PREPARATION OF COMPOUND AND CRYSTALLINE FORMS THEREOF

FIELD OF THE INVENTION

The present invention belongs to the field of medicinal chemistry, and more specifically provides a preparation method of a therapeutic compound and its crystallized form.

BACKGROUND

TEA domain transcription factors (TEAD proteins) are effectors for oncogenic YAP/TAZ signaling in cancers with deregulated Hippo tumor suppressor pathway signaling. The interaction between Yesl Associated Transcriptional Regulator (YAP1, aka YAP), WW domain-containing Transcription Regulator 1 (WWTR1, aka TAZ), a transcriptional coactivator paralog to YAP, and TEAD proteins was demonstrated both *in vitro* and *in vivo*. In both cases, the interaction of the proteins leads to increased TEAD transcriptional activity and expression of genes involved in proliferation, survival, angiogenesis, and other hallmarks of cancer. In normal tissues, intact Hippo signaling via MST1/2 and LATS1/2 kinases results in phosphorylation of YAP/TAZ, sequestration in the cytoplasm and proteosomal degradation. Inactivation of Hippo signaling as a result of somatic mutations in NF2, MST or LATS stabilizes YAP/TAZ and leads to nuclear translocation, association with TEAD, and activation of TEAD target genes. Genomic amplification of YAP/TAZ, or gene fusions resulting in constitutively active YAP/TAZ, are alternative mechanisms of Hippo pathway deregulation. In addition, activation of YAP/TAZ signaling has been described as a mechanism of resistance to other targeted cancer therapies, such as inhibitors of EGFR and MEK.

Accordingly, inhibitors of TEAD1, 2, 3 and/or 4, can have great potential in the application of cancer therapy, both as monotherapy in cancers with deregulated Hippo pathway signaling and as combination therapy with other targeted agents.

The compound (*R, E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one as a covalent inhibitor of YAP/TAZ-TEAD oncoproteins is described in patent application No. PCT/US23/23957.

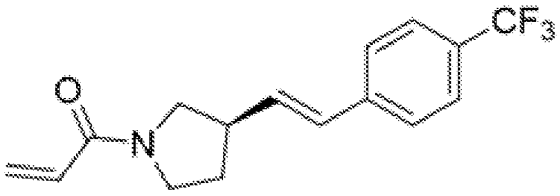
SUBSTITUTE SHEET (RULE 26)

DETAILED DESCRIPTION

It is to be understood that the embodiments disclosed herein are not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting.

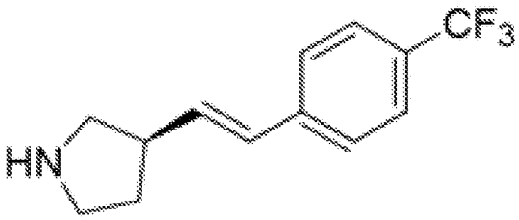
The present application provides a preparation method of compound (*R*, *E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one and its crystalline form.

Embodiment 1. A method for preparing a compound of formula I,



formula I,

wherein comprising the step of reacting a compound of formula 7



with prop-2-enoyl chloride or acrylic anhydride in an organic solvent with a base reagent.

Embodiment 2. the method of embodiment 1, wherein reaction conditions for preparing the compound of formula I are:

1) an organic solvent is selected from the group consisting of CH_2Cl_2, THF, 2-Methyl THF, 1,4-dioxane or ACN;

2) a base reagent is selected from the group consisting of $NaHCO_3$, Na_2CO_3, K_2CO_3, $KHCO_3$, Et_3N, iPr_2NEt, Pyridine, or N-Methyl morpholine;

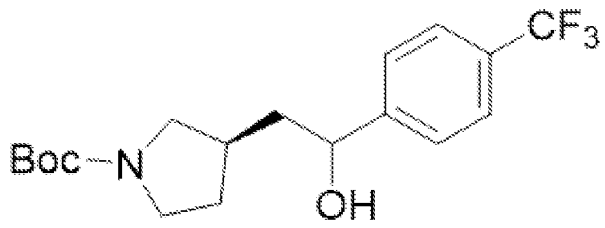
3) reaction time: 1 to 24 hours;

4) reaction temperature: 0 to 25°C.

Embodiment 3. The method of embodiment 1 or 2, wherein the compound of formula 7 is obtained by removing an amino protecting group.

Embodiment 4. The method of embodiment 3, wherein the amino protecting group is selected from the group consisting of the group consisting of 2-(trimethylsilyl)ethoxycarbonyl (Teoc), 1-methyl-1-(4- biphenyl)ethoxycarbonyl (Bpoc), tert-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), and benzyloxycarbonyl (Cbz).

Embodiment 5. The method of embodiment 3, wherein the compound of formula 7 is obtained by removing of the amino protecting group and dehydration of the compound of formula 6,



formula 6.

Embodiment 6. The method of embodiment 5, wherein reaction conditions for preparing the compound of formula 7 are:

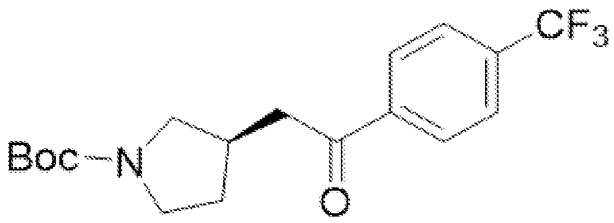
1) a solvent is selected from the group consisting of 2-Me-THF or 1,4-dioxane;

2) reaction time: 1 to 10 hours;

3) reaction temperature: 80 to 100 °C;

4) equivalents of H_2SO_4: 1 to 10 eq.

Embodiment 7. The method of embodiment 5, wherein the compound of formula 6 is obtained by reducing the compound of formula 5,



formula 5.

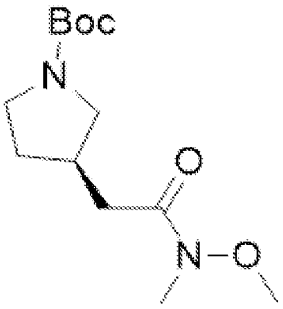
Embodiment 8. The method of embodiment 7, wherein reaction conditions for preparing the compound of formula 6 are:

1) a solvent is selected from the group consisting of MeOH, EtOH or THF;

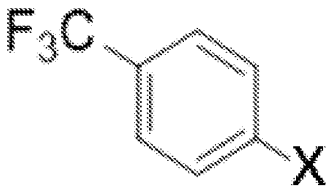
2) a reducing agent is selected from the group consisting of $NaBH_4$, $NaB(CN)H_3$ or DIBAL-H;

3) equivalents of reducing reagents: 0.3 to 1.2eq.

Embodiment 9. The method of embodiment 7, wherein the compound of formula 5 is obtained by reacting the compound of formula 3 with the lithium reagent generated from n-BuLi and the compound of formula 4,



formula 3 and

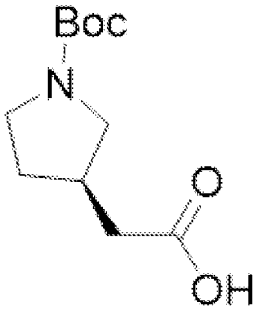


formula 4,

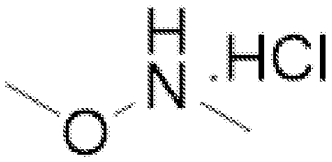
X in the compound of formula 4 is Br or I; and

a reaction solvent is selected from the group consisting of THF, 2-Me-THF, diethyl ether and 1,4-dioxane.

Embodiment 10. The method of embodiment 9, wherein the compound of formula 3 is obtained by reacting the compound of formula 1 with the compound of formula 2,



formula 1 and



formula 2

wherein reaction conditions are:

1) a solvent is selected from the group consisting of THF, 2-Me-THF, 1,4-dioxane, DMF or CH_2Cl_2;

2) reaction temperature: 0 to 25°C;

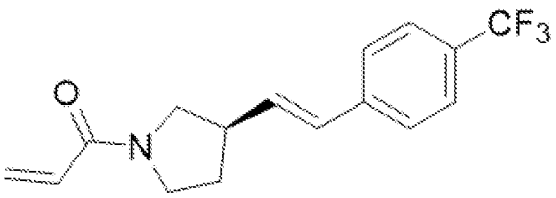
3) reaction time: 1 to 24h;

4) a coupling reagent is select from CDI, HATU, HBTU/HOBT, or EDCI;

5) a base is selected from the group consisting of Et_3N, iPr_2NEt or N-methyl morpholine;

6) equivalents of coupling reagents: 1 to 2 eq.

Embodiment 11. A crystalline form of a compound represented by formula I:



formula I,

wherein the crystalline form is characterized by X-ray powder diffraction peaks at 2θ angles (± 0.2°) 20.4°, 21.1°, and 21.7°.

