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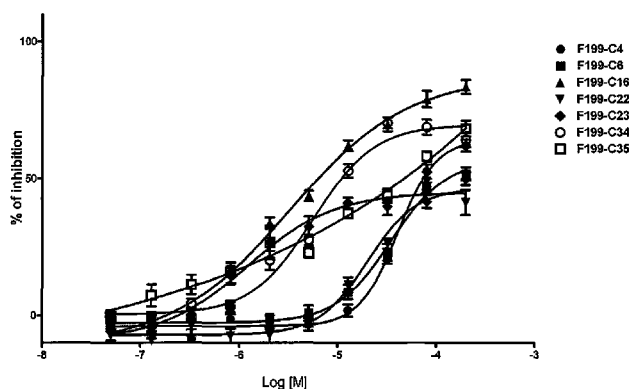
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[Continued on next page]

(54) Title: CDK5 INHIBITORS AND THERAPEUTIC USES THEREOF

FIG. 4

Drug	IC ₅₀ (μM)	Maximum inhibition
F199-C4 (gallic acid)	40.6	64.19% at 200 μM
F199-C6 (rodafin)	34.4	56.61% at 200 μM
F199-C16 (litvinolin)	3.24	87.5% at 200 μM
F199-C22 (6-O-galloyl rosin, novel structure)	20.1	46.5% at 80 μM
F199-C23 (4'-methoxyherbacetin)	1.21	45% at 20 μM
F199-C34 (6-O-galloyl arbutin)	5.63	70% at 80 μM
F199-C35 (epigallocatechin-3-gallate)	14.72	68.4% at 200 μM

(57) Abstract: Natural occurring inhibitors of cyclin-dependent protein kinase 5 (Cdk5), isolated from the root of *Rhodiola rosea* are structurally different from the known Cdk inhibitors. They show selectivity among different Cdk and efficacy to inhibit Cdk via a mixed-type of inhibition, which should lead to less toxicity and side-effects. They are useful in preventing and treating diseases and disorders associated with aberrant Cdk5 activities, such as acute and/or chronic pain, neuropathic pain, diabetes mellitus, cancer, neurodegenerative diseases and neuropathological disorders.



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CDK5 INHIBITORS AND THERAPEUTIC USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims priority from U.S. Provisional Application No. 61/282,039, filed December 7, 2009.

FIELD OF THE INVENTION

[2] This invention relates to new Cdk inhibitors isolated from natural sources for therapeutic uses. More particularly, it relates to compounds that are naturally occurring Cdk5 inhibitors from the plant *Rhodiola rosea* and their biological activities as Cdk5 inhibitors.

BACKGROUND OF THE INVENTION

[3] With today's high throughput chemical synthetic technologies and high efficiency screening methodologies, it may not be difficult to find chemical compounds that show promising biological effects at the cellular level in the laboratory. However, the rate of these compounds becoming clinically useful is very low due to a number of factors. A key factor is their toxicity and side effects, which are often severe and may not be tolerable by the human body during later stages of drug development. In this respect, new compounds discovered from natural sources that have been used therapeutically for thousands of years have received renewed

attention due to the fact that they have been consumed by humans for a long time and thus, their toxicity and side effects are more likely to be more tolerable than purely synthetic compounds.

[4] Cyclin-dependent kinases (Cdks) belong to a family of proline-directed serine/threonine kinases that play important roles in controlling cell cycle progression and transcriptional control. Activation of Cdks requires the association with specific regulatory subunits, cyclin, and requires the phosphorylation at specific threonine residues on Cdks. Cdk1, 2, 3, 4 and 6 play important roles in regulating the transition of different cell cycle phases. Cdk1 is a mitotic Cdk, whereas Cdk2, 4 and 6 are interphase Cdks that play regulatory roles in the progression of quiescent G1 to S phases. Therefore, abnormal activation of Cdks leads to cell cycle deregulation such as continued cell proliferation or unrestrained cell cycle re-entry, and subsequent development of diseases such as cancers (Shapiro, 2006; Malumbres & Barbacid, 2009). Cyclin-dependent kinase 5 (Cdk5), a proline-directed serine/threonine kinase, is unique due to its indispensable role in neuronal development and function. Despite its structural homology with other Cdks, Cdk5 may not be involved in the regulation of cell cycle. Activation of Cdk5 is dependent on its association with two specific activators, p35 and p39, which are expressed in neuronal cells. Therefore, while Cdk5 is expressed ubiquitously in cells, it is mainly active in postmitotic neurons due to the restricted expression of p35 and p39 (Tsai et

al., 1993; Zheng et al., 1998).

[5] Cdk5 plays a diverse physiological role in neural cells, including neuronal migration (Xie et al., 2003) and axon guidance (Connell-Crowley et al., 2000) during early neural development as well as synapse formation and synaptic plasticity (Cheung et al., 2006; Lai and Ip, 2009). However, more recently, Cdk5 has also been found to play important roles outside the central nervous system such as pain signaling that involves the sensory pathways (Pareek et al., 2006), and in modulating glucose-stimulated insulin levels in pancreatic beta cells, (Wei et al., 2005). Due to its key physiological roles, uncontrolled activity of Cdk5 has been linked to various diseases/disorders such that Cdk5 has emerged as a potential molecular target for therapeutic drugs. In neurons, Cdk5 deregulation triggers neuronal apoptosis (Cheung and Ip, 2004), suggesting that aberrant regulation of Cdk5 activity is responsible for the progression of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Aberrant Cdk5 activity, for example, is also linked to cancer development, progression and metastasis such as prostate and thyroid carcinoma (Strock et al., 2006; Lin et al., 2007).

[6] The two major pathological hallmarks of AD are the accumulation of senile plaques and neurofibrillary tangles in the diseased brain. The deregulation of Cdk5 is caused by the presence of p25, a cleavage product of p35 generated under pathological conditions (Patrick et al., 1999). Accumulation of p25 protein is found in

the brains of AD patients (Patrick et al., 1999). Recent findings also indicate that Cdk5 is one of the key kinases that regulate the formation of senile plaques (Monaco, 2004) and neurofibrillary tangles (Cruz et al., 2003).

[7] Another major neurodegenerative disease that links to Cdk5 is PD). Pathologically, PD is characterized by motor impairment due to the progressive death of selected populations of neurons, especially the dopaminergic neurons in the substantia nigra pars compacta (Muntane et al., 2008). In a PD mouse model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), elevated expression and activity of Cdk5 have been reported to be correlated with dopaminergic neurons cell death (Smith et al., 2003; Qu et al., 2007). Moreover, it is of interest to note that inhibition of Cdk5 results in an increase in dopamine release, which may help ameliorate PD progression (Chergui et al., 2004). Cdk5 has also been implicated in a plethora of other neurodegenerative diseases and neurological disorders such as Huntington's disease (Anne et al., 2007), Amyotrophic Lateral Sclerosis (ALS; Bajaj et al., 1998) and ischemic injury (Wang et al., 2003).

[8] More recently, aberrant Cdk5 activity has been linked to the pathogenesis of diabetes mellitus (type-2 diabetes). p35, the activator of Cdk5, is present in pancreatic beta cells and its activity negatively modulates insulin release in response to glucose (Wei and Tomizawa, 2007). A sustained increase in p35 protein and Cdk5 activity is reported in murine pancreatic beta cells upon high glucose exposure

(Ubeda et al., 2006). Moreover, inhibition of Cdk5 activity by chemical inhibitors increases insulin secretion in cultured beta cells and in a mouse model of diabetes in a glucose-dependent manner (Ubeda et al., 2006). These findings are consistent with the observation that p35^{-/-} mice exhibit enhanced insulin secretion upon glucose challenge (Wei et al., 2005). Cdk5 is thought to act through the regulation of the Ca²⁺ channel activity or regulation of insulin gene expression during glucotoxicity (Wei et al., 2005; Ubeda et al., 2006). Thus, Cdk5 inhibitors could be potential therapeutic agents for the treatment of type-2 diabetes (Kitani et al., 2007).

[9] Cdk5 has also been emerging as a major potential target for analgesic drugs. Cdk5/p35 has been indirectly linked to nociceptive pathways. For example Cdk5 regulates the activation of mitogen activated protein kinase (MAPK) in nociceptive neurons potentially modifying the hyperalgesia that results in increased MAPK activity. Cdk5 has also been implicated in other pain pathways such as calcium calmodulin kinase II, delta FosB, the NMDA receptor and the P/Q type voltage-dependent calcium channel. Furthermore, studies suggest that Cdk5 inhibitors may be of benefit in the management of acute pain. Cdk5/p35 is shown to be involved in the processing of pain while its inhibition reduces the responsiveness of normal pain pathways (Pareek et al., 2006; Pareek and Kulkarni, 2006). More specifically, peripheral inflammation in rats induces an increase in Cdk5 activity. While p35 transgenic mice with elevated Cdk5 activity are more sensitive to painful stimuli,

p35^{-/-} and conditional Cdk5^{-/-} mice with markedly reduced Cdk5 activity show delayed response to pain stimuli (Pareek et al., 2006; Pareek et al., 2007). Cdk5 also regulates mitogen-activated protein kinase1/2 (MEK1/2) /1M activity through a negative feedback loop during a peripheral inflammatory response (Pareek and Kulkarni, 2006). In addition, transient receptor potential vanilloid 1 (TRPV1), a ligand-gated cation channel that is activated by heat, protons and capsaicin, was recently identified as a substrate of Cdk5 (Pareek et al., 2007). Since phosphorylation of TRPV1 by Cdk5 regulates the functions of TRPV1 during pain signaling, it is believed that Cdk5 could serve as a new molecular target for developing analgesic drugs.

[10] Since Cdk5 is associated with various diseases, screening of inhibitors that target Cdk5 may help identify potential drug leads. However, to date, only a few Cdk5 inhibitors have been identified and they are far from being ready for clinical evaluation for neuro-indications. Roscovitine, a member of the 2,6,9-substituted purine analogs, is one of the Cdk5 inhibitors in development but it also targets Cdk1, Cdk2, Cdk7 and Cdk9 (Meijer et al., 1997). Currently, roscovitine is in phase-2 clinical trials for non-small cell lung carcinoma, breast cancer, and B-cell malignancies. The indirubin family is another class of Cdk inhibitors that has its roots in Chinese medicine. The bis-indole indirubin is an active component of Ganggui Longhui Wan, a traditional Chinese medicine recipe used for the treatment

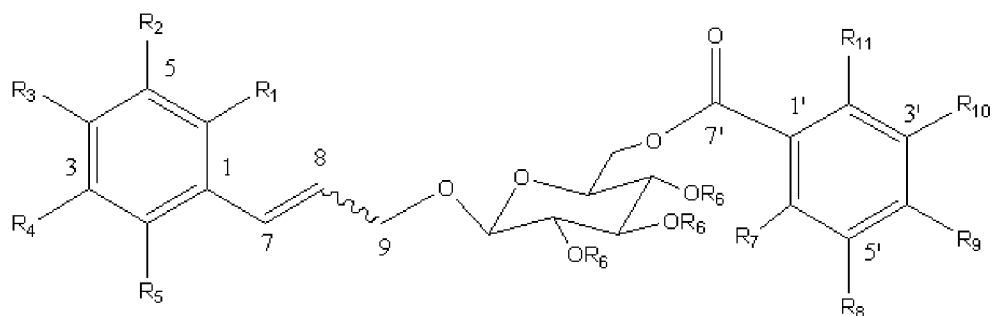
of leukemia and other chronic illnesses due to its antimitotic and antitumor activities (Leclerc et al., 2001). Indirubin inhibits various kinases including Cdk1, Cdk5 as well as glycogen synthase kinase-3 beta (GSK3 β). GSK3 β , together with Cdk5, is believed to be responsible for the hyperphosphorylation of TAU observed in AD.

[11] Clearly, the scientific literature strongly indicates that Cdk5 inhibitors are promising therapeutic agents for the treatment of pain and in the management of type-2 diabetes, and may also be useful in treating neurodegenerative diseases and neurological disorders. Yet, there is a lack of promising candidate compounds that can effectively inhibit Cdk5 over other Cdks.

SUMMARY OF THE INVENTION

[12] One object of the present invention is to provide a butanol fraction of *Rhodiola rosea* root having excellent Cdk5 inhibitory activity, and a composition comprising the same.

[13] Another object of the present invention, accordingly, is to provide a number of natural occurring compounds showing inhibitory effects on Cdk5 with high specificity. All the compounds are isolated and purified from a natural herb *Rhodiola rosea*. Some of those compounds are novel and have a structure of formula (I):



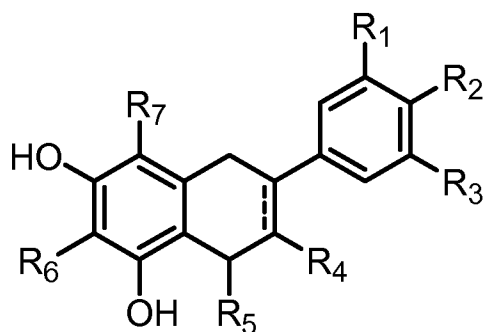
Formula I

[14] where R₁ -R₁₁ are each independently unsubstituted (i.e., being hydrogen) or substituted with the same or independently different substituents deemed suitable by a person of ordinary skill in the art. Example is as follows: F199-C22. A “substituent” is a commonly used term in organic chemistry and is understood by a person of ordinary skill in the art to be an atom or group of atoms substituted in place of a hydrogen atom on the parent chain of a hydrocarbon. It is within ordinary skill of the art to know what a particular substituent can be at a given position on the parent chain of hydrocarbon or the backbone without undue experimentation. Thus, for the present invention and the claims thereof, the term “substituent” means a suitable replacement of the hydrogen atom on the parent chain of a hydrocarbon, which is obvious to or easily determined by a person of ordinary skill in organic chemistry without needing undue experimentation. Examples of possible substituent for R₁ -R₅ may be hydroxyl, alkoxyl, acyl, halogen, alkyl, heteroalkyl, arylalkyl, cycloalkyl, cycloalkyl-alkyl, haloalkyl, heterocycloalkyl or aryl. For R₆, the

substituent may be acyl, alkyl, heteroalkyl, arylalkyl, cycloalkyl, cycloalkyl-alkyl, haloalkyl, heterocycloalkyl or aryl, except Ph-CO. For R₇-R₁₁, the substituent may be hydroxyl, alkoxy, acyl, halogen, alkyl, heteroalkyl, arylalkyl, cycloalkyl, cycloalkyl-alkyl, haloalkyl, heterocycloalkyl or aryl. The wavy line denoted by  indicates the carbon to which the wavy line is attached has a configuration of cis, trans or a mixture thereof.

