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Synthesis and Kinase Inhibitory Activity of 3′-(*S*)-*epi*-K-252a

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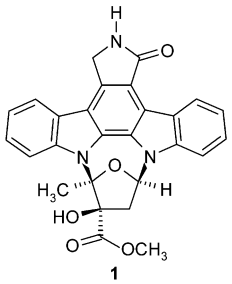
Abstract—The 3′-*epi* diastereomer of K-252a was synthesized with the goal of evaluating the stereochemical requirements of the 3′-sugar alcohol on kinase inhibitory activity. Inverting the 3′-alcohol resulted in a 20 nM inhibitor of VEGFR2 and a 1 nM inhibitor of TrkA tyrosine kinase.

The microbial derived indolocarbazole natural product (+)K-252a has gained significant attention over the past several years due to the variety of functional activities which it possesses. (+)K-252a (**1**), originally isolated from the culture broth of *Nocardiopsis* sp.,[1] inhibits a number of serine/threonine and tyrosine kinases such as protein kinase C (PKC), cAMP-dependant protein kinase (PKA), myosin light chain kinase (MLCK) and trkA in the nanomolar range.[2] The numerous functional activities of (+)K-252a include antitumor,[3] antibiotic,[4] antiinflammatory,[5] and neuronal survival promoting.[6]

The key structural attributes of glycosylated K-252a are the indolo[2,3-*a*]carbazole, a fused pyrrolo[3,4-*c*] lactam and a furanose sugar linked via two *N*-glycosidic bonds. A total synthesis of (+)K-252a confirmed the stereochemistry of the molecule to be 1′(*R*), 3′(*R*), 4′(*S*).[7] Our efforts with optimizing the activities of (+)K-252a derivatives led to three analogues advancing into clinical trials: CEP-2563 and CEP-701, trkA tyrosine kinase inhibitors for prostrate and pancreatic cancers respectively, and CEP-1347, an MLK inhibitor in phase II for the treatment of Parkinson's disease.[8] Further examination of this template led to the development of a route to the K-252a diastereomer-**2** to assess the contribution and role of the 3′-sugar alcohol on kinase potency and selectivity.

We report here the first synthesis and kinase inhibitory profile of the 1′(*R*), 3′(*S*), 4′(*S*)-K-252a diastereomer (**2**).



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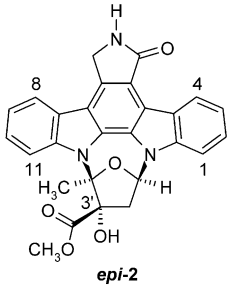

***epi*-2**

The synthetic strategy focused on the chemical transformation of **1** to **2** via ketone **4** (Scheme 1). K-252a was reduced with lithium borohydride to yield a diol, followed by periodic acid oxidation to ketone **4** in 83% yield.[9] The key steps in the synthesis were formation of the required C-3′ stereocenter through cyanide addition to the hindered ketone **4**, and subsequent transformation of nitrile to methyl ester. A number of cyanide reagents were evaluated and the diasteriomeric ratios of the cyanohydrin products were determined by HPLC (Table 1).

Tetrabutylammonium cyanide in dichloromethane–dioxane provided the highest yield of the desired cyanohydrin 3′-(*R*)-**5b** as a 1:1 mixture with isomer **5a**. Potassium cyanide and TMSCN gave lower yields while diethylaluminum cyanide resulted only in starting ketone. The low yields may result from difficulty in the isolation of the cyanohydrin products due to instability and reversibility. Attempts to separate the mixture of nitrile diastereomers chromatographically resulted in retrograde conversion to ketone **4**. To circumvent this, the mixture (*S*-**5a**/*R*-**5b**) was immediately subjected to a Pinner reaction (HCl_g, MeOH-dioxane, 0 °C), the result

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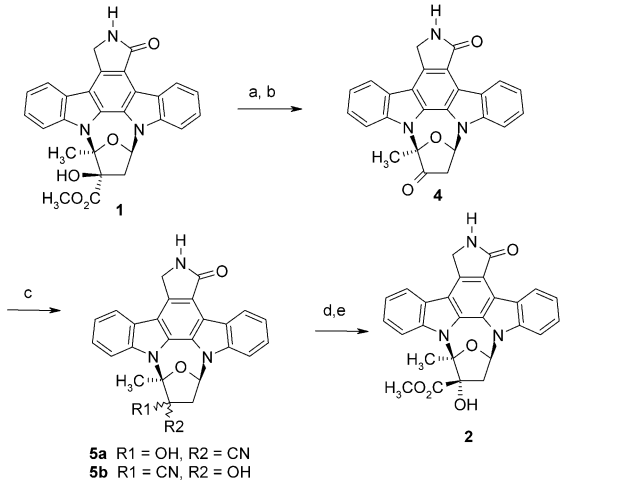

Scheme 1. (a) $LiBH_4$, THF, rt, 81%; (b) H_5IO_5, THF, rt 83%; (c) $nBu_4N^+CN^-$, CH_2Cl_2, 0 °C– rt, 60%; (d) HClg , CH_3OH–dioxane; (e) 6 N HCl.

