Communication to the Editor

The Design, Synthesis and Preliminary Pharmacokinetic Evaluation of d3-Poziotinib Hydrochloride

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Received February 15, 2019; accepted March 13, 2019

To establish a synthetic route to d3-poziotinib hydrochloride. Treatment of 4-chloro-7-hydroxyquinazolin-6-yl pivalate (1) with d3-methyliodide afforded the etherization product, which reacted with 3,4-dichloro-2-fluoroaniline to generate the key intermediate d3-4-(3,4-dichloro-2-fluorophenylamino)7-methoxyquinazolin-6-yl pivalate (2). Followed the de-protection reaction, the nuleophilic substitution (SN1,2) reaction with tert-butylicarbonyl (TSP), and the de-protection reaction of t-butoxycarbonyl (Boc) group, and the amide formation reaction with acrylyl chloride, d3-poziotinib was obtained, which was converted to hydrochloride salt by treatment with concentrated hydrochloric acid (HCl). Starting from a known compound 4-chloro-7-hydroxyquinazolin-6-yl pivalate (1), after 7 steps transformation, d3-poziotinib hydrochloride was obtained with a total yield of 9.02%. The structure of d3-poziotinib hydrochloride was confirmed by 1H-NMR, 13C-NMR, and high resolution (HR)-MS. Meanwhile, the in vitro microsomal stability experiment showed that d3-poziotinib had a longer half time (t1/2 = 4.6h) than poziotinib (t1/2 = 3.5h).

Key words poziotinib; d3-poziotinib; metabolic closure

INTRODUCTION

Poziotinib (Fig. 1, HM781-36B) is a pan-human epidermal growth factor receptor (HER) inhibitor. It was orally administered alone or in combination with cytotoxic chemotherapy agents for treatment of gastric cancer, non-small cell lung cancer (NSCLC)2 and breast cancer.3 In the preclinical studies, it was found that poziotinib in vivo could be converted to an active metabolite M2 (Fig. 1) through O-demethylation metabolic pathway,4 and the M2 was considered to be the main cause of the drug’s major side effects such as severe diarrhea, vomiting, etc.

Metabolic closure is a common strategy to improve the stability of drug metabolism. By blocking the drugs’ metabolic sites, the clearance rate of drugs could be delayed, and the production of active metabolites was decreased, so the effect time of drugs could be prolonged.

Deuterate is a stable non-radiation isotope of hydrogen. Due to the similarities of hydrogen and deuterate in space and shape, deuterated drugs should always keep the original biological activities and selectivity. Meanwhile, due to the bond strength of C–D bond is stronger than C–H bond, deuterated drugs are always bearing following advantages: 1. decrease the clearance, prolong the half time, and decrease side-effects; 2. decrease the metabolism of drugs in guts and liver, increase the therapeutic effects; 3. decrease the production of harmful metabolites, enhance the tolerance of drug.5,6

The first U.S. Food and Drug Administration (FDA) approved deuterated drug was Austedo, a deuterated tetranaqine, which was used for treatment of Huntington’s disease-related dance symptoms.7 CTP-656, deuterated Ivacaftor, was an orphan drug developed by Concert for the treatment of cystic fibrosis.8 In order to decrease the production of undesirable metabolites, and enhance the patients’ tolerance of drugs, deuterated poziotinib was designed and synthesized in this article.

MATERIALS AND METHODS

Routine monitoring of reaction was performed by TLC using pre-coated GF254 TLC plate. 1H-NMR was recorded on a Bruker AVANCE 400 spectrometer at 400MHz with tetramethylsilane used as an internal reference. MS were performed with electron spray ionization (ESI) mode. High-resolution (HR)-MS were recorded on an Agilent 6210 ESI/time-of-flight (TOF) mass spectrometer. Melting points (mp) were recorded on a Büchi B-540 melting point apparatus and were uncorrected. Flash column chromatographic separation was achieved using a silica gel from Qingdao Ocean Chemical (200 to 300mesh) with a particle size from 54 to 74µm using ethyl acetate and hexane (or petroleum ether) as the eluent.

Materials All reagents were purchased from commercial sources and were used as received. 3,4-dichloro-2-fluoroaniline (Maya, China), d3-methyliodide (CD3I) (Aldrich, U.S.A.), N-tert-butoxycarbonyl (Boc)-4-hydroxyxypiperidine (J&K, China), 7N ammonia/Methanol (Aldrich), acrylyl chloride (Energy Chemical, China), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Acros, U.S.A.), tert-butylicarbonyl (TSP), 4-(tosyloxy)piperidine-1-carboxylate (TSP, 5): prepared by literature method.9) white solid, mp 97.9–98.7°C; 4-chloro-7-hydroxyquinazolin-6-yl pivalate (1): prepared by literature methods.10) white wax.