Embodiment 12. The crystalline form of embodiment 11, wherein the crystalline form is further characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 15.2° and 23.0°.

Embodiment 13. The crystalline form of embodiment 12, wherein the crystalline form is further characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 13.6°, 14.0°, 16.5° and 17.0°.

Embodiment 14. The crystalline form of any one of embodiments 11-13, wherein the crystalline form is characterized by an X-ray powder diffraction pattern substantially similar to FIG. 1.

Embodiment 15. The crystalline form of any one of embodiments 11-14, wherein the crystalline form is at least 60% a single crystalline form, at least 70% a single crystalline form, at least 80% a single crystalline form, at least 90% a single crystalline form, at least 95% a single crystalline form, or at least 99% a single crystalline form by weight.

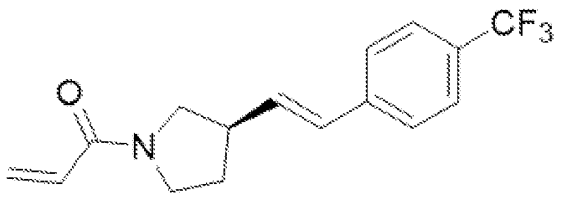
Embodiment 16. The crystalline form of any one of embodiments 11-15, wherein the crystalline form has a chemical purity of at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% by weight.

Embodiment 17. The crystalline form of any one of embodiments 11-16, wherein the crystalline form is characterized by a differential scanning calorimetry (DSC) curve that comprises an endotherm at about 87±2°C, preferably about 86.9-88.6°C.

Embodiment 18. The compound crystalline form of embodiment 17, wherein the crystalline form is characterized by the DSC curve substantially similar to FIG. 2.

Embodiment 19. The crystalline form of any one of embodiments 11-18, wherein the crystalline form is an anhydrate.

Embodiment 20. A crystalline form of a compound represented by formula I:



formula I,

wherein the crystalline form characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 17.3° and 18.3°.

Embodiment 21. The crystalline form of embodiment 20, wherein the crystalline form further characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 20.4°, 21.1° and 21.7°.

Embodiment 22. The crystalline form of embodiment 20 or embodiment 21, wherein the crystalline form is characterized by an X-ray powder diffraction pattern substantially similar to the curve in FIG. 3.

Embodiment 23. A method to form the crystalline form according to any one of embodiments 11-22, the crystalline form is formed by evaporation or evaporation crystallization, wherein the solvent used in any one of Embodiments 11-19 is selected from the group consisting of tetrahydrofuran (THF), acetonitrile (ACN), methyl tert-butyl ether (MTBE), the solvent used in any one of Embodiments 20-22 is selected from the group consisting of acetone, ethyl acetate (EA), methyl ethyl ketone(MEK) and ethanol.

Embodiment 24. A pharmaceutical composition, comprising a pharmaceutically effective amount of the crystalline form according to any one of embodiments 11-22 and a pharmaceutically acceptable carrier.

Embodiment 25. A method of treating cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the compound crystalline form of any one of embodiments 11-22 or the pharmaceutical composition of embodiment 24; or

the compound crystalline form of any one of embodiments 11-22 or the pharmaceutical composition of embodiment 24, for use as a medicament for treating cancer in a subject in need thereof; or

use of the compound crystalline form of any one of embodiments 11-22 or the pharmaceutical composition of embodiment 24 in the manufacture of a medicament for treating cancer in a subject in need thereof.

Embodiment 26. The method or use of embodiment 25, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, ovarian cancer, pancreatic ductal adenocarcinoma (PDAC), glioblastoma, gastric cancer, cervical cancer, colon cancer, endometrial cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, leukemia, non-Hodgkin's lymphoma, prostate cancer, rectal cancer, malignant melanomas, alimentary/gastrointestinal tract cancer, liver cancer, skin cancer, lymphoma, malignant pleural mesothelioma (MPM), kidney cancer, muscle cancer, bone cancer, brain cancer, eye or ocular cancer, rectal cancer, colorectal cancer, cervical cancer, oral cancer, benign and malignant tumors, stomach cancer, corpus uteri, testicular cancer, renal cancer, throat cancer, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's Sarcoma, Kaposi's Sarcoma, basal cell carcinoma and squamous cell carcinoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, angiosarcoma, hemangioendothelioma, Wilms Tumor, neuroblastoma, mouth/pharynx cancer, esophageal cancer, larynx cancer, neurofibromatosis, tuberous sclerosis, hemangiomas, and lymphangiogenesis, preferably, the cancer is selected from the group consisting of glioblastoma, gastric cancer, colorectal cancer, pancreatic ductal adenocarcinoma (PDAC), and malignant pleural mesothelioma (MPM).

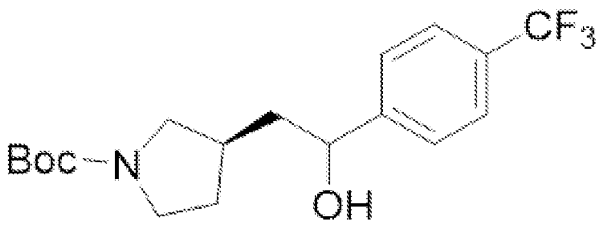
Embodiment 27. A method of inhibiting TEAD1, 2, 3, and/or 4 in a subject or in a sample, comprising administering to the subject a therapeutically effective amount of the compound crystalline form of any one of embodiments 11-22 or the pharmaceutical composition of embodiment 24, or

the compound crystalline form of any one of embodiments 11-22 or the pharmaceutical composition of embodiment 24, for use as a medicament for inhibiting TEAD1, 2, 3, and/or 4 in a subject or in a sample; or

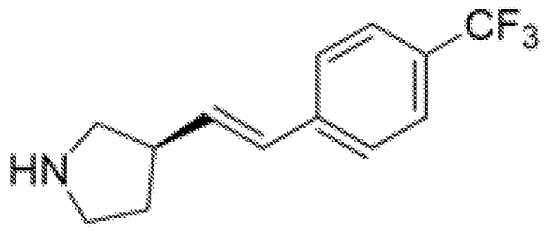
use of the compound crystalline form of any one of embodiments 11-22 or the pharmaceutical composition of embodiment 24 in the manufacture of a medicament for inhibiting TEAD1, 2, 3, and/or 4 in a subject or in a sample,

preferably, TEAD1 and 3 are inhibited selectively over TEAD2 and 4.

Embodiment 28. A compound represented by the formula of compound 6 or 7,



formula 6,



formula 7.

Definitions

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure.

The term "about" will be understood by persons of ordinary skill in the art. Whether the term "about" is used explicitly or not, every quantity given herein refers to the actual given value, and it is also meant to refer to the approximation to such given value that would be reasonably inferred based on the ordinary skill in the art. Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X" As used herein , and unless otherwise specified, the terms "about" and "approximately," when used in connection with temperatures, doses, amounts, or weight percent of ingredients of a composition or a dosage form, mean a dose, amount, or weight percent that is recognized by those of ordinary skill in the art to provide a pharmacological effect equivalent to that obtained from the specified

dose, amount, or weight percent. Specifically, the terms "about" and "approximately," when used in this context, contemplate a dose, amount, or weight percent within 15%, within 10%, within 5%, within 4%, within 3%, within 2%, within 1%, or within 0.5% of the specified dose, amount, or weight percent.

As used herein, "crystalline" refers to a solid form of Compound I, wherein there exists long-range atomic order in the positions of the atoms. The crystalline nature of a solid can be confirmed, for example, by examination of the X-ray powder diffraction pattern. If the XRPD shows sharp intensity peaks in the XRPD then the compound is crystalline. A solid form of Compound I that is "crystalline" is a solid that is completely crystalline or partially crystalline, and encompasses solids that are at least 80% crystalline, 85% crystalline, 90% crystalline, 95% crystalline, and 99% crystalline, by weight. Crystalline solids are ordered arrangements of molecules that possess distinguishable crystal lattice or unit cell, and consequently have definable long range ordering. In some embodiments, substantially crystalline refers to a solid form of Compound I that is at least 80% crystalline. In other embodiments, substantially crystalline refers to a solid form that is at least 90% crystalline.

As used herein, chemical purity refers to extent to which the disclosed form is free from materials having different chemical structures. Chemical purity of the compound in the disclosed crystal forms means the weight of the compound divided by the sum of the weight of the compound plus materials/impurities having different chemical structures multiplied by 100%, i.e., percent by weight. In one embodiment, the compound in the disclosed crystalline forms has a chemical purity of at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% by weight.

