ELSEVIER

International Journal of Pharmaceutics 239 (2002) 207–211

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Note

Preformulation studies of a novel camptothecin anticancer agent, CKD-602: physicochemical characterization and hydrolytic equilibrium kinetics

Jae-Hyun Kim *, Seok-Kyu Lee, Jong-Lae Lim, Hee-Jong Shin, Chung Il Hong

DDS Research Lab, Pharmaceutical Research Labs, CKD Research Institute, Chong Kun Dang Pharm., Chonan PO Box 74, Chonan 330-600, South Korea

Received 12 January 2002; received in revised form 25 February 2002; accepted 3 March 2002

Abstract

(20*S*)-7-(2-isopropylamino)ethylcamptothecin·HCl (CKD-602), a new camptothecin (CPT) anticancer agent, is a pale yellowish crystalline compound. DSC thermogram exhibited a melt endotherm near 270 °C, and CKD-602 was found to be slightly hygroscopic. The solubility of CKD-602 in deionized water was 8.22 mg/ml, and two pK_a values were measured to be 2.32 and 9.15, respectively. A pH-dependent partition coefficient behavior in octanol-buffer was observed. CKD-602 in solid state was stable over the range of temperature and humidity, but decomposed slightly by light. The hydrolysis of CKD-602 occurred reversibly and rapidly in aqueous buffer solutions. The conversion rate constants (k_f: from the lactone to the carboxylate and k_r: from the carboxylate to the lactone) and the final equilibrium ratio (K_{eq}) between two species were dependent on the pH of aqueous solutions.

Keywords: Preformulation; (20*S*)-7-(2-isopropylamino)ethylcamptothecin HCl; Anti-cancer agent; pH-dependent partition coefficient; Reversible hydrolysis

The unique mechanism of camptothecin (CPT), inhibiting the function of topoisomerase I-DNA complex, led to the development of semi-synthetic analogues, Irinotecan (Campto®) and Topothecan (Hycamtin®), for the treatment of ovarian and colorectal cancer, respectively (Wall et al., 1966; Hsiang et al., 1989; Creemers et al., 1994). However, the high chemical reactivity of the α-hydroxy carbonyl group in the terminal lactone ring of CPT analogues, absolutely required for cytotoxic activity, induces a rapid equilibration between the lactone form and the biologically inactive ring-opened carboxylate form in neutral and alkaline media (Wani et al., 1980; Fassberg and Stella, 1992; Wall and Wani, 1995).

(20*S*)-7-(2-isopropylamino)ethylcamptothecin·HCl (CKD-602, Fig. 1), a new semi-synthetic analogue of CPT, has shown a considerable anti-

* Corresponding author. Tel.: +82-41-529-3319; fax: +82-41-558-3004.

E-mail address: jhjaykim@dreamwiz.com (J.-H. Kim).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00099-6

tumor activity at the preclinical stage and is currently under the clinical development (Jew et al., 1998; Ahn et al., 2000).

For the physicochemical characterization, DSC determination was carried out heating from 20 to 330 °C (in a sealed pan at a rate of 5 °C/min), and the changes in moisture content at various humidity conditions (23–94% RH) were observed.

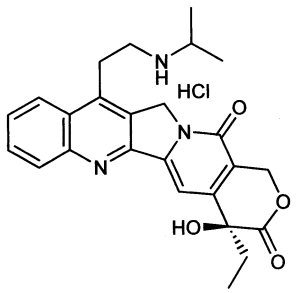


Fig. 1. Chemical structure of CKD-602.

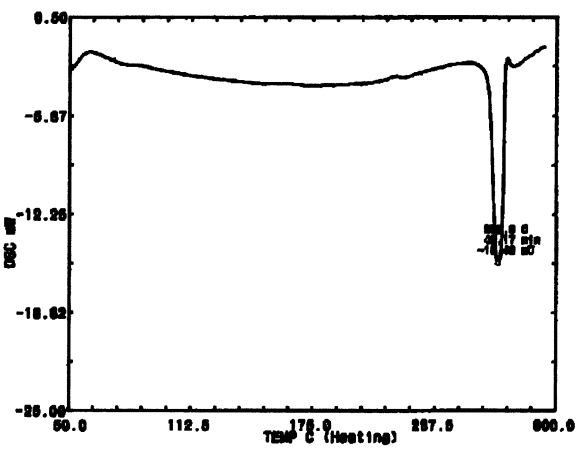


Fig. 2. DSC thermogram of CKD-602.

