Synthesis of a New Pyridine Pharmaceutical Intermediate in Potential Anti-cancer of HDACI

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ABSTRACT: In order to obtain better anti-cancer activity of HDACI, new HDCIs analog and a pyridine pharmaceutical intermediate were designed by comparing the molecule of FK228 and Largazole and introducing the pyridine intermediate to the cyclic tetrapeptide pharmacophore. The synthetic route of HDCIs analog and the pyridine intermediate were designed. The HDCIs analog and the pyridine intermediate were all synthesized, and the optimum conditions of synthesizing the pyridine intermediate were got by using parallel test. The pyridine intermediate can be used to manufacture new potential HDACIs by forming new structure of the polypeptide. Due to the special structure of the pyridine intermediate, it can also be widely used in other medical field. Through our work we have designed synthetic routes of the pyridine intermediate and discussed the optimum conditions. And we provide a new synthesis method of cyclopeptide HDCIs.

Keywords: FK228 analogue; HDACIs; pyridine; tetrapeptide

1 INTRODUCTION

Pyridine compound is a high value-added fine chemical product. Its chemically active can participate in a variety of chemical reactions. Pyridine compounds chlorinated, fluoride and ammonia oxidation can be prepared for a variety of intermediates in the pharmaceutical synthesis and fine chemical industries that have been widely used. Pyridine compounds are currently used in medicine, pesticides, spices, feed additives, adhesives and synthetic materials. Such compounds are being used to develop the most widely-used range of fine chemical products global manufactures pyridine compounds of $ 50 billion in annual sales. In the field of medicine, pyridine can be used to produce cephalaxin, prednisone dexamethasone acetate, sulfa sulfuric acid piperazine, hydrocortisone, idoxuridine, progesterone, norfloxacin, vitamin A, D2 or D3, the fourth generation of cephalosporins, and more than 40 kinds of synthetic materials of commonly-used medicines.

For a long time, cancer has been considered to be the result of a wide variety of genetic and genomic alterations, such as amplifications, translocations, deletions and point mutations [1]. But recent research showed cancer development is not only related to the genetic changes but also involves epigenetic changes. Epigenetics is concerned with the inheritance of information based on gene-expression levels. The main epigenetic modifications in humans are DNA methylation and posttranslational histone modifications.