[15] Of course, as it is understood by people of ordinary skill in the art, those natural occurring compounds may be produced by synthetic processes in the laboratory or in an industrial scale. It is further understood that those identified compounds may be made in the forms of a pharmaceutically acceptable salt, prodrug, hydrate and isomers thereof as it may be deemed suitable by the users under their particular situations.

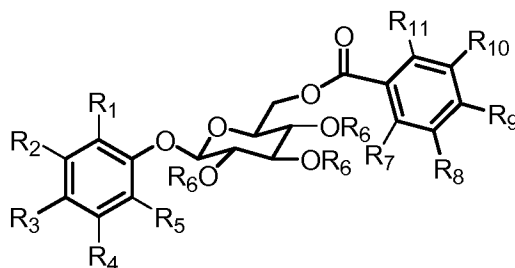
[16] Another object of the present invention is to provide a method of inhibiting Cdk5 activities, specifically, inhibiting Cdk5/p25 and Cdk5/p35 activities, with high specificity. The objected is achieved by contacting the enzyme with the compounds isolated from a natural herb *Rhodiola rosea*. Some of the compounds are of formula II or III:



Formula II

[17] where $R_1 - R_7$ are each independently unsubstituted (i.e., being hydrogen) or substituted with the same or independently different substituents deemed suitable by a person of ordinary skill in the art. Examples of the compounds are as follow:

F199-C6, F199-C16, F199-C23, F199-C35, F199-C42, and F199-C57.



Formula III

[18] where $R_1 - R_{11}$ are each independently unsubstituted (i.e., being hydrogen) or substituted with the same or independently different substituents deemed suitable by a person of ordinary skill in the art. Example of the compounds is as follow: F199-C34. There are other Cdk5 inhibitors of the present invention which are not of a structural formula II or III. Examples are as follows: F199-C4 and F199-C22. Detailed

information about the structure of the compounds referenced here is provided later in this specification.

[19] A further object of the present invention is to provide a method for treating or preventing diseases that are caused by aberrant changes in Cdk5's activities. Examples of such diseases are acute and chronic pain, diabetes mellitus, cancer, neurodegenerative disease and neuropathological disorders (such as AD, PD, ALS and Huntington's disease). The object is achieved by administering a therapeutically effective amount of compounds to patients as specified in the foregoing.

[20] In addition to the effect on Cdk5, the treatment method of the present invention also protects neuronal cells from neurotoxicity and apoptosis induced by the amyloid beta peptide ($A\beta$). $A\beta$ is a cleavage product derived from the amyloid precursor protein (APP), which accumulates as extracellular or senile plaques, the hallmark of AD. While the actual cause of AD remains elusive, $A\beta$ has been implicated in many reports to play a part in the initiation and progression of the disease. Additionally, studies have shown that $A\beta$ is neurotoxic, resulting in neuronal loss and subsequent memory loss and cognitive impairment.

[21] The various features of novelty which characterize the invention are pointed out with particularity in the claims annexed to and forming a part of this disclosure. For a better understanding of the invention, its operating advantages, and specific

objects attained by its use, reference should be made to the drawings and the following description in which there are illustrated and described preferred embodiments of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[22] FIG. 1 presents the dose response curve of Cdk5/p25 and Cdk5/p35. Different concentrations of Cdk5/p25 or Cdk5/p35 were incubated with substrates and ATP for 1 hour. The assay was performed in quadruplicates.

[23] FIG. 2 shows inhibitory effect of roscovitine on Cdk5 kinase activity in a dose-dependent manner. Roscovitine at various concentrations were incubated with 30 ng/ml of Cdk5/p25 or Cdk5/p35. The assay was performed in quadruplicates.

[24] FIG. 3 shows inhibitory effect of the compounds of the present invention. Compounds (30 μ M) were incubated with 30 ng/ml of Cdk5/p25 or Cdk5/p35. Roscovitine at 30 μ M has 100% inhibition to both Cdk5/p25 and Cdk5/p35. Assays were performed in quadruplicates and data was averaged from 4 independent experiments

[25] FIG. 4 shows dose dependent inhibitory effects on Cdk5/p25 activity by compounds of the present invention. Assays were done in quadruplicates and data was averaged from 4 independent experiments

[26] FIG. 5 shows a protective effect on rat cortical neurons against A β

excitotoxicity by compound F199-C6. Embryonic rat cortical neurons (7 DIV) were pre-treated with various concentrations of compounds (3-50 μ M) and then co-incubated with A β ₂₅₋₃₅ (10 μ M). After overnight incubation, the MTT assay was performed. Cell survival was calculated as a percentage compared to the vehicle control (DMSO). Assays were conducted in duplicates and data were compared with student-t test. * = P < 0.05.

[27] FIG. 6 shows a protective effect on rat cortical neurons against A β excitotoxicity by compound F199-C34 under the conditions as described for FIG. 5.

[28] FIG. 7 shows a protective effect on rat cortical neurons against A β excitotoxicity by compound F199-C22 under the conditions as described for FIG. 5.

[29] FIG. 8 shows differential inhibitory effects of F199-C16 on different Cdk complexes.

[30] FIG. 9 shows differential inhibitory effect of F199-C23 on different Cdk complexes.

[31] FIG. 10 shows differential inhibitory effect of F199-C34 on different Cdk complexes.

[32] FIG. 11 shows the mode of inhibition of F199-C16 and F199-C23 on Cdk5/p25 complex.

[33] FIG. 12 shows the effect of F199-C16 on the paw-licking response in an animal model of pain.

[34] FIG. 13 shows the effects on Cdk5/p25 of total extract and various fractions of *Rhodiola rosea* of the present invention.

DETAILED DESCRIPTION OF PARTICULAR EMBODIMENTS OF THE INVENTION

Preparation of Total Extract

[35] The air-dried roots of *Rhodiola rosea* were harvested in October, 2005 at Yili, Xin-Jiang, China and purchased from Sichuan Medicinal Herb Ltd. (Chengdu, China) in October, 2005. The air-dried roots of *Rhodiola rosea* (300 g) was first dried and immersed in 1.5 L 70% ethanol (EtOH, material to solvent ratio at 1 to 5) for 30 mins. The herb-solvent mixture was then refluxed 3 times for 2 hours each. The extract was filtered and the filtrate was evaporated to dryness to give 44.0 g of the total extract (TE).

Preparation of Refined Fraction (F199-BU)

[36] The TE (44.0 g) was dissolved/suspended in 300 mL water and sequentially extracted with chloroform (CF, 300 mL) and water-saturated butanol (BU, 300 mL) at 1 to 1 volume ratio. Extraction with each different solvent was repeated 5 times and the solvent extracts were filtered and dried to give fractions CF (14.5 g), BU (17.6 g), and water (WA, 10.2 g). HPLC parameters for F199-BU detection: HPLC conditions:

High-performance liquid chromatography-diode array detection (HPLC-DAD) method has been developed for the analysis of and the quality control of F199-BU batch 090725T and F199-BU batch 080602T. Waters HPLC system consisting of a 600 pump, a 717 auto-sampler and a UV/VIS Photodiode Array 2996 Detector was used for all analyses. Chromatographic separations were carried out on a SunFire C18 column (Particle size 5 μm , 4.6 mm $\times$ 150.0 mm) with acetonitrile (as Solvent A) and water (as Solvent B) in the mobile phase at a flow rate of 1.0 ml/min at room temperature. A gradient elution was applied from 10% to 60% of solvent A starting from 0 to 45 min (0~35 min, 10% to 50% ACN; 35~40 min, 50% to 60% ACN; 40~45 min, 60% to 10% ACN). Samples were filtered through a 0.45 μm Millipore syringe filter unit. Twenty microliter samples were injected for HPLC analysis.

Isolation of F199 Compounds

[37] Compounds of this invention, with the identification code beginning with "F199", are isolated from *Rhodiola rosea* as described below, using a variety of reactions known to the skilled artisan. It is understood that one skilled in the art will also recognize that alternative methods may be employed to isolate the target compounds of this invention.

[38] The air-dried roots of *Rhodiola rosea* were re-fluxed three times with 95% aqueous EtOH (35 L, 35 L, 35 L, 2h/times). The 95% EtOH extract was concentrated

in vacuo to give a residue (900 g). The residue was suspended in H₂O and then partitioned successively with petroleum ether, ethyl acetate and n-BuOH. Evaporation of these fractions resulted in a total of 90 g petroleum ether extract, 250 g EtOAc extract, and 360 g n-BuOH extract, respectively.

[39] Then, the EtOAc extract (250 g) was first fractionated using a silica gel column eluted with the mixture of CHCl₃–MeOH with ascending polarity, and finally eluted with methanol to obtain 108 fractions (Fr.1-Fr.108). Depending on the TLC (Thin Layer Chromatography) pattern, similar fractions were combined. Compounds gallic acid (F199-C4, 18 mg) (from fraction Fr.21-22), rhodalin (F199-C6, 22 mg), litvinolin (F199-C16, 36 mg) (from Fr. 62-69), 6-O-galloyl rosin (F199-C22, 10 mg), 4'-methoxyherbacetin (F199-C23, 22 mg) (from Fr. 72-75), 6-O-galloyl arbutin (F199-C34, 17 mg), and epigallocatechin-3-gallate (F199-C35) (from Fr. 89-90) were isolated using repetitive column chromatography (silica gel or Sephadex LH-20) and recrystallization methods.

[40] A part of n-BuOH extract (200 g) was subject to silica Gel column chromatography, eluting with EtOAc/EtOH/H₂O with a ratio of 20:2:1, 16:2:1, 10:2:1, 6:2:1, 4:2:1, and then 66 fractions were obtained. Compounds kaempferol-3-O- β -D-xylopyranosyl(1 $\rightarrow$ 3) β -D-glucopyranoside (F199-C42, 9 mg) (from Fr. 12-14), and isomericitrin (F199-C57, 6 mg) (from Fr. 41-54) were isolated using repetitive column chromatography (silica gel or Sephadex LH-20, finally purity with preparative

HPLC).

Cdk5 Kinase Assay and Kinetic Studies

[41] Cdk5 kinase assay was performed as described in the instruction manual (Invitrogen, cat. no. PV3673). This assay employs a FRET-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage. The assay uses a synthetic peptide substrate (the Z'-LYTE™ peptide substrate) that is labeled with a donor fluorophore (coumarin) and an acceptor fluorophore (fluorescein) to make up a FRET pair. In the primary reaction, Cdk5 transfers the γ -phosphate of ATP to a serine/threonine residue on the substrate, while the presence of a kinase inhibitor in the primary reaction suppresses phosphorylation. In the secondary reaction, a site-specific protease (the Development Reagent) is added and cleaves non-phosphorylated Z'-LYTE™ peptide substrate at a substantially higher rate than phosphorylated substrate. Cleavage disrupts FRET between the donor and acceptor fluorophores on the non-phosphorylated substrate, while uncleaved phosphorylated substrate maintains FRET. The effectiveness of the compounds on inhibiting the Cdk5 activity is then measured by calculating the emission ratio.

[42] Cdk5 kinase assay was performed by adding Z'-LYTE™ peptide substrate and ATP together with Cdk5/p25 or Cdk5/p35 kinase solution in a 384-well black

polystyrene plate (Corning 3676). The plate was then incubated for one hour at room temperature. Development solution was then added in the dark for another hour. Finally, stop solution was added and the coumarin and fluorescein emission signals were measured on a fluorescence plate reader (Tecan, infinite F500) (excitation: 400 nM, emission 445 and 520 nM, respectively). To calculate the activity of cdk5/p25 and Cdk5/p35, Z'-LYTE phosphopeptide was used as a 100% phosphorylation control while nonphosphorylated Z'-LYTE peptide is used as a 0% phosphorylation control to establish the maximal and minimal emission ratio values which enable to calculate the percentage of kinase phosphorylation. To determine the inhibitory effect on cdk5 activity, fractions and compounds to be tested were included in the kinase reaction. Zero inhibition was determined by using DMSO (solvent) instead of the compounds in the reaction. Roscovitine, a well-known cdk5 inhibitor, was used as the positive control for the inhibition of kinase activity. Assays were done in quadruplicates and data was averaged from at least 4 independent experiments. Similar experimental protocols were performed for Cdk1/cyclin B and Cdk2/cyclin A kinase activity study. Activity of each kinase complex was evaluated by titration, and the resultant value of EC_{30} was used in the inhibitory study. Since activity of kinase complexes varies with different batches of preparation, EC_{30} will be determined in each new batch of kinases. IC_{50} of the compounds on kinase complexes was determined in dose-dependent studies from an average of 3-5 individual experiments. Kinetic studies of

compounds were performed by Cdk5/p25 kinase assays with various concentrations of ATP and tested compounds. The kinetic parameters were determined by a nonlinear least-squares program using GraphPad Prism 5 software.

A β Assay

[43] Cortical neurons at 7 DIV in culture isolated from embryonic day 18 rats were used as cell models in the assay. Toxicity caused by A β 25-35 peptide in these cells was evaluated by MTT detection after 2 days incubation. The A β assay was performed to investigate the ability of the invention to protect cortical neurons from cell death induced by exogenous A β 25-35 peptide addition. Assays were done in duplicates and data was averaged from at least 3 independent experiments.

Animal Model of Pain

[44] Adult ICR mice (6-8 week-old) habituated in the experimental room for 3 days before the testing. Testing was conducted from 9 am to 3 pm. Mice were randomly grouped and weighed. Mice were pretreated with a single dose of F199-C16 (10-100 mg/kg, intraperitoneal injection) and then injected with 10 μ l of 2.5% formalin in the subplantar of the right hind paw. The licking time (in seconds) was recorded during 0-10 minutes (early phase) and in 11-40 minutes (late phase) after formalin injection. The whole process was monitored and recorded by a digital monochrome video camera. Data were expressed as the mean $\pm$ SEM. (* p <0.05; ANOVA, with Student-

Newman Keuls test).

