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Design, synthesis and biological evaluation of deuterated nintedanib for improving pharmacokinetic properties

Ruixue Xu,[a†] Miao Zhan,[a†] Lingling Peng,[a] Xuehai Pang,[b] Jun Yang,[a] Tao Zhang,[a] Hongxia Jiang,[a] Lifeng Zhao,[a*] and Yuanwei Chen[a,b*]

Nintedanib is a novel triple angiokinase inhibitor that inhibits three growth factors simultaneously. Deuterated derivatives of nintedanib at certain metabolically active sites were prepared and evaluated *in vitro* and *in vivo*. In particular, deuterated compound SKLB-C2202 had significantly improved pharmacokinetic properties compared with nintedanib. These efforts lay the foundation for further investigating the druggability of SKLB-C2202.

Keywords: Nintedanib; triple angiokinase inhibitor; deuterium isotope effect; antitumor activity

Introduction

Nintedanib, also known as BIBF 1120, a potent indolinone derivative, is a small molecule triple angiokinase inhibitor that simultaneously inhibits three growth factors: vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR).[1,2] Angiogenesis is a crucial endogenous pathway in both healthy and diseased tissues. In a healthy human body, it can help in wound-healing, but in excess in diseased tissues, it also can create many new blood vessels to supply diseased tissues with oxygen and nutrients.[3] So angiogenesis can be a therapeutic target.[4] VEGF plays a key role in multiple signaling pathways that control survival, mitogenesis, migration, and differentiation of endothelial cells and create blood vessels. An over-expression of VEGF generally has been seen as associated with tumor progression.[5] VEGF-receptor inhibitors have been developed by many companies,[6] and several entities have already been approved, such as sorafenib,[7] sunitinib,[8] vandetanib,[9] axitinib,[10] regorafenib,[11] cabozantinib, and[12] ponatinib.[13] PDGFR[14] and FGFR[15] both control the migration and adherence of cells and provide support and stability to vessel walls. All three growth factors are crucially involved in the formation of blood vessels, and suppression of them can play an important role in the prevention of tumor growth and spread.

Nintedanib is currently being investigated in a number of indications including advanced non-small cell lung cancer, small cell lung cancer, prostate cancer, breast cancer, ovarian cancer, cervix cancer, endometrial cancer, thyroid cancer, liver cancer, acute myeloid leukemia, and just recently, it was approved by the Food and Drug Administration (FDA) for the treatment of idiopathic pulmonary fibrosis.[16]

Nintedanib, an orally administered drug,[17] has a distinct pharmacodynamic feature in *in vitro* tests. This is a sustained pathway inhibition, suggesting slow receptor off-kinetics, but it is rapidly metabolized by methylester cleavage *in vivo*, leading to a short mean half-life.[1] In healthy humans, the major metabolite $\mathbf{M_1}$ is achieved by methyl ester cleavage, $\mathbf{M_2}$ is formed by oxidative *N*-demethylation on the piperazine ring, $\mathbf{M_3}$ is generated by double demethylation as previously mentioned, and $\mathbf{M_4}$ by oxidation of the piperazine moiety and conjugation (Figure 1).[18]

On the basis of this information, we considered whether the metabolism rate could be slowed down to get a longer mean half-life by blockade of metabolically active sites. We hypothesized that the goal could be achieved through minimal structure modification, keeping the advantages of nintedanib and overcoming its drawbacks at the same time. Accordingly, the hydrogen atoms at the metabolically active sites were substituted for deuterium.

Deuterium is a stable naturally-occurring, non-radioactive isotope of hydrogen. Due to the different atomic properties of deuterium the C-D, the C-D bond is stronger than the C-H bond.[19] Exchanging hydrogen with deuterium in functional groups can result in many benefits. First, the metabolic shunting may reduce the formation of undesirable metabolites or increase the formation of desired active metabolites. Second, deuteration can reduce the rate of systemic clearance resulting in a longer half-life. It can even maintain similar systemic exposure with decreased peak levels and increased trough levels. Finally, decreased pre-systemic metabolism results in higher

[a]*State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu, 610041, China*

[b]*Hinova Pharmaceuticals Inc, Suite 801, Building C1, #88 South KeYuan Road, Chengdu Tianfu Life Science Park, Chengdu, 610041, China*

