i ± SD) [µmol/L]
JR-AB2-000 1.64 ± 0.31 (1.46 ± 0.25)
JR-AB2-011 0.36 ± 0.17 (0.19 ± 0.07)
% inhibition
inhibitor log [µM]
IC_{50} ± S.D. (µM)
Rictor 0.35 ± 0.21
Raptor >100
mLST8 >100
Deptor >100
JR-AB2-011 log [µM]
IC_{50} ± S.D. (µM)
Rictor 1.77 ± 0.29
Raptor >100
mLST8 >100
Deptor >100
JR-AB2-000 log [µM]

FIG. 5A

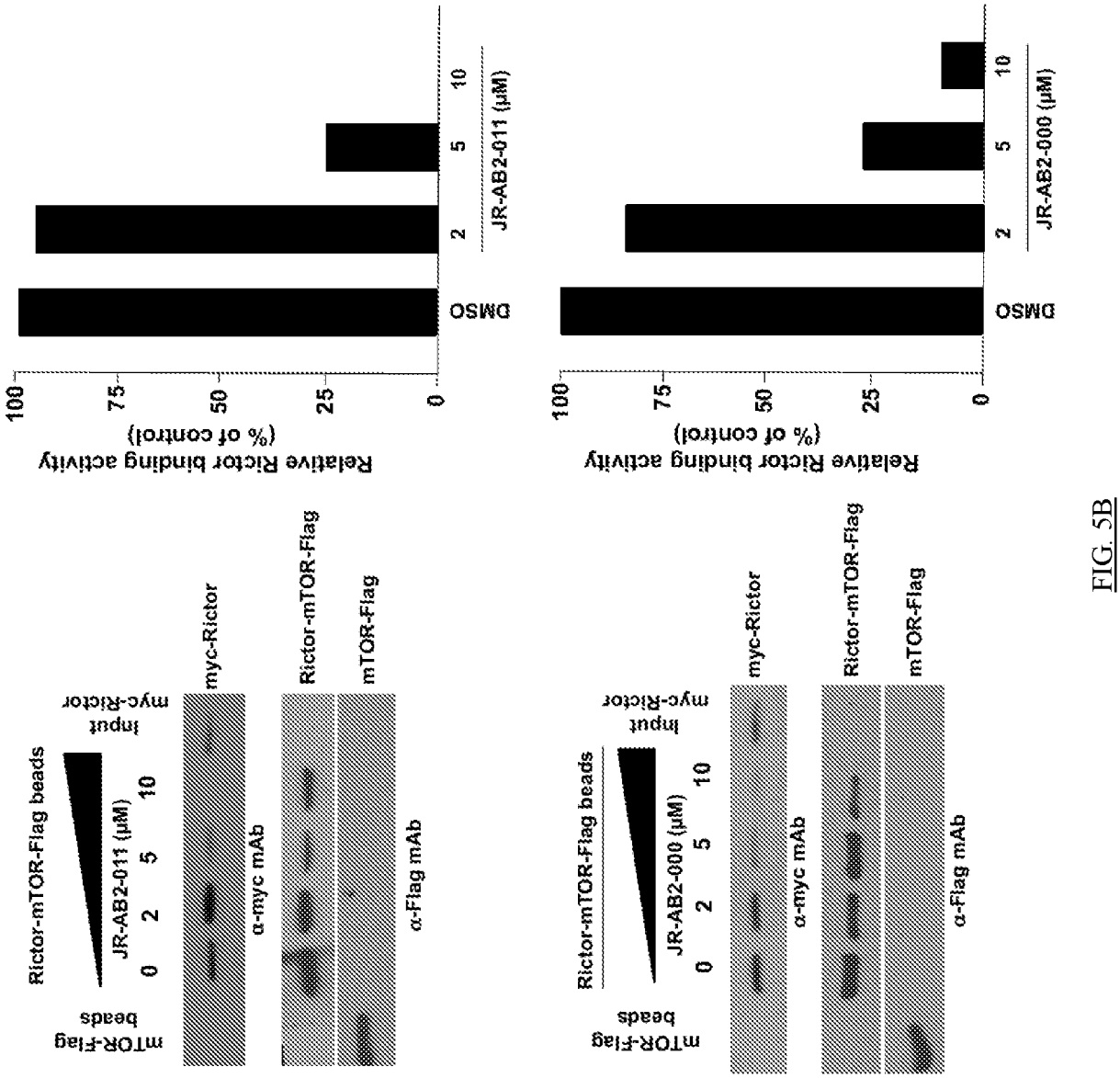

Relative Rictor binding activity
(% of control)
100
75
50
25
0
DMSO
2
5
10
JR-AB2-011 (μM)
JR-AB2-000 (μM)
Rictor-mTOR-Flag beads
mTOR-Flag beads
input
myc-Rictor
myc-Rictor
Rictor-mTOR-Flag
mTOR-Flag
α-myc mAb
α-Flag mAb