Kinase Activity of Cdk5/p25 and Cdk5/p35

Kinase activity of Cdk5/p25 and Cdk5/p35 was measured using the Z'-LYTE™ Kinase Assay Kits (Invitrogen) as described above. The measurement data are shown in FIG. 1, which is in a form of dosage response curves. Different concentrations of Cdk5/p25 or Cdk5/p35 were incubated with substrates and ATP for 1 hour. As shown in FIG. 1, the kinase activity, indicated as the percentage of phosphorylation, increases with the increased concentration of the enzyme Cdk5/p25 and Cdk5/p35. As shown by the curve, EC₅₀ for Cdk5/p25 is about 35.8 ng/ml Cdk5/p25 is about 34.4 ng/ml.

Inhibitory Effects of Roscovitine on Cdk5 Measured in a FRET-based Assay

[45] Roscovitine (Ros) is a well-known Cdk5 kinase inhibitor. Its inhibitory effect on Cdk5/p35 and Cdk5/p25 was examined using Z'-LYTE™ Kinase Assay Kits (Invitrogen) as the positive control. FIG. 2 shows that roscovitine causes a decrease in Cdk5/p25 and Cdk5/p35 kinase activity in a dose-dependent manner, with IC₅₀ = 0.16 μM comparable to that reported in literature (Meijer et al., 1997).

Inhibitory Effects on Cdk5 of Compounds of the Present Invention

[46] Using the FRET-based Cdk5 kinase assay method described above, the compounds of the present invention were examined for their inhibitory effects on

Cdk5 kinase activity. The amount of Cdk5/p25 and Cdk5/p35 used for screening Cdk5 inhibitors is EC_{30} of the kinase complex and the EC_{30} was determined with a titration study of kinase complex. The effectiveness of the compounds on inhibiting Cdk5 activity was then measured by comparing the percent phosphorylation of peptide substrates by Cdk5 kinase in the presence of test compounds and percent phosphorylation of peptide substrates by Cdk5 without test compounds.

[47] As shown in FIG. 3, eleven compounds isolated from *Rhodiola rosea* (F199) showed inhibitory effects on the kinase activity of Cdk5/p25 and Cdk5/p35, albeit to different extents. F199-C1 (daucosterol), F199-C22 (6-O-galloyl rosin, novel structure) and F199-C42 (kaempferol-3-O- β -D-xylopyranosyl(1 $\rightarrow$ 3) β -D-glucopyranoside) showed weak inhibition (<25%) on Cdk5 activity. F199-C4 (gallic acid), F199-C6 (rodalin), and F199-C57 (isomericitrin) showed a moderate inhibition (25-50%), while F199-C16 (litvinolin), F199-C23 (4'-methoxyherbacetin), F199-C34 (6-O-galloyl arbutin), and F199-C35 (epigallocatechin-3-gallate) exhibited a strong inhibition (50-75%). F199-C1 (daucosterol) and F199-C2 (kaempferol) served as controls since they have been reported to moderately inhibit Cdk5 activity.

IC₅₀ and Maximal Inhibition

[48] Seven natural compounds isolated from *Rhodiola rosea* (F199) were selected for

IC₅₀ and maximal inhibition determination. Various concentrations of the F199 compounds were incubated with Cdk5/p25. The effectiveness of the compounds on inhibiting Cdk5 activity was then calculated by comparing the percent phosphorylation of peptide substrates by Cdk5/p25 in the presence of test compounds and percent phosphorylation of peptide substrates by Cdk5/p25 without test compounds. The results are presented in Table 1 and FIG. 4, which show that, of the seven compounds tested, F199-C16 (litvinolin) and F199-C34 (6-O-galloyl arbutin) have the lowest IC₅₀ and highest maximum inhibition to Cdk5/p25.

Table 1

Compound	IC ₅₀ (μM)	Maximum inhibition
F199-C4 (gallic acid)	40.6	64.19% at 200 μM
F199-C6 (nodalin)	34.4	56.61% at 200 μM
F199-C16 (litvinolin)	3.24	87.5% at 200 μM
F199-C22 (6-O-galloyl rosin, novel structure)	20.1	46.5% at 80 μM
F199-C23 (4'-methoxyherbacetin)	1.21	45% at 20 μM
F199-C34 (6-O-galloyl arbutin)	5.63	70% at 80 μM
F199-C35 (epigallocatechin-3-gallate)	14.72	68.4% at 200 μM

Effect of Anti-Excitotoxicity Induced by Aβ

[49] Exogenous addition of Aβ initiates cell death of neuronal cultures via apoptosis. The MTT assay, a well-known cell death assay, was performed to study the ability of the compounds of the present invention to prevent Aβ-induced excitotoxicity in primary cortical neuronal cells. Embryonic rat cortical neurons (7

DIV) were pre-treated with various concentrations of the compounds (3-50 μ M) and then co-incubated with A β 25-35 (10 μ M). After overnight incubation, the MTT assay was performed. Cell survival was calculated as a percentage compared to the vehicle control (DMSO). Assays were conducted in duplicates and data were analyzed with student-t test. * = $P < 0.05$. The results of exemplary compounds, F199-C6, F199-C34 and F199-C22 are presented in FIG. 5, FIG. 6 and FIG. 7, respectively. It is shown that those compounds at about 30 and 50 μ M significantly promoted neuronal cell survival against A β toxicity compared to the DMSO control. These results demonstrate that the compounds of the present invention have significant effects in reducing the excitotoxicity induced by the A β .

Selectivity among Different Cdks

[50] F199 compounds showed different selection or specificity among different types of Cdks, which can be useful information in developing therapeutic agents for different indications associated with different Cdks. FIG. 8 shows that while F199-C16 inhibited Cdk1/cyclin B complex potently, it exhibited milder inhibition on both Cdk5/p35 and Cdk5/p25. Inhibitory effect of F199-C16 on Cdk2/cyclin A was minimal. As shown in FIG. 9, F199-C23 inhibited similarly on Cdk1/cyclin B, Cdk5/p35 and Cdk5/p25 complexes, but its inhibitory effect on Cdk2/cyclin A was minimal. For F199-C34, while showing potent inhibitory effect on the Cdk5/p25 complex, it showed less effect on Cdk1/cyclin B, Cdk2/cyclin A, and Cdk5/p35

complexes.

Mixed-type of Inhibition on Cdk5/p25 Complex

[51] The inhibition mode of compounds, F199-C16 and F199-C23, at 0.5, 1, 2 and 4 μ M was examined by measuring their effects on the pathological Cdk5/p25 complex in the presence of increasing concentrations of ATP using the FRET assay. FIG. 11 shows that both F199-C16 and F199-C23 exhibited a typical type of mixed inhibition on Cdk5/p25 complex, while roscovitine displayed a typical type of competitive inhibition.

Reduction of Paw-licking Response in Pain Model

[52] The effect of F199-C16 was examined in an animal model of pain. The pain was induced by injecting formalin into the paw of the mouse. As shown in FIG. 12, F199-C16 reduced the licking time of right hind paw in a dose-dependent manner. At 100 mg/kg, the compound significantly reduced the licking time in both early (0-10 min) and late (11-40 min) phases of formalin-induced pain.

Effects of Total Extract and Various Fractions on Cdk5

[53] Total extract (TE) of *Rhodiola rosea* was prepared as described above. TE was then further fractionated using solvent partition, and 3 fractions, chloroform (CF), butanol (BU) and water (WA) were obtained. As shown in FIG. 13, F199-TE, F199-CF and F199-BU inhibited the pathological Cdk5/p25 and Cdk1/cyclin B more effectively

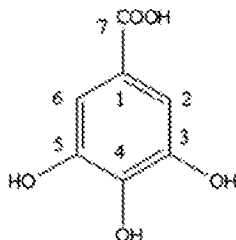
than Cdk5/p35. While the IC_{50} of F199-BU on Cdk1/cyclin B and Cdk5/p25 were 0.045 and 0.035 ng/ μ l, respectively, the IC_{50} on Cdk5/p35 was 0.11 ng/ μ l.

HPLC Chromatograms of F199-BU

[54] The refined fraction (F199-BU) prepared from *Rhodiola rosea* extract (F199-TE) exhibited Cdk5 inhibitory effect as described in FIG. 13. The protocol to prepare the refined fraction was described as the above. As shown in FIG. 14, the HPLC chromatograms of two batches of F199-BU (090725T and 080620T) were largely overlapped, indicating the fraction is highly reproducible and stable using our propriety fractionation procedures.

Characterization of Compounds isolated from *Rhodiola rosea*

Gallic Acid (F199-C4):

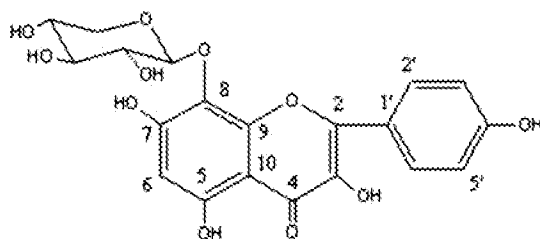


[55] This compound has a molecular weight of 170 and molecular formula of $C_7H_6O_5$ on the basis of 1H and ^{13}C NMR data in the following table, and is determined to be gallic acid.

The ^1H and ^{13}C NMR data of compound gallic acid

Position	^1H NMR	^{13}C NMR
1		121.78
2	7.05 (1H, s)	110.16
3		146.16
4		139.38
5		146.16
6	7.05 (1H, s)	110.16
7		170.15

Rhodalin (F199-C6):

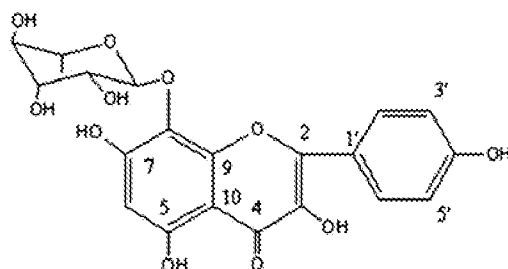


[56] This compound has a molecular weight of 434 and molecular formula of $\text{C}_{20}\text{H}_{18}\text{O}_{11}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be rhodalin.

The ^1H and ^{13}C NMR data of compound rhodalin (CD_3OD)

Position	^1H NMR	^{13}C NMR
2		148.02
3		137.03
4		177.12
5		149.89
6	6.23 (1H, s)	99.31
7		149.90
8		126.07
9		148.02
10		104.29
1'		123.67
2'	8.34 (1H, d, $J=8.8$ Hz)	131.16
3'	6.89 (1H, d, $J=8.8$ Hz)	116.12
4'		160.41
5'	6.89 (1H, d, $J=8.8$ Hz)	116.12
6'	8.34 (1H, d, $J=8.8$ Hz)	131.15
Xyl		
1''	4.69 (1H, d, $J=7.6$ Hz)	104.30
2''	3.60 (1H, m)	75.16
3''	3.40 (1H, m)	77.47
4''	3.60 (1H, m)	70.92
5''	3.93 (1H, dd, $J=5.2, 11.2$ Hz) 3.22 (1H, t, $J=11.2$ Hz)	67.40

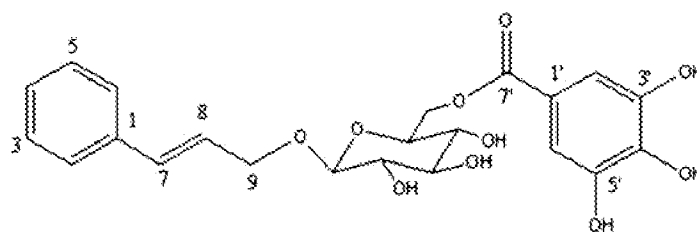
Litvinolin (F199-C16):



[57] This compound has a molecular weight of 448 and molecular formula of $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be litvinolin.

The ^1H and ^{13}C NMR data of compound litvinolin (DMSO)

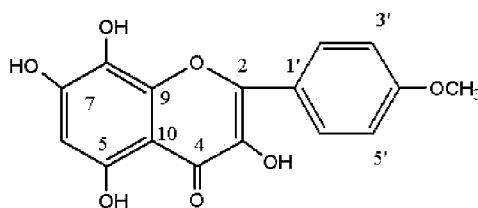
Position	^1H NMR	^{13}C NMR
2		147.44
3	8.78 (1H, brs OH)	135.81
4		176.41
5	11.90 (1H, brs OH)	151.66
6	6.59 (1H, s)	98.38
7	9.45 (1H, brs OH)	148.02
8		127.13
9		144.54
10		104.61
1'		121.83
2'	8.15 (1H, brs)	129.83
3'	6.95 (1H, brs)	115.46
4'	10.13 (1H, brs, OH)	159.39
5'	6.95 (1H, brs)	115.46
6'	8.15 (1H, brs)	129.83
Rha		
1''	5.48 (1H, brs)	99.49
2''	3.95 (1H, m)	70.11
3''	3.82 (1H, m)	70.11
4''	3.34 (1H, m)	71.78
5''	3.82 (1H, m)	69.93
6''	1.23 (3H, brs)	17.91

6-O-Galloyl Rosin (F199-C22):

[58] This compound has a molecular weight of 448 and molecular formula of $\text{C}_{22}\text{H}_{24}\text{O}_{10}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be 6-O-galloyl rosin.

The ^1H and ^{13}C NMR data of compound 6-*O*-galloyl rosin (CD_3OD)

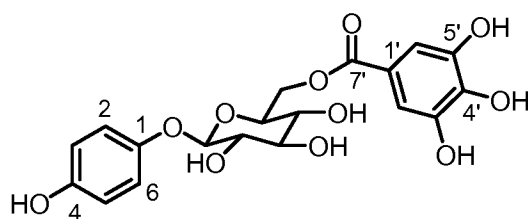
Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
1		138.29	Glc-1	4.40 (1H, d, $J=8.0$ Hz)	103.38
2	7.34 (1H, d, $J=7.6$ Hz)	127.74	Glc-2	3.26 (1H, m)	75.76
3	7.24 (1H, t, $J=7.6$ Hz)	129.72	Glc-3	3.33 (1H, m)	78.72
4	7.18 (1H, brd, $J=7.6$ Hz)	128.87	Glc-4	3.43 (1H, m)	70.97
5	7.24 (1H, t, $J=7.6$ Hz)	129.72	Glc-5	3.53 (1H, m)	75.33
6	7.34 (1H, d, $J=7.6$ Hz)	127.74	Glc-6	4.55 (1H, dd, 12.0, 2.0) 4.44 (1H, dd, 12.0, 5.6)	65.02
7	6.57 (1H, d, $J=16.0$ Hz)	134.43	1'		121.70
8	6.33 (1H, ddd, $J=16.0$, 5.6, 1.2 Hz)	126.59	2'	7.11 (1H, s)	110.43
9	4.42 (1H, m) 4.27 (1H, m)	72.04	3'		146.78
			4'		140.00
			5'		146.78
			6'	7.11 (1H, s)	110.43
			7'		168.50

4'-Methoxyherbacetin (F199-C23):

[59] This compound has a molecular weight of 316 and molecular formula of $\text{C}_{16}\text{H}_{12}\text{O}_7$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be 4'-methoxyherbacetin.