Table 1
Solubility of CKD-602 in various solvents at 25 °C

Solvent	Solubility (mg/ml)
Acetic acid, glacial	10.70 ± 0.08
Methanol	4.13 ± 0.32
Ethanol	1.11 ± 0.10
Deionized water	$8.22^{a} \pm 0.18$
Acetonitrile	0.057 ± 0.004

[a] Presented as the total solubility of lactone and carboxylate.

Solubility in various solvents was measured, and solid-state stability test was performed in several controlled conditions. pK_a values and apparent partition coefficients (P_{app}) were also determined in the range of pH 2–12 by a potentiometric titration instrument (Takács-Novák et al., 1997). The hydrolysis was investigated at 25 °C in various buffer solutions over the range of pH 2.26–8.36 by HPLC at 254 nm.

The DSC thermogram of CKD-602 produced a melt endothermic peak at about 270 °C in Fig. 2, and CKD-602 was found to be slightly hygroscopic and converted to its hydrate form due to water-adsorption. CKD-602 was fairly soluble in acetic acid and deionized water, but hardly soluble in acetonitrile (Table 1). The results of the solid-state stability test in Table 2 revealed that CKD-602 was stable over the range of temperature and humidity, but slightly decomposed by light. Two pK_a values were estimated to be 2.32 ± 0.05 for pK_{a1} and 9.15 ± 0.02 for pK_{a2}, and the dissociation scheme was proposed in Scheme 1.

The partition of CKD-602 between an octanol (o) and an aqueous buffer (w) phase was also expressed in Scheme 1, where the true microscopic partition coefficients, P_I, P_{II}, P_{III} and P_{IV} for each species, were given. The overall apparent partition coefficient (P_{app}) can be expressed by Eq. (1):

$$P_{app} = \frac{[\mathbf{I}]_o + [\mathbf{II}]_o + [\mathbf{III}]_o + [\mathbf{IV}]_o}{[\mathbf{I}]_w + [\mathbf{II}]_w + [\mathbf{III}]_w + [\mathbf{IV}]_w} \quad (1)$$

The fractions of **III** and **IV** are negligible at the range of pH 1–4, and those of **I** and **II** negligible at the range of pH 8–12, so P_{app} in each region can be expressed by Eqs. (2) and (3):

$$P_{app}\left(1 + \frac{K_{a1}}{a_H}\right) = P_{\mathbf{I}} + P_{\mathbf{II}}\left(\frac{K_{a1}}{a_H}\right) \quad (2)$$

$$P_{app}\left(1 + \frac{a_H}{K_{a2}}\right) = P_{\mathbf{IV}} + P_{\mathbf{III}}\left(\frac{a_H}{K_{a2}}\right) \quad (3)$$

From the plots of $P_{app}(1 + K_{a1}/a_H)$ versus (K_{a1}/a_H) and $P_{app}(1 + a_H/K_{a2})$ versus (a_H/K_{a2}), $P_{\mathbf{I}}$, $P_{\mathbf{II}}$, $P_{\mathbf{III}}$ and $P_{\mathbf{IV}}$ were obtained to be 0.14, 2.69, 57.03 and 1.22, respectively ($K_{a1} = 4.79 \times 10^{-3}$ and $K_{a2} = 7.08 \times 10^{-10}$). At the range of pH 4–8, the pH-dependent interconversion region, the frac-

Table 2
Stability of CKD-602 in solid state

Storage condition[a]	Appearance	Remaining (%)
Initial	Pale yellowish crystalline powder	100
25 °C, 60% RH, 12 months	Pale yellowish crystalline powder	99.52
40 °C, 75% RH, 6 months	Pale yellowish crystalline powder	99.27
Sunlight, 3 months	Deep yellowish crystalline powder	90.26