The term "hydrate" refers to a crystalline Compound I where a stoichiometric or non-stoichiometric amount of water is incorporated into the crystal structure. A hydrate is a solvate wherein the solvent incorporated into the crystal structure is water. The term "anhydrous" when used with respect to a compound means substantially no solvent incorporated into the crystal structure, e.g., less than 0.1% by weight as determined by Karl Fisher analysis. A compound that is anhydrous is referred to herein as an "anhydrate".

The term "amorphous" means a solid that is present in a non-crystalline state or form. Amorphous solids are disordered arrangements of molecules and therefore possess no distinguishable crystal lattice or unit cell and consequently have no definable long range ordering. Solid state ordering of solids may be determined by standard techniques known in the art, e.g., by X-ray powder diffraction (XRPD) or differential scanning calorimetry (DSC).

Amorphous solids can also be differentiated from crystalline solids e.g., by birefringence using polarized light microscopy.

A single crystalline form of Compound I means that (*R*, *E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one is present as a single crystal or a plurality of crystals, in which each crystal has the same crystalline form (*i.e.*, crystalline form I or II). When the crystal form is defined as a specified percentage of one particular single crystalline form of the compound, the remainder is made up of amorphous form and/or crystalline forms other than the one or more particular forms that are specified. In one embodiment, the crystalline form is at least 60% a single crystalline form, at least 70% a single crystalline form, at least 80% a single crystalline form, at least 90% a single crystalline form, at least 95% a single crystalline form, or at least 99% a single crystalline form by weight. Percent by weight of a particular crystal form is determined by the weight of the particular crystal form divided by the sum weight of the particular crystal, plus the weight of the other crystal forms present plus the weight of amorphous form present multiplied by 100%.

The 2-theta values of the X-ray powder diffraction patterns for the crystalline forms described herein may vary slightly from one instrument to another and also depending on variations in sample preparation and batch to batch variation. Therefore, unless otherwise defined, the XRPD patterns and/or the 2-theta peak values recited herein are not to be construed as absolute and can vary by ± 0.2 degrees. The 2-theta values provided herein were obtained using Cu, k-Alpha radiation.

Temperature values, e.g., for DSC peak temperatures and DSC onset temperatures herein may vary slightly from one instrument to another and also depending on variations in sample preparation, the rate of temperature increases over the course of the experiment, batch to batch variation of the material, and other environmental factors. Therefore, unless otherwise defined, temperature values recited herein are not to be construed as absolute and can vary by ± 5 degrees.

"Substantially the same XRPD pattern" or "an X- ray powder diffraction pattern substantially similar to" a defined figure means that for comparison purposes, at least 90% of the peaks shown are present. It is to be further understood that for comparison purposes some variability in 2-theta peak positions from those shown is allowed, such as ± 0.2 degrees. It should be understood that when the expression "characterized by X-ray powder diffraction peaks at 2θ angles (± 0.2°)" followed by a list of 2-theta peak positions that the ± 0.2 degrees applies to each and every one of the peak positions listed.

Pharmaceutical Formulations and Methods of Treatment and Administration

The term "treatment" is used interchangeably herein with the term "therapeutic method" and refers to both 1) therapeutic treatments or measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic conditions, disease or disorder, and 2) and prophylactic/ preventative measures. Those in need of treatment may include individuals already having a particular medical disease or disorder as well as those who may ultimately acquire the disorder *(i.e.*, those needing preventive measures).

"Treating" or "treatment" of any condition or disorder refers, in some or any embodiments, to ameliorating a condition or disorder that exists in a subject, including prophylactically. In another embodiment, "treating" or "treatment" includes ameliorating at least one physical parameter, which may be indiscernible by the subject. In yet another embodiment, "treating" or "treatment" includes modulating the condition or disorder, either physically *(e.g.,* stabilization of a discernible symptom) or physiologically *(e.g.*, stabilization of a physical parameter) or both. In yet another embodiment, "treating" or "treatment" includes delaying the onset of the condition or disorder. In yet another embodiment, "treating" or "treatment" includes the reduction or elimination of either the condition (e.g. cancer) or one or more parameters (e.g. tumor size or tumor growth rate) of the condition (e.g. cancer), or to retard the progression of the condition (e.g. cancer) or of one or more parameters (e.g. tumor size or tumor growth rate) of the condition (e.g. cancer), or to reduce the severity of the condition (e.g. cancer) or of one or more parameters (e.g. tumor size or tumor growth rate) of the condition (e.g. cancer). In yet another embodiment, "treating" or "treatment" includes administering a compound described herein prophylactically. In some embodiments, "Treating" or "treatment" of a disease includes: (1) inhibiting the disease, i.e., arresting (i.e., stabilizing) or reducing the development of the disease or its clinical symptoms; or (2) relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

The term "subject" as used herein refers to any individual or patient to which the subject methods are performed. Generally, the subject is human, although as will be appreciated by those in the art, the subject may be an animal.

The terms "therapeutically effective amount" "effective dose" "therapeutically effective dose", "effective amount," and the like refer to the amount of a subject compound or composition that will elicit the biological or medical response in a tissue, system, animal, individual, or human that is being sought by administering said compound.

Also disclosed herein are pharmaceutical compositions comprising compound crystalline forms disclosed herein. The term "pharmaceutically acceptable carrier" refers to a non-toxic carrier that may be administered to a patient, together with a compound of this disclosure, and which does not destroy the pharmacological activity thereof.

The compound crystalline forms of this disclosure may be employed in a conventional manner for controlling the disease described herein, including, but not limited to, cancer. Such methods of treatment, their dosage levels and requirements may be selected by those of ordinary skill in the art from available methods and techniques. For example, the compounds of this disclosure may be combined with a pharmaceutically acceptable adjuvant for administration to a patient suffering from cancer in a pharmaceutically acceptable manner and in an amount effective to treat cancer.

Alternatively, the compound crystalline forms of this disclosure may be used in compositions and methods for treating or protecting individuals against the diseases described herein, including but not limited to cancer, over extended periods of time. The compound crystalline forms may be employed in such compositions either alone or together with other compounds of this disclosure in a manner consistent with the conventional utilization of such compounds in pharmaceutical compositions. For example, a compound crystalline form of this disclosure may be combined with pharmaceutically acceptable adjuvants conventionally employed in vaccines and administered in prophylactically effective amounts to protect individuals over an extended period of time against the diseases described herein, including, but not limited to, cancer.

As used herein, the terms "combination," "combined," and related terms refer to the simultaneous or sequential administration of therapeutic agents in accordance with this disclosure. For example, a described compound crystalline form may be administered with another therapeutic agent simultaneously or sequentially in separate unit dosage forms or together in a single unit dosage form. Accordingly, the present disclosure provides a single unit dosage form comprising a described compound crystalline form, an additional therapeutic agent, and a pharmaceutically acceptable carrier, adjuvant, or vehicle. Two or more agents are typically considered to be administered "in combination" when a patient or individual is simultaneously exposed to both agents. In many embodiments, two or more agents are considered to be administered "in combination" when a patient or individual simultaneously shows therapeutically relevant levels of the agents in a particular target tissue or sample (e.g., in brain, in serum, etc.).

When the compound crystalline forms of this disclosure are administered in combination therapies with other agents, they may be administered sequentially or concurrently to the patient. Alternatively, pharmaceutical or prophylactic compositions according to this disclosure comprise a combination of a compound crystalline form described herein and another therapeutic or prophylactic agent. Additional therapeutic agents that are normally administered to treat a particular disease or condition may be referred to as "agents appropriate for the disease, or condition, being treated."

The compounds utilized in the compositions and methods of this disclosure may also be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those, which increase biological penetration into a given biological system (e.g., blood, lymphatic system, or central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and/or alter rate of excretion.

According to an embodiment, the compositions of this disclosure are formulated for pharmaceutical administration to a subject or patient, e.g., a mammal, preferably a human being. Such pharmaceutical compositions are used to ameliorate, treat or prevent any of the diseases described herein including but not limited to cancer in a subject

Agents of the disclosure are often administered as pharmaceutical compositions comprising an active therapeutic agent, i.e., and a variety of other pharmaceutically acceptable components. See Remington's Pharmaceutical Science (15th ed.， Mack Publishing Company, Easton, Pa., 1980). The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, depending on the formulation desired, pharmaceutically acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

In some embodiments, the present disclosure provides pharmaceutically acceptable compositions comprising a therapeutically effective amount of one or more of a described compound crystalline form， formulated together with one or more pharmaceutically

acceptable carriers (additives) and/or diluents for use in treating the diseases described herein, including, but not limited to cancer. While it is possible for a described compound crystalline form to be administered alone, it is preferable to administer a described compound crystalline form as a pharmaceutical formulation (composition) as described herein. Described compound crystalline forms may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

When compound crystalline forms described herein are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1% to 99.5% of active ingredient in combination with a pharmaceutically acceptable carrier. In some embodiments, 0.5% to 90% of active ingredient can be used.