The ^1H and ^{13}C NMR data of compound 4'-methoxyherbacetin (DMSO)

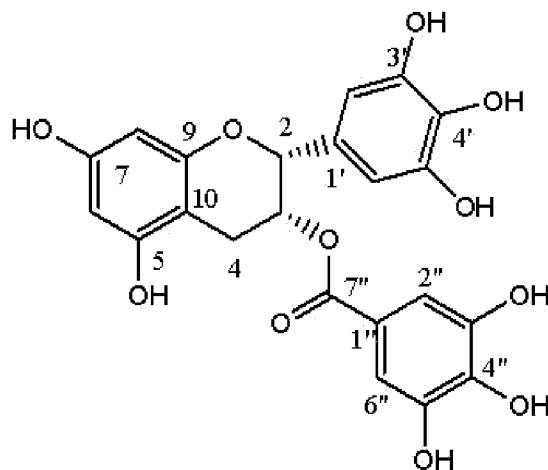
Position	^1H NMR	^{13}C NMR
2		154.53
3		136.00
4		178.18
5		155.00
6	6.49 (1H, s)	95.82
7		148.80
8		127.89
9		145.47
10		105.10
1'		124.23
2'	8.22 (1H, d, J=8.0 Hz)	131.31
3'	6.93 (1H, d, J=8.0 Hz)	116.57
4'		160.94
5'	6.93 (1H, d, J=8.0 Hz)	116.57
6'	8.22 (1H, d, J=8.0 Hz)	131.31
4'-OCH ₃	3.97	57.28

6-O-Galloyl Arbutin (F199-C34):

[60] This compound has a molecular weight of 424 and molecular formula of $\text{C}_{19}\text{H}_{20}\text{O}_{11}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be 6-O-galloyl arbutin.

The ^1H and ^{13}C NMR data of compound 6-*O*-galloyl arbutin (CD_3OD)

Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
1		153.96	Glc-5	3.68 (1H, m)	75.14
2	6.90 (1H, d, $J=8.8$ Hz)	116.88	Glc-6	4.57 (1H, brd, $J=11.6$ Hz) 4.41 (1H, dd, $J=11.6, 6.8$ Hz)	65.05
3	6.60 (1H, d, $J=8.8$ Hz)	119.62	1'		121.57
4		152.56	2'	7.11 (1H, s)	110.46
5	6.60 (1H, d, $J=8.8$ Hz)	119.62	3'		146.71
6	6.90 (1H, d, $J=8.8$ Hz)	116.88	4'		140.09
Glc-1	4.70 (1H, d, $J=6.8$ Hz)	103.98	5'		146.71
Glc-2	3.38 (1H, m)	75.70	6'	7.11 (1H, s)	110.46
Glc-3	3.44 (1H, m)	78.07	7'		168.43
Glc-4	3.46 (1H, m)	71.99			

Epigallocatechin-3-Gallate (F199-C35):

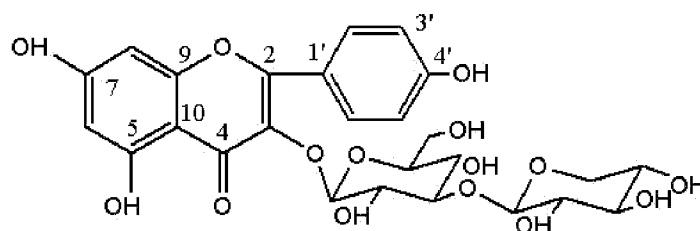
[61] This compound has a molecular weight of 458 and molecular formula of $\text{C}_{22}\text{H}_{18}\text{O}_{11}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be epigallocatechin-3-gallate.

The ^1H and ^{13}C NMR data of compound epigallocatechin-3-gallate (CD_3OD)

Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
2	4.96 (1H, s)	78.77	3'		146.84
3	5.52 (1H, brs)	70.11	4'		133.94
4	2.97 (1H, dd, J=17.2, 4.4 Hz) 2.83 (1H, dd, J=17.2, 2.4 Hz)	27.00	5'		146.84
5		158.02	6'	6.50 (1H, s)	107.06
6	5.95 (1H, s)	96.70	1''		121.68
7		157.98	2''	6.94 (1H, s)	110.44
8	5.95 (1H, s)	96.05	3''		146.44
9		157.38	4''		139.95
10		99.61	5''		146.44
1'		130.98	6''	6.94 (1H, s)	110.44
2'	6.50 (1H, s)	107.06	7''		167.83

Kaempferol-3-O- β -D-Xylopyranosyl(1 $\rightarrow$ 3) β -D-Glucopyranoside

(F199-C42):

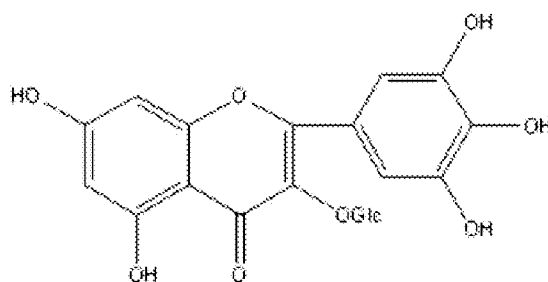


[62] This compound has a molecular weight of 580 and molecular formula of $\text{C}_{26}\text{H}_{28}\text{O}_{15}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be kaempferol-3-O- β -D-xylopyranosyl(1 $\rightarrow$ 3) β -D-glucopyranoside.

The ^1H and ^{13}C NMR data of compound kaempferol-3-*O*- β -D-xylopyranosyl(1 $\rightarrow$ 3) β -D-glucopyranoside (CD₃OD)

Position	^1H NMR	^{13}C NMR	Position	^1H NMR	^{13}C NMR
2		158.52	5'	6.88 (1H, d, J=8.4 Hz)	116.43
3		135.14	6'	8.04 (1H, d, J=8.4 Hz)	132.47
4		179.73	Glc-1	5.46 (1H, d, J=7.6 Hz)	100.92
5		158.52	Glc-2	3.22 (1H, m)	75.08
6	6.18 (1H, brs)	99.94	Glc-3	3.34 (1H, m)	82.47
7		165.83	Glc-4	3.33 (1H, m)	71.22
8	6.37 (1H, brs)	94.82	Glc-5	3.50 (1H, m)	77.24
9		163.19	Glc-6	4.29, 3.96 (each, 1H, m)	62.56
10		105.96	Xyl-1	4.75 (1H, d, J=6.8 Hz)	105.53
1'		122.99	Xyl-2	3.50 (1H, m)	75.18
2'	8.04 (1H, d, J=8.4 Hz)	132.47	Xyl-3	3.52 (1H, m)	78.41
3'	6.88 (1H, d, J=8.4 Hz)	116.43	Xyl-4	3.53 (1H, m)	71.16
4'		161.55	Xyl-5	3.85, 3.16 (each, 1H, m)	66.83

Isomericitrin (F199-C57):



[63] This compound has a molecular weight of 460 and molecular formula of $\text{C}_{21}\text{H}_{16}\text{O}_{12}$ on the basis of ^1H and ^{13}C NMR data in the following table, and is determined to be isomericitrin.

The ^1H NMR data of compound isomericitrin (DMSO)

Position	^1H NMR	Position	^1H NMR
2		3'	
3		4'	
4		5'	
5		6'	7.29 (1H, s)
6	6.20 (1H, d, J=2.0 Hz)	Glc-1	5.23 (1H, d, J=7.6 Hz)
7		Glc-2	3.35 (1H, m)
8	6.38 (1H, d, J=2.0 Hz)	Glc-3	3.47 (1H, m)
9		Glc-4	3.41 (1H, m)
10		Glc-5	3.51 (1H, m)
1'		Glc-6	3.73 (1H, dd, J=12.0, 2.4 Hz) 3.61 (1H, dd, J=12.0, 4.8 Hz)
2'	7.29 (1H, s)		

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in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? J Biol Chem 276, 251-260.

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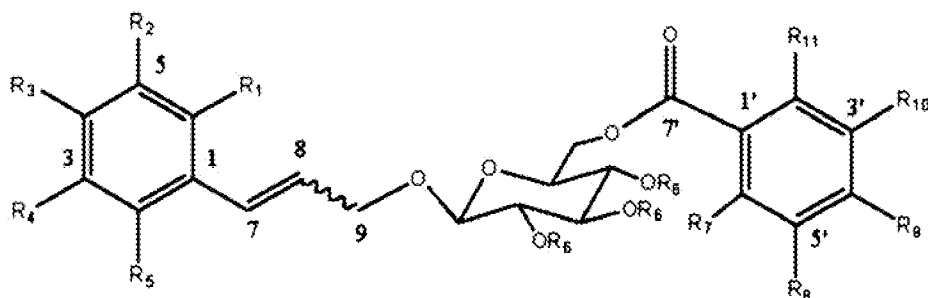
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- [97] While there have been described and pointed out fundamental novel features of the invention as applied to a preferred embodiment thereof, it will be understood

that various omissions and substitutions and changes, in the form and details of the embodiments illustrated, may be made by those skilled in the art without departing from the spirit of the invention. The invention is not limited by the embodiments described above which are presented as examples only but can be modified in various ways within the scope of protection defined by the appended patent claims.

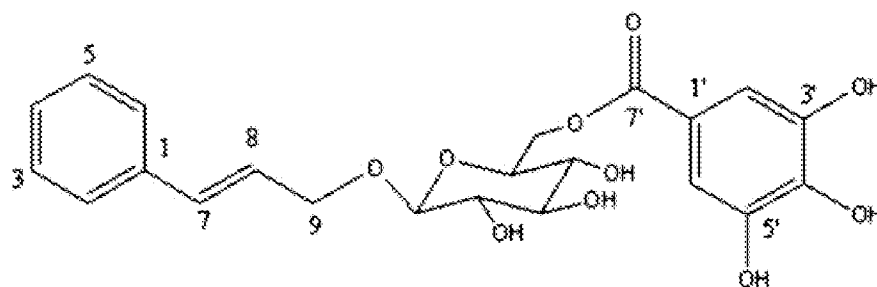
WHAT IS CLAIMED IS:

1. A compound comprising a backbone structure as follows:



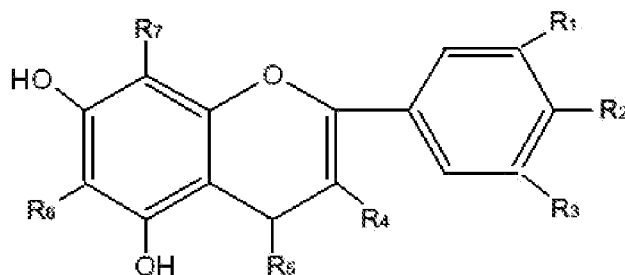
wherein R_{1-11} are independently same or different, being independently hydrogen or a substituent.

2. The compound of claim 1, comprising a structure of the following formula:



3. A pharmaceutical composition, comprising a pharmaceutically effectively amount of compound of claim 1 and one or more pharmaceutically acceptable excipients.
4. The pharmaceutical composition of claim 3, comprising a pharmaceutically effectively amount of compound of claim 2 and one or more pharmaceutically acceptable excipients.
5. A method of inhibiting Cdk5 enzyme in human cells, comprising a step

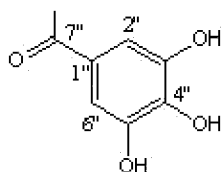
contacting said Cdk5 enzyme with an amount of a compound of the following formula:



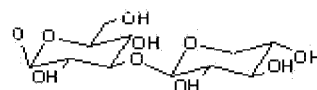
wherein R_{1-7} are independently same or different, being independently hydrogen or a substituent.

6. The method of claim 5, where said compound is selected from the group consisting of F119-C6, F199-C16, F199-C23, F199-C35, F199-C42 and F199-C57.
7. The method of claim 5, where R_1 , R_2 and R_3 of said compound are independently H, OH or OCH_3 .
8. The method of claim 5, wherein R_4 of said compound is OH or OR_{12} , where R_{12}

is a glucose group,



or



9. The method of claim 5, wherein R_7 of said compound is OH or O-Glucose.
10. The method of claim 5, wherein R_5 of said compound is H or $=O$.
11. The method of claim 5, which is for treating or preventing a disease or disorder associated with an aberrant Cdk5 activity.

12. The method of claim 11, wherein said disease or disorder is neuropathic pain, stroke, brain trauma, epilepsy or neurodegenerative diseases.
13. The method of claim 12, wherein said neurodegenerative diseases is amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease or Huntington's disease.
14. The method of claim 5, wherein said compound is defined in claim 1.
15. The method of claim 5, wherein said compound is defined in claim 2.
16. A method of inhibiting Cdk5 enzyme in human cells, comprising a step contacting said Cdk5 enzyme with an amount of a substance selected from the group consisting of F199-C4, F199-C34 and F199-BU.
17. The method of claim 16, which is for treating or preventing a disease or disorder associated with an aberrant Cdk5 activity.
18. The method of claim 17, wherein said disease or disorder is neuropathic pain, stroke, brain trauma, epilepsy or neurodegenerative diseases.
19. The method of claim 18, wherein said neurodegenerative diseases is amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease or Huntington's disease.