FIG. 5B

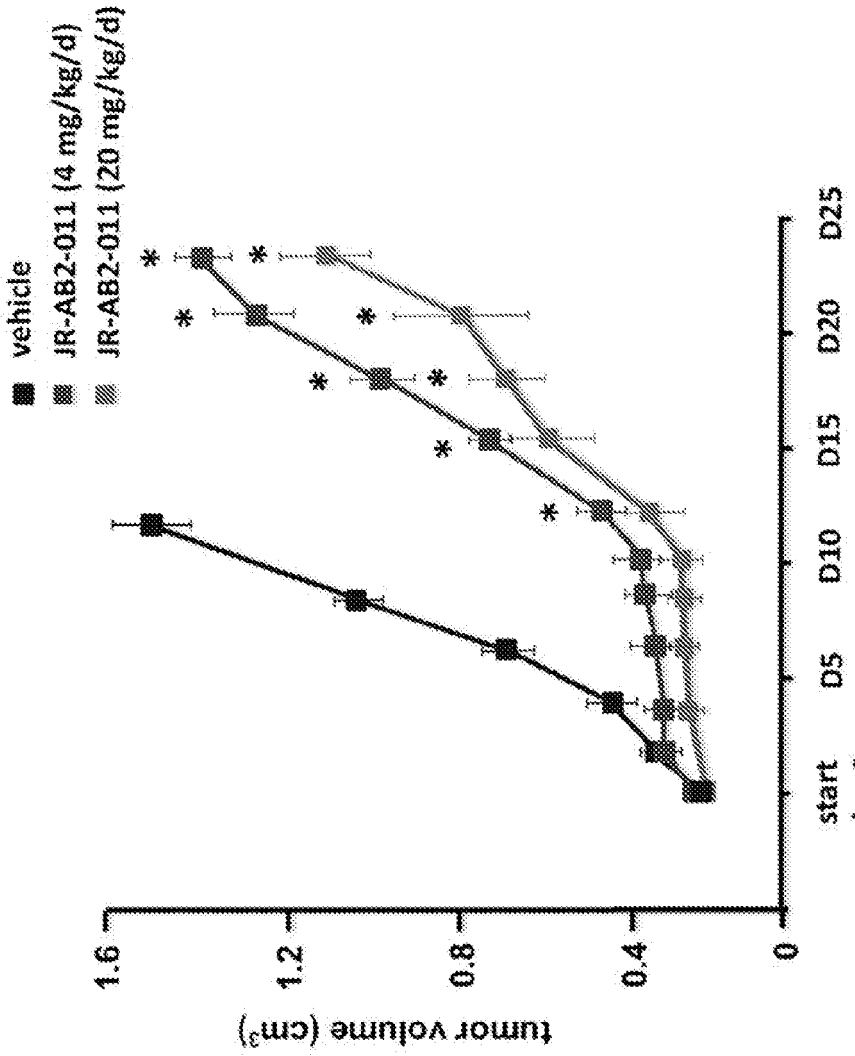

vehicle
JR-AB2-011 (4 mg/kg/d)
JR-AB2-011 (20 mg/kg/d)
tumor volume (cm³)
1.6
1.2
0.8
0.4
0
start
day 0
D5
D10
D15
D20
D25
*

