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Research on the synthesis and characterization of abiraterone acetate

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Abiraterone acetate is an orally administered, selective inhibitor of the 17a-hydroxylase and C17,20-lyase enzymatic activities of cytochrome P450 (CYP) 17. The convenient protocol for the synthesis of Abiraterone acetate from dehydroiso and rosterone 3-acetate via a four-step reaction includes converting Ketone carbonyl into hydrazine, an Iodine reaction, cross-coupling and zcetylation. A total conversion of 51-55% for Abiraterone acetate, was accomplished. The structure of Abiraterone acetate was characterized by ^{1}HNMR, ^{13}C NMR, IR, MS, HPLC and elemental analysis. The reaction conditions of the route was studied to reduce the cost and avoid the formation of by-products and make the route suitable for large-scale production.

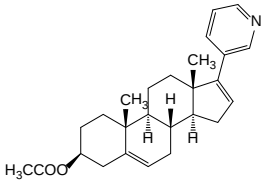
Keywords: Abiraterone acetate; synthesis ; Structure.

INTRODUCTION

Abiraterone acetate (Zytiga, chemical structure shown Fig.1) is an orally administered, selective inhibitor of the 17a-hydroxylase and C17,20-lyase enzymatic activities of cytochrome P450 (CYP) 17. CYP17 is required for androgen biosynthesis, with androgen receptor signaling crucial in the progression from primary to metastatic prostate cancer [1-4]. Previously called hormone-resistant or (hormone-refractory prostate cancer) – i.e., prostate cancer not responding to androgen deprivation or treatment with androgen receptor antagonists. In addition, on the Pharmaceuticals market the drug under the trade name Abiratas, Cadila Pharmaceuticals markets the drug as Abretone and Glenmark Pharmaceuticals as Abirapro.

Abiraterone acetate (brand names Zytiga, Abiratas, Abretone, Abirapro) is a steroidal antiandrogen, specifically an androgen synthesis inhibitor, used in combination with prednisone in metastatic castration-resistant prostate cancer (It is a product of the active agent abiraterone and is marketed by Janssen Biotech under the trade name Zytiga.

Abiraterone acetate is a CYP3A4 substrate and hence should not be administered concurrently with strong CYP3A4 inhibitors such as (ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole) or inducers such as phenytoin, carbamazepine, rifampin, rifabutin, rifapentine, phenobarbital. It also inhibits CYP1A2, CYP2C9, and CYP3A4 and likewise should not be taken concurrently with substrates of any of these enzymes that have a narrow therapeutic index.



(3S,8R,9S,10R,13S,14S)-10,13-dimethyl-17-(pyridin-3-yl)-2,3,4,7,8,9,10,11,12,13,14,15-dodecahydro-1H-cyclopentaphenanthren-3-yl acetate
Chemical Formula: $C_{26}H_{33}NO_2$
Exact Mass: 391.25
Molecular Weight: 391.55
m/z: 391.25 (100.0%), 392.25 (28.5%), 393.26 (4.3%)
Elemental Analysis: C, 79.76; H, 8.50; N, 3.58; O, 8.17

Fig. 1. Chemical structure of Abiraterone acetate.

This article is aimed at the development in the USA of Abiraterone acetate, including its pharmacological properties, although the synthesis of Abiraterone acetate has been discussed together with the problems from a different viewpoint such as the reaction rate, conversion, uncertain properties and derivate product harmful for humans or the environment.

In this research [5-9], dehydroiso and rosterone 3-acetate was used as the starting material with a specific synthetic pathway shown in Scheme 1. The final products were characterized by 1H Nuclear Magnetic Resonance (1H NMR), 13C Nuclear Magnetic Resonance (13C NMR), Infrared Spectroscopy (IR), Mass Spectroscopy (MS) and elemental analysis.