[a] Transparent vials containing accurately weighed CKD-602 were stored in light-protected chambers with controlled temperature and humidity or near a south facing window after sealing.

tions of **I** and **IV** are negligible, so P_{app} can be rearranged as follows by combining the relationships in Eqs. (6) and (7):

$$P_{app} = \frac{P_{II}(P_{III}+1) + 10^{1.01\,pH-6.50}P_{III}(PII+1)}{(P_{III}+1) + 10^{1.01\,pH-6.50}(P_{II}+1)} \quad (4)$$

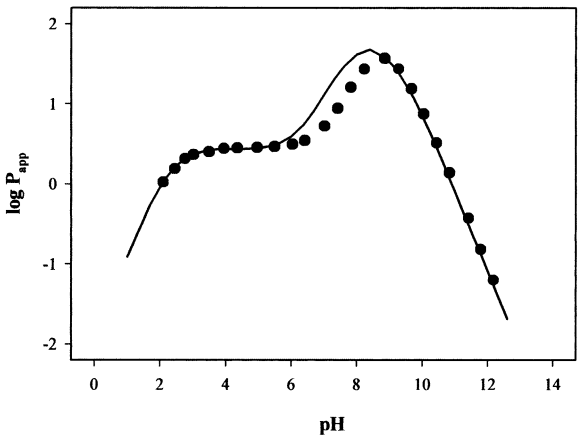
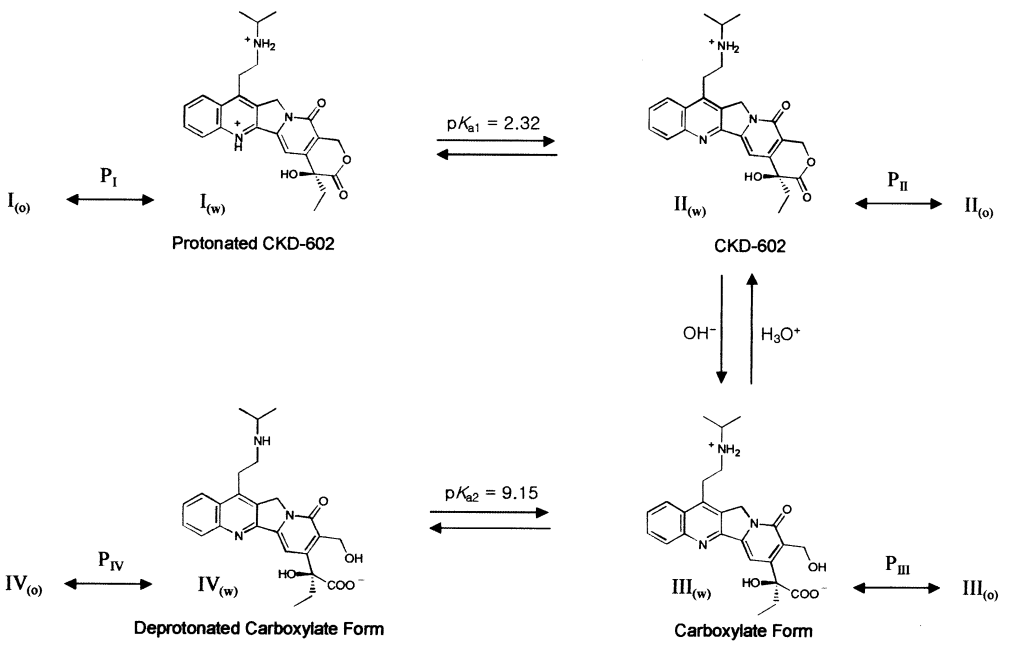


Fig. 3. Apparent partition coefficient (log P_{app})-pH profile of CKD-602. The line was fitted by Eqs. (2)–(4).

Plots of log P_{app} against pH in Fig. 3 showed pH-dependent partition coefficient behavior of CKD-602.

During the kinetic study, CKD-602 (**II**) was found to be major one at pH < 5, but the carboxylate form (**III**) was major at pH > 7, which is consistent with the results of other CPT ana-



Scheme 1.