Table 1. Reaction conditions and yields of cyanohydrin **5b**

Entry	Reaction conditions	Recovered **4** (%)	**5a**, **5b** (1:1) (%)
1	KCN, CH_3CO_2H, THF, MeOH	40	40
2	Et_2AlCN, CH_2Cl_2	95	
3	TMSCN, $ZnCl_2$, CH_2Cl_2	80	20
4	Bu_4NCN, CH_2Cl_2/dioxane		60

of which afforded one major product with a mass corresponding to K-252a (*m*/*e* 467) although with a more polar HPLC retention time.

Full analysis confirmed the structure to be the 3′-(*S*) diastereomer **2**. Under the acidic Pinner reaction conditions, the 3′-(*S*)-cyanohydrin **5a** eliminates CN back to ketone **4**, while the 3′-(*R*) isomer **5b** is converted to 3′-(*S*)-*epi*-K-252a **2**. K-252a shows an HPLC retention time of 13.12 min compared to 12.74 min for 3′-(*S*)-*epi*-K-252a.[10]

The assignment of **2** relative to **1** was made using ^1H and ^{13}C NMR along with COSY, and HETCOR experiments. Several differences were observed between the two compounds in their proton NMR spectra. The methyl ester of K-252a was observed at δ 3.93 (DMSO-d_6) compared to δ 3.30 on 3′-*epi*-K-252a (**2**). The difference in these shifts can be explained by the three dimensional structure which was generated by molecular modeling. The methyl ester of 3′-*epi*-K-252a (**2**) is oriented up in the face of the indolocarbazole ring, while on K-252a (**1**) the ester group is below the plane of the sugar. An NOE experiment with 3′-*epi*-K-252a was conducted in which the methyl ester was irradiated. An NOE of 8% was observed for H-11 (δ 7.66) and 11% for H-10 (δ 7.47). In a similar experiment with K-252a an NOE was not observed with H-10 or H-11 since the methyl ester is spatially distant from the aromatic ring. A significant field effect is observed from the ester and 4′-H_b on **1** and 4′-H_a on *epi*-**2**. The chemical shifts are outlined in Figure 1.

The kinase inhibitory data shown in Table 2 reveals that 3′-*epi*-K-252a (**2**) is 2–10 times more potent than K-252a for inhibition of PKC, VEGFR2 and TrkA. K-252a inhibits the tyrosine kinases TrkA, the high affinity neurotrophin receptor, and VEGFR2 with IC_{50} values of 13 and 43 nM, respectively.[11] *Epi*-K-252a (**2**) shows an increase of about 10-fold (IC_{50} 1.2 nM) in inhibition of TrkA and 2-fold (IC_{50} 19 nM) for VEGFR2.[12] K-252a inhibits the serine-threonine kinases PKC and MLK1 with IC_{50} values of 250 and 22 nM, respectively.[13] In addition, *epi*-K-252a (**2**) is about 2-fold (IC_{50} 114 nM) more potent for PKC while activity for MLK1 is essentially equivalent (IC_{50} 25 nM). One possible explanation for the increased inhibitory activity of 3′-*epi*-K-252a for PKC, VEGFR2 and TrkA could be that the 3′-*epi* OH may be involved in a favorable hydrogen bonding interaction that is not accessible to (+)K-252a due to geometry. Modeling studies are on going and will be included in a full structure–activity publication.

In conclusion, we report the first synthesis of the 3′-(*S*)-*epi*-K-252a diastereomer in a four-step synthesis from K-252a. Inversion of the alcohol to the 3′-*epi*-isomer resulted in a 19 nM VEGFR2 inhibitor and a 1 nM TrkA inhibitor revealing valuable stereochemical requirements at the sugar moiety of K-252a for binding to these kinases.

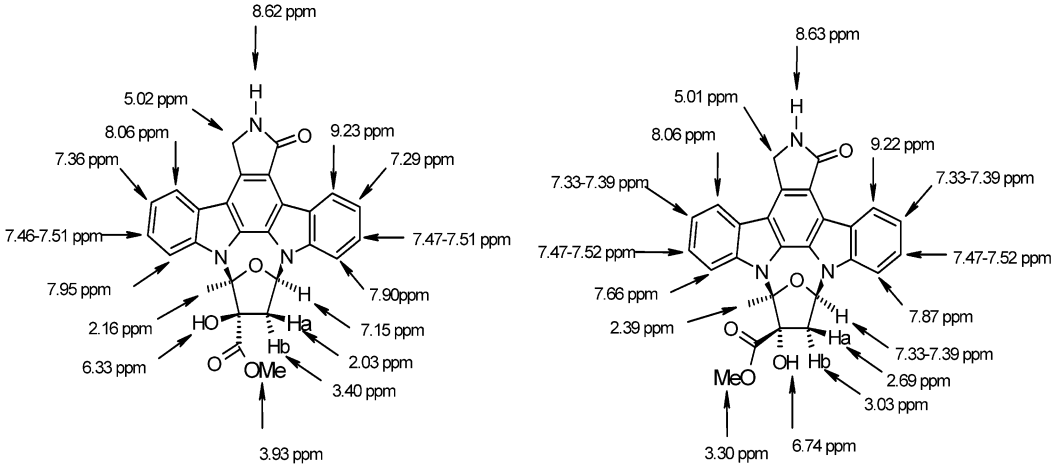

Figure 1. ^{1}NMR chemical shift assignments.