Preparations described herein may be given orally, nasally, as by, for example, a spray, parenterally, intravaginally, intracistemally, rectally, or topically, as by powders, ointments or drops, including buccally and sublingually. They are of course given in forms suitable for the relevant administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administrations are preferred.

Regardless of the route of administration selected, compound crystalline forms described herein which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present disclosure, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the disclosure may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of

The compound crystalline forms of the disclosure can be administered in combination with one or more additional therapeutic agents. The phrases “combination therapy”, “combined with” and the like refer to the use of more than one medication or treatment simultaneously to increase the response. The TEAD1, 2, 3, and/or 4 inhibitor of the present disclosure might for example be used in combination with other drugs or treatment in use to treat cancer. In various aspects, the compound crystalline form is administered prior to, simultaneously with or following the administration of the chemotherapeutic agent.

In another embodiment, the present disclosure is directed to the use of compound crystalline forms of Formula I, and/or a pharmaceutically acceptable salt thereof in the treatment of any cancer indication where YAP is localized in the nucleus of the tumor cells, including, but is not limited to, lung, thyroid, ovarian, colorectal, prostate, pancreas, esophagus, liver, breast, and skin cancer.

In still another embodiment, the disclosed compound crystalline forms or pharmaceutical composition thereof can disrupt YAP interactions with TEAD1, 2, 3, and/or 4. In certain embodiments, the disclosed compound crystalline forms or pharmaceutical composition thereof can prevent YAP from binding to TEAD1, 2, 3, and/or 4. In some embodiments, the disclosed compound crystalline forms or pharmaceutical composition thereof can compete with YAP for binding to TEAD1, 2, 3, and/or 4. In some embodiments, the disclosed compound crystalline forms or pharmaceutical composition thereof can bind to TEAD1, 2, 3, and/or 4. In some embodiments, the disclosed compounds or pharmaceutical composition thereof can bind to TEAD1 and 3. In some embodiments, the disclosed compounds or pharmaceutical composition thereof can selectively bind to TEAD1 and 3 over that of the other TEAD isoforms.

By “pharmaceutically acceptable” it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. For example, the carrier, diluent, or excipient or composition thereof may be administered to a subject along with a TEAD1, 2, 3, and/or 4 inhibitor of the disclosure without causing any undesirable biological effects or interacting in an undesirable manner with the TEAD1, 2, 3, and/or 4 inhibitor of the pharmaceutical composition in which it is contained.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the XRPD pattern of crystalline form I.

Figure 2 is the DSC curve of crystalline form I.

Figure 3 is the XRPD pattern of crystalline form II prepared by acetone.

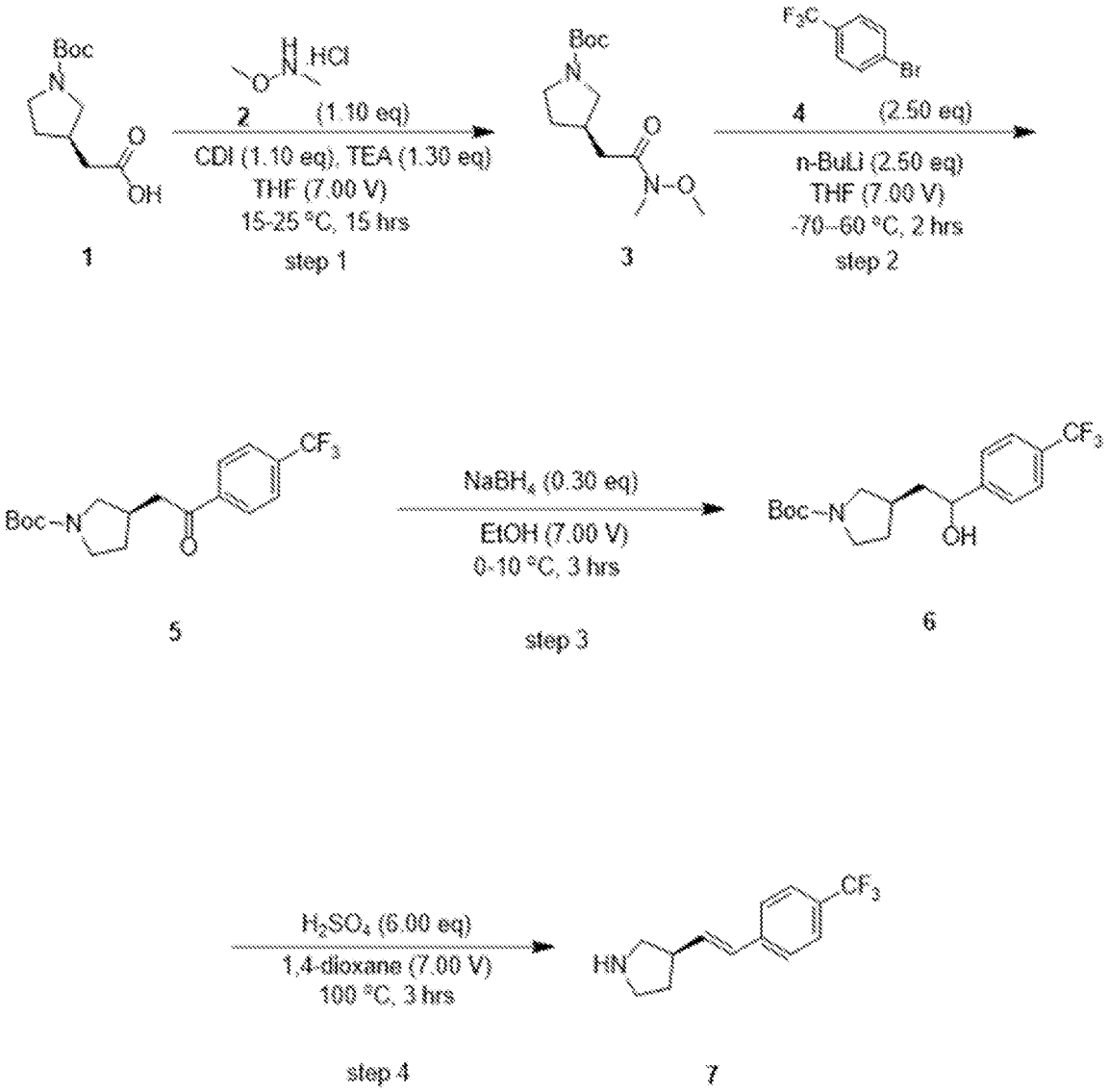
EXAMPLES

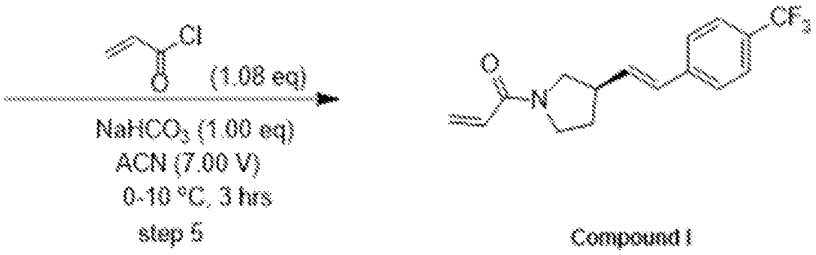
Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, it will be understood that modifications and variations

are encompassed within the spirit and scope of the instant disclosure. The preferred methods and materials are now described.

Example 1

A preparation method of **Compound I** (*R, E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one





Step 1 Synthesis of *tert*-butyl (*R*)-3-(2-(methoxy(methyl)amino)-2-oxoethyl)pyrrolidine-1-carboxylate 3

THF (21.0 L, 7.00 v/w) was charged into a reactor (50.0L) at 15 to 25°C, then (*R*)-2-(1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl)acetic acid **1** (3.0 kg, 13.1 mol. 1.00 eq.), TEA (1.72 kg, 17.0 mol, 1.30 eq.) and CDI (2.33 kg, 14.4 mmol, 1.10 eq.) were added into the reactor subsequently at 15 to 25°C. The mixture was stirred for 2 hours at 15 to 25°C. Then *N,O*-dimethylhydroxylamine hydrochloride **2** (1.40 kg, 14.4 mol, 1.10 eq.) was charged into the reactor at 15 to 25°C. The resulting solution was stirred for 15 hours at 15 to 25°C, and HPLC showed the reaction was completed. The reaction solution was filtered and the wet cake was washed with EtOAc (6.00 L, 2.00 v/w). The filtrate was concentrated to 2.00 to 3.00V, and EtOAc (15. 0 L, 5.00 v/w) was added at 15 to 25°C. The organic phase was washed with HCl aqueous solution (1N, 1.50 L, 5.00 v/w) (pH value was 3-4). The organic phase was separated and washed with NaCl solution (1.50 L, 15.0% w/w, 5.00 v/w). The organic phase was concentrated to afford *tert*-butyl (*R*)-3-(2-(methoxy(methyl)amino)-2-oxoethyl)pyrrolidine-1-carboxylate **3** (3.03 kg, 85% yield) as a brown oil with 94.5% purity.