2 MATERIALS AND METHODS

Histones are small basic proteins that are rich in amino-acids, lysine and arginine [2]. Histone acetyltransferase (HAT) and histone deacetylase (HDAC) can make histone N-terminal amino acid residue be acetylated or deacetylated in vivo, both enzymes work together to determine histone acetylation levels, and they also regulate expression of gene and some other cellular processes, and studies have shown that acetylation and deacetylation process and the occurrence of cancer are closely linked. Therefore, histone deacetylase (HDACs) are targets for anticancer drugs. Histone deacetylase inhibitor (HDACi) has become a kind of hot areas of research. Histone deacetylase inhibitor (HDACi) has become a kind of hot areas of research. Histone deacetylase inhibitor can improve the level of p21 and other genes expression by increasing cell level of histone acetylation. The process of tumor cells proliferation has showed a very good inhibitory effect, and the inhibitor can in-
duce tumor cell differentiation and/or the occurrence of apoptosis, so it has the effect of inhibiting tumor [5, 6]. Romidepsin (Istodax), a cyclic peptide, has been recently approved as an HDAC inhibitor against CTCL, indicating that cyclic peptides are pharmacophores of interest in this field. Histone deacetylases (HDACs) are promising targets for such a novel NB therapy. HDACs catalyze the removal of acetyl groups from the lysine residues of proteins including the core nucleosomal histones. Histone tails contain 40 lysine residues, which are acetylated by HATs. Acetylation induces a conformational change within chromatin, allowing the transcriptional machinery access to DNA thus promoting gene expression. HDACs repress the gene expression by deacetylating the lysine tails, allowing the positively charged lysines to be tightly bound to the negatively charged DNA and denying the transcriptional machinery access to genes, thereby repressing gene expression. Thus, these post-translational modifications play a key role in directing gene expression, and can create a phenotype that is unrelated to changes in DNA. Histone deacetylases play a prominent role in the regulation of gene transcription by histone deacetylation. They are involved in the remodeling of chromatin, and consequently, in the regulation of gene expression. There is growing evidence that perturbation of epigenetic balances promotes the initiation and progression of cancer. HDAC inhibitors have been found to have therapeutic properties in many different human tumor cell lines, including those derived from the bladder, breast, prostate, lung, ovary and colon among others, indicating that the inhibition of HDAC activity may be a viable strategy for the treatment of cancers. HDAC inhibitors can be divided into short-chain fatty acids, hydroxamic acids, cyclopeptide HDACis, benzamides, electrophilic ketones, trithiocarbonate and other categories; however, in this article we focus on the cyclic peptides. Cyclic peptides were the most complex in all the HDACis. Surface recognition structure of macrocyclic inhibitors given the complex structure, but also to ensure the inhibitor with the enzyme molecule interactions more fully, this may be one of the reasons to ensure cyclic peptide inhibitor activity. Like most other inhibitors, the structure of cyclic peptide HDACis is divided into three domains [7], which are surface recognition domain, linker domain and Zinc binding domain. FK228 (formerly named FR901228), also known as depsipeptide, is produced by Chromobacterium violaceum and shows potent in vivo antitumor activity. FK228 received FDA approval in 2009 for the treatment of cutaneous T-cell lymphoma. The naturally occurring FK-228 displays impressive anticancer and anti-angiogenesis activities through the HDAC inhibition, and it is now approved as a cancer drug. FK228 is a bicyclic depsipeptide with intramolecular disulfide bridge, which is reductively activated after uptake into the cells generating the sulfhydryl groups. Inspired by the stability of FK228 due to its prodrug nature [12], several HDAC inhibitors with the disulfide bond have been proposed with more simplified structure for the ease of synthetic study. In the past decade, a considerable number of synthetic analogs have been reported as cyclic-tetrapeptide HDAC inhibitors. Main nucleus of 4-aniline quinazoline and hydroxylamine part of histone acetyl off enzyme (HDAC) inhibitor fragments are connected. By this way, they developed an EGFR inhibitors, ErbB2 and HDAC targets, the acetylation of EGFR phosphorylation and HDAC has certain inhibitory effect. Predecessors by the way of connecting hydroxylamine fragments at site-6,7 of 4-aminoquinazolinone obtained good active compound such as compound 1and 2, therefore, we imagined that if at site-4'- of 4-aniline quinazoline was substituted by different hydroxyl oxime acid group to get some new compounds. Largazole is a natural cyclic peptide which can selectively inhibit Class I histone deacetylase and show remarkable selectivity between transformed and non-transformed cells. The design of these analogs has been done either by changing the amino acids sequence in the cyclic tetrapeptide framework to achieve high affinity of the inhibitors with the enzyme surface or by the introduction of different type of functional groups as zinc ligands to achieve a strong binding affinity to the enzyme’s active site. The functional groups introduced are mostly full of electron. And more functional groups have been introduced to the non-peptide HDAC inhibitors. In order to develop HDAC inhibitors with various functional groups, we replaced the epoxyketone moiety of chlamydocin scaffold with a pyridine ring. As the first part of this paper, we have designed and synthesized 2 cyclic peptide compounds as HDACi based on the structures of Largazole and FK228. We tried to derive novel artificial amino acids and a pyridine ring to introduce them into the chlamydocin scaffold [9].

![Figure 1. Structural regions of cyclopeptide HDCIs.](image-url)

By comparing the structures of FK228 and Largazole, we designed and synthesized other compounds. The cyclic tetrapeptide structure in surface recognition domain was changed by introducing an amino acid containing a pyridine ring for a fuller contact with the surface and enzymes. The pyridine intermediate has a similar structure with amino acid, and it can be widely used in the medical field, especially as cyclic peptide intermediate fragment deacetylase inhibitors as FK228 analogues. Details are shown as follows in Figure 1.
Since the molecules have a cyclic tetrapeptide structure, we split the molecule into three different fragments and then they are connected end to end by a peptide bond or ester bond [14, 15]. Through the retrosynthesis the molecule can be split into three different parts as Figure 2 shows by breaking the amide bond and one ester bond. The zinc domain and the linker can be synthesized by fragment 1. The cyclic peptide was divided into valine and fragment 3. So the amino acid containing the pyridine ring becomes the key intermediate in the total synthesis of molecule DCE-3529415. Details are shown as follows in Figure 3.

Figure 2. Retrosynthesis of DCE-3529415.