FIG. 1

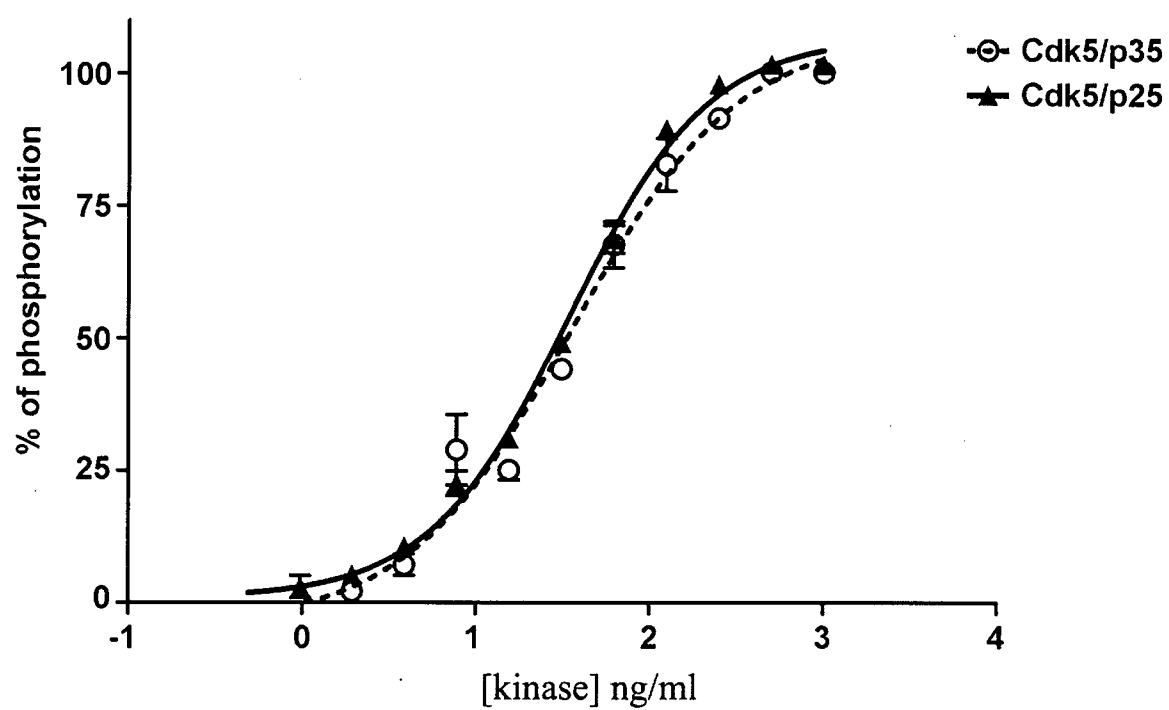


FIG. 2

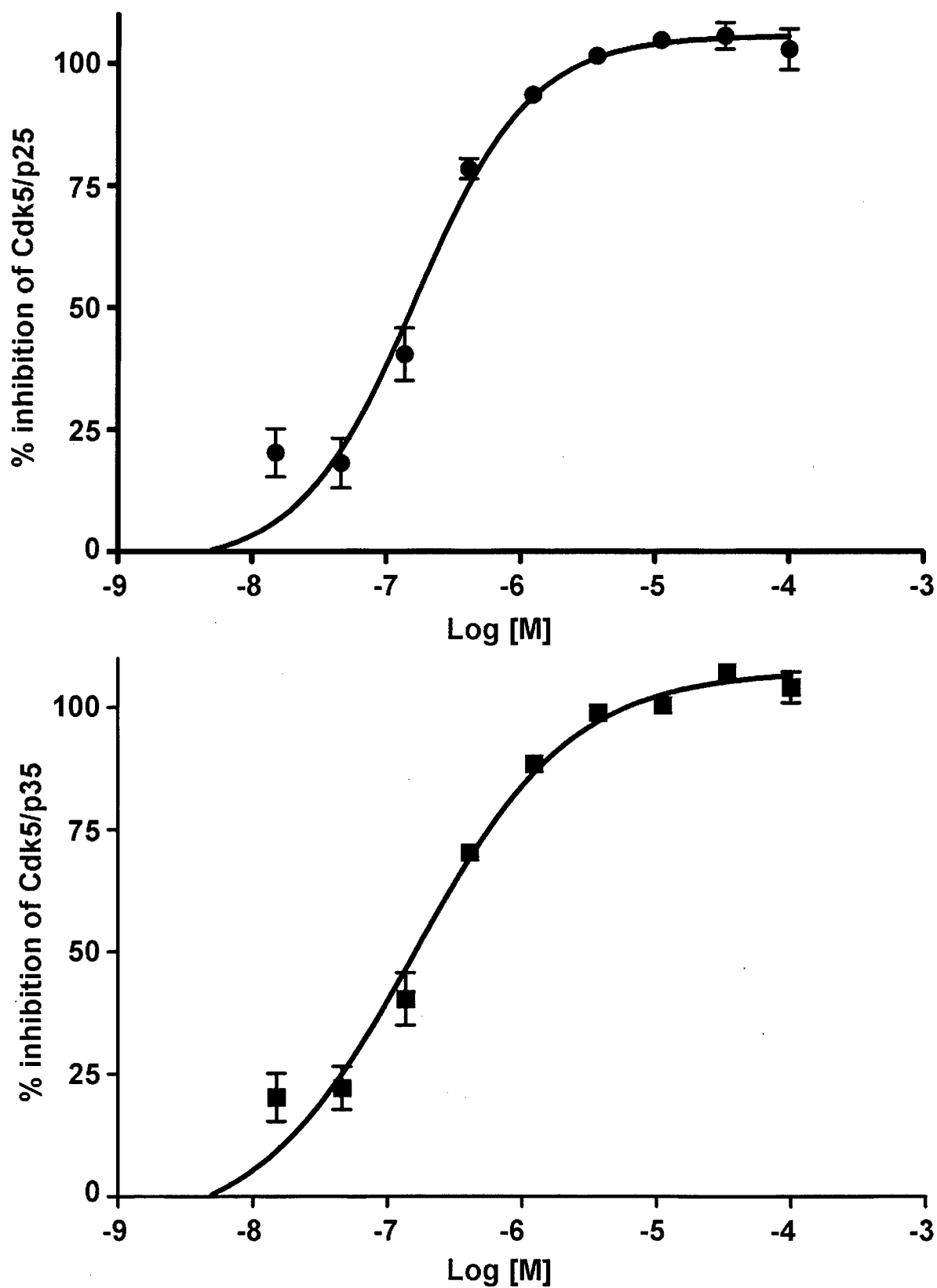


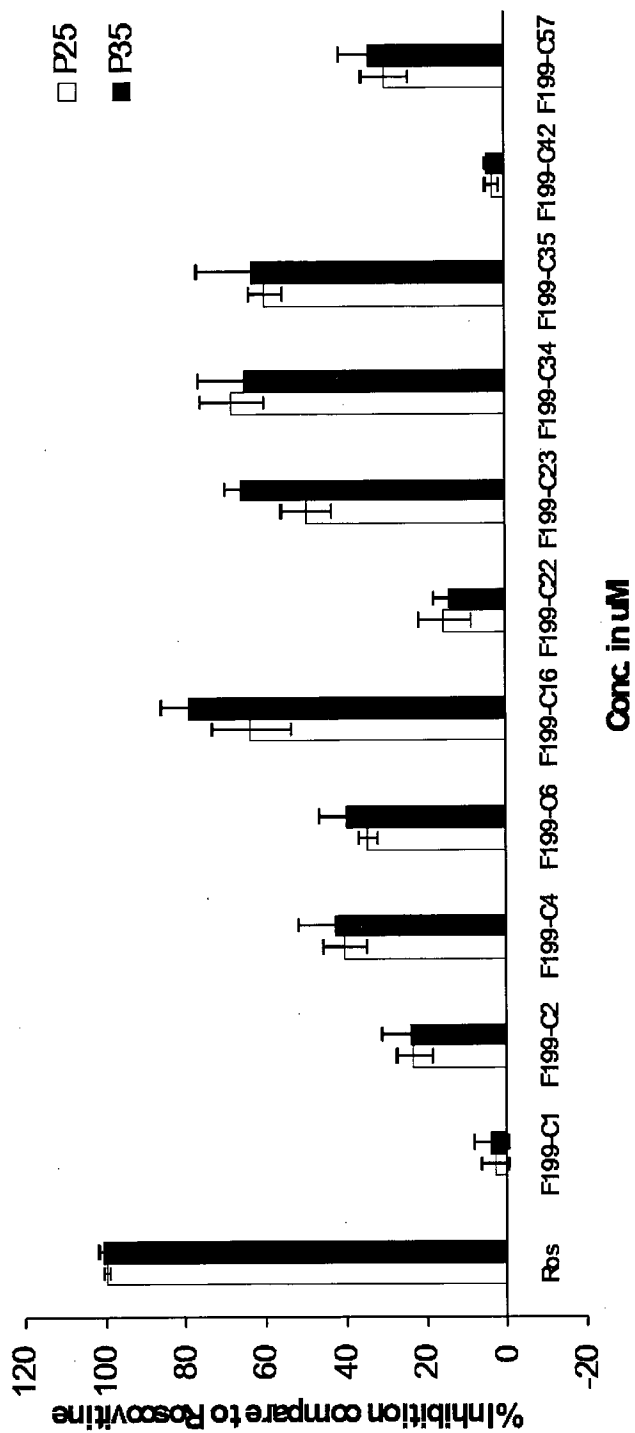
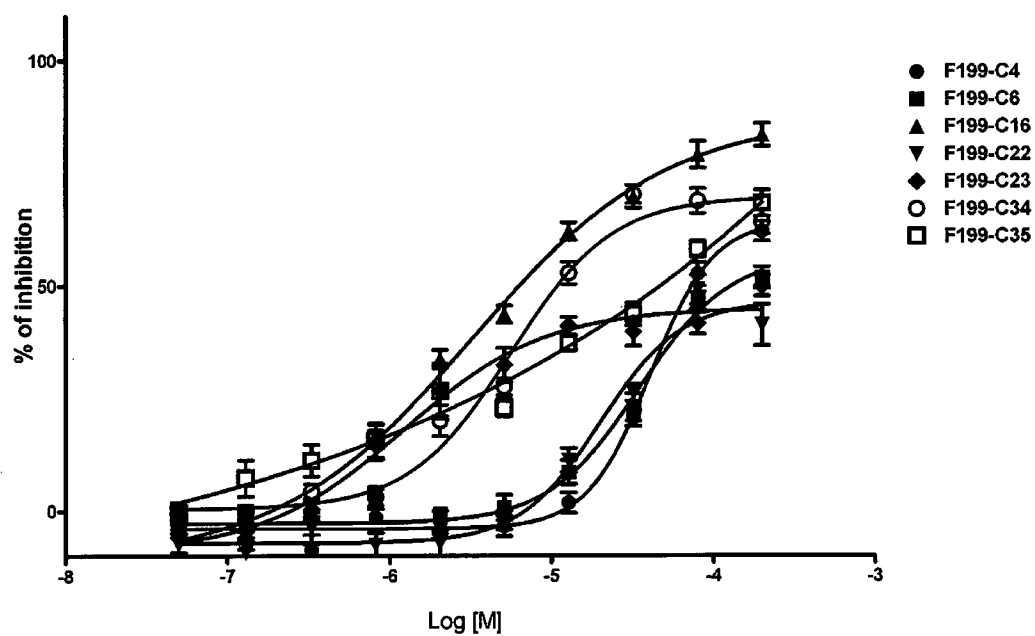
FIG 3.

FIG. 4

Drug	IC ₅₀ (uM)	Maximum inhibition
F199-C4 (gallic acid)	40.6	64.19% at 200 μM
F199-C6 (rodalinal)	34.4	56.61% at 200 μM
F199-C16 (litvinolin)	3.24	87.5% at 200 μM
F199-C22 (6-O-galloyl rosin, novel structure)	20.1	46.5% at 80 μM
F199-C23 (4'-methoxyherbacetin)	1.21	45% at 20 μM
F199-C34 (6-O-galloyl arbutin)	5.63	70% at 80 μM
F199-C35 (epigallocatechin-3-gallate)	14.72	68.4% at 200 μM

