Pharmacokinetics and biodistribution of the cyclin-dependent kinase inhibitor -CR8- in mice

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Abstract

Background: CR8 is a second generation inhibitor of cyclin-dependent kinases derived from roscovitine. CR8 was shown to be 50–100 fold more potent than roscovitine in inducing apoptosis in different tumor cell lines. In the present investigation, we have established an analytical method for the quantification of CR8 in biological samples and evaluated its bioavailability, biodistribution and pharmacokinetics in mice.

Methods: A liquid chromatography method utilizing UV-detection was used for the determination of CR8. CR8 was administered either orally (100 mg/kg) or i.v. (50 mg/kg) and the animals were sacrificed at different time points. Blood samples and organs were collected, after which the pharmacokinetic parameters were calculated for plasma and organs.

Results: CR8 was eluted at 5 minutes in the high performance liquid chromatography system used. The LLOQ detection was 0.10 μg/ml and linearity was observed within the 0.10-10 μg/ml range (r² > 0.998). The accuracy and precision were >86%, while the recovery from plasma was >95%. CR8 was stable for 2 months at room temperature in both solution and plasma. CR8 pharmacokinetics was fitted to a two-compartment open model after oral administration and to a one compartment model after i.v. injection. The elimination half-life was about 3 hours. Organ exposure to CR8 (expressed as % AUC organ vs. AUC plasma) was highest in liver (205%), adipose tissue (188%) and kidney (150%) and low in bone marrow (30%) and brain (15%) as compared to plasma. The oral bioavailability of CR8 was found to be essentially 100%.

Conclusions: We have developed a rapid and simple method for the analysis of CR8. CR8 pharmacokinetics pattern showed 100% bioavailability, long half-life and limited distribution to brain and bone marrow, which may allow systemic exposure higher than the IC50 reported for cell death in tumor cell lines. CR8 displays favorable pharmacological properties and is therefore a good candidate for future clinical studies.

Keywords: Roscovitine, CR8, Pharmacokinetics, Cyclin-dependent kinase inhibitors, Kinase inhibitors, Biodistribution, HPLC

Background

Roscovitine is a selective cyclin-dependent kinase (CDK) 1, 2, 5, 7, and 9 inhibitor [1-4] shown to be effective against several tumors. At present, roscovitine is undergoing Phase II clinical trials in cancer patients (non-small cell lung cancer, nasopharyngeal cancer). The drug has limited side effects and toxicity compared to conventional chemotherapy [5]. However, roscovitine is shown to have a short elimination half-life and rapid metabolism, which leads to suboptimal exposure in clinical trials. Recently, 2-[(R)-(1-Ethyl-2-hydroxyethylamino)-6-(4-(2-pyridyl)benzyl)-9-isopropylpurine (CR8; Figure 1) was introduced as a second generation cyclin-dependent kinase inhibitor derived from roscovitine [6]. CR8 is shown to be about 50-fold more potent compared to roscovitine in inducing apoptosis in different tumor cell lines including ALL and CLL [7].

Several in-vivo and in-vitro studies have reported promising results due to the high efficacy of CR8. Bukanov et al. show CR8 to be effective against autosomal dominant polycystic kidney disease by blocking the renal and hepatic cystogenesis in mice [8]. Another study reports that CR8 can induce apoptotic tumor cell death in the neuroblastoma cell line, which is one of the
most frequent solid tumors in children [9]. CR8 is also proven to be a neuro-protector in experimental traumatic brain injury and has significantly reduced the lesion volume in rats subjected to moderate spinal cord contusion injury [10,11]. Moreover, CR8 is shown to increase the number of surviving neurons after spinal cord injury and to decrease the posttraumatic elevation of biochemical markers of apoptosis in an animal model [12].

Generally, the final effects of drugs in-vivo are influenced by many factors, such as systemic exposure, distribution in various organs, metabolism, lipophilicity, and protein binding. Bettayeb et al. show the optimal concentrations of CR8 needed to obtain a desirable pharmacological effect in-vitro in different cell lines to be about 0.7 μM [7], which is far below that reported for roscovitine (14.6 μM) [4].