The main challenges in the synthesis include the asymmetric construction of the hydroxyl mercapto heptenoic acid unit, the 16-membered cyclic depsipeptide ring itself (that is, four amino acids and hydroxyl mercapto heptenoic acid), and the intramolecular oxidative coupling of the thiol moieties to produce the stable prodrug form of the molecule. Using this method, Wen et al. utilized a lactamization as an alternative route to cyclization in 2008. Yurek-George et al. [13-16] examined the importance of several features of the romidepsin structure. To fragment 3, as the former through breaking the amide bond, the molecule can be divided to 2 parts, one is threonine. The hydroxyl of threonine can react with MsCl and then be removed by DABCO, so the double bond can form [14]. Details are shown as follows in Figure 4. The other one is an amino acids analogue with pyridine ring. The key to the amino acids analogue is to introduce synthetic carboxyl and amino functional groups. So we choose the 2, 6-dimethylpyridine as the raw material. Reacting with KMnO₄ the one methyl of 2, 6-dimethylpyridine can be oxidized to carboxyl and the other methyl can stay the same [17]. Then the methyl can be transferred into benzyl reacting with NBS by radical reaction which is the material of Gabriel reaction in the next step changing the benzyl to amino. Details are shown as follows in Figure 5.

Figure 4. Retrosynthesis of fragment 3 of DCE-3529415.

Figure 5. Synthesis of fragment 3 of DCE-3529415.

3 EXPERIMENTAL SECTION

All solvents were A.R. grade and purchased from Shanghai Chemical Reagent Company. Melting points were determined on a WPS-2A capillary apparatus and were uncorrected. The 1HNMR and spectra were recorded on a Bruker Avance III 500 NMR spectrometer using TMS as an internal standard and chemical shifts were given in ppm with tetramethylsilane (TMS). The HRMS spectra were acquired on a Solari X-70FT-MS apparatus. TLC was carried out on silica gel plates.
Compound 19 (21.43g, 0.2mol) dissolved in 1000mL H2O (not completely dissolve and not affect the reaction) was added KMnO4 47.41g. The solid was added in for ten times, and the interval between two times is 30min. After adding solid raising the temperature to 70°C, reflux for 5h. After the material was completely disappeared, the reaction solution was cooled to room temperature, filtered. The filtrate was evaporated to dryness, and then 200mL HCl (70%) was added to the solid dissolved. Extract the solution with ethanol, the solution was evaporated to be dryness and recrystallization, giving product (17.3g).

Compound 19 (15g, 0.11mol) was dissolved in 500mL methanol; thionyl chloride was dropped into methanol at 0°C. After the dropwise addition the reaction apparatus was brought to room temperature, reacting for 5 hours. Methanol was removed by rotary evaporation to give a white solid. The solid was dissolved in 200mL of sodium hydroxide solution at a concentration of 1mol/L. The pH value was adjusted to 9. Ethyl acetate was added to the solution and then was extracted. The organic phase was collected and dried. The solvent was evaporated under reduced pressure to give the product 19” (10g).

Compound 19’ (10g, 0.066mol) was dissolved in 60mL CCl4, NBS 14.1g (0.079mol) was added under reduced pressure to give the crude product. The crude product was recrystallized to give pure product (17.3g).

Compound 19’’ (10g, 0.066mol) was dissolved in 60mL CCl4, NBS 14.1g (0.079mol) was added under stirring. AIBN was added for 3 times about 5g (0.03mol). After the addition reflux at 80°C for 6 hours, add a certain amount of water and stir for 15min, the water is separated from CCl4 then dried. The CCl4 was removed under reduced pressure to give a pale yellow compound 980.8mg. At 0 °C, the pale yellow compound (900mg, 2.02mmol) in DMF (10mL) was added with DABCO (2.27g), and reaction was carried out at room temperature 2h, quenched with saturated ammonium chloride, extracted with dichloromethane, washed with water and saturated brine, concentrated, dried over anhydrous sodium sulfate, and concentrated by column chromatography to give compound 6 (1.48 g, 51%).

At 0 °C, under the protection of Ar gas, the compound 24 (1.0 g, 2.72mmol) in DCM (10mL) were sequentially added DMAP (16.6 mg), TEA (1.13 mL), MsCl (0.63 mL), Reaction was carried out at room temperature overnight, quenched with saturated ammonium chloride and extracted with dichloromethane, washed with water and saturated brine, and concentrated, dried over anhydrous sodium sulfate, and concentrated by column chromatography to give compound 6 (1.48 g, 51%).