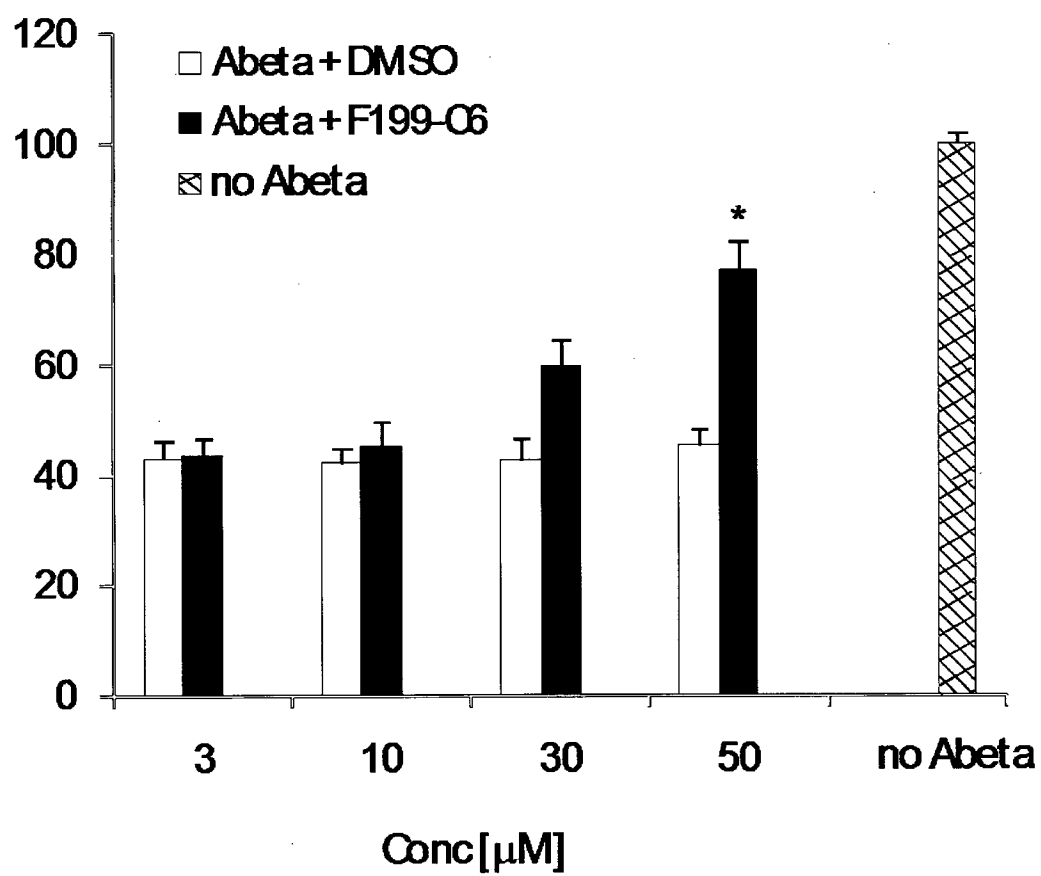
FIG. 5

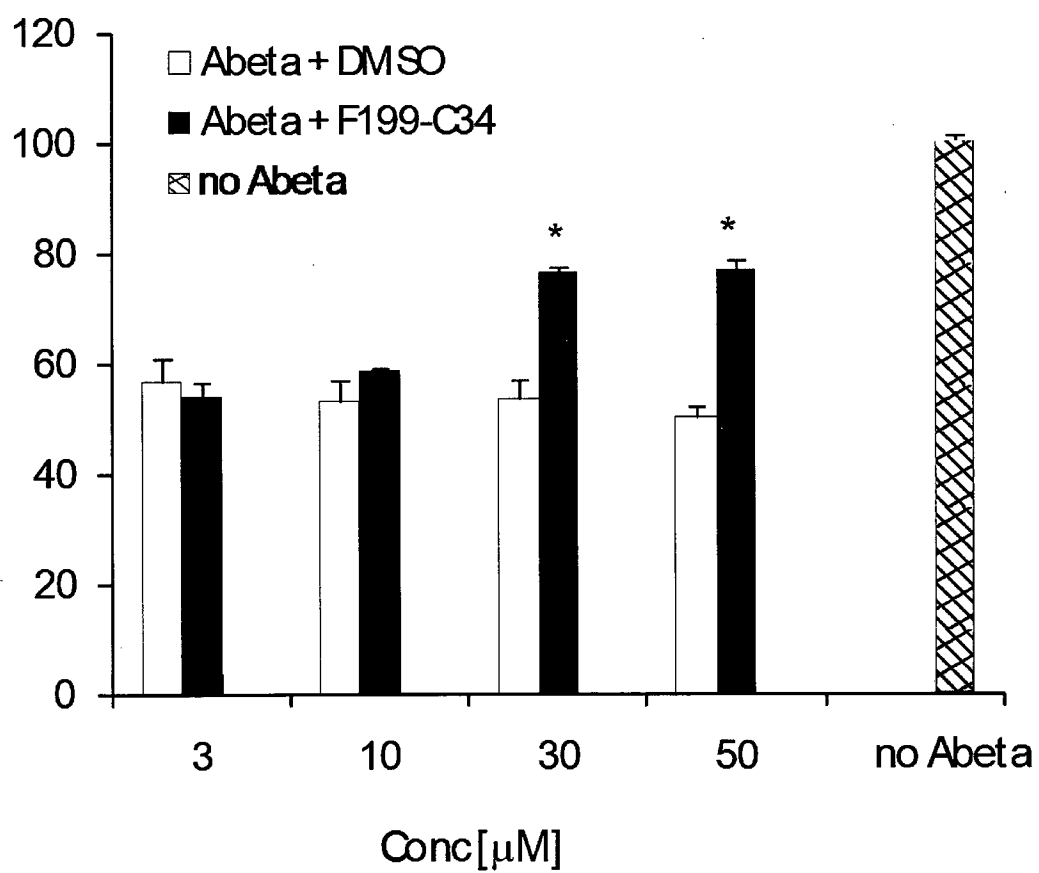
FIG. 6

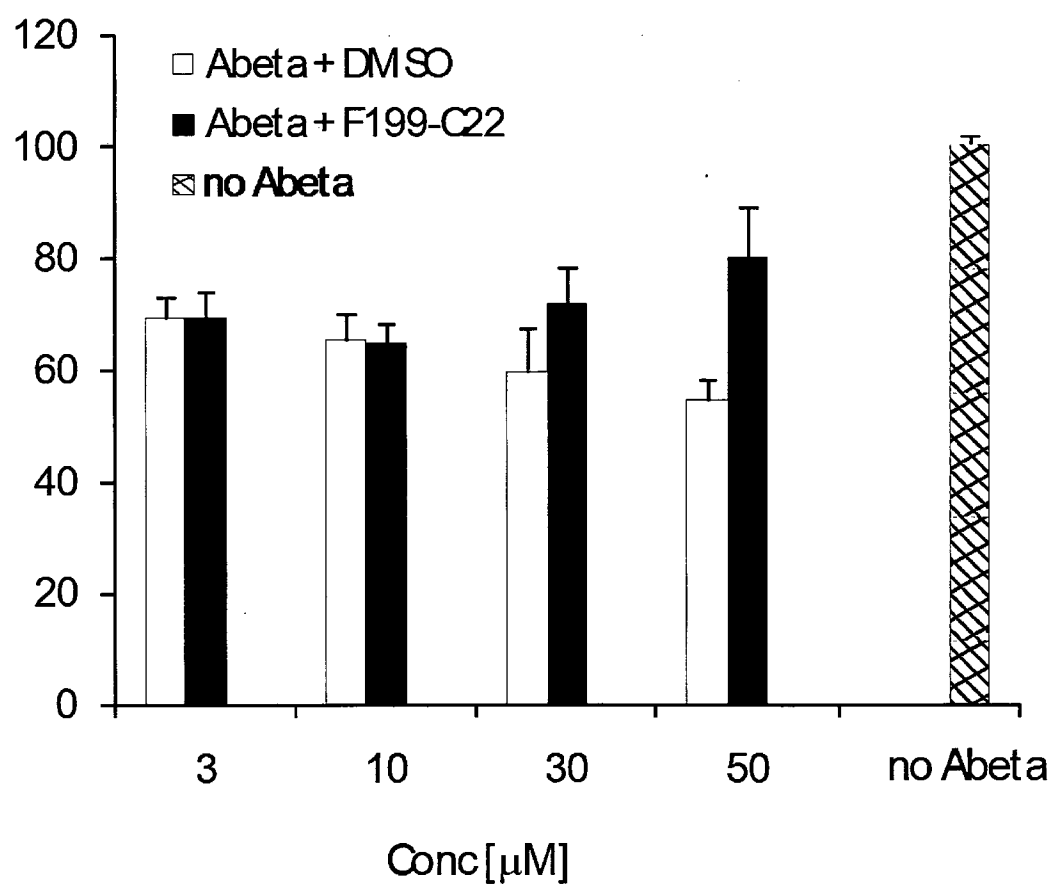
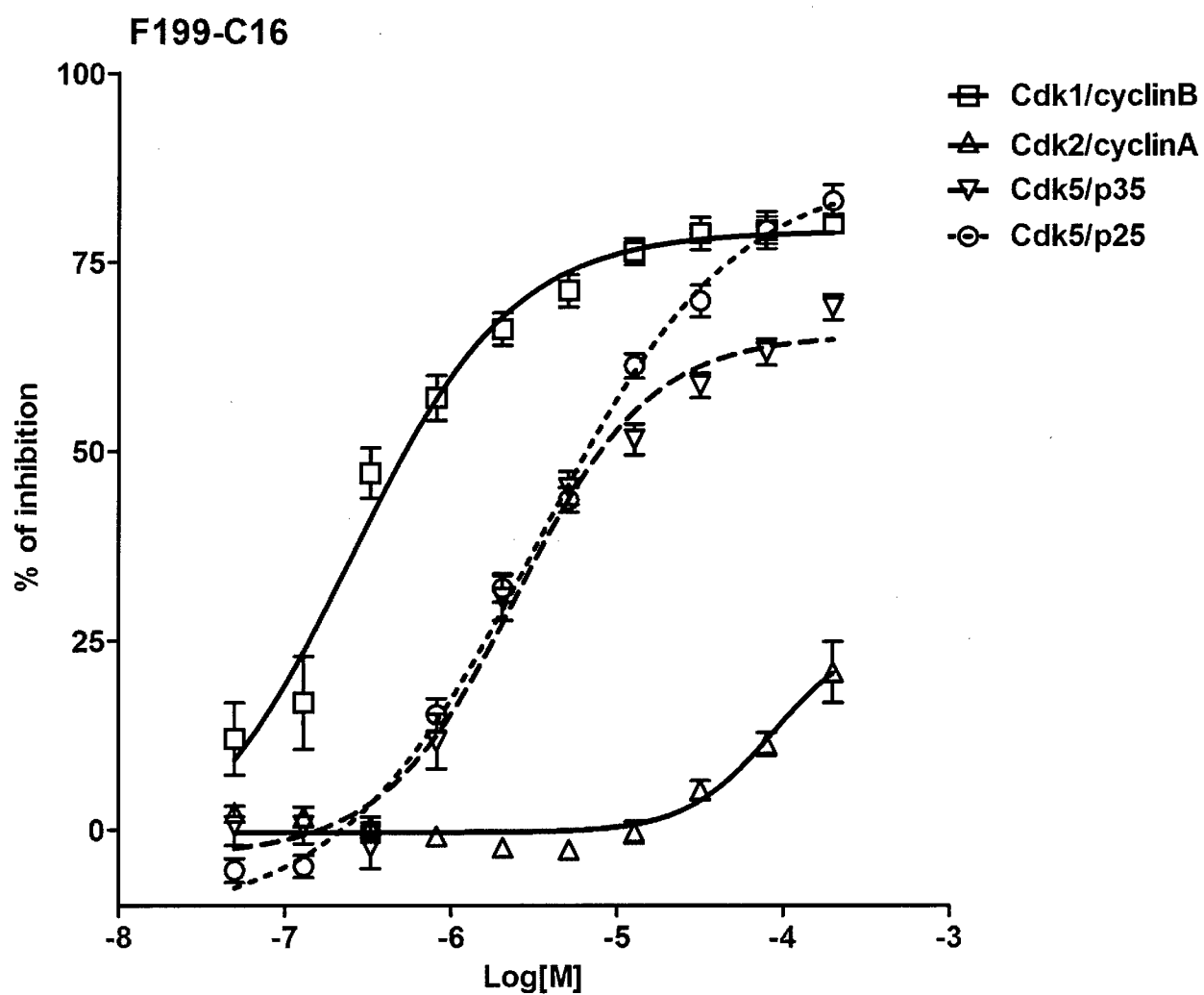
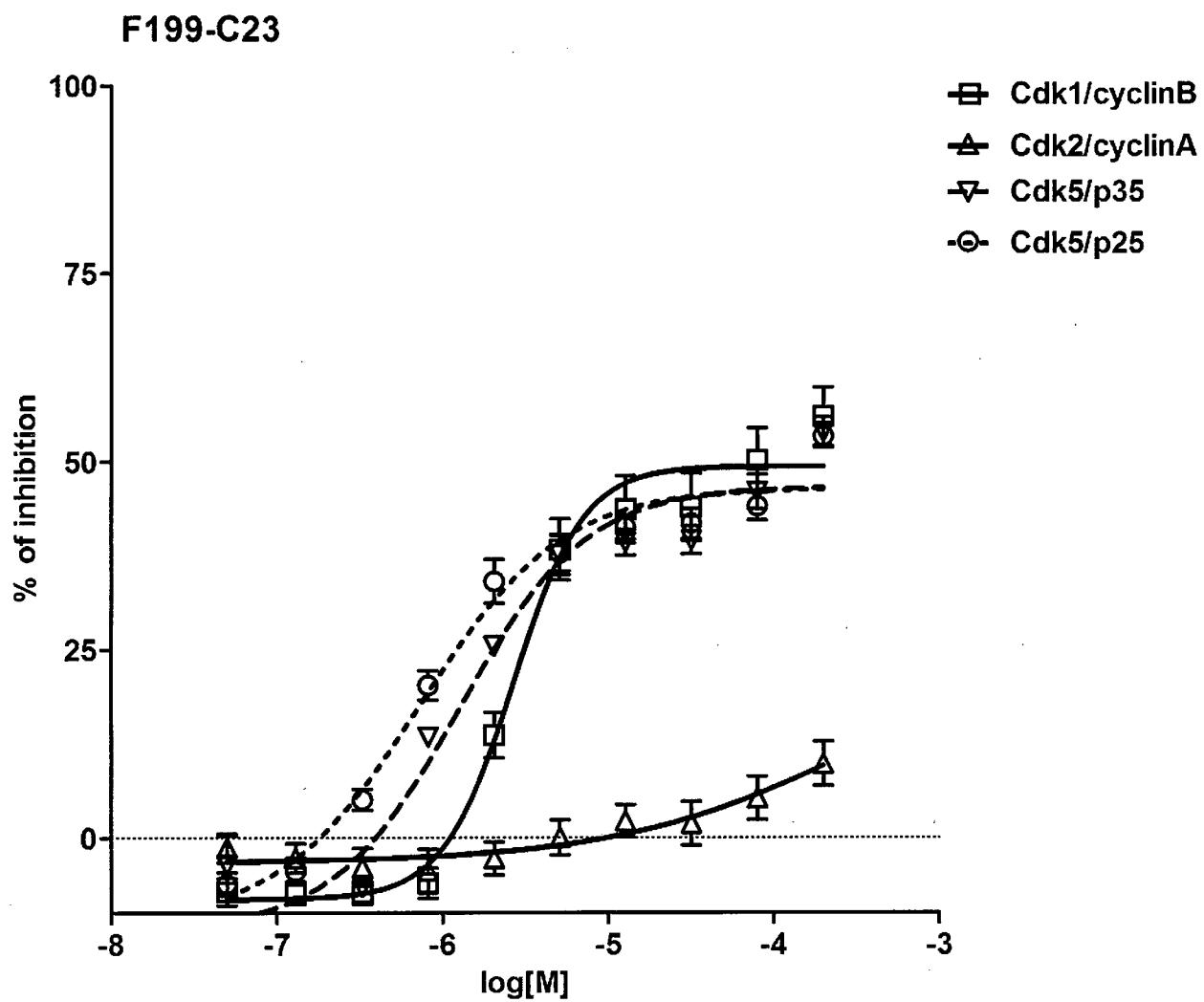
FIG. 7

