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Research Paper

Identification, Synthesis and Characterization of Related Substances of Aliskiren Hemifumarate, an Antihypertensive Drug

N. Uday Kumar^{a*}, N. M. Sekhar^a, G. Srinivas^a, D. Kiran Kumar^a, A. Roopa^a, V. Prabhakar Reddy^b, Rakeshwar Bandichhor^a.

^aDepartment of Research and Development, Integrated Product Development, Innovation Plaza, Dr. Reddy's Laboratories Ltd., Survey Nos. 42, 45, 46 & 54, Bachupally, Qutubullapur, R. R. Dist-500 072, A.P., India.

^bDepartment of chemistry, Osmania University, Hyderabad, A.P., India.

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Abstract: Aliskiren Hemifumarate is the first in a new class of orally effective, nonpeptide direct renin inhibitors developed for the treatment of hypertension. It is approved by the U.S. Food and Drug Administration in 2007 for the treatment of hypertension and related cardiovascular diseases.^[1-2] During the synthesis of Aliskiren Hemifumarate, we have observed eleven related substances, namely lactone amine, schiff base, ether cleavage related substances-1, 2, 3, enantiomer, diastereomers-1, monomethyl, N-alkyl, N-methyl and dihydroxy aliskiren. The present work describes the synthesis, isolation and characterization of these related substances (impurities).

Introduction

Aliskiren is used as Hemifumarate salt and has molecular formula $C_{30}H_{53}N_3O_6 \cdot 0.5 C_4H_4O_4$ and molecular weight 609.8 (free base-551.8). The commonly used dose of Aliskiren Hemifumarate is 150 mg per day.

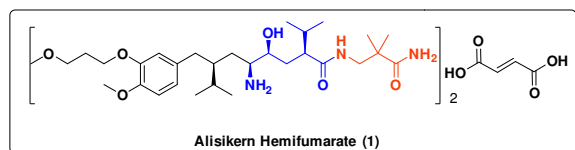


Figure 1. Structure of Aliskiren Hemifumarate.

Corresponding Author*

E-mail: udaykumarn@drreddys.com

The presence of related substances in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products. Therefore, it is necessary to study the impurity profile of the API to be used in the manufacturing of a drug product. International conference on Harmonization (ICH) guidelines recommends identifying and characterizing all related substances that are present at a level of 0.10% (based on the daily dose).^[3] In this context, we have under taken a comprehensive study to synthesize and characterize the related substances in Aliskiren Hemifumarate API. These related substances are

- (2S,4S,5S,7S)-N-(3-amino-2-methyl-3-oxopropyl)-5-azido-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methylnonanamide fumarate (Monomethyl Aliskiren **7**).
- (2R,4S,5S,7S)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methylnonanamide (Diastereomer **8**).
- (2S,4S,5S,7S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4,5-dihydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methylnonanamide (Dihydroxy **9**).
- (2S,4S,5S,7S)-5-amino-N-(3-((3-amino-2,2-dimethyl-3-oxopropyl)amino)-2,2-dimethyl-3-oxopropyl)-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methylnonanamide (N-alkylated **10**).
- (2S,4S,5S,7S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methyl-5-(propan-2-ylideneamino)nonanamide (schiff's base **11**).
- (2R,4R,5R,7R)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methylnonanamide (Enantiomer **12**).
- (2S,4S,5S,7S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-2-isopropyl-7-(4-methoxy-3-(3-methoxypropoxy)benzyl)-8-methyl-5-(methylamino)nonanamide (N-methylated **13**).
- (2S,4S,5S,7S)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-7-(3-hydroxy-4-methoxybenzyl)-2-isopropyl-8-methylnonanamide (metabolite-**14**).
- (2S,4S,5S,7S)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-7-(3-(3-hydroxypropoxy)-4-methoxybenzyl)-

2-isopropyl-8-methylnonanamide (metabolite-**15**).

- (2S,4S,5S,7S)-5-amino-N-(3-amino-2,2-dimethyl-3-oxopropyl)-4-hydroxy-7-(4-hydroxy-3-(3-methoxypropoxy)benzyl)-2-isopropyl-8-methylnonanamide (metabolite-**16**).

- (3S,5S)-5-((1S,3S)-1-amino-3-(4-methoxy-3-(3-methoxypropoxy)benzyl)-4-methylpentyl)-3-isopropylidihydrofuran-2(3H)-one (metabolite-**17**).