**Correspondence to: Yuanwei Chen and Lifeng Zhao, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu, 610041, China. E-mail: ywchen@scu.edu.cn; lifengzhao@scu.edu.cn*

[†] *These authors contributed equally*

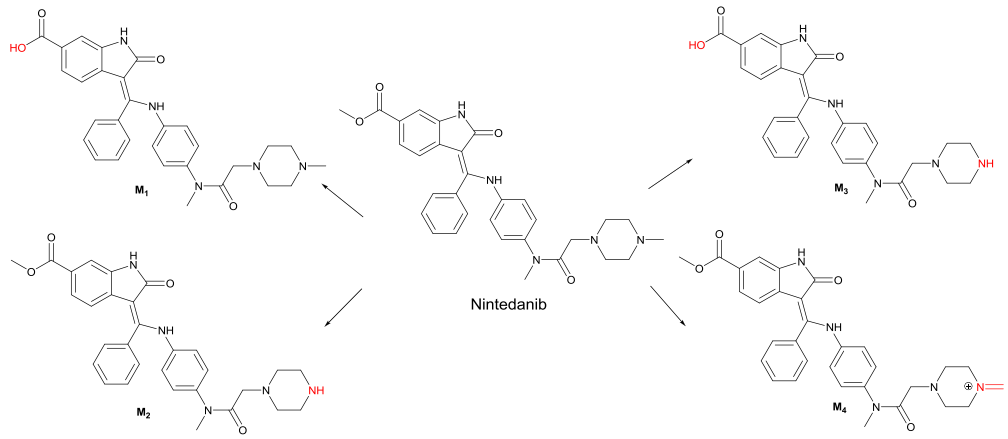


Figure 1. The major metabolic pathways of nintedanib. This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr

bioavailability of unmetabolized drug, which means a smaller dosage can achieve the same exposure.[20,21] Many studies have already reported several deuterium-labeled entities,[22–28] and some of these have entered clinical trials including CTP-354,[20] CTP-449,[29] SD-254, and[21] SD-809.[19] The Auspex drug, SD-809, significantly reduced the incidence of uncontrolled movements in patients with Huntington's disease compared with a placebo, according to results from a phase III study announced recently, and it is expected to seek US approval for SD-809 in the middle of this year.[19]

Our approach was based on the primary kinetic isotope effect.[30,31] As nintedanib's major metabolic pathways are methyl ester cleavage and oxidative *N*-demethylation, we therefore replaced the hydrogen atoms at the active sites with deuterium as shown in Table 1. (SKLB-C2201, SKLB-C2202 and SKLB-C2203).

Experimental

General methods

All commercial reagents and anhydrous solvents were obtained from commercial sources and were used without further purification, unless otherwise specified. Reactions were monitored by thin layer chromatography (TLC) on glass plates coated with silica gel containing fluorescence indicator reagents. ^{1}H NMR and ^{2}H NMR spectra were recorded on Bruker Avance III 400 (400 MHz for ^{1}H NMR and 61.4 MHz for ^{2}H NMR). Chemical shifts (δ) are expressed in ppm and are internally referenced (^{1}H NMR: 0.00 ppm for TMS in $CDCl_3$, 4.70 ppm for DSS in D_2O. ^{2}H NMR: 7.26 ppm for $CDCl_3$ in $CHCl_3$, 8.32 ppm for $CDCl_3$ in DMSO). Deuterium content was determined by comparison with the integration of both deuterated and non-deuterated position. Deuterium incorporation was assigned by ^{2}H NMR. The mass spectrometry (MS) systems used were the Quattro Premier XE mass spectrometer and a Q-TOF premier mass spectrometer (Waters Micromass, Milford, Massachusetts, USA), which were used for LC/MS analysis and accurate mass detection, respectively. Both were equipped with a standard ESI source.

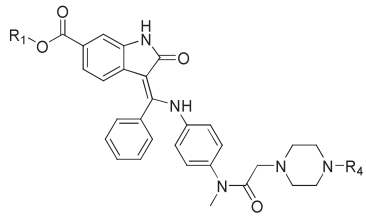
Details of synthesis and biological assays are described in the Supporting Information.