FIG. 8



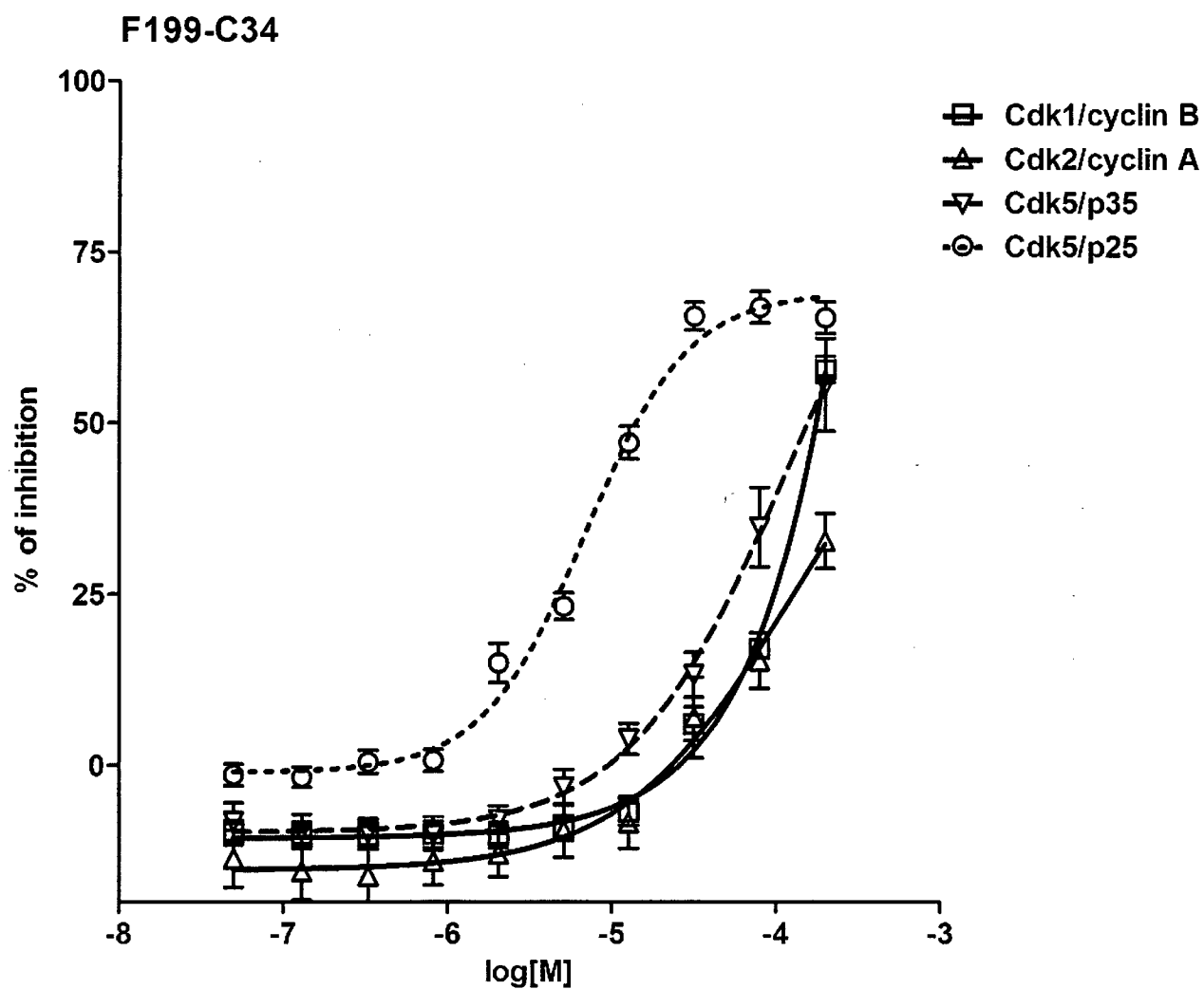
IC50 (μM)	F199-C16
Cdk1/cyclin B	0.25
Cdk2/cyclin A	87.5
Cdk5/p35	2.5
Cdk5/p25	3.31

FIG. 9



IC50 (μM)	F199-C23
Cdk1/cyclin B	2.68
Cdk2/cyclin A	>200
Cdk5/p35	1.26
Cdk5/p25	0.73

FIG. 10



IC50 (μM)	F199-C34
Cdk1/cyclin B	>200
Cdk2/cyclin A	119.1
Cdk5/p35	116.1
Cdk5/p25	6.98

FIG. 11

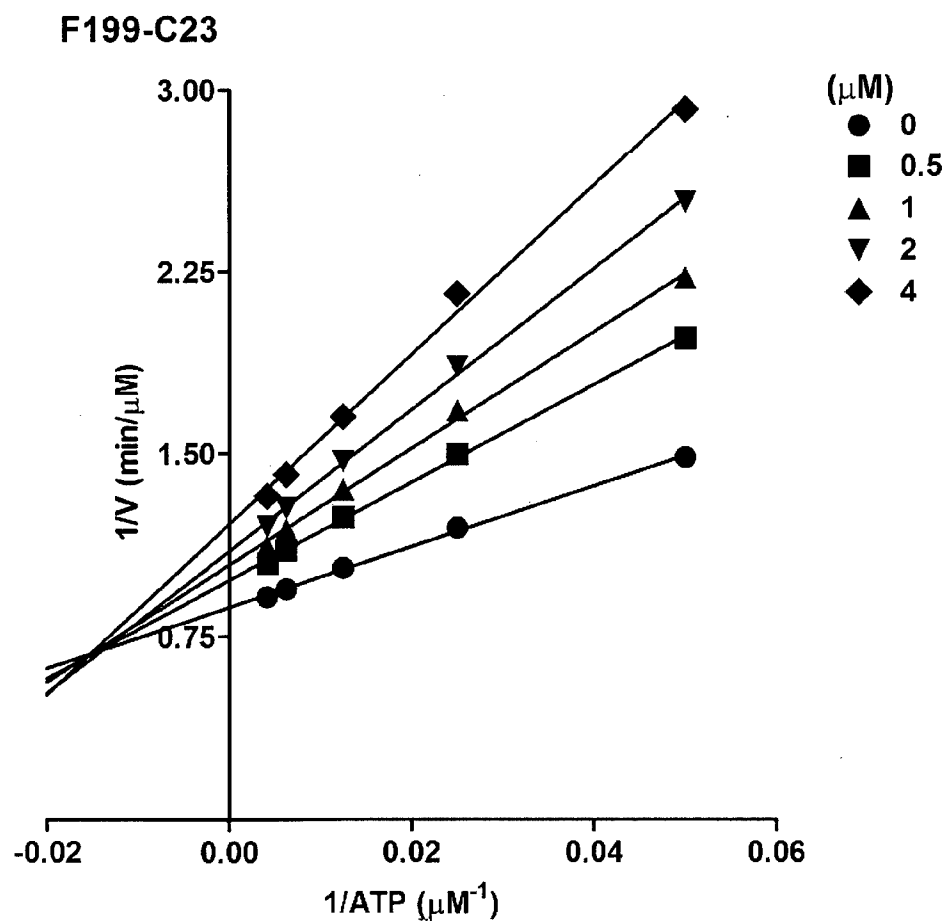
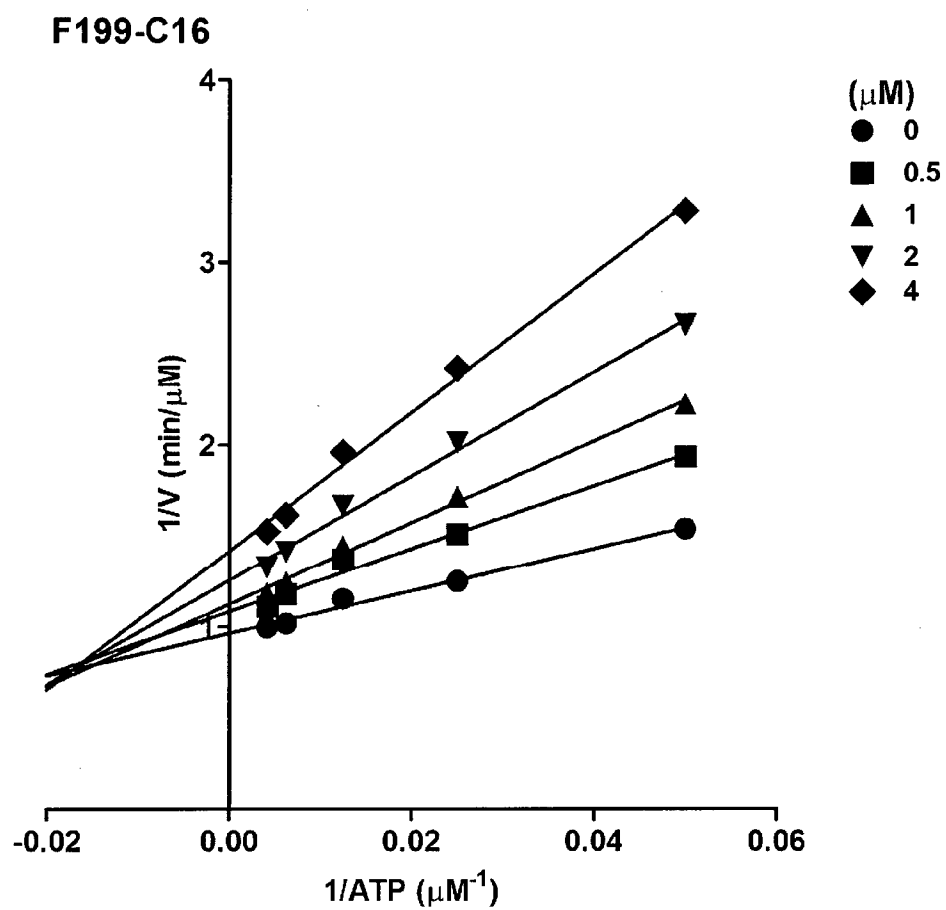


FIG. 11 (cont.)