Despite these results obtained in cell cultures, the pharmacokinetic profile of CR8 needs to be analyzed prior to further preclinical and possibly clinical investigations. In the present investigation we have established and validated an analytical method for the quantitative determination of CR8 according to standard bioanalytical guidelines. We have also investigated the pharmacokinetics profile and the tissue distribution of CR8 in mice. The present results are encouraging in terms of further development of CR8 as a drug candidate.

**Methods**

**Chemicals and reagents**

Tetrahydrofuran and methanol of high performance liquid chromatography (HPLC) grade were obtained from Merck (Darmstadt, Germany), Tween20 and dimethyl sulfoxide (DMSO) from Sigma-Aldrich (Stockholm, Sweden). All other reagents and solvents were of HPLC analytical grade. S-CR8 was dissolved in DMSO as a 75 mg/ml stock solution and was stored at −20°C. Serial dilutions were prepared from the main stock for the calibration curves, as quality control samples and for administration to mice. (R)-roscovitine and (S)-CR8 were synthesized as described previously [13].

**Instrumentation and chromatographic conditions**

The HPLC system consisted of a Gilson 234 auto-injector equipped with a 100 μl loop, an LKB 2150 pump (Pharmacia inc., Sweden), an “LDC analytical spectrometer 3200” UV-detector (USA) and a CSW 32 chromatography station integrator. Separation was performed on a Zorax SB-CN column (3.5 μm × 4.6 mm × 150 mm) from Agilent (USA), while the column was maintained at room temperature during analysis.

The mobile phase consisted of tetrahydrofuran: 25 mM phosphate buffer pH = 2.6, ionic strength = 0.022 (20:80, v/v). Flow rate was set at 0.9 ml/min, running time at 8 min for standard curve and quality controls and at 15 min for animal samples to elute all the metabolites. Injection volume was 50 μl and the UV wavelength used was 305 nm. Standard curves were prepared from spiked pooled human plasma, and were linear in the range of 0.1 - 10 μg/ml.

**Standard solutions and controls**

Pooled heparin plasma from healthy donors was obtained from the Blood Transfusion Center, Karolinska University Hospital. Serial standard dilutions in 50 mM Hydrochloric acid were prepared from the stocks for the calibration curves and for the quality controls.

Working standard solutions of CR8 in plasma at 10, 8, 6, 5, 4, 3, 2, 1, 0.5, 0.25 and 0.1 μg/ml were prepared by adding 50 μl from the standard stocks to 950 μl plasma to reach 1 ml containing the final concentrations. The calibrators were prepared on the day of each run.

Four quality control samples at concentrations of 0.75, 1.5, 3.5 and 7.0 μg/ml were also prepared in triplicate and stored at room temperature, +4°C and −20°C for stability testing.

The quality control samples were repeated using mouse plasma in triplicate using the same concentrations as in human plasma.

**Sample preparation from plasma**

70 μl plasma was added to 140 μl methanol and vortexed for 15 sec. Samples were centrifuged at 10000 g for 10 minutes, and 50 μl from the supernatant were injected into the HPLC system.

**Bioanalytical method validation**

**Selectivity**

A selective method should involve no interference from endogenous compounds, metabolites and/or different degradation products related to the drug [14]. We confirmed that our method involved no such interference by running blank biological samples from untreated mice and mice receiving only the drug vehicle [15].
**Calibration curve**

A calibration curve was constructed from eight standard points of CR8 at concentrations ranging from 0.1 - 10 µg/ml, including lower limit of quantification (LLOQ). The peak areas of CR8 were plotted against the concentrations. Linearity was assessed by a weighted least-squares regression analysis and a correlation coefficient ($r^2$) of 0.99 or better was deemed acceptable. The LLOQ was set as the lowest measurable concentration with acceptable accuracy and precision not more than 20% of the expected values [16].

**Precision and accuracy**

The precision of the method was defined as the percent relative standard deviation (% SD) calculated from triplicate measurements [(standard deviation)/mean value] × 100. The accuracy was defined as the percent relative error (% E) of the mean of the replicate measurements in relation to the theoretical values [(measured value - nominal value)/nominal value] × 100 [16,17]. Precision and accuracy were determined by analyzing quality control samples (QCs) prepared at four concentrations (0.75, 1.5, 3.5 and 7.0 µg/ml) in pentaplicate on three consecutive days [18].