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INSTRUMENTS AND EXPERIMENTS

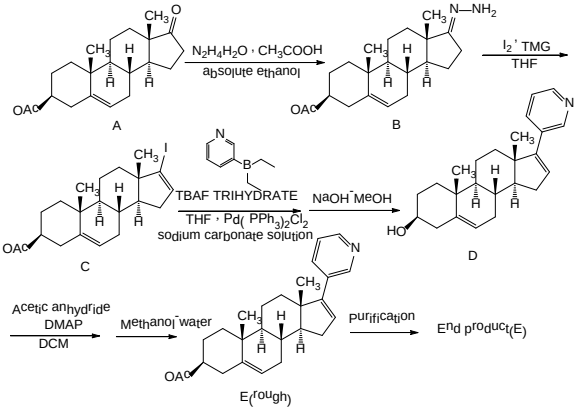
Instruments

A Nicolet 170 SX type infrared spectrometer (KBr tablet); a BRUKER AV - 500 nuclear magnetic resonance instrument (DMSO-d6+TFA-d); an AGILENT 1200 LC-MSD mass spectrometer and Elementar Vario EL Ⅲ type element analyzer were employed. Analytical reagents were used in the analysis and for synthesis.

Preparation of different compounds

Preparation of compound B

Anhydrous ethanol (50000 g, 1085.3 mol), compound A (10000 g, 30.26 mol), hydrazine hydrate (2188.75 g, 40.3 mol) and glacial acetic acid (350g, 5.80 mol) were added in the reactor. The reaction temperature was fixed at 20~30˚C, the mixture was stirred to a complete dissolution for 60 min (TLC monitoring, VEA: VHex = 2:5, ARf about 0.9, BRf about 0.15, phosphomolybdic acid-a color development reagent), with detection every 15 minutes until the raw material reacted completely.



Scheme 1. Synthesis pathway of Abiraterone acetate.

The mixture was cooled to 0~5˚C, the washing process added the DCM (dichloromethane,65000g) with precooling of the purified water (80000g) at 2~8˚C, the organic phase was dewatered by washing stratification in anhydrous sodium sulfate for about 30 min, the dewatering process at the temperature 0~10˚C and gas protection by N2. Washing the filter cake of filtration with a small amount of DCM, the merged filtrate evaporated to dryness under a reduced pressure and removed the DCM（the heated water evaporated at less than 30˚C, because B is an unstable intermediate）since the mixture was no obvious liquid in the reactor. After stirring, THF (tetrahydrofuran, 20000g) was immediately added and dissolved for 30 min (and a vacuum drained the residual gas of DCM) a faint yellow solution resulted with the mixture preserved at 0~10˚C and a gas protection by N_2 at the next step given a melting point of 204-206˚C.

Preparation of compound C

THF (40000 g, 554.7 mol) and iodine (15000 g, 59.1 mol) were added in the reactor by gas protection of N2, cooled to -5~5˚C, then slowly adding TMG (1,1,3,3-Tetramethylguanidine, 13500 g, 117.2 mol), attention must be drawn to the fact that this process was intensely exothermic and therefore the temperature was maintained under 10 ˚C. The final mixture was stirred for 10 min and then Compound B and the THF solution were added to the reactor, the temperature was maintained at 5 ~ 5˚C and added drop by drop over a period of 6h. The final drops completed the reaction for 20 minutes (the reaction was monitored by TLC ,VEA: VHex = 2:5, A Rf is about 0.15, with phosphomolybdic acid as a color development reagent). Washing filter cake by filtration with a small amount of THF, the merged filtrate evaporated to dryness under reduced pressure and removed the THF at 45~ 55 ˚C to about a 25-30 L, residue mixture after a reflux reaction for 9h. Following HPLC detection, the diiodide compound concentration was established at less than 0.1 %, evaporated to a dry state under reduced pressure with the THF removed at 45~ 55 ˚C. Until completely dissolved DCM (36 kg) was added and stirred as a mixture.

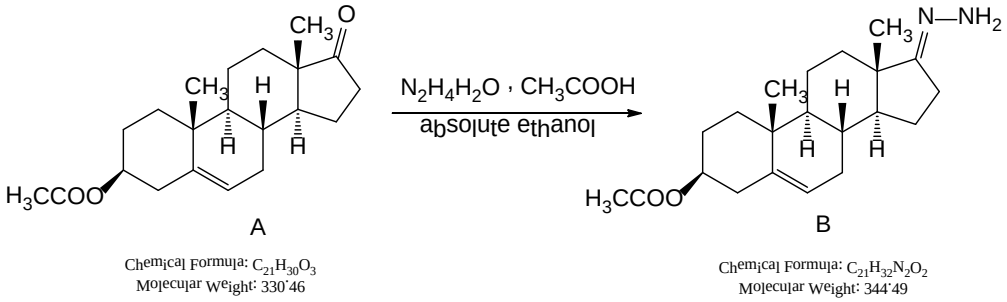
The reaction solution was poured into a precooled 0.6 M of sulfuric acid aqueous solution (50000g, 2~8˚C).