Table 3
Hydrolysis rate constants for CKD-602 in various buffer solutions ($\mu = 0.4$) at 25 °C

pH	Buffer	Concentration (M)	k_{obs} (per min)[a]
2.26	HCl	0.005	0.8601[c]
3.25	Acetate	0	3.80×10^{-2}[b]
		0.05	5.14×10^{-2}[c]
		0.10	6.26×10^{-2}[c]
		0.15	7.66×10^{-2}[c]
3.63	Acetate	0	2.62×10^{-2}[b]
		0.05	2.77×10^{-2}[c]
		0.10	2.89×10^{-2}[c]
		0.15	3.05×10^{-2}[c]
4.21	Acetate	0	9.07×10^{-3}[b]
		0.05	1.01×10^{-2}[c]
		0.10	1.24×10^{-2}[c]
		0.15	1.28×10^{-2}[c]
4.74	Phosphate	0	4.59×10^{-3}[b]
		0.05	5.24×10^{-3}[c]
		0.10	6.54×10^{-3}[c]
		0.15	6.87×10^{-3}[c]
5.16	Phosphate	0	1.40×10^{-3}[b]
		0.05	3.06×10^{-3}[c]
		0.10	5.49×10^{-3}[c]
		0.15	6.76×10^{-3}[c]
5.68	Phosphate	0	5.70×10^{-4}[b]
		0.05	1.95×10^{-3}[d]
		0.10	3.63×10^{-3}[d]
		0.15	4.86×10^{-3}[d]
6.13	Phosphate	0	–[f]
		0.05	1.40×10^{-3}[d]
		0.10	2.37×10^{-3}[d]
		0.15	4.59×10^{-3}[d]
6.60	Phosphate	0	6.10×10^{-4}[b]
		0.05	2.14×10^{-3}[d]
		0.10	2.99×10^{-3}[d]
		0.15	4.86×10^{-3}[d]
7.11	Phosphate	0	3.00×10^{-3}[b]
		0.05	4.29×10^{-3}[d]
		0.10	7.59×10^{-3}[d]
		0.15	7.88×10^{-3}[d]
7.62	Phosphate	0	9.30×10^{-3}[b]
		0.05	1.14×10^{-2}[e]
		0.10	1.28×10^{-2}[e]
		0.15	1.53×10^{-2}[e]
8.36	Borate	0	0.1737[b]
		0.05	0.4877[e]
		0.10	0.7347[e]
		0.15	1.0822[e]

[a] Two runs were performed and the mean was indicated.
[b] k_{obs} extrapolated to zero buffer concentration.
[c] The average value determined from monitoring the disappearance of the carboxylate and the appearance of the lactone.
[d] The average value determined from monitoring the appearance and disappearance of the carboxylate, and the appearance and disappearance of the lactone.
[e] The average value determined from monitoring the disappearance of the lactone and the appearance of the carboxylate.
[f] Not applicable.

logues. The mechanism of lactone ring cleavage has been known to involve acyl cleavage due to the hyperchemical reactivity of the α-hydroxyl group in the lactone ring (Fassberg and Stella, 1992).

The rate constants (k_{obs}) were calculated from the slopes of linear relationship in Eq. (5), where A_{eq}, A_t and A_0 are the concentrations after the completion of reactions (> 3 half-lives), at time t and at time zero, respectively:

$$\ln\left\{\frac{A_t - A_{eq}}{A_0 - A_{eq}}\right\} = -k_{obs}t \quad (5)$$

Values of k_{obs} ($= k_f + k_r$) in each buffer solution were listed in Table 3, where buffer concentration contributed to the hydrolysis rate by general acid/base catalysis. Thus, the rate in a phosphate buffer (pH 5.68) was about 8.5-fold faster at 0.15 M than in unbuffered solution (i.e. the intercept of the buffer plot). The acidic and basic species of HCl solution and borate buffer solutions had a more catalytic effect than those in other buffer solutions.