Chemistry Staring from a known compound 4-chloro-7-hydroxyquinazolin-6-yl pivalate (1, Chart 1), the hydroxyl group was converted to corresponding methyl ether 2 by treatment with d3-methyl iodide under basic condition. Compound 4 was obtained after 2 steps transformation: 1: replacement of chloride group in compound 2 by 2-fluoro-3,4-dichloroaniline; 2: de-protection of Piv group by ammonia/methanol. Under basic condition, treatment of TSP (5) with compound 4, the key intermediate 5 was afforded with moderate yield. Followed the removal of Boc group by concentrated hydrochloric acid (HCl), amide formation reaction by treatment with acry-
lyl chloride, and salt formation with concentrated HCl, d3-poziotinib hydrochloride (7) was obtained as yellowish powder.

**In Vitro Microsomal Stability Test**  To a solution of rat liver microsomes solution 2.0 mL (1.0 mg/mL), was added the solution of test compounds 2.0 mL. After mixing, the solution was divided into 3 parts, and incubated for 3 min at 37°C. When a solution of 10 µL of 400 mM NADPH regeneration solution was added to above mixture, aliquots sample were taken and terminated with 100 µL of cold methanol at 0, 5, 15, 30, 60, 120 min. Following centrifugation, the supernatant was added to above mixture, aliquots sample were combined, dried, filtered, and concentrated. The residue was purified by a chromatography to afford the second pure solid (yield: 67.44%). The filtrate was concentrated, and the residue was purified by a chromatography to afford the second crop of product.  \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta_{\text{ppm}}\): 11.16 (brs, 1H, ArNH), 8.39 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.58 (m, 2H, ArH), 7.23 (s, 1H, ArH), 4.68–4.73 (m, 1H, OCH), 3.67–3.71 (m, 2H, NCH\(_2\)), 3.25 (m, 2H, NCH\(_2\)), 1.98–2.01 (m, 2H, CH\(_2\)), 1.57–1.67 (m, 2H, CH\(_2\)), 1.42 (s, 9H, OC(CH\(_3\))\(_3\)).

**d3-tert-Butyl-4-(4-(3,4-dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-yloxy)piperidine-1-carboxylate (6)**  To a solution of compound 3 (2.9 g, 6.6 mmol) in methanol, was added ammonia solution 30 mL (7 N in methanol). After addition, the reaction mixture was allowed to stir at room temperature for 10 h. The yellow precipitate was collected and dried to afford the de-protection product quantitatively, which used in next step directly.

To a solution of TSP (5) (1.21 g, 3.4 mmol), and K\(_2\)CO\(_3\) (0.7 g, 5.1 mmol) in DMF (4 mL), was added above yellow compound (0.6 g, 1.7 mmol). The reaction mixture was allowed to stir for 24 h at 67–70°C till TLC showed that all starting material consumed. After cooling to room temperature, water (4.8 mL) was added to the reaction mixture slowly. The resulting mixture was stirred for 3 h at room temperature, and the solid was collected by filtration and washed with water (2×10 mL). After drying, compound 5 was obtained as off-white to pale yellow solid (43.57%).  \(^1\)H-NMR (DMSO-\(d_6\), 400 MHz) \(\delta_{\text{ppm}}\): 9.6 (brs, 1H, ArNH), 8.39 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.58 (m, 2H, ArH), 7.23 (s, 1H, ArH), 4.68–4.73 (m, 1H, OCH), 3.67–3.71 (m, 2H, NCH\(_2\)), 3.25 (m, 2H, NCH\(_2\)), 1.98–2.01 (m, 2H, CH\(_2\)), 1.57–1.67 (m, 2H, CH\(_2\)), 1.42 (s, 9H, OC(CH\(_3\))\(_3\)).

**RESULTS**

**d3-4-Chloro-7-methoxyquinazolin-6-yl Pivalate (2)**  To a solution of compound 1 (4.5 g, 16.1 mmol), potassium carbonate (K\(_2\)CO\(_3\)) (6.7 g, 48.3 mmol) in \(N,N\)-dimethylformamide (DMF) (50.0 mL), was added CDI (1.5 mL, 24.1 mmol) slowly at 0°C. After addition, the reaction mixture was allowed to stir at room temperature for 16 h till TLC showed that all starting material consumed. The reaction mixture was partitioned between water and ethyl acetate, the organic layers were combined, dried, filtered, and concentrated. The residue was purified by chromatography to afford the title compound as white powder (60.67%).  \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta_{\text{ppm}}\): 8.95 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.40 (s, 1H, ArH), 3.25 (m, 2H, NCH\(_2\)), 1.42 (s, 9H, OC(CH\(_3\))\(_3\)).