The organic layer was washed with purified water twice (40000g * 2), then dried over sodium sulfate. The solvent was concentrated under reduced pressure to 10 L. Anhydrous ethanol (40000g) was added to the mixture, concentrated under reduced pressure at 10~25 ˚C for 4 h, with precipitation into a white solid, mixing and crystallization for 1h at a controlled temperature of 0~5 ˚C. Yielding a crude compound C, with 10.5 kg of a light yellow to white powder after washing and stratification, the organic phase obtained after washing the anhydrous sodium sulfate and drying. The filter cake with a small amount of methylene chloride leaching and merging the filtrate, reduced the pressure concentration to about 50 l, adding the anhydrous ethanol (40000g) and concentrated for 4h, with a feed liquid, at the same time continued to concentrate the methylene chloride. Rejection of the filter cake after precooling the ethanol - water (V/V=5:1, 0~5˚C) beating time, with two raw intermediates, the light yellow to white powder being about 63 kg. The power was added to the

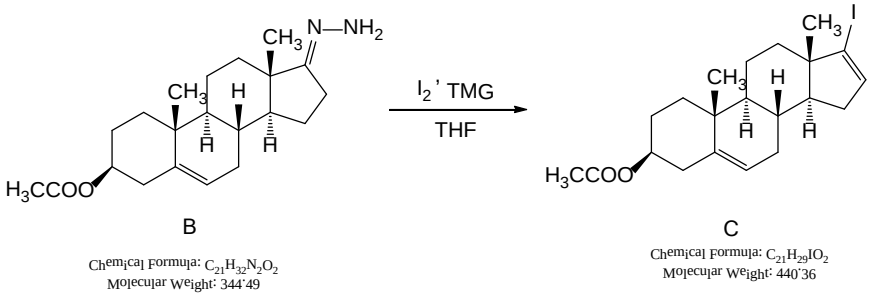
anhydrous ethanol(25000g), heated at 45~50˚C, stirred for 60 min and cooled at 0~10˚C, crystallizing after stirring for 1h, the solid obtained after filtration was air dried at 45~50˚C for 6h to obtain a white powder compound C (10000g), with dry weight loss of less than 0.5%. Step 1 and step 2 yield a total of 71 % with a range: 70% ~ 80%, at a melting point of 172-177˚C.

Preparation of compound D

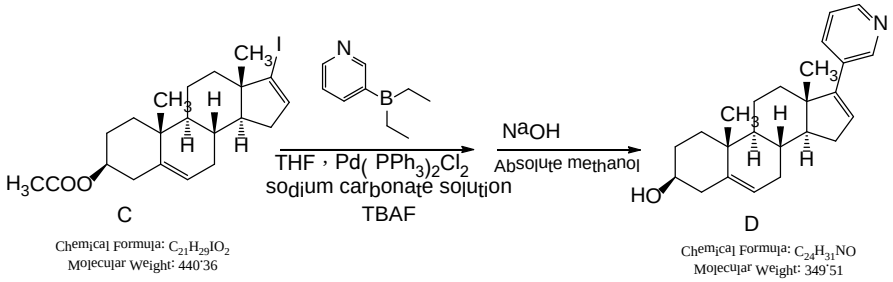
Diethyl(3 - pyridine borane)(3500 g, 3.7 mol), Pd(PPh3)2Cl2(175.4 g, 0.25 mol), TBAF (Tetrabutylammonium fluoride trihydrate, 7870, 24.94 mol) and C (10000 g, 22.71 mol) were stirred with dry THF (35200 g, 488.12 mol) in the reactor at RT(room temperature) for 20min. Then (16.7 %, 31000 g) of sodium carbonate solution were added and heated for a reflux reaction at 65 ~70˚C in a water bath, on the surface the reaction mixture become red brown for 75min.