**Recovery**

Absolute recovery is defined as percentage of reference compound that is measured to the exact amount of compound added to a blank buffer [15]. Recovery was computed by comparing responses in triplicate of extracted low, middle and high QCs (0.75, 1.5 and 7.0 µg/ml, respectively) samples with those of extracted solvent to which CR8 has been added at the same nominal concentration [14].

Relative recovery is defined as percentage of the amount of drug measured from the plasma compared to the amount measured from pure solvent e.g. 50 mM hydrochloric acid (HCl) [15]. The low, medium and high controls (0.75, 1.5 and 7.0 µg/ml, respectively) were prepared in pooled plasma and in 50 mM HCl. Samples were run in triplicate and recovery was calculated as the percentage of (plasma peak area/methanol peak area) × 100.

**Stability**

The stability of CR8 was determined in plasma samples using concentrations of 0.75, 1.5, 3.5 and 7.0 µg/ml (in triplicate). The samples were kept at room temperature, +4°C and −20°C and analyzed twice weekly for 2 months. Peak areas of all the samples were compared to peak areas obtained at time zero.

**Animals**

All experiments in this study were approved by the Stockholm Southern Ethics Committee for Animal Research and were conducted in accordance with the Animal Protection Law, the Animal Protection Regulation and the Regulation for the Swedish National Board for Laboratory Animals.

Female BALB/c mice 8–10 weeks old and weighing 18–21 g were obtained from Scanbur, Sollentuna, Sweden. The mice were allowed to acclimatize to their surroundings for one week before starting treatment. The mice were fed pelleted food and water *ad libitum*. CR8 was dissolved in DMSO: 50 mM HCl (10:90 v/v). The first group of mice received i.v. doses of CR8 (50 mg/kg body weight), the second received oral doses of CR8 (100 mg/kg body weight) and three control mice received vehicle alone. Three mice were sacrificed at each time point and blood samples were collected by cardiac puncture into heparinized tubes at 10, 20, 30, 60 min, 2, 4, 6, 8 h post administration. Blood samples were centrifuged immediately after collection at 3000 g for 5 min at 4°C and the plasma was prepared as mentioned previously. Supernatants were collected and all samples were frozen at −20°C directly after preparation until further analysis by HPLC. Other organs including liver, spleen, kidneys, brain, adipose tissue and lungs were removed, washed and snap frozen immediately in liquid nitrogen. Bone marrow was flushed from both femurs and mononuclear cells were counted and frozen. The samples were stored at −20°C to avoid any metabolic activity.

**Tissue sample preparation**

Samples from different organs were homogenized by probe sonication in sodium perborate (PBS) (1:3 – 1:5 w/v) for 2 min and vortexed for 1 min. 100 µl of the homogenate was added to 200 µl methanol, vortexed for 1 min and centrifuged at 10000 g for 10 min. 50 µl of the supernatant were injected into the HPLC system.

Femurs were removed and cleaned; bone marrow was flushed with 0.3 ml of PBS and single cell suspension was prepared by gentle flushing through needle and syringe. Nucleated cells were counted using Türk solution and bone marrow was stored at −20°C until assay.

Quality control samples were run together with the corresponding mouse samples in duplicate.

**Pharmacokinetics**

Parameters including distribution volume of the central compartment, elimination rate constant, plasma maximum concentration and micro constants were estimated. Clearance (Cl) and distribution volume at the steady state were calculated from the primary parameters. The plasma concentration versus time curves (AUC) were calculated from the model derived parameters, and the elimination half-lives were calculated from the slope of the terminal elimination phase. Bone marrow...
data were derived after adjustment of concentrations to 10⁶ cells (= approx. 1 g of tissue). The pharmacokinetic modeling was performed using WinNonlin version 5.2 (Pharsight, USA).