MS (ESI, m/z), 217.1 $[M\text{-}^{t}Bu+1]^{+}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.68 (d, 3H), 3.64 – 3.58 (m, 1H), 3.49 – 3.41 (m, 1H), 3.34 – 3.25 (m, 1H), 3.19 (d, 3H), 2.98 – 2.92 (m, 1H), 2.67 – 2.58 (m, 1H), 2.51 (m, 2H), 2.14 – 2.04 (m, 1H), 1.62 – 1.55 (m, 1H), 1.49 (d, 9H).

Step 2 Synthesis of *tert*-butyl (*R*)-3-(2-oxo-2-(4-(trifluoromethyl)phenyl)ethyl)pyrrolidine-1-carboxylate 5

THF (4.5 L, 3.00v/w) was charged into a reactor (50.0L) at 15 to 25°C, and the solution was cooled to -60 to -70°C. n-BuLi (5.51 L, 2.50 M, 13.8 mol, 2.50 eq.) was added dropwise into the reactor at -60 to -70°C, then 1-bromo-4-(trifluoromethyl)benzene (3.10 kg, 13.8 mol, 2.50 eq.) **4** in THF solution (1.50 L, 1.00 v/w) was added dropwise into the reactor at -60 to -70°C .

After the addition was completed, the reaction mixture was stirred at -60 to -70°C for 1 hour. *tert*-Butyl (*R*)-3-(2-(methoxy(methyl)amino)-2-oxoethyl)pyrrolidine-1-carboxylate **3** (1.50 kg, 5.51 mol, 1.00 eq.) in THF solution (1.50 L, 1.00 v/w) was then added dropwise into the reactor at -60 to -70°C. The mixture was stirred for 2 hours at -60 to -70°C. HPLC shows the reaction was completed. HCl aqueous solution (2.00 N, 7.50 L, 5.00 v/w) was added into the reactor dropwise at -60 to -70°C. The reaction was then warmed gradually to 0 to 10°C. EtOAc (10.5 L, 7.00 v/w) was charged into the mixture. The organic phase was separated, washed with $NaHCO_3$ aqueous solution (7.50 L, 5.00 v/w) followed by NaCl aqueous solution (7.50 L, 5.00 v/w). The organic phase was concentrated under the reduced pressure at 40 to 50°C. Heptane (10.5 L, 7.00 v/w) was charged into the reactor and the suspension was concentrated under the reduced pressure at 40 to 50°C until the volume was 2.00 to 3.00 V, then heptane (7.50 L, 5.00 v/w) was charged into the reactor again. The suspension was cooled to 0 to 10°C, and stirred for 2 hours. The suspension was filtered and the wet cake was washed with heptane (1.50 L, 1.00 v/w). Cake was dried in the oven at 40 to 50°C. *tert*-Butyl (*R*)-3-(2-oxo-2-(4-(trifluoromethyl)phenyl)ethyl) pyrrolidine-1-carboxylate **5** was obtained (1.68 kg, 82.0% yield) as an off-white solid with 100% purity.

MS (ESI, m/z), 302.1 $[M-^tBu+1]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.08 – 8.04 (m, 2H), 7.76 – 7.73 (m, 2H), 3.72 – 3.66 (m, 1H), 3.52 – 3.45 (m, 1H), 3.38 – 3.29 (m, 1H), 3.05 (d, *J* = 3.2Hz, 2H), 2.99 – 2.85 (m, 1H), 2.82 – 2.70 (m, 1H), 2.21 – 2.11 (m, 1H), 1.67 – 1.52 (m, 1H), 1.46 (s, 9H).

Step 3 Synthesis of *tert*-butyl (3*R*)-3-(2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl) pyrrolidine-1-carboxylate 6

EtOH (17.5 L, 7.00 v/w) was charged into a reactor (50.0L) at 15 to 25°C, then *tert*-butyl (*R*)-3-(2-oxo-2-(4-(trifluoromethyl)phenyl)ethyl) pyrrolidine-1-carboxylate **5** (2.50 kg, 7.00 mol, 1.00 eq.) was added. The suspension was cooled to 0 to 10°C, and $NaBH_4$ (79.5 g, 2.10 mol, 0.30 eq.) was added to the reactor at 0 to 10°C. The resulting mixture was stirred for 1 hour at 0 to 10°C, and HPLC showed the reaction was completed. HCl aqueous solution (1.50 L, 0.60 v/w) was added dropwise to the reactor at 0 to 10°C. The suspension solution was filtered and the wet cake was washed with EtOAc (5.00 L, 2.00 v/w). Water (12.5 L, 5.00 v/w) was added to the above mixture and the solution was concentrated to 5.00 to 6.00 V. EtOAc (17.5 L, 7.00 v/w) was added. The organic phase was separated and washed with NaCl aqueous solution (12.5 L, 5.00 v/w). The organic phase was concentrated under the reduced pressure at 40 to 50°C. Heptane (12.5 L, 5.00 v/w) was added into the reactor. The suspension was stirred for 2 hours

at 15 to 25°C, then filtered. The wet cake was washed with heptane (2.50 L, 1.00 v/w), and dried in the oven at 40 to 50°C. *tert*-Butyl (3*R*)-3-(2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl) pyrrolidine-1-carboxylate **6** (2.25 kg, 90% yield) was obtained as an orange solid with 100% purity.

MS (ESI, m/z), 382.1 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.62 - 7.60 (m, 2H), 7.49 - 7.47 (m, 2H), 4.78 - 4.77 (m, 1H), 3.64 - 3.40 (m, 2H), 3.22 - 3.20 (m, 1H), 2.97 - 2.59 (m, 2H), 2.02 - 1.50 (m, 5H), 1.43 (s, 9H).

Step 4 Synthesis of (*R,E*)-3-(4-(trifluoromethyl)styryl)pyrrolidine 7

1,4-Dioxane (17.5 L, 7.00 v/w) was charged into a reactor at 15 to 25°C, then concentrated H_2SO_4 (4.09 kg, 41.8 mol, 6.00 eq.) was added into 1,4-dioxane at 15 to 25°C. *tert*-Butyl (3*R*)-3-(2-hydroxy-2-(4-(trifluoromethyl)phenyl)ethyl)pyrrolidine-1-carboxylate **6** (2.50 kg, 6.96 mol, 1.00 eq.) was added into the reactor at 15 to 25°C. The resulting suspension was stirred for 2 hours at 15 to 25°C, then heated to 95 to 105°C. The reaction solution continued to stir for 3 hours at 100°C, and HPLC showed the reaction was completed. The solution was cooled to 15 to 25°C, and poured into water (12.5 L, 5.00 v/w) at 15 to 25°C. The solution was concentrated to 5.00 to 7.00 V, and cooled to 0 to 10°C. The mixture was adjusted with NaOH aqueous solution (40% w/w) to pH around 11 to 12 at 0 to 10°C. The suspension was filtered and solid was washed with CH_2Cl_2/MeOH (7.50 L, 3.00 v/w, 6/1). The filtrate was added CH_2Cl_2/MeOH (17.50 L, 3.00 v/w, 6/1). The organic layer was separated. Aqueous layer was extracted with CH_2Cl_2/MeOH (17.50 L, 3.00 v/w, 6/1). The organic phase was combined and washed with water (12.5 L, 5.00 v/w). The organic phase was concentrated under the reduced pressure to afford (*R,E*)-3-(4-(trifluoromethyl)styryl)pyrrolidine **7** (1.53 kg, 91.5% yield) as a brown solid with 91.5% purity.

MS (ESI, m/z), 242.1 $[M+1]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.47 -7.45 (m, 2H), 7.36 - 7.34 (m, 2H), 6.39-6.32 (m, 1H), 6.23-6.17 (m, 1H), 3.15-3.10 (m, 1H), 2.98 - 2.96 (m, 2H), 2.83 - 2.65 (m, 3H), 2.02 - 1.97 (m, 1H), 1.61 - 1.55 (m, 1H).