FIG. 6A

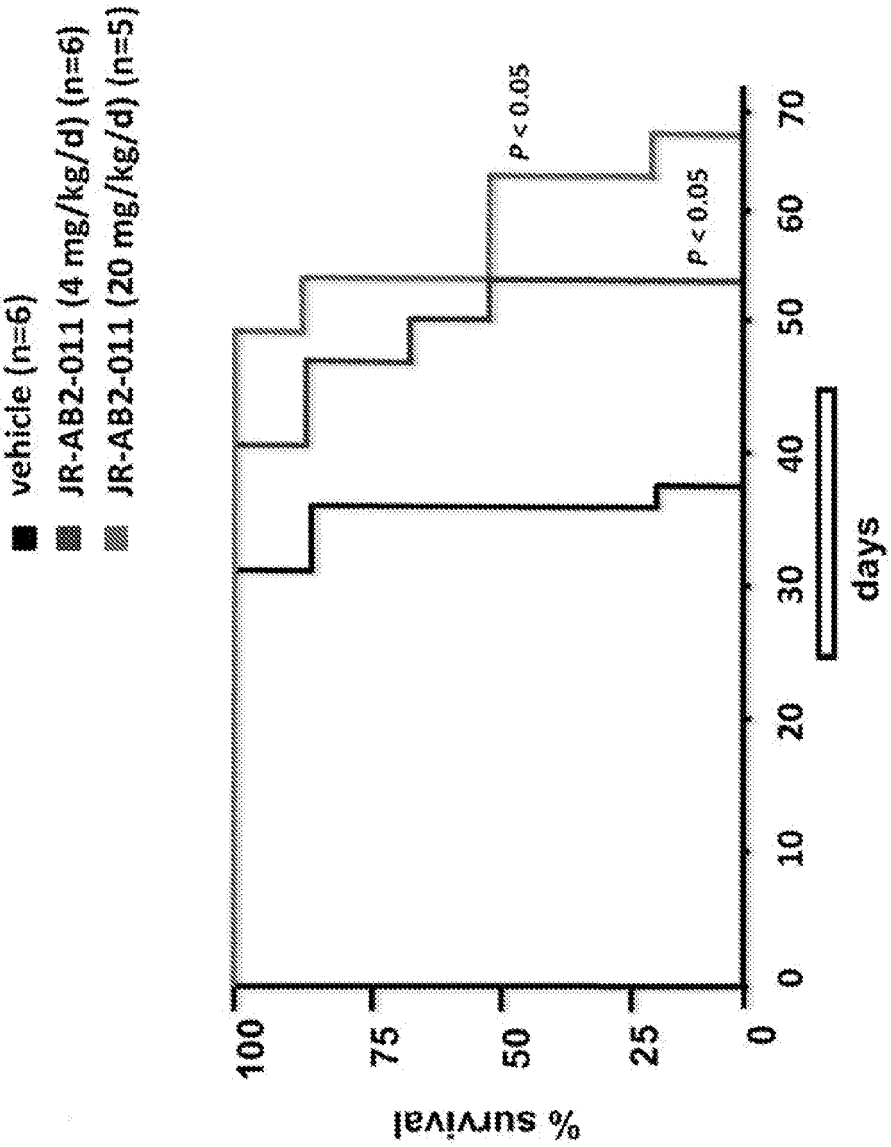

vehicle (n=6)
JR-AB2-011 (4 mg/kg/d) (n=6)
JR-AB2-011 (20 mg/kg/d) (n=5)
P < 0.05
P < 0.05
% survival
100
75
50
25
0
0
10
20
30
40
50
60
70
days

FIG. 6B

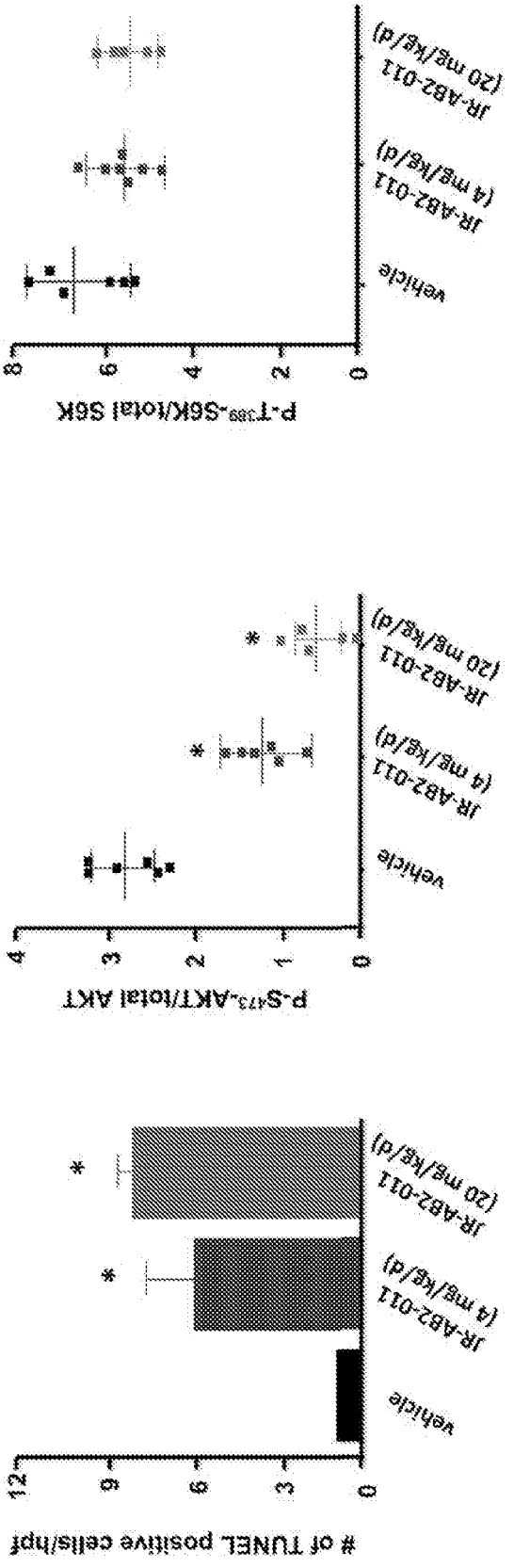

P-T389-S6K/total S6K
8
6
4
2
0
vehicle
JR-AB2-011 (4 mg/kg/d)
JR-AB2-011 (20 mg/kg/d)
P-S473-AKT/total AKT
4
3
2
1
0
vehicle
JR-AB2-011 (4 mg/kg/d)
JR-AB2-011 (20 mg/kg/d)
# of TUNEL positive cells/hpf
12
9
6
3
0
vehicle
JR-AB2-011 (4 mg/kg/d)
JR-AB2-011 (20 mg/kg/d)