Oral bioavailability (F) defined as the fraction of the oral administered dose of CR8 that reaches the systemic circulation was calculated according to the following formula:

\[
F = \frac{[AUC]_{po} \times \text{dose}_{IV}}{[AUC]_{IV} \times \text{dose}_{po}}
\]

Calculations and statistics
The peak areas of CR8 were plotted versus the corresponding nominal concentrations of the standards, and the standard curve was calculated by linear regression. CR8 concentrations in mouse plasma, organs and quality control samples were calculated from the resulting curves. All values are presented as mean ± S.D.

Results
Analysis of CR8
A precise and accurate method was developed to detect the drug in plasma and tissue samples in order to investigate the pharmacokinetics of CR8. CR8 is a lipophilic compound; however, due to the presence of the 2-pyridyl ring attached to the benzyl ring, its lipophilicity is lower than that of the parent analogue roscovitine. DMSO was found to be a suitable solvent for very high concentrations of CR8, and 50 mM HCl was found to be a suitable solvent for standard concentrations in QCs (Table 1).

Recovery
The absolute recovery of CR8 in plasma was 97 ± 6%, 95 ± 5.0% and 98 ± 4% at the concentrations of 0.75, 1.5 and 7.0 μg/ml, respectively, while the relative recoveries of the drug for the same concentrations in plasma compared to that obtained from 50 mM HCl were 99 ± 3, 103 ± 2 and 96 + 5%, respectively. The recovery from plasma was almost completely with low variability. Consequently, plasma could be a suitable matrix for the regular calibrations needed to analyze patient/animal materials in future in-vivo experiments [21].

Stability
The drug proved to be highly stable after 2 months at room temperature, +4°C or −20°C, with no significant decrease in the concentration of the QC samples (stability found to be approximately 97 ± 3.5%).

CR8 Metabolites
Five CR8 metabolites were separated and found to absorb UV at the same wavelength as CR8. These metabolites were named M1-M5 and appeared in the chromatogram in the following order: M1 at 4.23 minutes, M2 at 5.79 minutes, M3 at 8.13 minutes, M4 at 9.95 minutes and M5 at 11.81 minutes (Figure 4). The first and fifth metabolites (M1 & M5) appeared rapidly with maximum concentration at 20 minutes and slowly disappeared until 8 hours. The metabolite M4 increased by time to reach maximum concentration at 60 minutes then disappeared. The metabolites M2 and M3 increased and accumulated with time until 8 hours post administration (Figure 4).

Pharmacokinetics of CR8
To conduct the CR8 pharmacokinetic studies, a simple HPLC-UV method for the determination of CR8 in human plasma was developed and validated. The pharmacokinetics of CR8 were then investigated in mice and
found to fit a two-compartment open model after oral administration and a one compartment bolus model after i.v. administration (Figure 5) using Gauss-Newton criteria. The CR8 AUC was about 100 $\mu$g.hr/ml when 100 mg/kg was administered orally and about 45 $\mu$g.hr/ml when 50 mg/kg was administered intravenously. However, the estimated $C_{\text{max}}$ was over 100 $\mu$g/ml after i.v. administration, which is 4 fold higher compared to that observed after oral administration (Table 2).

Absorption and distribution were rapid and the maximum concentrations were found at 30 min. Most importantly, the elimination half-life was estimated to be about 3 hours.

Exposure to concentrations much higher than the reported average $IC_{50}$ value (0.7 $\mu$M) [7] was found to last more than 8–10 hours.

The oral bioavailability of CR8 was calculated to be 108 ± 9%.

**Biodistribution of CR8**

CR8 was widely distributed into all tissues, and the time-concentration curves (AUCs) were fitted to one compartment for all tissues (Table 3).

Higher exposure was found in the liver (the tissue/plasma exposure ratio was 200%), adipose tissue (180%) and kidneys (150%) compared to that observed as systemic exposure in plasma. Lung and spleen showed a tissue/plasma ratio of 80% and 50%, respectively (Figure 6) as compared to plasma.

**CR8 distribution to the bone marrow**

Exposure to CR8 was about 30% in bone marrow compared to in plasma when the concentrations were normalized to tissue weight/g (Figure 6).