Table 2. Kinase activity (IC_{50}, nM)[a]

Entry	PKC	TrkA	VEGFR2	MLK1
K252a **1**	250±25	13±4	43±16	22±5
epi-K-252a **2**	114[b]	1.2±0.2	19±2	25±4

[a]Experiment run in triplicate.
[b]Single experiment.

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References and Notes

1. Kase, H.; Iwahashi, K.; Matsuda, Y. *J. Antibiot*. **1986**, *39*, 1059.
2. Kase, H.; Iwahasi, K.; Matsuda, Y.; Yamada, K.; Takahashi, M.; Murakata, C.; Sato, A.; Kanedo, M. *Biochem. Biophys. Res. Commun*. **1987**, *142*, 436.
3. Omura, S.; Sasaki, Y.; Iwai, Y.; Takeshima, H. *J. Antibiot*. **1995**, *48*, 535.
4. Sezaki, M.; Sasaki, T.; Nakazawa, T.; Takeda, U.; Iwata, M.; Watanabe, T.; Koyama, M.; Kai, F.; Shomura, T.; Kojima, M. *J. Antibiot*. **1985**, *38*, 1437.
5. Ohmori, K.; Ishii, H.; Manabe, H.; Satoh, H.; Tamura, T.; Kase, H. *Arzneimittelforschung* **1988**, *38*, 809.
6. (a) Knusel, B.; Hefti, F. *J. Neurochem*. **1992**, *59*, 1987. (b) Kaneko, M.; Saito, Y.; Saito, H.; Matsumoto, T.; Matsuda, Y.; Vaught, J. L.; Dionne, C. A.; Angeles, T. S.; Glicksman, M. A.; Neff, N. T.; Rotella, D. P.; Kauer, J. C.; Mallamo, J. P.; Hudkins, R. L.; Murakata, C. *J. Med. Chem*. **1997**, *40*, 1863.
7. Wood, J. L.; Stoltz, B. M.; Dietrich, H.-J.; Pflum, D. A.; Petsch, D. T. *J. Am. Chem. Soc*. **1997**, *119*, 9641.
8. (a) Camoratto, A. M.; Jani, J. P.; Angeles, T. S.; Maroney, A. C.; Sanders, C. Y.; Murakata, C.; Neff, N. T.; Vaught, J. L.; Isaacs, J. T.; Dionne, C. A. *Int. J. Cancer* **1997**, *72*, 673. (b) Saporito, M.; Hudkins, R.; Maroney, A. *Progress in Medicinal Chemistry,* Elsevier Science: Oxford **2002**, Vol. 40, p 23. (c) Murakata, C.; Kaneko, M.; Gessner, G.; Angeles, T. S.; Ator, M. A.; O'Kane, T. M.; McKenna, B. A. W.; Thomas, B. A.; Mathiasen, J. R.; Saporito, M. S.; Bozyczko-Coyne, D.; Hudkins, R. L. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 147.
9. Murakata, C.; Sato, A.; Takahashi, M.; Kobayashi, E.; Morimoto, M.; Akinaga, S.; Hirata, T.; Mochida, K.; Kase, H.; Yamada, K.; Iwashashi, K. U.S. Patent 4,923,986, 1990; *Chem. Abstr*. **1988**, *110*, 75861e.
10. Zorbax RX C-8 column with an elution program of 10–100% acetonitrile/water with 0.1% TFA over 40 min at 1.6 mL/min.
11. (a) Gingrich, D. E.; Hudkins, R. H. WO 02/17914, 2002. *Chem. Abstr*. **2002**, *136*, 216643m and references cited within. (b) PKC activity was assessed using the Millipore Multiscreen TCA "in plate" assay described in: Pitt, A. M.; Lee, C. *J. Biomol. Screening* **1996**, *1*, 47.
12. (a) Ruggeri, B. A.; Miknyoczki, S. J.; Singh, J.; Hudkins, R. L. *Curr. Med. Chem*. **1999**, *6*, 845. (b) Angeles, T. S.; Steffler, C.; Bartlett, B. A.; Hudkins, R. L.; Stephens, R. M.; Kaplan, D. R.; Dionne, C. A. *Anal. Biochem*. **1996**, *236*, 49.
13. Maroney, A. C.; Finn, J. P.; Connors, T. J.; Durkin, J. T.; Angeles, T.; Gessner, G.; Xu, Z.; Meyer, S. L.; Savage, M. J.; Greene, L. A.; Scott, R. W.; Vaught, J. L. *J. Biol. Chem*. **2001**, *276*, 25302.