FIG. 6C

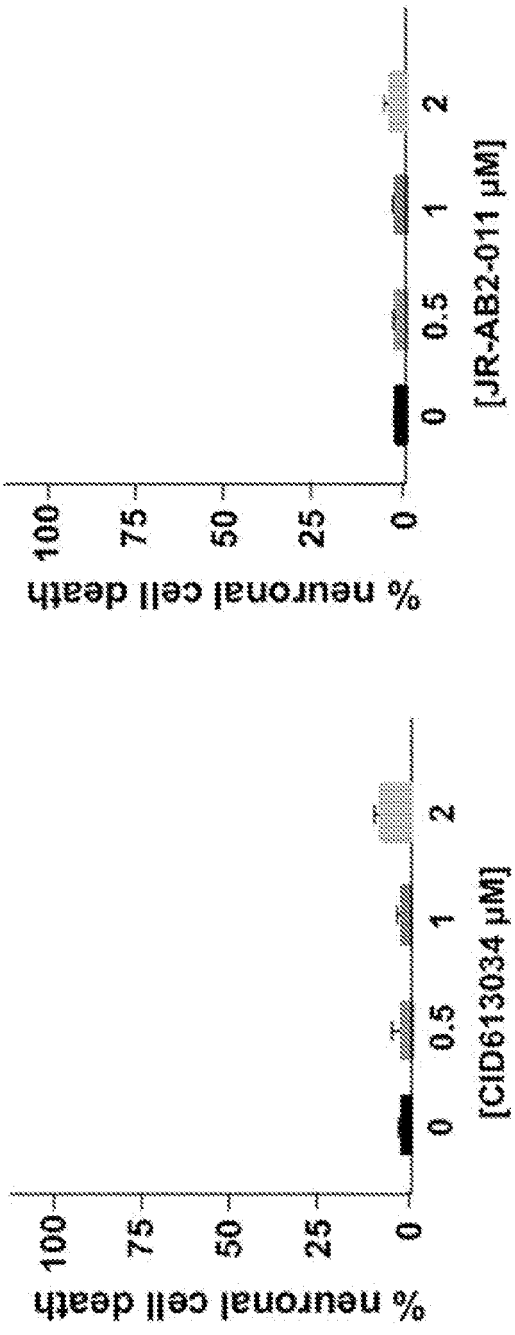

% neuronal cell death
100
75
50
25
0
0
0.5
1
2
[CID613034 µM]
% neuronal cell death
100
75
50
25
0
0
0.5
1
2
[JR-AB2-011 µM]