This exposure is higher than that reported as $IC_{50}$ required for the elimination of leukemic cell lines in about 6 h (data not shown).
CR8 distribution to the brain

CR8 was found to pass the blood–brain barrier (BBB) and the concentration in the brain was about 15% of that in plasma. Despite this low tissue/plasma ratio, the concentration reached a value above the reported CDK5/p25 and casein kinase 1 (CK1) inhibitory IC₅₀ values for more than 6 hours.

Discussion

Recently, the tri-substituted purine CR8 was introduced as a selective second generation CDK inhibitor analogue of roscovitine [6,7]. *In-vitro* studies have shown that CR8 is 2–4 fold more potent as a CDK inhibitor and 50–100 fold more cytotoxic in tumor cell lines [7]. However, this enhanced *in-vitro* activity of CR8 needs to be confirmed *in-vivo* in animal models prior to further development toward clinical trials in patients. The future investigation and development of this purine requires an accurate analytical method for the quantitative determination of its pharmacokinetics pattern. In the present investigation we have developed and validated a new analytical method for the determination of CR8 in plasma and tissue samples. Our data showed that the method is selective and accurate. The acceptance criteria for the method validation are well in accordance with internationally accepted criteria [15,18]. Moreover, the volume required for the analysis is only 50 μl of plasma, a favorable factor when considering plasma sampling from small laboratory animals.

The present method was employed for studying the pharmacokinetics and biodistribution of the drug in mice. It is of great importance to use a single, suitable analytical method for both pre-clinical and clinical studies of newly introduced drugs, such as CDK inhibitors, that are promising for the treatment of several diseases including cancer. One important factor is that the

Table 1 Precision and accuracy of the CR8 analysis (n = 3-5 each)

| Concentration (µg/ml) | Between batch | Within batch Batch1 | Batch2 | Batch3 | Batch1 | Batch2 | Batch3 |
|-----------------------|--------------|---------------------|--------|--------|--------|--------|--------|
| 0.1 (LLOQ)            | 3.7          | 4.2                 | 3.4    | 4.8    | -16    | -15.4  | -8.5   |
| 0.75 (QC1)            | 7.7          | 1.4                 | 1.8    | 1.9    | 5.5    | -12.9  | -1.2   |
| 1.5 (QC2)             | 6.9          | 1.5                 | 1.7    | 0.8    | 4.3    | -6.7   | 3.0    |
| 3.5 (QC3)             | 4.9          | 0.2                 | 0.6    | 1.1    | -9.1   | -9.9   | -0.6   |
| 7.0 (QC4)             | 3.3          | 0.8                 | 2.0    | 1.3    | -3.5   | -7.4   | -4.1   |

Figure 4 HPLC chromatograms of CR8 and its metabolites. Chromatograms showing CR8 and its five metabolites at different time points after oral administration, (red) 20 minutes post administration, (blue) 60 minutes post administration, (green) 8 hours post administration, (black) blank.
The present method does not require work up procedures, a favorable factor for clinical studies where large numbers of samples are generated and speed of analysis is important.

The pharmacokinetics profile (especially the oral bioavailability and the elimination half-life) constitutes a set of critical factors, strongly influencing whether CR8 should enter preclinical and clinical trials as an anti-tumor agent. Good oral absorption and appropriate biological half-life allowing enough exposure to the drug to produce pharmacological activity and anti-tumor effect [22], and sufficient elimination allowing minimal toxicity, are indeed crucial for further development of an anti-cancer drug.