**d3-4-(3,4-Dichloro-2-fluorophenylamino)-7-methoxyquinazolin-6-yl Pivalate (3)**  A solution of compound 2 (2.9 g, 9.76 mmol), 3,4-dichloro-2-fluoroaniline (2.1 g, 11.7 mmol) in acetonitrile (20 mL) was stirred at 90 ± 5°C for 8 h till TLC showed that all starting material consumed. The reaction mixture was cooled to room temperature, and the white precipitate was isolated and washed with acetonitrile (2×20 mL) and dried to afford the title compound as white solid (yield: 67.44%). The filtrate was concentrated, and the residue was purified by a chromatography to afford the second crop of product.  \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \(\delta_{\text{ppm}}\): 11.16 (brs, 1H, ArNH), 8.78 (s, 1H, ArH), 8.43 (s, 1H, ArH), 7.67 (s, 1H, ArH), 7.50–7.54 (t, 1H, J = 8.2 Hz, ArH), 7.20–7.23 (dd, 1H, J = 8.7, 1.5 Hz, ArH), 1.52 (s, 9H, OC(CH\(_3\))\(_3\)).

**d3-Poziotinib Hydrochloride (7)**  To a solution of compound 6 (8.0 g, 14.9 mmol) in acetone (80 mL), was added conc. HCl (14.4 mL) slowly. After addition, the reaction mixture was allowed to stir at room temperature for 5 h, till TLC showed that all starting material consumed. The precipitate was isolated, washed with acetone (2×10 mL), and dried to afford the de-protection intermediate (7.4 g, 97.6%) as yellow solid, which used in following step directly.

To a solution of above solid (8.7 g, 17.0 mmol) in tetra-
hydrofuran (THF) (61 mL), was added a solution of sodium bicarbonate (NaHCO₃) (5.71 g, 68 mmol) in water (87 mL) under vigorous stirring. The resultant solution was cooled to 0–3°C, and a solution of acryly chloride (1.6 mL, 19.9 mmol) in THF (61 mL) was added through an addition funnel within 0–3°C, and a solution of acryly chloride (1.6 mL, 19.9 mmol) in THF (61 mL) was added through an addition funnel within 30 min. After addition, the reaction mixture was allowed to stir for 30 min at such temperature till TLC showed the completion of the reaction. Water (140 mL) was added to the reaction mixture, the aqueous solution was extracted with ethyl acetate (3×100 mL). The organic layers were combined, dried, filtered, and concentrated. The residue was purified by column chromatography to afford the d₃-poziotinib free base as pale yellow foam (5.93 g, 70.73%).

A hot solution of d₃-poziotinib free base (5.8 g, 11.7 mmol) in methanol (50 mL) was acidified to pH = 2–3 by conc. HCl. The reaction mixture was allowed to stir at room temperature for 24 h. The precipitate was isolated, washed with acetone (10 mL), and dried to afford d₃-poziotinib hydrochloride as pale yellow powder (4.6 g, 74.2%). mp 230–232°C.

Fig. 2. The Results of in Vitro Microsomal Stability Test

Fig. 3. The NMR Spectra Comparison of Poziotinib (a) and d₃-Poziotinib (b)

Fig. 4. The NMR Spectra Comparison of d₃-Poziotinib (a) and Poziotinib (b)

D3-Poziotinib was more metabolic stable, and had a longer half time.

DISCUSSION

The only difference between poziotinib and d₃-poziotinib is the methyl group on the quinazoline ring. So on the NMR spectrum, poziotinib and d₃-poziotinib should share same signals except the methyl group. Figure 3 compares the ¹H-NMR and ¹³C-NMR of poziotinib hydrochloride (a) with d₃-poziotinib (b). In the ¹H-NMR spectra, the poziotinib has a single methyl-peak signal near 4 ppm, and the d₃-poziotinib has no signal at this location. Meanwhile, in the ¹³C-NMR spectra, poziotinib has a methyl signal peak at 56.59 ppm, however, d₃-poziotinib has no signal in this position.

CONCLUSION

Starting from a known compound 4-chloro-7-hydroxyquinazolin-6-yl pivalate (I), after 7 steps transformation, d₃-poziotinib hydrochloride was obtained with a total yield of 9.02% (conditions not optimized). The reaction conditions of this synthetic route were mild. Meanwhile, the in vitro microsomal stability assay showed that the half time of d₃-poziotinib was a little longer than Poziotinib, which means that d₃-poziotinib was more metabolic stable than Poziotinib. The further pharmacological and toxicological investigation of d₃-poziotinib was still undergoing, and the results will report soon.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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