FIG. 7

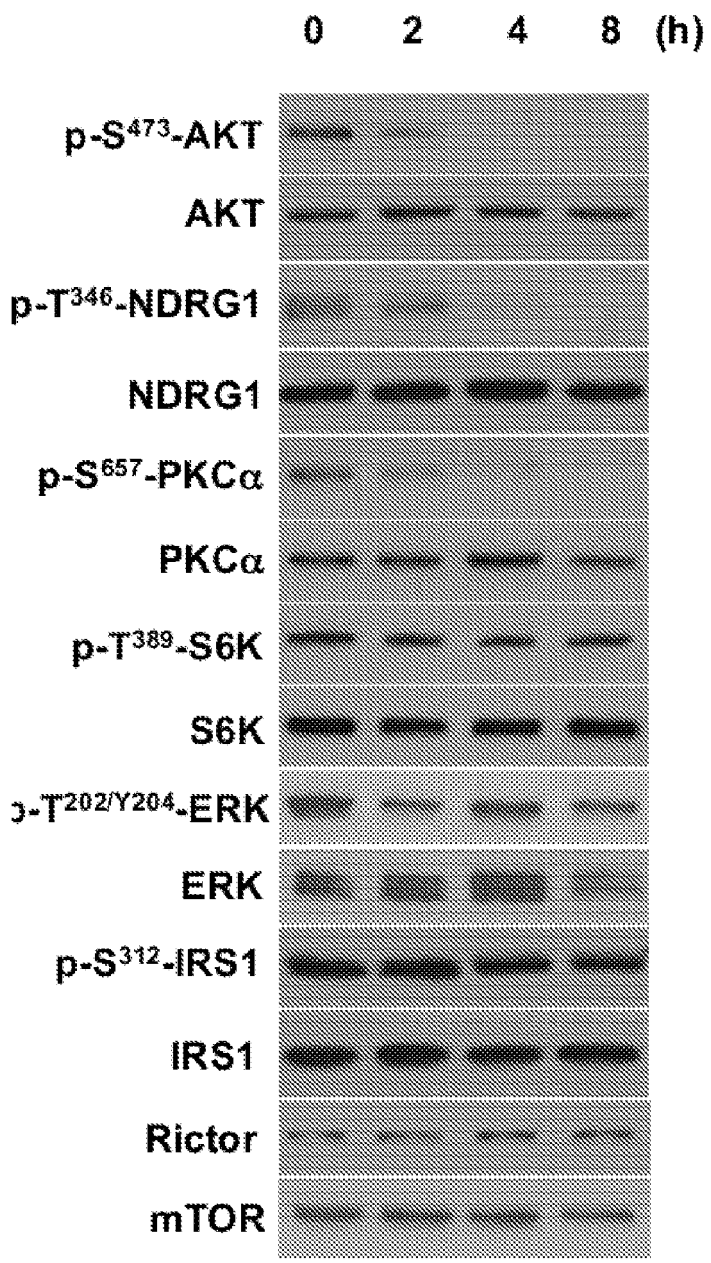

0 2 4 8 (h)
p-S473-AKT
AKT
p-T346-NDRG1
NDRG1
p-S657-PKCα
PKCα
p-T389-S6K
S6K
p-T202/Y204-ERK
ERK
p-S312-IRS1
IRS1
Rictor
mTOR

FIG. 8A

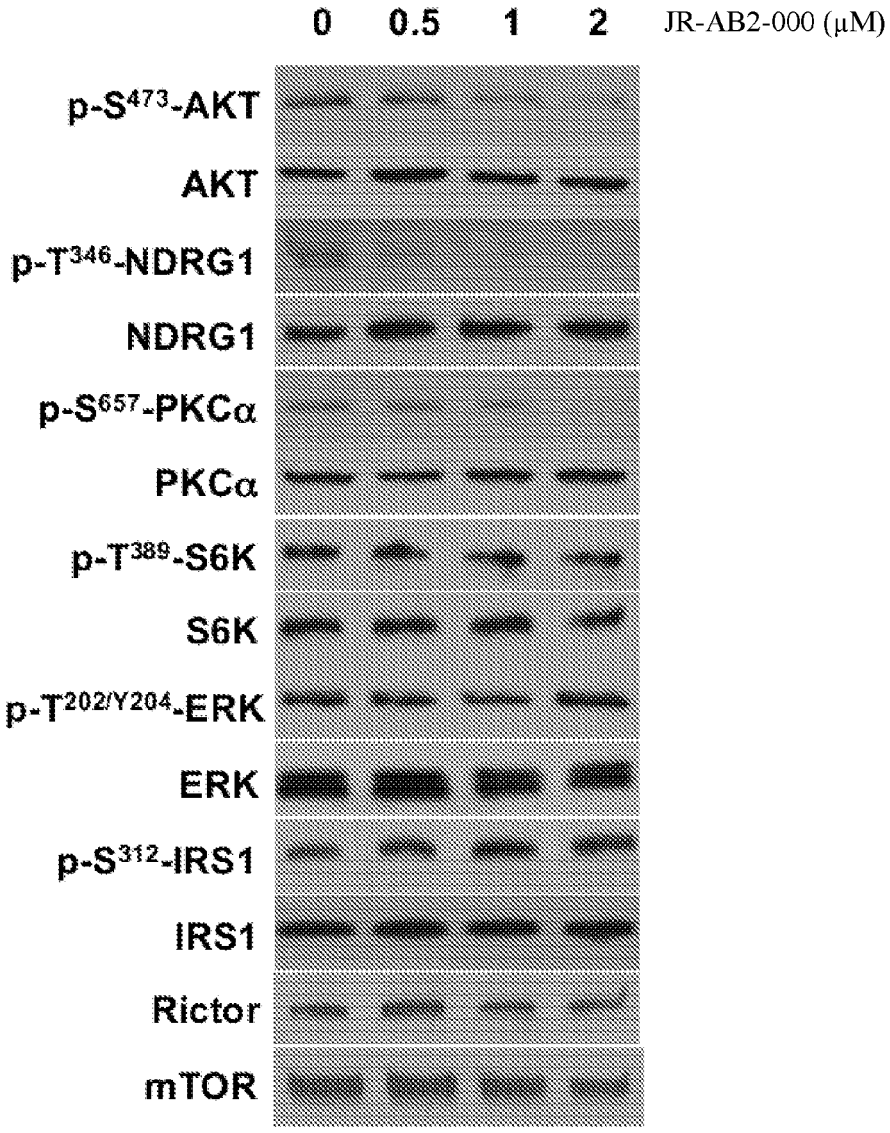

0
0.5
1
2
JR-AB2-000 (μM)
p-S473-AKT
AKT
p-T346-NDRG1
NDRG1
p-S657-PKCα
PKCα
p-T389-S6K
S6K
p-T202/Y204-ERK
ERK
p-S312-IRS1
IRS1
Rictor
mTOR