Results and discussion

The deuterated analogs of nintedanib were synthesized starting from the corresponding indolinones and aromatic amines as building blocks. As depicted in Scheme 1, **11a** and **11b** were obtained by a six-step procedure consisting of an esterification of 3-nitrobenzoic acid, followed by a nucleophilic substitution using **4a** or **4b**, then one-step hydrogenation-intramolecular amidation, condensation with trimethyl orthobenzoate after protecting with chloracetyl, and finally deprotection of the protecting group.

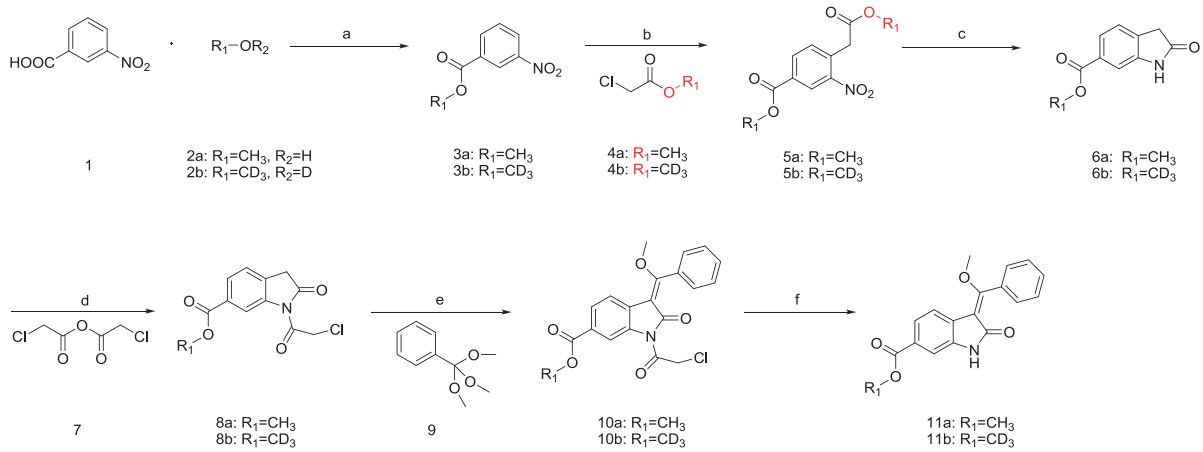
As described in Scheme 2, aromatic amines, **16a** and **16b**, were obtained by a three-step procedure. Acylation of *N*-methyl-4-nitroaniline **12** with chloroacetic anhydride **7** afforded compound **13**, followed by nucleophilic substitution with 1-methyl piperazine **14a** or 1-methyl-*d3*-piperazine **14b** to give compounds **15a** and **15b**, which were reduced to afford the title compound.

Table 1. VEGFR-2, FGFR-1, and PDGFR-α inhibition of nintedanib and its derivatives

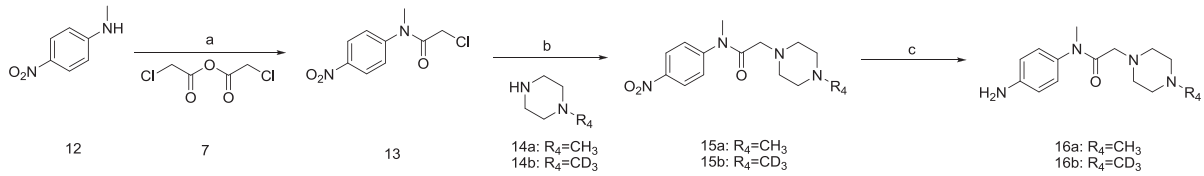


Compound	R_1	R_4	IC50 (nM)		
			VEGFR-2	FGFR-1	PDGFR-α
Nintedanib	CH_3	CH_3	3.1	81	5.5
SKLB-C2201	CD_3	CH_3	3.1	75	5.5
SKLB-C2202	CH_3	CD_3	2.9	67	5.5
SKLB-C2203	CD_3	CD_3	4.3	79	4.4