Roscovitine, which was introduced as one of the earliest CDK inhibitors, is at the present time undergoing phase II clinical trials in advanced non-small cell lung cancer and nasopharyngeal cancers [23]. Roscovitine was shown to have good oral bioavailability and low systemic toxicity profile [5,24]. However, the anti-tumor responses observed were modest, most probably a consequence of the drug’s short half-life and rapid metabolism to inactive metabolites [25]. Generally, exposure to the drugs is an important factor in the exertion of their anti-tumor activity. It has been shown that the optimum effect of roscovitine and the closely related analogue, N&N1, on tumor cell lines occurs after 8–16 hours of exposure [26-28]. The systemic exposure to roscovitine (expressed as AUC in plasma) in adult animals and humans did not allow a plasma concentration to be maintained above the reported anti-tumor IC$_{50}$ (15–17 μM) for more than 1–2 hours, even when the maximum tolerated doses were administered [5]. The only exception was in young rats, most probably due to immature metabolism [29]. The present results showed that the systemic exposure expressed as plasma AUC of CR8 (after single dose administration of 100 mg/kg) was, for 10 hours, far higher than the anti-tumor IC$_{50}$ values found in-vitro (0.3 μg/mL). Thus, second generation analogues of roscovitine might, in addition to increased cell potency, solve the problem of insufficient exposure observed in roscovitine. The higher systemic exposure might in part be explained by the longer half-life (approximately 3 h) found in female BALB/c mice in the present study. In this respect CR8 differs from both roscovitine [27,30] and its close analogue N&N1 [26],

| PK parameters | Oral   | i.v.     |
|---------------|--------|---------|
| AUC (h.μg/ml) | 96.34 ± 10.53 | 44.35 ± 3.3 |
| Tα (h)        | 1.3 ± 0.43   | 0.11 ± 0.01 |
| Tβ (h)        | 3.1 ± 0.35   | 2.95 ± 0.11 |
| C$_{max}$ (μg/ml) | 34.3 ± 1.94 | 163.9 ± 0.25 |
| Vss (ml)      | 21 ± 3       | 44 ± 4   |
| CL (ml/h)     | 21 ± 2       | 23 ± 2   |
| Bioavailability | $\frac{96.43 \times 50}{(44.35 \times 100)} \times 100 = 108\%$ |

AUC: Area under time plasma concentration curve.
Tα: First elimination half-life.
Tβ: Terminal elimination half-life.
C$_{max}$: Maximum reached concentration.
Vss: Distribution volume at steady state.
CL: Clearance.
which both have a reported half-life of approximately 1 hour when given i.v. at the same dosage.

CR8 was found to have approximately 100% oral bioavailability, which means the drug is suitable for oral route intake and that the first pass effect is negligible.

CR8 distributed rapidly to all the peripheral tissues. The highest tissue distribution, as compared to plasma AUC, was found mainly in elimination organs, namely liver (200%) and kidneys (200%). These results encourage the hope that CR8 could perhaps be used to treat liver and kidney tumors, since it was found to induce apoptotic cell death in both hepatic and renal tumor cell lines [7]. Moreover, CR8 showed high distribution to the adipose tissue (180%) compared to that found in plasma, a factor that should be considered in dose scheduling in obese patients.

Surprisingly, CR8 showed higher distribution to the bone marrow (30%) compared to roscovitine, which was shown to pass the bone marrow barrier by only 1.5% [28] while the biodistribution of CR8 in the spleen was about 40%. Moreover, the cytotoxicity of CR8 against leukemic cell lines has been reported [7]. Altogether, these results suggest the possible use of CR8 in treating leukemia and lymphomas. However, the high biodistribution to the bone marrow may cause myelosuppression, which was not detected in roscovitine [28]. Further studies are currently ongoing in our laboratory to explore the cytotoxic effect of CR8 on normal hematopoietic progenitors. Moreover, we have observed five different metabolites with different polarities that have appeared with different kinetics throughout the CR8 kinetics. Urgent studies are needed to identify and quantify these metabolites, and to establish their pharmacological activates and toxicities.

In the present study, we have also shown that CR8 passes the BBB (concentration reached is 15% of that in plasma) compared to that reported for roscovitine (30%). This exposure of the brain to CR8 is nevertheless higher than that required for inhibition of its neuronal targets like CDK5 and CK1 (IC₅₀ values: 0.12 μM and 0.61 μM, respectively) [7]. This fact may benefit the drug during further studies in neurodegenerative animal models and brain tumor models.