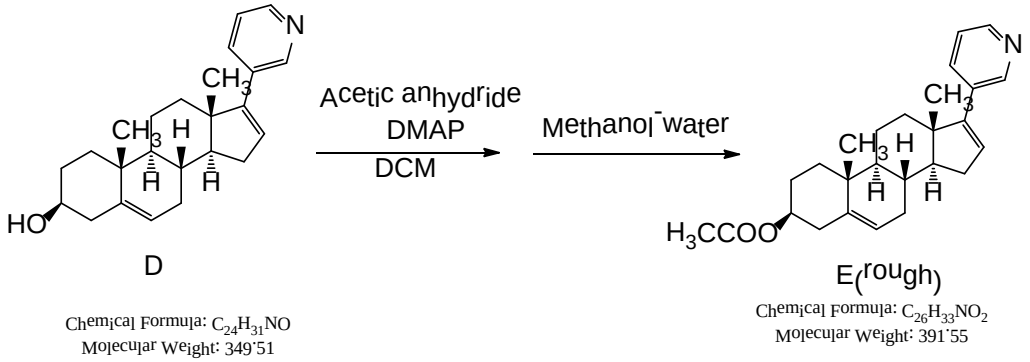
Scheme 2. Synthesis pathway of Compound B



Scheme 3. Synthesis pathway of Compound C



Scheme 4. Synthesis pathway of Compound D.



Chemical Formula: $C_{24}H_{31}NO$
Molecular Weight: 349.51

Chemical Formula: $C_{26}H_{33}NO_2$
Molecular Weight: 391.55

Scheme 5. Synthesis pathway of Compound E.

The reaction was monitored by TLC for a period of 15 minutes, (VEA: VPE = 1:8, Rf of Borane is about 0.85, Rf of C is about 0.8, phosphomolybdic acid as a color development reagent). After the complete disappearance of compound C the reaction mixture was cooled to 25～35˚C adding active carbon, then after stirring for about 30minutes the mixture was collected by filtration. The organic phase of the mixture was added, 8% of sodium hydroxide methanol solution (22000g) at 25～35˚C, the reaction was monitored by TLC over a period of 15 minutes (VEA: VHex = 1:2 and Rf of D is about 0.2, UV detected), after the complete disappearance of compound C, the reaction halts.

The reaction mixture crystalized after being stirred for 1h at 0~5˚C. Methanol (14000 g) was added to the filtration cake and stirred for 30 minutes, then purified water (17000g) was added and stirred for 30 minutes. The cake of filtration was washed by water-methanol (v/v=1,31000g). The cake of filtration was washed by precooled THF(0～5˚C), the solid obtained by filtration was force air dried at 47~52˚C for 6h to gain the white color of the crude solid compound D, the dry weight loss was less than 2%. Crude compound D was added to the THF (20000g), the mixture was stirred and refluxed for 90 minutes cooled and stirred at 0～5˚C, after crystallization for 1h the white solid compound D (5400g) was obtained, with a dry weight loss of less than 2% and a melting point of 225-230˚C.

Preparation of compound E

DCM(45000 g, 529.8 mol), compound D (5400 g, 15.45 mol), DMAP (4-Dimethylaminopyridine, 200 g, 1.63 mol) and Acetic Anhydride (2500 g, 24.29 mol) were stirred for 16h at 15～35˚C, the reaction was monitored by HPLC (compound D less than 0.2%). Activated carbon (120g) was added and stirred for 30 minutes, the liquid of filtration was then washed with saturated sodium bicarbonate water twice. The organic phase was dewatered by stratification of the anhydrous sodium sulfate after about 30 minutes and the liquid of filtration was then evaporated under a reduced pressure at 20～30˚C for a reduced volume of 50L. Methanol (18000 g) was added to the mixture at less than 15˚C, evaporated under reduced pressure for 4h, crystalizing into a white solid. At the time of adding the purified water (23000 g) the mixture was stirred crystalizing after 30 minutes. The cake of filtration was dried under reduced pressure for 6h, in order to obtain the white solid rough compound E (5100g), with a dry weight loss of less than 2%.