The compound 23 (2.0g, 7.93mmol) and H-Thr-OMe (1.6g, 9.52mmol) in DCM (40mL) was added PyBop (4.13g, 7.93mmol) and DIPEA (5.3mL), Reaction was carried out at room temperature overnight, quenched with saturated ammonium chloride and extracted with dichloromethane, washed with water and saturated brine, and concentrated, dried over anhydrous sodium sulfate, and concentrated by column chromatography to give compound 6 (1.48 g, 51%).
carboxylic acid

1HNMR(501MHz,CDCl3)δ12.1(s,1H),8.52(d,J=8.3Hz,1H),8.35(d,J=8.6Hz,1H),7.99(d,J=6.9Hz,1H),4.55(s,2H),1.33(s,9H) ESI-HRMS calcd for C12H16N2O4 [M + H]+,253.12

Threine,N-[[6-[[1,1-dimethylethoxy]carbonyl]amino]methyl]-2-pyridinyl[carbonyl]-, methyl ester
1HNMR(501MHz,CDCl3)δ8.55(d,J=8.7Hz,1H),8.50(d,J=8.7Hz,1H),8.01(d,J=9.7Hz,1H),7.81(m,2H),5.1(d,J=6.1Hz,1H), 4.7(d, J=6.1Hz,1H),4.4(s,2H), 3.3(s,3H), 2.3(m,1H),1.3(s,9H) ESI-HRMS calcd for C17H25N3O6 [M + H]+,368.18

2-Butenoicacid,2-[[6-[[1,1-dimethylethoxy]carbonyl]amino]methyl]-2-pyridinyl[carbonyl]aminol, methyl ester
1HNMR(501MHz,CDCl3)δ8.12(d,J=7.4Hz,1H),7.85(s,1H),7.83(d,J=8.7Hz,1H),7.02(s,1H),4.48(d,J=5.6Hz,2H), 1.89(s,3H),1.57(s,3H),1.43(d,J=29.6Hz,9H) ESI-HRMS calcd for C17H23N3O5 [M+H]+,350.17

2-Butenoicacid,2-[[6-[[1,1-dimethylethoxy]carbonyl]amino]methyl]-2-pyridinyl[carbonyl]aminol
1HNMR(501MHz,CDCl3)δ9.50(d,J=7.4Hz,1H),7.85(s,1H),7.43(d,J=50.9Hz,1H),7.02(s,1H),4.48(d,J=22.2Hz,2H),1.89(s,3H),1.43(d,J=29.6Hz,9H). ESI-HRMS calcd for C16H21N3O5 [M+H]+,336.16

4 RESULTS AND CONCLUSION

During the process for preparing compound 19', we found that the equivalent of KMnO4 has a greater impact on the yield of the product. So we carried out experiments at different equivalents, and the results are shown as follows. The chart showed the yield of 19' declining with the increasing equivalent of NBS. Because, the dibromide increases very quickly with the increasing equivalent of NBS, the 1.2 equiv. of NBS was chosen to be the optimum condition.

Data in Table 1 showed the equivalent of KMnO4 are the key to control yield of 6-Methyl-2-Pyridincarboxylic acid. With the increasing equivalent of KMnO4, more and more 6-Methyl-2-Pyridinecarboxylic acid are transferred into 2,6-pyridinedicarboxylic acid. With the increasing equivalent of NBS, more and more 6-Methylpicolinic acid methyl ester r are transferred into 2-pyridinecarboxylic acid, 6-(dibromomethyl)-methyl ester and 2-pyridinecarboxylic acid,6-(trbromomethyl)- methyl ester. According to Figure 7, the A point which is 1.2 equivalent of NBS was chosen to be the optimum condition.

In summary, by means of comparing the structures of FK228 and Largazole, we designed and synthesized several compounds. The cyclic tetrapeptide structure in surface recognition domain was changed by introducing an amino acid containing a pyridine ring for a fuller contact with the surface and enzymes. Through the retrosynthesis analysis, a pyridine pharmaceutical intermediate was designed and synthesized. The pyridine intermediate has a similar structure with amino acid, it can be widely used in the medical field, especially as cyclic peptide intermediate fragment deacetylase inhibitors as FK228 analogues. The key intermediate fragment for the entire molecule cyclic tetrapeptide structure plays an important role in the surface recognition domain. Through continuous improvement and exploration of the conditions, we

Table 1. Equivalent of KMnO4 effect on yield.

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Table 2. Equivalent of NBS effect on yield.

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found more optimal conditions. And this has important implications for the synthesis of analogues of FK228, and the development of new drugs is also important.

ACKNOWLEDGMENTS

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REFERENCES