Analytical methods have been reported for the determination of metabolites of Aliskiren Hemifumarate in biological fluids and pharmaceutical formulations.^[4] However, a detailed synthetic procedure is not reported for metabolites and other related substances. Best of our Knowledge a detailed impurity profile study is not yet cited anywhere, except metabolites and monomethyl Aliskiren.^[4,5]

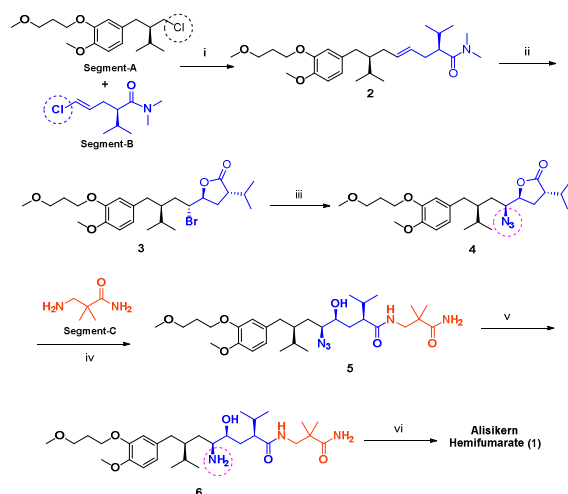


Figure 2. Synthesis of Aliskiren Hemifumarate **1**. Reagents and conditions: (i) (a) for RMgX preparation: Mg, THF, 1,2-dibromomethane, 60-65 °C, (b) for coupling: Fe(acac)₃, NMP, 0-5 °C, 10% HCl solution, toluene, 72 %; (ii) NBS, H₃PO₄, THF, 0-5 °C, 5% NaHSO₃, ethyl acetate, 5% NaHCO₃, DM water, sat. NaCl solution, 72 %; (iii) NaN₃, tripropylene

glycol (TPG), DM water, 80-85 °C, dimethylpropyl amine, Toluene, 52 %; (iv) **Segment-C**, TEA, 85-90 °C, ethyl acetate, DM water, sat NaCl solution, 90%; (v) 10 % Pd/C, methanol, methanolic NH₃, 5-6 Kg/cm² H₂ gas pressure, 25-35 °C, 90 %; (vi) fumaric acid, methanol, acetonitrile, 25-35 °C, 90 %.

Results and Discussion

Aliskiren Hemifumarate **1** has been synthesized as per **Fig 2**. with slight modification of reported synthesis.^[6] Coupling of **Segment-A** Grignard reagent with **Segment-B** using catalytic amount of Fe(acac)₃, NMP to afford **2** in 72% yield. Streoselective bromo lactonization of **2** was carried out using NBS, H₃PO₄ in THF solvent to gives **3** in 72% yield. Nucleophilic substitution of bromo with azide of **3** in S_N2 manner using sodium azide in presence of TPG to afford **4** in 52% yield. Condensation of **Segment-C** (3-amino-2,2-dimethylpropanamide) and **4** in presence of 2-hydroxy pyridine and TEA gives open azido Aliskiren **5** in 90% yield. Reduction of **5** using 5% Pd/C in presence of MeOH.NH₃ and MeOH gives Aliskiren Free base **6** in 90% yield. Final step is salt formation of **6** with fumaric acid in presence of MeOH:CH₃CN to afford Aliskiren Hemifumarate **1** in 90% yield.

Monomethyl Aliskiren 7

Monomethyl Aliskiren **7**, forms due to the presence of 3-amino-2-methylpropanamide **20** in the **Segment-C** and carry through the synthetic process to give this related substance in the final product. Monomethyl Aliskiren is prepared starting with 2-cyanoacetamide **18** according to **Fig. 3**. Methylation of **18** upon reaction with DMS in presence of K₂CO₃ in toluene solvents to afford 2-methyl cyano acetamide **19** in 78%

yield, which subjected for catalytic hydrogenation with Raney Ni in methanol to afford **20** in 95% yield. This **20** on lactone aminolysis with **5** in presence of 2-hydroxy pyridine and TEA afforded **21** in 80% yield, which on catalytic hydrogenation with Pd/C in methanol, followed by salt formation with fumaric acid in presence of CH₃CN:MeOH solvents to afford **7** in 90% yield. The electro spray ionization (ESI) mass spectrum of **7** displayed peaks at *m/z* 538.0 [(M+H)⁺] in positive ion mode and as sodium adduct at *m/z* 560.0 [(M+H)+Na] and LC-MS-MS displayed peaks at *m/z* 538.3 and daughter ion peaks at *m/z* 520.5, 503.5, 486.1, 436.7, 422.4, 404.3 and 117.20. The *m/z* 117.20 represent there was only one methyl group in the **Segment-C**. In ¹H NMR spectrum of compound **7**, corresponding doublet peak of mono-methyl at δ 1.15 ppm is present. The DEPT spectra displayed 7 negative signals due to seven methylene groups and 18 positive peaks corresponding to six methyl groups and twelve methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3350 cm⁻¹ (NH & OH stretching) and 1663 cm⁻¹ (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ¹³C NMR spectrum. The assigned structure for compound **7** is clearly confirmed by the spectral data.