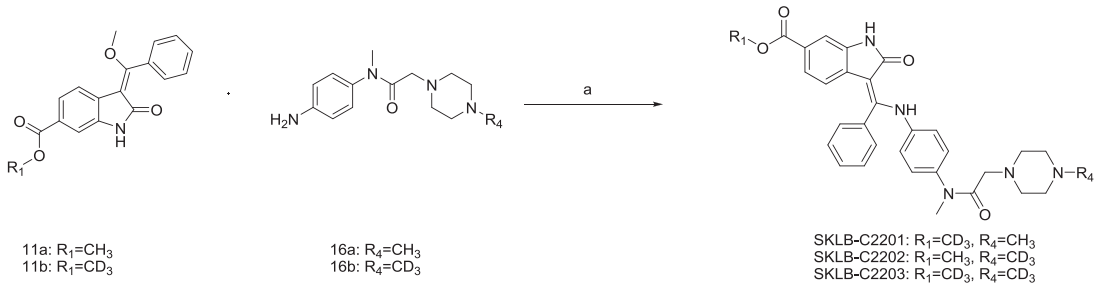
VEGFR, vascular endothelial growth factor receptor; PDGFR, platelet derived growth factor receptor; FGFR, fibroblast growth factor receptor.



Scheme 1. Synthesis of intermediates **11a** and **11b**. Reagents and conditions: (a) conc. sulfuric acid; (b) Potassium tert-butylate, DMF; (c) Pd/C, acetic acid; (d) Toluene, reflux; (e) Acetic anhydride, toluene, 90–120 °C; and (f) KOH, methyl alcohol. This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr



Scheme 2. Synthesis of intermediates 16a, 16b. Reagents and conditions: (a) Ethyl acetate, reflux; (b) Toluene, 30–50 °C; and (c) H_2, Pd/C. This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr



Scheme 3. Synthesis of nintedanib analogs. Reagents and conditions: (a) Methanol, reflux.

Table 2. The *in vivo* pharmacokinetic parameters of nintedanib and deuterated analogs

Compound	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-t} (mg/L*h)	$AUC_{0-\infty}$ (mg/L*h)
Nintedanib	2.03	2.00	232	843	1036
SKLB-C2201	2.90	2.00	132	556	785
SKLB-C2202	3.05	2.00	556	1339	1564
SKLB-C2203	1.58	2.00	211	650	735

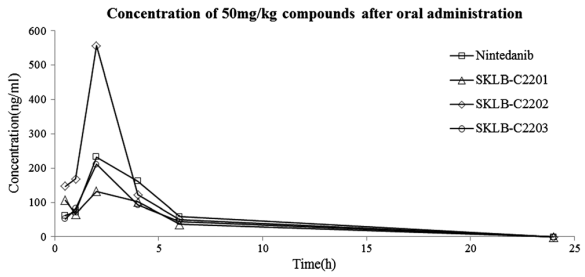


Figure 2. Plasma concentration of nintedanib analogs after oral administration (50 mg/kg).

As shown in Scheme 3, deuterium-labeled analogs SKLB-C2201, SKLB-C2202, and SKLB-C2203 were obtained by condensation of indolinones 11a and 11b with amines **16a** and **16b**.

The compounds were screened using a set of kinase selective assays and all derivatives tested inhibited VEGFR-2, FGFR-1 and PDGFR-α *in vitro*. The results are summarized in Table 1. As expected, the deuterated analogs of nintedanib remain in an almost equal inhibitory activity against the three kinases tested compared with its parent drug. All compounds tested show a

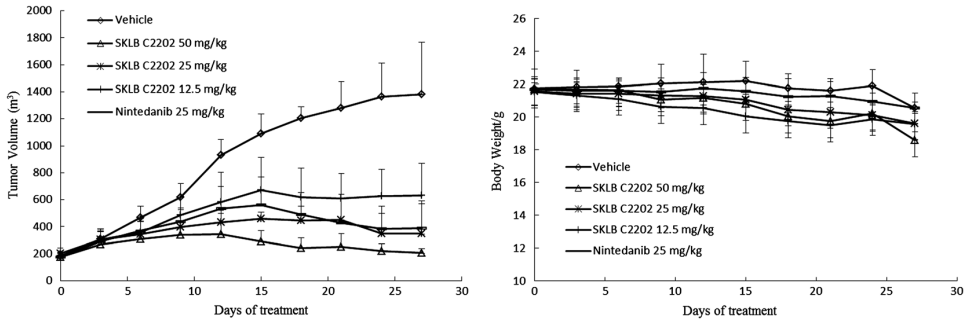


Figure 3. SKLB-C2202 inhibits human tumor xenograft growth in a model of a human head and neck SCC (FaDu) (left). Body weight changes of mice (right); Vehicle is control group of 0.5% hydroxyethyl cellulose.

single-digit inhibition activity against VEGFR-2 and PDGFR-α and a double-digit activity against FGFR-1. This may be attributable to the similar size of hydrogen and deuterium atom and hence the maintenance of similar activity.