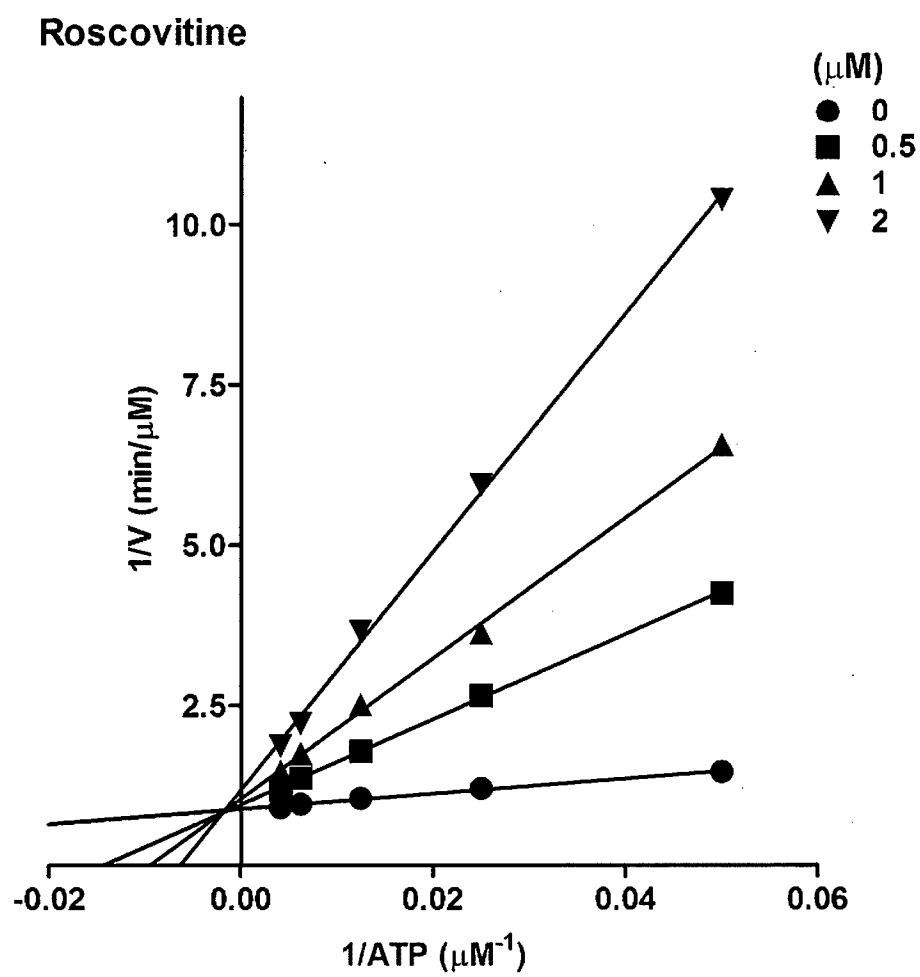


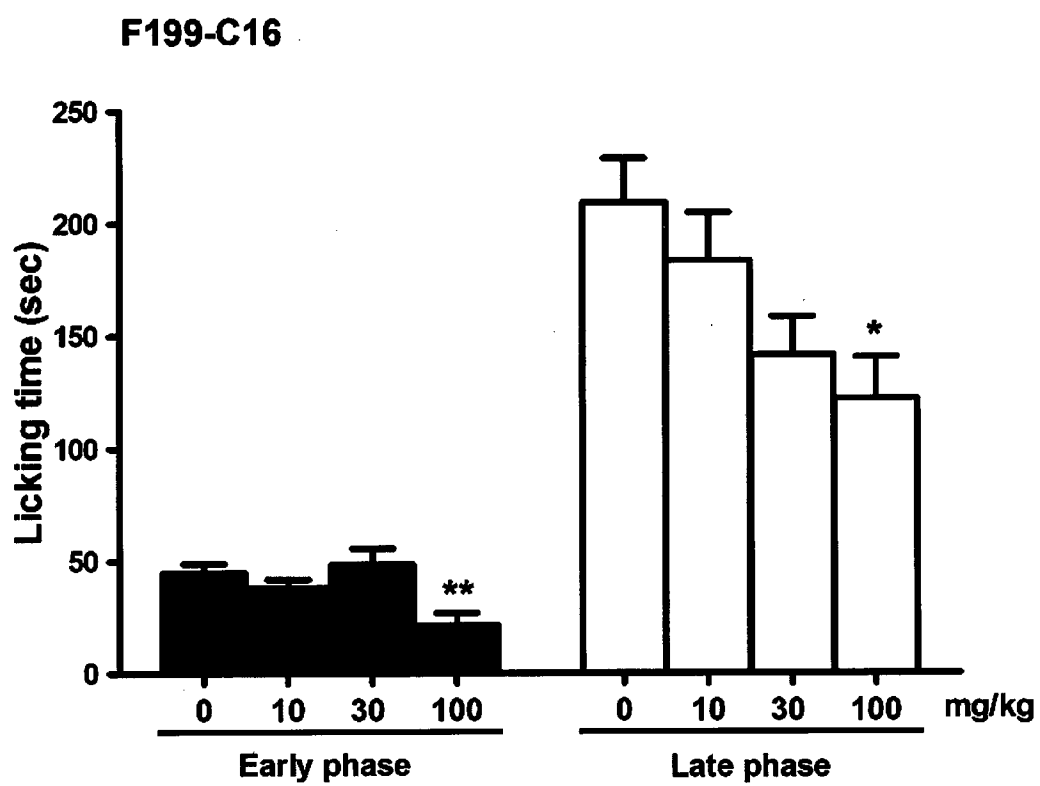
FIG. 12

FIG. 13

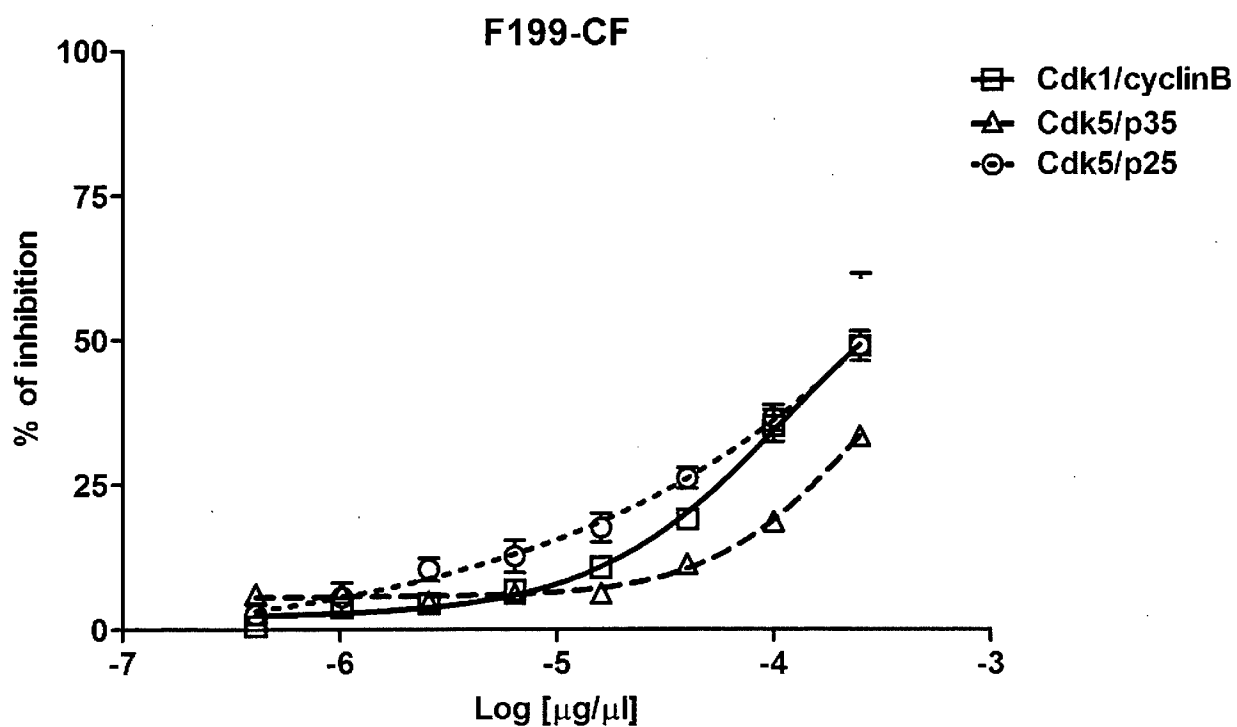
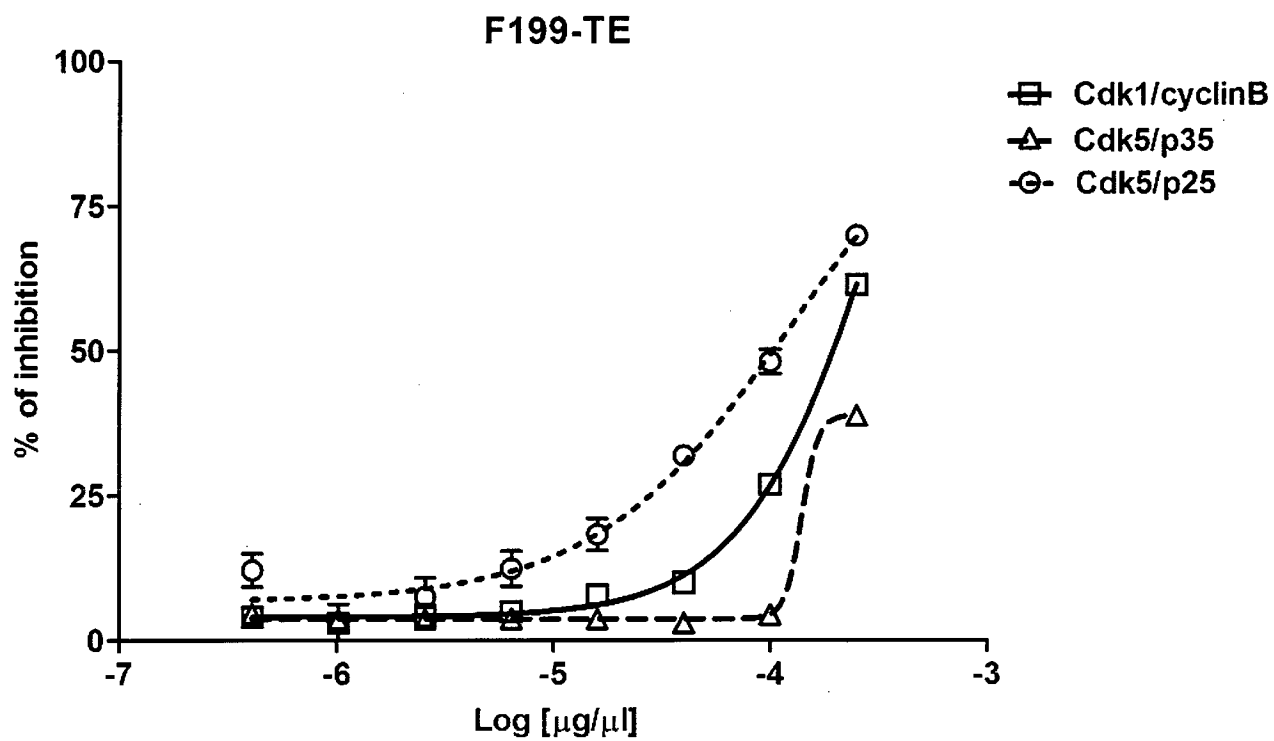
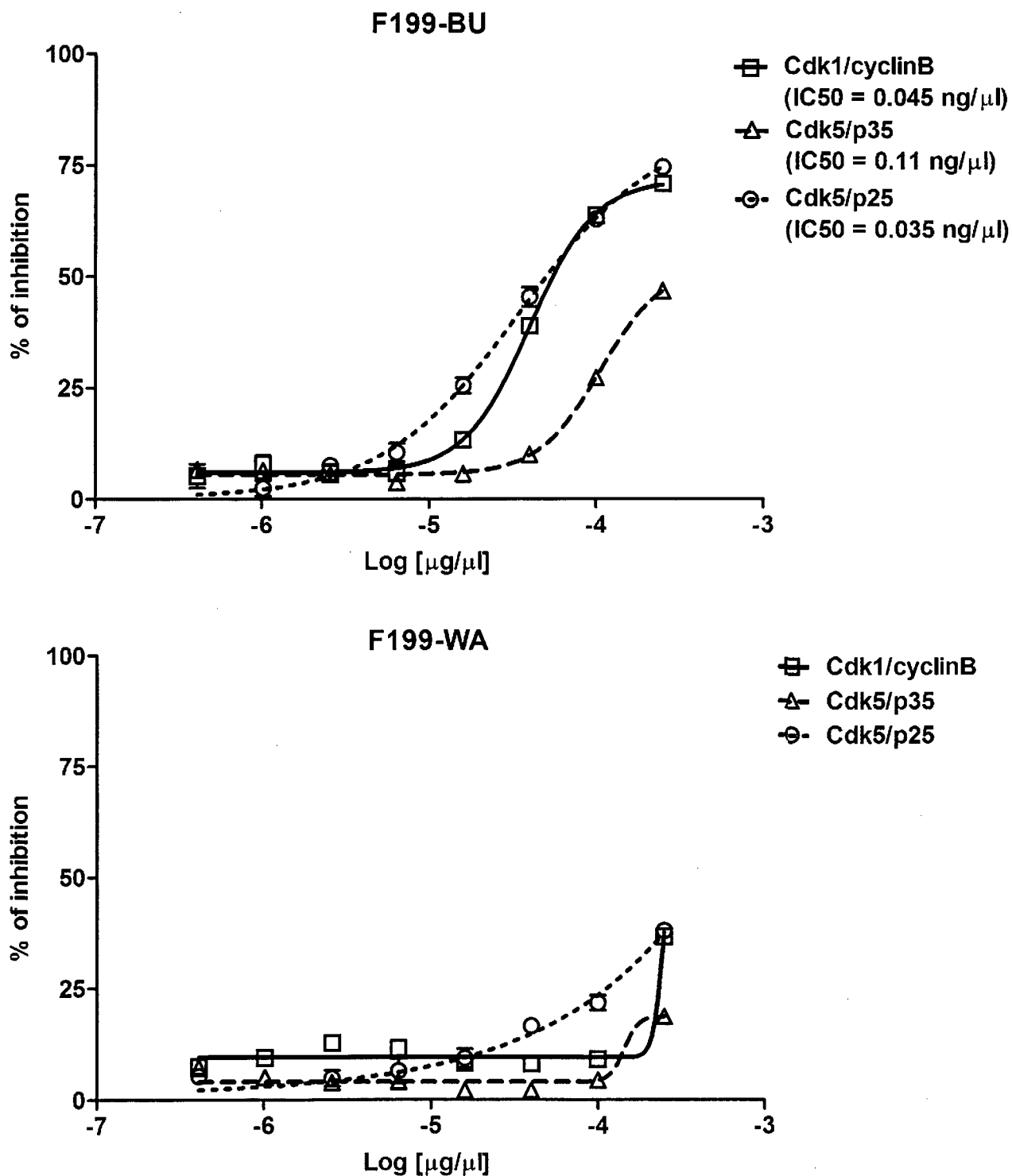


FIG. 13 (cont.)



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/001982

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: C07H3/-; C07H13/-; C07D311/-; A61K31/-; A61P35/-; A61P25/-

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)

WPI, EPODOC, CNPAT, CNKI, CAPLUS, CA: CDK5; rhodiola rosea, extract+; benzopyran; neurodegenerative; cancer; flavonol; glycoside, gallic acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN1534040A(SHANGHAI NABEI BIOTECHNOLOGY CO. LTD.), 06 Oct. 2004(06.10.2004), page 1	1-4, 14-19
A	ALIOTTA Giovanni ET AL. THREE BIOLOGICALLY ACTIVE PHENYLPROPANOID GLUCOSIDES FROM MYRIOPHYLLUM VERTICILLATUM. Phytochemistry, Vol. 31, No.1, 1992, pages 109-111	1-4, 14-19
X	GERALD, Zapata-Torres ET AL. Effects of Natural Flavones and Flavonols on the Kinase Activity of Cdk5. Journal of Natural Products, Vol. 67, No. 3, 2004, pages 416-420	5-13, 16-19

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:	“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
“A” document defining the general state of the art which is not considered to be of particular relevance	“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
“E” earlier application or patent but published on or after the international filing date	“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
“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)	“&”document member of the same patent family
“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	

Date of the actual completion of the international search 13 Feb. 2011(13.02.2011)	Date of mailing of the international search report 17 Mar. 2011 (17.03.2011)
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer DAI, Nianzhen Telephone No. (86-10)82245638

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/001982

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 5-19

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 5-19 are directed to the methods for treatment of human cells by therapy. But the search has been carried out and based on the following subject matter: the use of the compounds according to claims 5-19 in the preparation of medicaments for treating or preventing the disease or disorder associated with an aberrant Cdk5 activity.

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I: Claims 1-4, 14-15 relate to compounds of glycosides, pharmaceutical compositions thereof, the use for preparation of medicaments thereof; Claims 16-19 relate to the use of F199-C34(see page 30 in description) in the preparation of medicaments.

Group II: Claims 5-13 relate to the use of flavonols in the preparation of medicaments; Claims 16-19 relate to the use of F199-BU(the active components in F199-BU are F199-C42 and F199-C57, see pages 14-17, 32-33) in the preparation of medicaments.

Group III: Claims 16-19 relate to the use of F199-C4("Gallic Acid", see page 25) in the preparation of medicaments.

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

The main structures of active compounds in Groups I-III(glycosides, flavonols, Gallic Acid) are different, and also the active compounds do not belong to the same class of compounds recognized in the technical field. Hence, the application does not meet the requirements of unity of invention.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2010/001982

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN1534040A	06.10.2004	None	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2010/001982

Continuation of:

CLASSIFICATION OF SUBJECT MATTER:

C07H3/04(2006.01)i

C07H13/08(2006.01)i

C07D311/08(2006.01)i

A61K31/352(2006.01)i

A61K31/19(2006.01)i

A61K31/7016(2006.01)i

A61K31/7004(2006.01)i

A61P35/00(2006.01)i

A61P25/00(2006.01)i

A61P25/28(2006.01)i