FIG. 8B

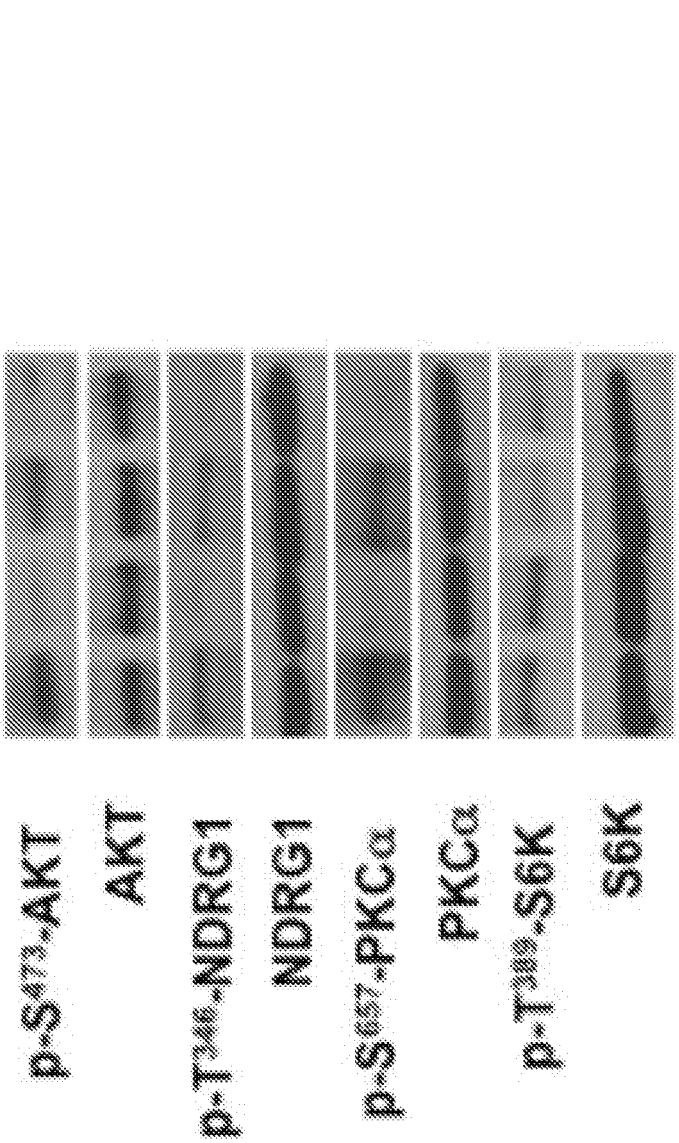

U87
LN229
JR-AB2-011 (1 μM)
p-S473-AKT
AKT
p-T346-NDRG1
NDRG1
p-S657-PKCα
PKCα
p-T389-S6K
S6K

FIG. 9

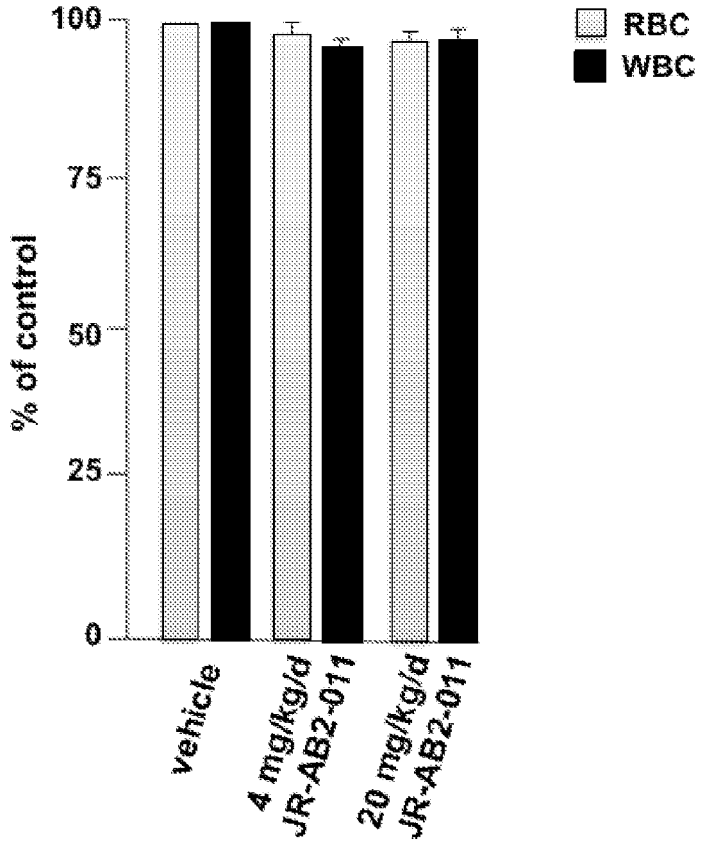

100
75
50
25
0
% of control
RBC
WBC
vehicle
4 mg/kg/d JR-AB2-011
20 mg/kg/d JR-AB2-011