The equilibrium ratio (K_{eq}) of two species at any given pH value can be written as follows:

$$K_{eq} = \frac{[\mathbf{III}]_{eq}}{[\mathbf{II}]_{eq}} = \frac{k_f}{k_r} \quad (6)$$

where $[\mathbf{II}]_{eq}$, $[\mathbf{III}]_{eq}$, k_f and k_r are the equilibrium concentration of the lactone form and the carboxylate form, and the conversion rate constant from the lactone to the carboxylate and from the carboxylate to the lactone, respectively. Plots of $\log K_{eq}$ as a function of pH in the range of pH 4.21–7.62 gave the following:

$$\log K_{eq} = 1.01\ \mathrm{pH} - 6.50 \quad (7)$$

where the slope of unity suggested that an inverse proportionality existed between K_{eq} and a_H. Knowing the values of k_{obs} and K_{eq} enabled calculations of k_f and k_r. Fig. 4 indicated that k_f was dependent on hydroxide ion activity at $\mathrm{pH} \geq 5$, and k_r on hydronium ion activity at $\mathrm{pH} \leq 6$.

In conclusion, some physicochemical properties and hydrolytic equilibrium kinetics of CKD-

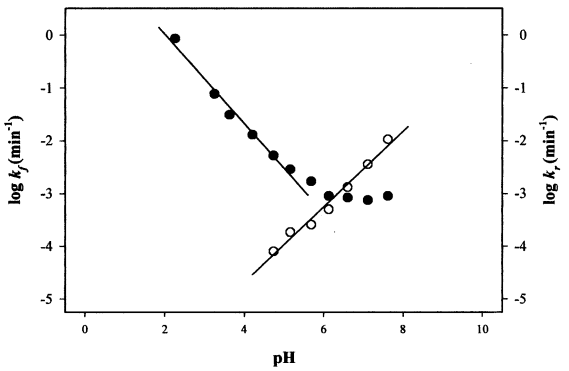


Fig. 4. pH-rate profile for the hydrolysis of CKD-602. k_f (○); k_r (●).

602 were investigated, and these results could provide useful data for the biopharmaceutical study of the compound required for the optimum drug product development in a parenteral or oral dosage form.

Acknowledgements

The authors gratefully acknowledge Dr S.K. Ahn and J.W. Lee for generously supplying CKD-602 and Dr S.S. LEE for viewing the manuscript critically.

References

Ahn, S.K., Kim, J.K., Hong, C.I., 2000. CKD-602. Drugs Future 25, 1243–1246.

Creemers, G.J., Lund, B., Verweij, J., 1994. Topoisomerase I inhibitors: topotecan and irenotecan. Cancer Reat. Rev. 20, 73–96.

Fassberg, J., Stella, V.J., 1992. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. J. Pharm. Sci. 81, 676–684.

Hsiang, Y.H., Liu, L.F., Wall, M.E., Wani, M.C., Nicholas, A.W., Manikumar, G., Kirschenbaum, S., Silber, R., Potmesil, M., 1989. DNA topoisomerase **I**-mediated DNA cleavage and cytotoxicity of camptothecin analogues. Cancer Res. 49, 4385–4389.

Jew, S.S., Kim, M.G., Kim, H.J., Rho, E.Y., Park, H.G., Kim, J.K., Han, H.J., Lee, H., 1998. Synthesis and in vitro cytotoxicity of C(20)(*RS*)-camptothecin analogues modified at both B(or A) and E ring. Bioorg. Med. Chem. Lett. 8, 1797–1800.

Takács-Novák, K., Avdeef, A., Box, K.J., 1997. Potentiometric pK_a determination of water insoluble compounds: validation study in methanol/water mixtures. Int. J. Pharm. 151, 235–248.

Wall, M.E., Wani, M.C., 1995. Camptothecin and Taxol: discovery to clinic—thirteenth Bruce F. Cain Memorial Award Lecture. Cancer Res. 55, 753–760.

Wall, M.E., Wani, M.C., Cook, C.E., Palmer, K.H., 1966. Plant antitumor agents. **I**. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. J. Am. Chem. Soc. 88, 3888–3890.

Wani, M.C., Ronman, P.E., Lindley, J.T., Wall, M.E., 1980. Plant antitumor agents. 18. Synthesis and biological activity of camptothecin analogues. J. Med. Chem. 23, 554–560.