Conclusions
We have developed and validated a simple, fast and selective quantitative analytical method which allowed us to analyze the pharmacokinetic profile and tissue distribution of the second generation CDK inhibitor CR8. CR8 was shown to have 100% bioavailability and longer half-life compared to roscovitine, which may allow higher systemic exposure than the IC₅₀ values for tumor cell death induction. The drug passes the BBB at a level sufficient to reach concentrations able to inhibit neuronal kinase targets. Bone marrow exposure was about 30% of that found as systemic exposure, a concentration potentially sufficient to eliminate leukemic cells. However, further studies are required to determine the myelosuppressive effect and hematotoxicity of CR8. The present study thus shows favorable pharmacokinetics properties of CR8 compared to its parent analogue roscovitine, encouraging further in-vivo studies and consideration of CR8 as a drug candidate.
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HS: carried out the experiments and drafted the manuscript. IE: performed the quality controls, and drafted the manuscript. LM: Provided the drug, drafted the manuscript. MH: Designed the experiment. All authors read and approved the final manuscript.

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References
1. Bach S, Knockaert M, Reinhardt J, Lozach O, Schmitt S, Baratte B, Koken M, Coburn SP, Tang J, Jung T, et al. Roscovitine targets, protein kinases and pyridoxal kinase. J Biol Chem 2005, 280:31208–31219.
2. Meijer L, Borgne A, Mulner O, Chong JP, Blow JJ, Inagaki N, Inagaki M, Delcros JG, Moulinoux JP: Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdck2 and cdks5. Eur J Biochem 1997, 243:527–536.
3. Meijer L, Raymond E. Roscovitine and selective other purines as kinase inhibitors. From starfish oocytes to clinical trials. Acc Chem Res 2003, 36:417–425.
4. Meijer LKB, Galons H: Roscovitine (CYC202, Seliciclib). In Monographs on Enzyme Inhibitors. CDK Inhibitors and their Potential as Anti-Tumor Agents. Edited by Smith PJ, Yue E. Boca Raton, FL, USA: CRC Press, Taylor & Francis; 2006.
5. Benson C, White J, De Bono J, O’Donnell A, Raynaud F, Cruickshank C, McGath H, Walton M, Workman P, Kaye S, et al. A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-roscovitine), administered twice daily for 7 days every 21 days. Br J Cancer 2007, 96:29–37.
6. Oumata N, Bettaeye K, Ferandin Y, Demange L, Lopez-Giral A, Goddard ML, Myrianthopoulos V, Mikros E, Flajolet M, Greengard P, et al: Roscovitine-derived, dual-specificity inhibitors of cyclin-dependent kinases and casein kinases 1. J Med Chem 2008, 51:2292–2302.
7. Bettaeye K, Oumata N, Echalier A, Ferandin Y, Endicott JA, Galons H, Meijer L: CRB1, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases. Oncogene 2008, 27:5791–5807.
8. Bukhanov NO, Moreno SE, Natoli TA, Rogers KA, Smith LA, Ledbetter SR, Oumata N, Galons H, Meijer L, Ibrahimagov-Beskrovaya O: CDK inhibitors R-roscovitine and S-CRB8 effectively block renal and hepatic cystogenesis in an orthologous model of ADPKD. Cell Cycle 2012, 11:4040–4046.
9. Bettaeye K, Baubæk D, Delheouze C, Loaez N, Hole AJ, Baumili S, Endicott JA, Dou-Rasay S, Bernard J, Oumata N, et al: CDK inhibitors roscovitine and CRB8 trigger McI-1 down-regulation and apoptotic cell death in neuroblastoma cells. Genes Cancer 2010, 1:369–380.
10. Kababdi SY, Stoica BA, Hamsann M, Loane DJ, Kharebava G, Murray II MG, Cabatbat RM, Faden AI: CRB8, a selective and potent CDK inhibitor, provides neuroprotection in experimental traumatic brain injury. Neurotherapeutics 2012, 9:405–421.