Step 5 (*R,E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one Compound I

Acetonitrile (17.5 L, 7.00 v/w) was charged into a reactor at 10 to 20°C, then (*R,E*)-3-(4-(trifluoromethyl)styryl)pyrrolidine **7** (2.50 kg, 10.4 mol, 1.00 eq.) and $NaHCO_3$ (0.81 kg, 10.4 mol, 1.00 eq) were added subsequently at 10 to 20°C. The suspension was cooled to 0 to 10°C, and prop-2-enoyl chloride (0.94 kg, 11.2 mol, 1.08 eq.) was added dropwise into the reactor at 0 to 10°C. The mixture was stirred for 3 hours at 0 to 10°C, and HPLC showed the reaction was

completed. The solution was filtered and the wet cake was washed with EtOAc (5.00 L, 2.00 v/w). The filtrate was concentrated to 1.00 to 2.00 V, then EtOAc (12.5 L, 5.00 v/w) was added. The organic solution was washed with $NaHCO_3$ aqueous solution (7.50 L, 3.00 v/w). The organic phase was concentrated to 1.00 to 2.00 V, then MTBE (12.5 L, 5.00 v/w) was added. The solution was concentrated to 1.00 to 2.00 V, and another portion of MTBE (12.5 L, 5.00 v/w) was added. The solution was concentrated to 2.00 to 3.00 V, and cooled to -10 to 0°C. The mixture was stirred for 4 hours at -10 to 0°C. The suspension was filtered and the wet cake was washed with MTBE (2.50 L, 1.00 v/w). The cake was dried in the vacuum oven at 30 to 40°C over 8 hours. (*R,E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one **Compound I** was obtained (3.50 kg, 64.9% yield) as an off-white crystalline solid with 98.3% purity and 100% ee.

MS (ESI, m/z), 296.10 $[M+1]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.58 - 7.55 (m, 2H), 7.45 - 7.43 (m, 2H), 6.55 - 6.41 (m, 3H), 6.27 (m, 1H), 5.71 - 5.68 (m, 1H), 3.89 - 3. 35 (m, 4H), 3.12 -3.00 (m, 1H), 2.23 - 1.81 (m, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ (ppm) -62.477, -62.492. Melting point, 87.8 - 88.6°C.

The Compound I of Example 1 is characterized by XRPD (Cu, k-Alpha, λ=1.54184Å). The measured XRPD pattern is shown in Figure 1, and its DSC curve is shown in Figure 2. The crystalline form prepared in Example 1 named Crystalline form I, which is characterized by X-ray powder diffraction peaks at 2θ angles (± 0.2°) 20.4°, 21.1°, 21.7°, 15.2°, 23.0°, 13.6°, 14.0°, 16.5° and 17.0°. In addition, the start and end of melting point of the product is measured three times and shown in Table 1, and the peaks of the XRPD pattern are shown in Table 2.

Table 1. The start and end of melting point of Compound I of Example 1

Items	1(°C)	2(°C)	3(°C)	Average value(°C)
Start of Melting	87.8	87.8	87.7	87.8
End of Melting	88.5	88.7	88.5	88.6

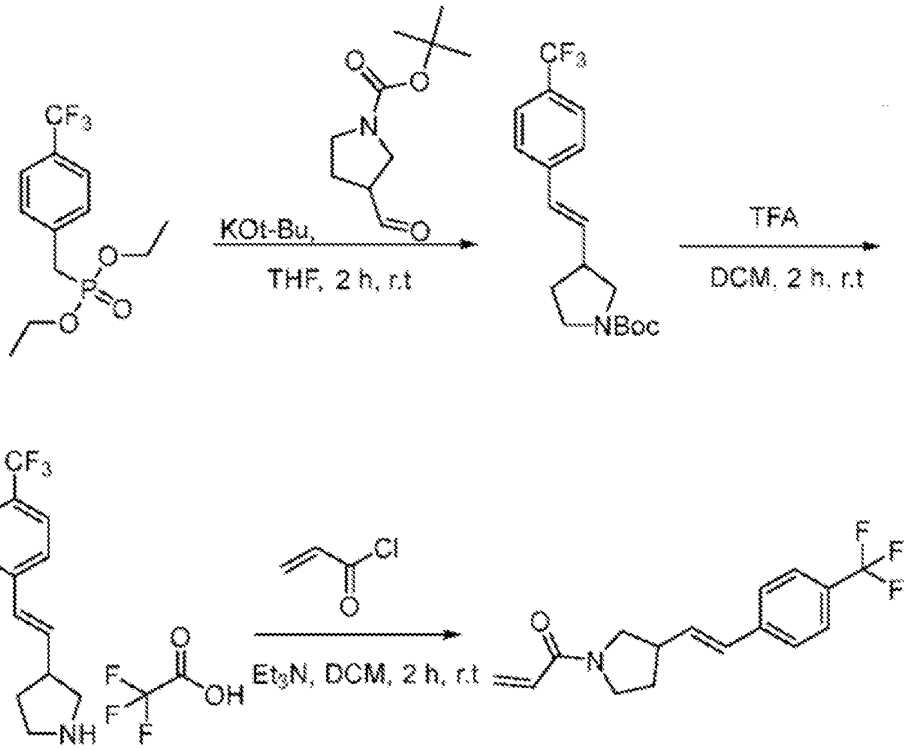
Table 2. XRPD Pattern Peaks of Compound I of Example 1

PEAK No.	2-Theta	d	BG	Height	Height%	Area	A%	FWHM
1	3.280	26.9342	23	231	3.7	9470	12.7	0.698

2	10.522	8.4078	98	189	3.0	2781	3.7	0.250
3	11,075	7.9888	100	113	1.8	1289	1.7	0.194
4	13.621	6.5009	101	255	4.1	4186	5.6	0.279
5	14.001	6.3255	101	1004	16.1	15165	20.4	0.257
6	14.657	6.0438	101	153	2.5	2510	3.4	0.278
7	15.218	5.8220	101	1 580	25.3	1 9643	26.4	0.211
8	16.461	5.3852	172	340	5.4	3041	4.1	0.152
9	17.000	5.2155	178	572	9.2	6124	8.2	0.182
10	17.583	5.0439	185	172	2.8	3076	4.1	0.304
11	17.960	4.9391	190	198	3.2	3927	5.3	0.336
12	19.320	4.5942	187	744	11.9	13435	18.1	0.307
13	19.660	4.5156	198	1021	16.3	13487	18.2	0.225
14	20.019	4.4353	198	596	9.5	12935	17.4	0.369
15	20.441	4.3447	198	4413	70.7	54113	72.8	0.208
16	21.099	4.2106	198	6245	100.0	74281	100.0	0.202
17	21.698	4.0957	198	2395	38.4	43624	58.7	0.310
18	22.299	3.9868	198	454	7.3	15577	21.0	0.583
19	22.961	3.8733	198	862	13.8	11093	14.9	0.219
20	23.462	3.7917	198	237	3.8	4448	6.0	0.319
21	23.820	3.7356	172	151	2.4	2073	2.8	0.233
22	24.339	3.6570	172	475	7.6	6685	9.0	0.239
23	25.678	3.4692	176	241	3.9	2863	3.9	0.202
24	26.139	3.4092	165	190	3.0	2641	3.6	0.237
25	26.959	3.3072	180	362	5.8	4112	5.5	0.193
26	27.562	3.2363	156	325	5.2	4087	5.5	0.214
27	28.080	3.1778	156	545	8.7	8854	11.9	0.276
28	28.421	3.1404	156	362	5.8	7296	9.8	0.343
29	29.379	3.0401	156	341	5.5	6064	8.2	0.302
30	30.118	2.9672	191	135	2.2	1391	1.9	0.175
31	30.721	2.9103	172	196	3.1	1 568	2.1	0.136
32	31.320	2.8560	184	137	2.2	1026	1.4	0.128
33	31.782	2.8155	174	303	4.9	4859	6.5	0.273
34	32.321	2.7698	172	131	2.1	2125	2.9	0.276
35	32.722	2.7368	177	77	1.2	333	0.4	0.073
36	33.236	2.6956	139	95	1.5	803	1.1	0.144
37	36.177	2.4829	160	106	1.7	1 449	2.0	0.232
38	36. 561	2.4577	153	76	1.2	908	1.2	0.203
39	37.543	2.3957	150	77	1.2	1 466	2.0	0.322

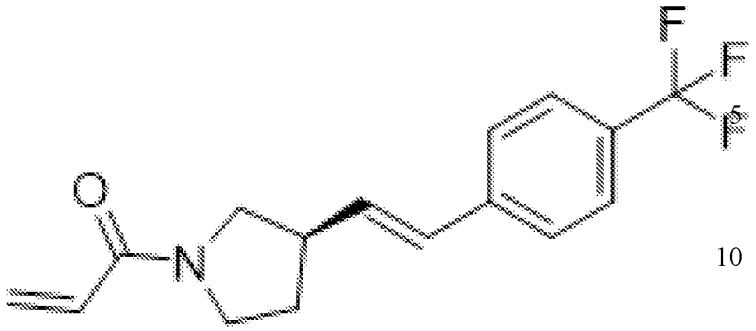
Example 2

Another preparation method of compound I (*R, E*)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one



The racemic product of the compound 1-{3-[(E)-2-[4-((trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one) is prepared according to patent application No. PCT/US23/23957. MS (ESI) mass calcd. for $C_{16}H_{16}F_3NO$, 295.10 m/z, found, 296.10 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d6) δ 7.74 - 7.62 (m, 4H), 6.69 - 6.45 (m, 3H), 6.18 - 6.09 (m, 1H), 5.74 - 5.62 (m, 1H), 3.89 - 3.51 (m, 3H), 3.23 - 2.96 (m, 2H), 2.18 - 2.01 (m, 1H), 1.95 - 1.69 (m, 1H), ^{19}F NMR (376 MHz, DMSO-d6) δ (ppm): -60.83.

