Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1873–1876

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PRODRUG ESTERS OF THE INDOLOCARBAZOLE CEP-751 (KT-6587)

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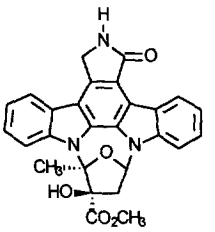
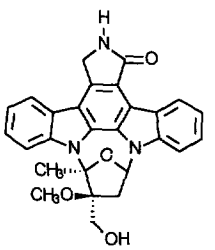
Received 2 March 1998; accepted 28 May 1998

Abstract: Prodrug esters of the indolocarbazole CEP-751 (KT-6587) were prepared with the goal of identifying water soluble, stable but cleavable forms for intravenous dosing. A dipeptide proform Lys-β-Ala (**16**, CEP-2563/KT-8391) was identified for advancement to clinical trials.

Formation of water-soluble ester prodrugs has long been understood as an efficient means of increasing aqueous solubility of poorly soluble drugs that contain a hydroxyl group. The most commonly used esters are those containing ionic or ionizable groups such as dicarboxylic acid hemiesters, phosphate esters, sulfate esters, and α-amino acid esters.[1] However, there remain apparent problems with these groups considering the properties of an ideal prodrug: (1) high water solubility at the pH of optimum stability; (2) sufficient stability for long term storage of injectable solutions; and (3) rapid, quantitative conversion in vivo to the parent drug. One factor leading to the failure of simple salt forming ester prodrugs is the often inherent poor solution stability. Ester prodrugs with exceptional solution stability can be designed by understanding the influence of the pro-moiety structure on the intrinsic liability of the ester linkage. A challenging aspect in the development of an ester stable at neutral pH is the added requirement of enzymatic (or non-enzymatic) in vivo conversion to the active form.



1 K-252a



2 CEP-751 (KT-6587)

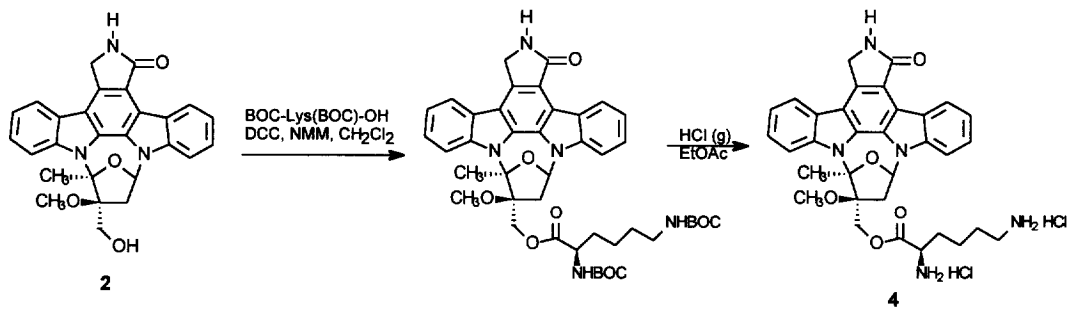
The indolocarbazole alkaloid K-252a (**1**) is an inhibitor of a number of serine/threonine kinases,[2] in addition to inhibition of the tyrosine kinase domain of the high affinity nerve growth factor (NGF) receptor *trk A*, as well as the related neurotrophin receptors *trk B* and *trk C*.[3] The role of NGF in the development and continued survival/maintenance of the adult nervous system is well established. Recent evidence supports an involvement of NGF in prostate cancer growth based upon the premise that prostatic carcinoma cells utilize an NGF/trk A autocrine mechanism for continued proliferation.[4] A detailed derivatization program of K-252a

0960-894X/98/$19.00
PII: S0960-894X(98)00328-X

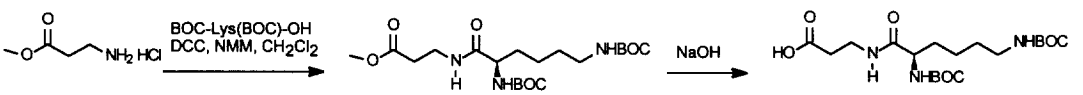
aimed at *trk A* inhibition identified hydroxymethyl derivative **2** (CEP-751/KT-6587) as a development candidate for the treatment of prostate cancer.[5] A significant problem limiting the usefulness of **2** (and the indolocarbazole class in general) as a therapeutic agent is its poor aqueous solubility (7 μg/mL). A method of intravenous delivery was essential to advance indolocarbazole **2** into clinical evaluation. This communication reports on the identification of a novel, solution stable, water soluble dipeptide prodrug form of **2**.

The esters were prepared in high yield using standard peptide coupling techniques with the alcohol **2** and an appropriate amino acid (DCC, DMAP, CH_2Cl_2, rt). In cases where N-protection was required a N-*t*-BOC-amino-acid was utilized as shown in Scheme 1 for the Lysinyl dihydrochloride derivative **4**. Removal of the BOC protecting group was accomplished with HCl(g) in EtOAc and the resulting amine hydrochlorides purified by recrystallization. The synthesis of BOC-Lys(BOC)-β-Ala-OH is outlined in Scheme 2. β-Alanine methyl ester hydrochloride was coupled with BOC-Lys(BOC)-OH (DCC, NMM, CH_2Cl_2, rt, 97%) to give the fully protected dipeptide. Hydrolysis of the methyl ester was accomplished using 1 equiv NaOH at 5 °C, followed by careful neutralization (0.5 M HCl, 5 °C) to give the di-*t*-BOC-acid in 80% yield as a white solid.