11. Wu J, Pajoosh-Ganj A, Stoica BA, Dinizo M, Guanciale K, Faden AI: Delayed expression of cell cycle proteins contributes to astroglial scar formation and chronic inflammation after rat spinal cord contusion. J Neuroinflammation 2012, 9:169.
12. Wu J, Kharebava G, Piao C, Stoica BA, Dinizo M, Sabirzhanov B, Hamsann M, Guanciale K, Faden AI: Inhibition of EZF1/CDK1 pathway attenuates neuronal apoptosis in vitro and confers neuroprotection after spinal cord injury in vivo. PLoS One 2012, 7:e4229.
13. Oumata N, Ferandin Y, Meijer L, Galons H: Practical synthesis of roscovitine and CRB8. Org Process Res Dev 2009, 13:641–644.
14. Bressolle F, Bremet-Pettit M, Audran M: Validation of liquid chromatographic and gas chromatographic methods. Applications to pharmacokinetics. J Chromatogr B Biomed Appl 1996, 6863–10.
15. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, Laytlow T, Viswanathan CT, Cook CE, McDowell RD: Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Eur J Drug Metab Pharmacokinet 1991, 16:249–255.
16. Hartmann C, Smeyers-Verbeke J, Massart DL, McDowell RD: Validation of bioanalytical chromatographic methods. J Pharm Biomed Anal 1998, 17:193–218.
17. Kanes HT, March C: Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis. Pharm Res 1993, 10:1420–1426.
18. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, et al: Bioanalytical method validation—a revisit with a decade of progress. Pharm Res 2000, 17:1551–1557.
19. Kanes HT, March C: Calibration and validation of linearity in chromatographic biopharmaceutical analysis. J Pharm Biomed Anal 1991, 9:911–918.
20. Kanes HT, Shiu G, Shah VP: Validation of bioanalytical methods. Pharm Res 1991, 8:421–426.
21. Midha KK, Rawson MJ, Hubbard JW: Individual and average bioequivalence of highly variable drugs and drug products. J Pharm Sci 1997, 86:1193–1197.
22. Lin JH, Lu AY: Role of pharmacokinetics and metabolism in drug discovery and development. Pharmaco Rev 1997, 49:403–449.
23. Fischer PM, Gianella-Barron A: Recent progress in the discovery and development of cyclin-dependent kinase inhibitors. Expert Opin Investig Drugs 2005, 14:457–477.
24. de la Motte S, Gianella-Barron A: Pharmacokinetic model of R-roscovitine and its metabolite in healthy male subjects. Int J Clin Pharmacol Ther 2004, 42:322–239.
25. McCue SJ, Stuart I: Metabolism of the trisubstituted purine cyclin-dependent kinase inhibitor seliciclib (R-roscovitine) in vitro and in vivo. Drug Metab Dispos 2007, 35(3):561–70.
26. Bettaeye K, Sallam H, Ferandin Y, Popowycz F, Fournet A, Hassan M, Schaler A, Bernard P, Endicott J, Joseph B, Meijer L: N-RN: a new class of cell death-inducing kinase inhibitors derived from the purine roscovitine. Mol Cancer Ther 2008, 7:2713–2724.
27. Raynaud F, Whittaker SR, Fischer PM, Scull M, Walton MI, Barrie SE, Garrett MD, Rogers P, Clarke SJ, Kelland LR, et al: in vitro and in vivo pharmacokinetic-pharmacodynamic relationships for the trisubstituted aminopurine cyclin-dependent kinase inhibitors olomoucine, bohemeine and CYC202. Clin Cancer Res 2005, 11:4875–4887.
28. Song H, Vita M, Sallam H, Tehranishi R, Nilsson C, Siden A, Hassan Z: Effect of the Cdk-inhibitor roscovitine on mouse hematopoietic progenitors in vivo and in vitro. Cancer Chemother Pharmacol 2007, 60:841–849.
29. Sallam H, Jimenez P, Song H, Vita M, Cedazo-Minguez A, Hassan M: Age-dependent pharmacokinetics and effect of roscovitine on Cdk5 and Erk1/2 in the rat brain. Pharmacol Res 2008, 58:312–37.
30. Nutley BP, Raynaud FJ, Wilson SC, Fischer PM, Hayes A, Goddard PM, McCue SJ, Jarman M, Lane DP, Workman P: Metabolism and pharmacokinetics of the cyclin-dependent kinase inhibitor R-roscovitine in the mouse. Mol Cancer Ther 2005, 4:125–139.

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