(R, E)-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one was obtained by chiral separation of the above racemic compound according to patent application No. PCT/US23/23957.



compound I

The racemic product was further separated by SFC (Column: CHIRAL ART Cellulose-SB, 2*25 cm, 5 μm; Mobile Phase A: Hex(0.5% 2M NH_3-MeOH)-HPLC, Mobile Phase B: IPA-HPLC; Flow rate: 20 mL/min; Gradient: 15% B to 15% B in 21 min; Wave Length: 220/254 nm; RTl(min): 14.491 (first isomer); Sample Solvent: EtOH-HPLC; Injection Volume: 0.4 mL; Number Of Runs: 2) to afford one stereoisomer **Compound I**, assigned as *R* (second eluting isomer), *(R, E)*-1-(3-(4-(trifluoromethyl)styryl)pyrrolidin-1-yl)prop-2-en-1-one as a white solid (3.8 mg). MS (ESI) mass calcd. for $C_{16}H_{16}F_3NO$, 295.10 m/z, found, 296.10 $[M+H]^+$). ^1H NMR (400 MHz, DMSO-*d6*) δ 7.74 - 7.62 (m, 4H), 6.69 - 6.45 (m, 3H), 6.18 - 6.09 (m, 1H), 5.74 - 5.62 (m, 1H), 3.89 - 3.51 (m, 3H), 3.23 - 2.96 (m, 2H), 2.18 - 2.01 (m, 1H), 1.95 - 1.69 (m, 1H). ^{19}F NMR (376 MHz, DMSO-d_6) δ (ppm): -60.82.

Example 3

Biological Activity Testing of Compound I

The ability of Compound I to inhibit TEAD-dependent transcription was assessed in the MCF7-TEAD-Luc reporter assay (BPS #60618). For data analysis, dose-dependent curves were plotted as "% inhibition" (% of DMSO-treated control wells), and were fitted by four-parametric model in GraphPad Prism to obtain IC50 values. TEADl-Luc MCF7: average IC50 value ≤10 nM, TEADl-Luc MCF7: maximum inhibition rate ≥75%.

The potency and efficacy of Compound I in inhibiting cell proliferation and viability were assessed in multiple cell lines. The average IC50 value measured in NCI-226 cells was less than 100 nM, and the maximum average inhibition rate was 50%-75%. The average IC50 value measured in H2052 cells was less than 100 nM, and the maximum average inhibition rate was 50%-75%. In the TEAD selectivity test, the inhibition percentages of 3μm Compound I against TEAD1, TEAD2, TEAD3, and TEAD4 were 94%, 57%, 98%, and 52% respectively.

Example 4

Preparation and Characterization of Crystalline Forms of Compound I of Example 2

Referring to Table 3, 5 mg of Compound I prepared in Example 2 was dissolved as a starting material in a specific solvent, and the solid obtained by evaporation crystallization was characterized by XRPD (Cu, k-Alpha λ=1.54184Å). The products prepared from THF (tetrahydrofuran), ACN (acetonitrile) and MTBE (methyl tert-butyl ether) are crystalline form I. The product prepared by Acetone, EA (ethyl acetate), MEK (methyl ethyl ketone) and ethanol had X-ray powder diffraction pattern different from that of crystalline form I and was named crystalline form II (see Figure 3 and Table 4). Crystalline form II is characterized by X-ray powder diffraction peaks at 2θ angles (± 0.2°) 17.3°, 18.3°, 20.4°, 21.1° and 21.7°. Crystalline form I and Crystalline form II had different XRPD pattern compared to the Compound I prepared in Example 2.

Table 3 Characterization of the products prepared by evaporation crystallization method

No.	Solvent	Solid (mg)	Volume (mL)	XRD
1	THF	5	0.2	Crystalline Form I
2	ACN	5	0.2	Crystalline Form I
3	MTBE	5	0.2	Crystalline Form I
4	Acetone	5	0.2	Crystalline Form II
5	EA	5	0.2	Crystalline Form II
6	MEK	5	0.2	Crystalline Form II
7	Ethanol	5	0.2	Crystalline Form II

Table 4 XRPD Pattern Peaks of Crystalline Form II of Example 2 via Acetone

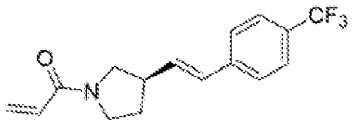
PEAK No.	Angle	d Value	Rel. Intensity	Intensity
Peak #1	6.656	13.2687	4.10%	107.375
Peak #2	10.346	8.54359	2.80%	75.1042
Peak #3	10.875	8.12884	2.00%	65.0005
Peak #4	13.431	6.58713	54.70%	483.737
Peak #5	13.857	6.38572	15.40%	170.127

Peak #6	15.04	5.8858	24.60%	242.523
Peak #7	16.298	5.43441	8.10%	116.962
Peak #8	16.846	5.25889	9.00%	127.492
Peak #9	17.292	5.12408	29.70%	294.281
Peak #10	17.79	4.98172	2.30%	76.8356
Peak #11	18.258	4.85509	27.70%	279.428
Peak #12	18.679	4.74666	7.00%	113.136
Peak #13	19.098	4.6433	7.70%	121.455
Peak #14	19.496	4.54945	14.10%	176.503
Peak #15	19.775	4.48599	33.00%	329.497
Peak #16	20.29	4.37319	79.20%	700.343
Peak #17	20.754	4.2765	10.00%	149.387
Peak #18	20.932	4.24043	100.00%	867.58
Peak #19	21.518	4.12634	29.60%	304.283
Peak #20	21.686	4.09468	10.40%	150.71
Peak #21	22.202	4.00082	5.10%	104.545
Peak #22	22.803	3.89662	30.30%	304.03
Peak #23	23.045	3.85628	13.20%	168.14
Peak #24	24.081	3.69264	8.40%	126.219
Peak #25	24.977	3.56218	3.40%	81.0157
Peak #26	25.496	3.49085	4.30%	86.0247
Peak #27	25.786	3.45226	1.60%	63.1609
Peak #28	26.561	3.35318	6.00%	97.8277
Peak #29	26.77	3.32753	10.20%	131.147
Peak #30	27.374	3.25547	6.90%	103.49
Peak #31	27.922	3.19279	6.50%	98.7953
Peak #32	28.252	3.15628	5.50%	90.3913
Peak #33	29.25	3.05084	5.30%	87.8315
Peak #34	29.917	2.9843	2.60%	64.1173
Peak #35	30.632	2.9162	4.10%	74.4063
Peak #36	31.616	2.82768	7.50%	104.365
Peak #37	32.161	2.78101	4.10%	76.0865
Peak #38	32.497	2.753	2.30%	60.5502
Peak #39	32.983	2.71354	1.90%	54.3043
Peak #40	34.188	2.62063	2.20%	57.4165
Peak #41	34.591	2.59101	2.40%	58.2956
Peak #42	36.369	2.4683	2.60%	61.6744
Peak #43	38.393	2.34271	3.70%	70.3168

CLAIMS

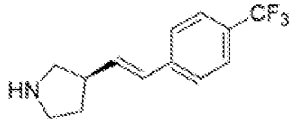
WHAT IS CLAIMED IS:

1. A method for preparing a compound of formula I,



formula I,

wherein comprising the step of reacting a compound of formula 7



with prop-2-enoyl chloride or acrylic anhydride in an organic solvent with a base reagent.

2. The method of claim 1, wherein reaction conditions for preparing the compound of formula I are:

1) an organic solvent is selected from the group consisting of CH_2Cl_2, THF, 2-Methyl THF, 1,4-dioxane or ACN;

2) a base reagent is selected from the group consisting of $NaHCO_3$, Na_2CO_3, K_2CO_3, $KHCO_3$, Et_3N, iPr_2NEt, Pyridine, or N-Methyl morpholine;

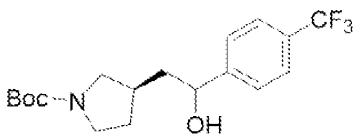
3) reaction time: 1 to 24 hours;

4) reaction temperature: 0 to 25°C.