Scheme 1



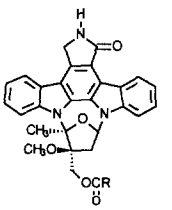
Scheme 2



The initial objective for the identification of potentially useful prodrugs was a minimum solubility of 1 mg/mL with less than 1% conversion to parent **2** over a 24 h period in: (1) water, (2) 10 mM sodium acetate (pH 3.6), (3) 10 mM sodium phosphate (pH 6.7), and (4) 10 mM Tris (pH 7.8) buffer solutions. Initially a set of α-amino acid ester hydrochlorides derived from Gly (**3**), Lys (**4**), His (**5**), Arg (**6**) and Glu (**7**) was profiled (Table 1). Lys **4** and Gly **3** were the only esters from this set to show modest stability (< 5% conversion to **2**) at both pH 3.6 and in water at ambient pH (**4** was < 1%), while all the derivatives were less stable in high pH buffers. The pH of a 1 mg/mL solution of **4** in water was ca. 3. Esters **9–11** were subsequently prepared in order to evaluate the steric effect on hydrolysis rate. Although not meeting our solution stability criteria, the N,N-dimethyl glycine ester **11** was greatly improved at higher pH compared to **3**. The niacin ester hydrochloride **12** and methiodide **13** proved to be poorly soluble and less stable, than desired, respectively.

Historically, α-amino acid esters are rapidly converted in vivo by plasma esterases but exhibit poor solution stability. A reason for this instability may be due to the strong electron withdrawing effect of the protonated α-amino group, destabilizing the ester bond towards hydrolysis.[6] To reduce this effect prodrugs were prepared with increasing distance between the ester function and the protonated amine in an attempt to furnish stability to non-enzymatic hydrolysis. The β-Ala **14** and dimethylaminobutyrate (DMAB) **15** displayed exceptional stability in the Na acetate buffer (pH 3.6). However, they were unstable in water and in the high pH buffers. The Lys-β-Ala dipeptide **16** (incorporating the solubilizing Lys **4** group with a β-Ala spacer) was prepared and found to display excellent aqueous solubility and stability.

Table 1. CEP-751-prodrug esters solution stability.



		Buffer Stability[a]			
Entry	Structure: CO-R[c]	Water	Acetate pH 3.6	Phosphate pH 6.7	Tris pH 7.8
3	Gly	5.5	4.5	54.4	37.9
4	Lys	0.9	4.3	63.0	53.0
5	His	12.8	14.5	13.0	27.1
6	Arg	20.5	4.9	13.8	24.4
7	Glu	9.4	8.1	13.8	24.4
8	$COCH_2CH_2CO_2H$	b	b	b	b
9	Ala	19.9	5.8	47.0	31.7
10	$COC(CH_3)_2NH_2$	10.6	1.3	22.5	13.1
11	$COCH_2N(CH_3)_2$	3.0	3.4	4.0	2.7
12	CO(2-Pyr)	b	b	b	b
13	$CO[2\text{-}(1\text{-}CH_3)Pyr^+]I^-$	45.6	2.9	65.7	78.4
14	$CO(CH_2)_2NH_2$ (β-Ala)	10.9	0	22.5	49.1
15	$CO(CH_2)_3N(CH_3)_2$	10.1	0	11.8	15.5
16	Lys-β-Ala	0	0	NT	NT

[a]24 h stability in the buffer indicated. Value shown represents the % of **2** formed after 24 h quantified by HPLC.
[b]solubility < 1 mg/mL.
[c]the amines were tested as HCl salts; **13** was the methiodide salt.
[d]NT = not tested.

Derivatives **14–16**, which were stable at pH 3.6, were further profiled in acetate buffer (pH 2–6, 24 h, Table 2). Esters **14** and **15** were stable at pH < 5, while at pH 6 hydrolysis was ca. 9% and 6%, respectively. However, the Lys-β-Ala ester (**16**) was stable up to pH 6 over 24 h. The maximum water solubility for **14–16** was also determined. Derivatives **14** and **15** displayed good water solubility (9.5 and 8 mg/mL), while the Lys-β-Ala **16** showed remarkable aqueous solubility of >200 mg/mL.

Table 2. Aqueous solubility and 24 h. pH stability of analogs **14 - 16** in acetate buffer[a]

	----------		pH	----------		
Entry	**2**	**3**	**4**	**5**	**6**	**H_2O[b]**
14	0	0	0	<0.9	8.8	9.5
15	0	0	0	<0.3	5.7	8
16	0	0	0	0	<0.1	>200

[a]1 mg/mL concentration. Value shown represents the % of **2** present @ 24 h measured by HPLC.
[b]maximum aqueous solubility, mg/mL.

Mammalian plasma and tissues contain a number of specific and nonspecific esterases that are capable of hydrolyzing ester linkages. Stability studies of esters **14–16** in various biological matrices were conducted. The esters were evaluated for conversion to parent in rat and human plasma and rat and human liver S-9 fractions (cytosol and microsomes), in addition to in vivo pharmacokinetic profiling in rats. CEP-2563 (**16**) showed a unique metabolic profile. Two pathways to for parent **2** were observed. The Lys residue cleaves to form β-Ala **14**, followed by hydrolysis of **14** to parent **2**, and also direct ester hydrolysis of **16** to **2**. A detailed pharmacokinetic profile of CEP-2563 (**16**) in animals and humans is the subject of a future publication.

In summary, a series of prodrug esters of the indolocarbazole clinical candidate CEP-751 (KT-6587) was prepared with the goal of identifying water soluble, stable and cleavable forms for intravenous dosing. A novel dipeptide, Lys-β–Ala (**16**, CEP-2563) was identified from this effort. Parent **2** is essentially insoluble in water while CEP-2563 dihydrochloride displayed exceptional aqueous solubility (>200 mg/mL) and stability. From these efforts, CEP-2563 has advanced to Phase I clinical trials.

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