The solid compound E (rough, 5000g) and active carbon (90g) were added together with acetonitrile (25000g), heated up at 75~80˚C, stirred for 60 min, then cooled below 35˚C. Then cooled continuously between 0 and 5˚C, stirring and crystallizing for 1h, obtaining a solid by filtration and drying under reduced pressure at 45~50˚C for 8h to obtain the white solid compound E(4500g), mp.143-147˚C.

The HPLC detection condition for the column was C18 (4.5* 250mm, 5μm), the mobile phase consisted of 0.05mol/L ammonium acetate (pH=2.0) the acetonitrile was 5:95, the detection wavelength was 254 nm, flow rate was 1.5mL/min, the concentration of Abiraterone acetate was less than 99.4%, HPLC spectrogram is shown in Fig.2.

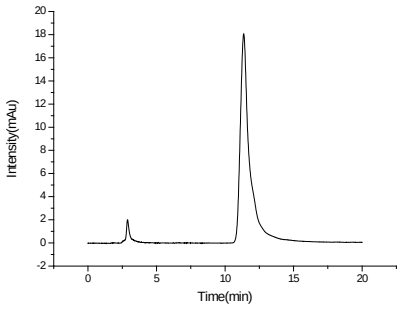


Fig. 2. HPLC spectrogram of Abiraterone acetate.

Compound E (Abiraterone acetate**)** ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.61 (d, *J* = 1.5 Hz, 1H), 8.49 – 8.41 (m, 1H), 7.66 – 7.61 (m, 1H), 7.21

(dd, *J* = 7.9, 4.8 Hz, 1H), 6.00 – 5.97 (m, 1H), 5.41 (d, *J* = 5.0 Hz, 1H), 4.66 – 4.56 (m, 1H), 2.33 (dd, *J* = 9.3, 3.6 Hz, 2H), 2.03 (s, 6H), 1.86 (dd, *J* = 15.4, 5.2 Hz, 2H), 1.80 – 1.70 (m, 2H), 1.63 – 1.53 (m, 3H), 1.08 (s, 3H), 1.04 (s, 3H).

ESI-MS m/z:392.22$[M+H]^+$.

RESULTS AND DISCUSSIONS

In this research, dehydroiso and rosterone 3-acetate as the starting material, reacted with hydrazine hydrate, iodine and Diethyl (3 - pyridine borane). Our attempt to synthesize compound B, was shown in Scheme 2. The Molar ratio of dehydroiso and rosterone-3-acetate and hydrazine hydrate is from 1 to 2, the best molar ratio was 1.42-1.50. The solvent of choice was absolute ethanol its weight ratio was 5, at the same time CH_3COOH was added, at a molar ratio of 0.18. The process for compound C as shown in Scheme 3, molar ratio compound B, iodine and TMG was 1.95 and 3.80, yielding about 75%. The route to Abiraterone acetate commercially available, was obtained for a 52% total yield.

CONCLUSION

The convenient protocol for the synthesis of Abiraterone acetate from dehydroiso and rosterone 3-acetate via a four-step reaction including Ketone carbonyl into hydrazine, an Iodine reaction, cross-coupling and zcetylation has been presented. High purity Abiraterone acetate, can be obtained after purification of the crude product. The total conversion of Abiraterone acetate was 52%. The reaction conditions of the route were studied in order to reduce the cost and avoid the formation of by-products and make the route suitable for large-scale production.

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ИЗСЛЕДВАНИЯ И ОХАРАКТЕРИЗИРАНЕ НА АБИРАТЕРОН-АЦЕТАТ

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(Резюме)

Абиратерон-ацетатът е селективен инхибитор на ензимната активност на 17а-хидролазата и С17,20-лиазата в цитохром Р450 (CYP) за орална употреба. Подходящият протокол за четири-етапната синтеза на абиратерон-ацетата от дехидро-изо- и ростерон-3-ацетат включва превръщането на кетонова карбонилна група в хидразин, реакция с йод, купелуване и ацетилиране. Постигнато е превръщане от 51-55% по отношение на абиратерон-ацетата. Структурата на абиратерон-ацетата е охарактеризирана чрез ^{1}Н ЯМР, ^{13}С ЯМР, ИЧ-, MS-спектроскопия, високо-ефективна течна хроматография и елементен анализ. Условията на реакцията по описания маршрут са изследвани с цел да се намалят разходите и да се избегне образуването на странични продукти за постигането на по-едромащабно производство.