The *in vivo* pharmacokinetic profiles of the deuterated materials were tested in healthy Balb/c mices. Nintedanib and its analogs were administered orally at a dose of 50 mg/kg. Blood samples were taken at 0.5, 1.0, 2.0, 4.0, and 24 h, and then drug plasma concentrations were quantified by LC-MS/MS. The pharmacokinetic parameters are shown in Table 2**,** and the plasma concentration is shown in Figure 2.

The major metabolic route of nintedanib results from methyl ester cleavage and the minor metabolic route from oxidative of the *N*-methylpiperidine ring, [1,18] We predicted that deuteration at both the methyl ester and the *N*-methyl on piperidine (SKLB-C2203) would slow down the hydrolysis and oxidation rate and therefore improve pharmacokinetic properties. However, our result showed that SKLB-C2203 had an almost identical pharmacokinetic profile to nintedanib with $AUC_{0\text{-}t}$ 650 and 843 (mg/L*h) and C_{max} 211 vs. 232 ng/ml, respectively. The deuteration of methyl ester alone (SKLB-C2201) resulted in a slightly worse AUC0-t than nintedanib. However, compound SKLB-C2202, the deutertion of *N*-methyl on piperidine has significantly better pharmacokinetic properties than nintedanib, with C_{max} of 2.39 times of nintedanib, $AUC_{0\text{-}\infty}$ of 1.51 times of nintedanib and improved $T_{1/2}$ (3.05 vs. 2.03 h). Better C_{max} indicated that SKLB-C2202 has good absorption. SKLB-C2202 has much better enhancement for PK profile, which indicated that the *N*-demethyl metabolite formation could be a significant overall factor in the pharmacokinetics.[18] Encouraged by the improved exposure, compound SKLB-C2202 was chosen for further antitumor activity studies *in vivo*. An animal model of human head and neck small cell carcinoma (FaDu) tumor xenografts in Balb/c mices was established. After 15 days inoculation, the average tumor volume reached 100–150 mm^3, the mice were randomly assigned to one vehicle control group, three SKLB-C2202 groups (50, 25, 12.5 mg/kg), and a nintedanib group (25 mg/kg). Each group contained seven mice. During a period of 28 days, the drug was administered per os at a frequency of once a day, and the tumor volume was measured every 3 days. As shown in Figure 3, SKLB-C2202 resulted in inhibition of tumor growth in three doses and shows dose proportionality. At a dosage of 50 mg/kg, the inhibition activity increased to 85.1% (treated versus control (T/C) values). At 25 mg/kg, the inhibitory activity is 74.9%, while with the same dose, its parent drug nintedanib has an antitumor activity of 72.0%. Although deuterated SKLB-C2202 has 50.9% better $AUC_{0\text{-}\infty}$ compared with parent nintedanib, the *in vivo* antitumor activity was not improved.

We also measured animal body weight change. As also shown in Figure 3, all groups are well tolerated during the treatment period. Even in the high-dose group (50 mg/kg), no significant weight loss was observed.

Conclusion

In conclusion, the substitution in deuterium for hydrogen in nintedanib did not adversely affect the binding affinity *in vitro*. Better pharmacokinetic properties *in vivo*, the deutrated analog SKLB-C2202 show significantly better properties than nintedanib and had a better plasma concentration which could prolong its half-life and improve the potency with a lower dosage. Finally, antitumor activity studies *in vivo*, SKLB-C2202 showed a better inhibition than nintedanib at the same dosage (25 mg/kg). When dosage was up to 50 mg/kg, the tumor-inhibitory rate went to 85.1%, with good animal tolerance. Further research more relevant to druggability is under investigation.

Acknowledgements

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Conflict of Interest

The authors did not report any conflict of interest.

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Supporting Information

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