3. The method of claim 1 or 2, wherein the compound of formula 7 is obtained by removing an amino protecting group.

4. The method of claim 3, wherein the amino protecting group is selected from the group consisting of the group consisting of 2-(trimethylsilyl)ethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), tert-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), and benzyloxycarbonyl (Cbz).

5. The method of claim 3, wherein the compound of formula 7 is obtained by removing of the amino protecting group and dehydration of the compound of formula 6,



formula 6.

6. The method of claim 5, wherein reaction conditions for preparing the compound of formula 7 are:

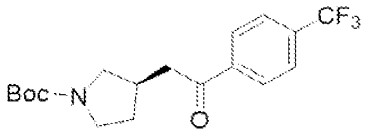
1) a solvent is selected from the group consisting of 2-Me-THF or 1,4-dioxane;

2) reaction time: 1 to 10 hours;

3) reaction temperature: 80 to 100 °C;

4) equivalents of H_2SO_4: 1 to 10 eq.

7. The method of claim 5, wherein the compound of formula 6 is obtained by reducing the compound of formula 5,



formula 5.

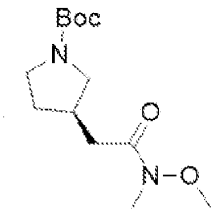
8. The method of claim 7, wherein reaction conditions for preparing the compound of formula 6 are:

1) a solvent is selected from the group consisting of MeOH, EtOH or THF;

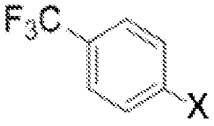
2) a reducing agent is selected from the group consisting of $NaBH_4$, $NaB(CN)H_3$ or DIBAL-H;

3) equivalents of reducing reagents: 0.3 to 1.2eq.

9. The method of claim 7, wherein the compound of formula 5 is obtained by reacting the compound of formula 3 with the lithium reagent generated from n-BuLi and the compound of formula 4,



formula 3 and

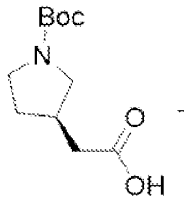


formula 4,

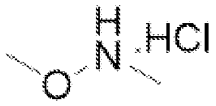
X in the compound of formula 4 is Br or I; and

a reaction solvent is selected from the group consisting of THF, 2-Me-THF, diethyl ether and 1,4-dioxane.

10. The method of claim 9, wherein the compound of formula 3 is obtained by reacting the compound of formula 1 with the compound of formula 2,



formula 1 and



formula 2

wherein reaction conditions are:

1) a solvent is selected from the group consisting of THF, 2-Me-THF, 1,4-dioxane, DMF or CH_2Cl_2;

2) reaction temperature: 0 to 25°C;

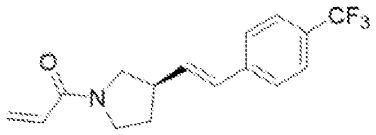
3) reaction time: 1 to 24h;

4) a coupling reagent is select from CDI, HATU, HBTU/HOBT, or EDCI;

5) a base is selected from the group consisting of Et_3N, iPr_2NEt or N-methyl morpholine;

6) equivalents of coupling reagents: 1 to 2 eq.

11. A crystalline form of a compound represented by formula I:



formula I,

wherein the crystalline form is characterized by X-ray powder diffraction peaks at 2θ angles (± 0.2°) 20.4°, 21.1°, and 21.7°.

12. The crystalline form of claim 11, wherein the crystalline form is further characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 15.2° and 23.0°.

13. The crystalline form of claim 12, wherein the crystalline form is further characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 13.6°, 14.0°, 16.5° and 17.0°.

14. The crystalline form of any one of claims 11-13, wherein the crystalline form is characterized by an X-ray powder diffraction pattern substantially similar to FIG. 1.

15. The crystalline form of any one of claims 11-14, wherein the crystalline form is at least 60% a single crystalline form, at least 70% a single crystalline form, at least 80% a single crystalline form, at least 90% a single crystalline form, at least 95% a single crystalline form, or at least 99% a single crystalline form by weight.

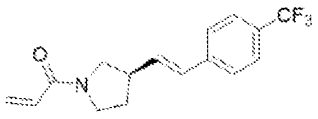
16. The crystalline form of any one of claims 11-15, wherein the crystalline form has a chemical purity of at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% by weight.

17. The crystalline form of any one of claims 11-16, wherein the crystalline form is characterized by a differential scanning calorimetry (DSC) curve that comprises an endotherm at about 87±2°C, preferably about 86.9-88.6°C.

18. The compound crystalline form of claim 17, wherein the crystalline form is characterized by the DSC curve substantially similar to FIG. 2.

19. The crystalline form of any one of claims 11-18, wherein the crystalline form is an anhydrate.

20. A crystalline form of a compound represented by formula I:



formula I,

wherein the crystalline form characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 17.3° and 18.3°.

21. The crystalline form of claim 20, wherein the crystalline form further characterized by X-ray powder diffraction peaks at 2θ angle (± 0.2°) 20.4°, 21.1° and 21.7°.

22. The crystalline form of claim 20 or claim 21, wherein the crystalline form is characterized by an X-ray powder diffraction pattern substantially similar to the curve in FIG. 3.

23. A method to form the crystalline form according to any one of claims 11-22, the crystalline form is formed by evaporation or evaporation crystallization, wherein the solvent used in any one of claims 11-19 is selected from the group consisting of THF, ACN, MTBE; the solvent used in any one of claims 20-22 is selected from the group consisting of acetone, ethyl acetate (EA), methyl ethyl ketone(MEK) and ethanol.

24. A pharmaceutical composition, comprising a pharmaceutically effective amount of the crystalline form according to any one of claims 11-22 and a pharmaceutically acceptable carrier.

25. A method of treating cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the compound crystalline form of anyone of claims 11-22 or the pharmaceutical composition of claim 24; or

the compound crystalline form of anyone of claims 11-22 or the pharmaceutical composition of claim 24, for use as a medicament for treating cancer in a subject in need thereof; or

use of the compound crystalline form of anyone of claims 11-22 or the pharmaceutical composition of claim 24 in the manufacture of a medicament for treating cancer in a subject in need thereof.

26. The method or use of claim 25, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, ovarian cancer, pancreatic ductal adenocarcinoma (PDAC), glioblastoma, gastric cancer, cervical cancer, colon cancer, endometrial cancer, head and neck cancer, lung cancer, melanoma, multiple myeloma, leukemia, non-Hodgkin's lymphoma, prostate cancer, rectal cancer, malignant melanomas, alimentary/gastrointestinal tract cancer, liver cancer, skin cancer, lymphoma, malignant pleural mesothelioma (MPM), kidney cancer,

muscle cancer, bone cancer, brain cancer, eye or ocular cancer, rectal cancer, colorectal cancer, cervical cancer, oral cancer, benign and malignant tumors, stomach cancer, corpus uteri, testicular cancer, renal cancer, throat cancer, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's Sarcoma, Kaposi's Sarcoma, basal cell carcinoma and squamous cell carcinoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, angiosarcoma, hemangioendothelioma, Wilms Tumor, neuroblastoma, mouth/pharynx cancer, esophageal cancer, larynx cancer, neurofibromatosis, tuberous sclerosis, hemangiomas, and lymphangiogenesis, preferably, the cancer is selected from the group consisting of glioblastoma, gastric cancer, colorectal cancer, pancreatic ductal adenocarcinoma (PDAC), and malignant pleural mesothelioma (MPM).

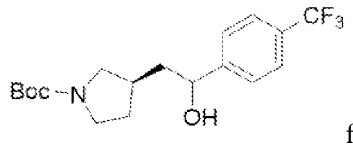
27. A method of inhibiting TEAD1, 2, 3, and/or 4 in a subject or in a sample, comprising administering to the subject a therapeutically effective amount of the compound crystalline form of any one of claims 11-22 or the pharmaceutical composition of claim 24, or

the compound crystalline form of anyone of claims 11-22 or the pharmaceutical composition of claim 24, for use as a medicament for inhibiting TEAD1, 2, 3, and/or 4 in a subject or in a sample; or

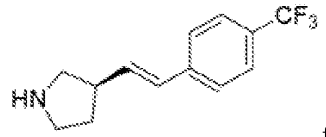
use of the compound crystalline form of anyone of claims 11-22 or the pharmaceutical composition of claim 24 in the manufacture of a medicament for inhibiting TEAD1, 2, 3, and/or 4 in a subject or in a sample,

preferably, TEAD1 and 3 are inhibited selectively over TEAD2 and 4.

28. A compound represented by the formula of compound 6 or 7,



formula 6,



formula 7.