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/026005

A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (2018.01) C07D 277/18, C07D 263/26, C07D 277/38, C07D 277/82, C07D 213/75, C07C 275/30, A61K 31/426, A61K 31/428, A61K 31/42, A61K 31/44, A61K 31/17, A61P 35/00

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

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

Databases consulted: Google Patents, CAPLUS, BIOSIS, EMBASE, MEDLINE, MARPAT, REGISTRY, PubMed, Google Scholar, DWPI
Search terms used: CID613034, mTOR*, mTORC2, inhib*, RICTOR (Rapamycin-insensitive companion of mammalian target of rapamycin), cancer, *proliferat*, glioblastom*, glioblastoma multiforme, GBM, tumor, neoplas*.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CAS Registry Number: 339352-38-4; CA Index Name: Urea, N'-(3,4-dichlorophenyl)-N-(4,5-dihydro-5-methyl-2-thiazolyl)-N-(3,4-dimethylphenyl)-; Entered STN: 05 Jun 2001. Also see PubChem CID: 613034, URL: <https://pubchem.ncbi.nlm.nih.gov/compound/613034>. 05 Jun 2001 (2001/06/05)	1-7,9-11,15-21
X	CAS Registry Number: 329182-61-8; CA Index Name: Urea, N'-(3,4-dichlorophenyl)-N-(4,5-dihydro-5-methyl-2-thiazolyl)-N-(4-fluorophenyl)-; Entered STN: 28 Mar 2001. 28 Mar 2001 (2001/03/28)	1-7,9-13,15-21
X	CAS Registry Number: 339352-39-5; CA Index Name: Urea, N'-(3,4-dichlorophenyl)-N-(4,5-dihydro-5-methyl-2-thiazolyl)-N-phenyl-; Entered STN: 05 Jun 2001. 05 Jun 2001 (2001/06/05)	1-7,9-11,15-21

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 Jul 2018	24 Jul 2018
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer SOMECH Erez Telephone No. 972-2-5651762

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International application No.
PCT/US2018/026005

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PubChem CID: 383613 (Create Date: 2005-03-26), URL: <https://pubchem.ncbi.nlm.nih.gov/compound/383613>. See Compound Name and Biological Test Results, specifically PubChem AID: 103 "NCI human tumor cell line growth inhibition assay. Data for the SK-OV-3 Ovarian cell line", Deposit Date: 2004-08-15 (also published in URL: <https://pubchem.ncbi.nlm.nih.gov/bioassay/103>). 26 Mar 2005 (2005/03/26) See PubChem CID: 383613 and the test results for this compound on bioassay PubChem AID: 103.	1-7,9-11,15-21,23, 26,30
Y		1-31
X	CAS Registry Number: 339352-36-2; CA Index Name: Urea, N'-(3,4-dichlorophenyl)-N-(4,5-dihydro-5-methyl-2-thiazolyl)-N-(3-methylphenyl)-; Entered STN: 05 Jun 2001. 05 Jun 2001 (2001/06/05)	1-7,9-11,15-21
X	PubChem CID: 383614 (Create Date: 2005-03-26), URL: <https://pubchem.ncbi.nlm.nih.gov/compound/383614>. See Compound Name and Biological Test Results, specifically PubChem AID: 25 "NCI Human Tumor Cell Line Growth Inhibition Assay. Data For The M14 Melanoma Cell Line", Deposit Date: 2004-08-15 (also published in URL: <https://pubchem.ncbi.nlm.nih.gov/bioassay/25>). 26 Mar 2005 (2005/03/26) See PubChem CID: 383614 and the test results for this compound on bioassay PubChem AID: 25.	1-7,9-11,15-21,23, 26,30
Y		1-31
X	CAS Registry Number: 312514-09-3; CA Index Name: Urea, N-(4,5-dihydro-5-methyl-2-thiazolyl)-N-(3,4-dimethylphenyl)-N'-phenyl-; Entered STN: 02 Jan 2001. 02 Jan 2001 (2001/01/02)	1-7,9-11,15,16, 19-21
X	PubChem CID: 383612 (Create Date: 2005-03-26), URL: <https://pubchem.ncbi.nlm.nih.gov/compound/383612>.See Compound Name and Biological Test Results, specifically PubChem AID: 25 "NCI Human Tumor Cell Line Growth Inhibition Assay. Data For The M14 Melanoma Cell Line", Deposit Date: 2004-08-15 (also published in URL: <https://pubchem.ncbi.nlm.nih.gov/bioassay/25>). 26 Mar 2005 (2005/03/26) See PubChem CID: 383612 and the test results for this compound on bioassay PubChem AID: 25.	1-7,15-21,23,26,30
Y		1-31
X	PubChem CID: 383609 (Create Date: 2005-03-26), URL: <https://pubchem.ncbi.nlm.nih.gov/compound/383609>. See Compound Name and Biological Test Results, specifically PubChem AID: 71 "NCI human tumor cell line growth inhibition assay. Data for the HCT-15 Colon cell line", Deposit Date: 2004-08-15 (also published in URL: <https://pubchem.ncbi.nlm.nih.gov/bioassay/71>). 26 Mar 2005 (2005/03/26) See PubChem CID: 383609 and the test results for this compound on bioassay PubChem AID: 71.	1-7,15-17,19-21,23, 26,30
Y		1-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/026005

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/138686 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US]) 02 Dec 2010 (2010/12/02) Claim 2, compound NSC672027, and page 40.	1-7,15-17,19-21,23, 26,27,30,31
Y		1-31
X	PubChem CID: 383608 (Create Date: 2005-03-26), URL: <https://pubchem.ncbi.nlm.nih.gov/compound/383608>. See Compound Name and Biological Test Results, specifically PubChem AIDs: 85, 87, 121, 155, 157, 161, 165, 167 and 175, Deposit Date: 2004-08-15 (each). 26 Mar 2005 (2005/03/26) See PubChem CID: 383608 and the test results for this compound on bioassay PubChem AIDs: 85,87,121,155,157,161,165,167,175.	1-7,9-11,19-21,23, 26,30
Y		1-31
X	WO 2010/075282 A1 (UNIVERSITY OF WASHINGTON [US]) 01 Jul 2010 (2010/07/01) Claim 5 and the last compound on page 57.	1-7,9-11,15,16, 19-21,23,26,30
Y		1-31
X	WO 2005/058842 A1 (LABORATOIRE THERAMEX [MC]) 30 Jun 2005 (2005/06/30) Examples 24, 25, 29 and 34	1-6,9,10,14-17,21, 23,26,30
Y		1-31
A	Holmes, B. et al. Mechanistic target of rapamycin (mTOR) inhibition synergizes with reduced internal ribosome entry site (IRES)-mediated translation of Cyclin D1 and c-MYC mRNAs to treat glioblastoma. Journal of Biological Chemistry (01 July 2016), Vol. 291, No. 27, pages: 14146-14159. 01 Jul 2016 (2016/07/01) The whole document.	1-31
X	CAS Registry Number: 371932-74-0; CA Index Name: Thiourea, N-(3-chloro-4-fluorophenyl)-N-(4,5-dihydro-5-methyl-2-thiazolyl)-N'-phenyl-; Entered STN: 27 Nov 2001. 27 Nov 2001 (2001/11/27)	1-7,9-12,15,16,19, 20,22
X	WO 2007/070568 A2 (DEKK-TEC INC. [US]) 21 Jun 2007 (2007/06/21) Claims 1, 5, 23, 24 and 44; pages 13 and 20, compounds DM-MPC-PEN and DM-DPC-PEN.	1-3,5,6,10,14,16,21, 23,26,27,30,31
Y		1-31
X	GB 1548397 A (LILLY INDUSTRIES LTD [GB]) 11 Jul 1979 (1979/07/11) Ex. 2, 4, 5, 19, 26, 27, 30 and 32.	1-8,11,16,21,23
X	CAS Registry Number: 355156-98-8; CA Index Name: Urea, N-(5-methyl-2-thiazolyl)-N,N'-diphenyl-; Entered STN: 07 Sep 2001. 07 Sep 2001 (2001/09/07)	1-11,15,16,19-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/026005

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	BENAVIDES-SERRATO, Angelica, et al. Specific blockade of Rictor-mTOR association inhibits mTORC2 activity and is cytotoxic in glioblastoma. PloS one (28 April 2017), 12.4: e0176599. Retrieved from URL: <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0176599>. 28 Apr 2017 (2017/04/28) The whole document.	1-31

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2018/026005

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
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WO 2010/075282 A1	01 Jul 2010	WO 2010075282 A1	01 Jul 2010
		US 2012040916 A1	16 Feb 2012
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International application No.
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/026005

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (2018.01) C07D 277/18, C07D 263/26, C07D 277/38, C07D 277/82, C07D 213/75, C07C 275/30, A61K 31/426, A61K 31/428, A61K 31/42, A61K 31/44, A61K 31/17, A61P 35/00