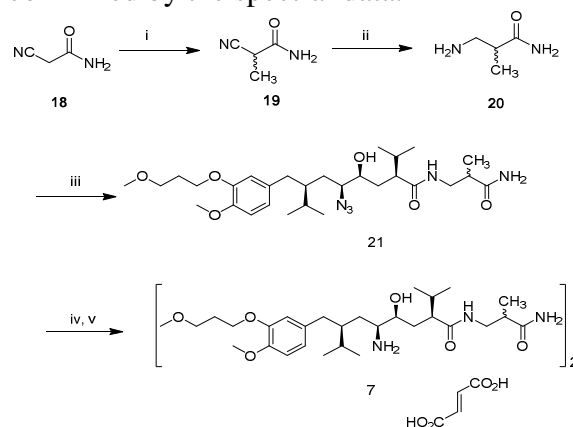


Figure 3. Synthesis of compound **7**.

Reagents and conditions: (i) K_2CO_3 , dimethyl sulfide (DMS), toluene, 55-60 °C, 7-8 h, 78 %; (ii) 100 % Raney Ni, 10 % MeOH.NH₃, MeOH, 6-7 Kg/cm² H₂, 55-60°C, 95%; (iii) **4**, 2-OH pyridine, TEA, 85-90°C, 14-16 h, 80 %, (iv) 10 % Pd/C, 10 % MeOH.NH₃, MeOH, 5-6 Kg/cm² H₂, 25-35 °C, 90%; (v) fumaric acid, MeOH, acetonitrile 25-35 °C, 90 %.

Diastereomer **8**

This related substance **8** originates while lactone aminolysis of compound **4** with **Segment-C**, and **8** have different configuration (*R*) for isopropyl group attached to amide functionality. This may be due to lactone aminolysis is a slow chemical conversion step, since during reaction there may be chances for the racemization of isopropyl group present in the lactone **4**. Hence, we enriched the precursor **22** impurity by maintaining the reaction at 100 °C with prolonged maintenance. The compound **22** was subjected for hydrogenation in presence of Pd/C in MeOH.NH₃ and MeOH to afford the corresponding diastereomer **8** in quantitative yield. Then compound **8** isolated by reverse phase preparative HPLC. The ESI mass spectrum of compound **8** displayed a signal at *m/z* 552.4 [(M+H)⁺] in positive ion mode and as sodium ion adduct at *m/z* 574.3 [(M+H)⁺+Na] and HRMS also supported the elemental count. ¹H NMR spectrum of compound **8** showed difference between the isopropyl group corresponding CH₃ peaks with respect to CH₃ peaks of Aliskiren free base. The DEPT spectra displayed 7 negative signals due to seven methylene groups and 17 positive peaks corresponding to six methyl groups and eleven methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3349 cm⁻¹ (NH & OH stretching) and 1662 cm⁻¹ (lactone

C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ¹³C NMR spectrum. The assigned structure for compound **8** is clearly confirmed by the spectral data.

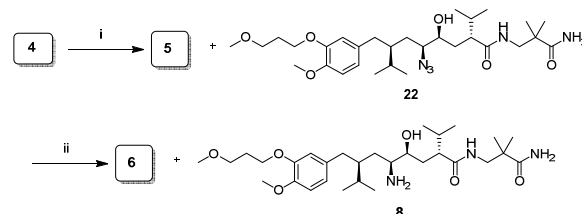


Figure 4. Synthesis of compound **8**. Reagents and conditions: (i) **Segment-C**, TEA, 85-90 °C; (ii) 10 % Pd-C, MeOH, 10% MeOH.NH₃, 5-6% H₂, 25-35 °C.

Dihydroxy Aliksiren **9**

Dihydroxy **9** is a process related substance, forms due to the presence of hydroxyl lactone **23**, while lactone aminolysis with **Segment-C**. Formation of **23** was identified during the bromolactonization of ene derivative **2**. The synthetic sequence for the preparation of this related substance **9** is depicted in **Fig.5**. Oxidative cyclization of bromo derivative **3** using hydrogen peroxide in presence of THF: H₂O to afford **23** in 75.8% yield, which subjected for lactone aminolysis with **Segment-C** to afford desired compound **9** in 87% yield. The ESI mass spectrum of impurity **9** displayed a peak at *m/z* 438 [(M+H)⁺] in positive ion mode and as sodium ion adduct at *m/z* 451 [(M+H)+Na]. In ¹H NMR spectrum of compound **9**, corresponding peaks of amine at δ 7.17, 6.58 were absent. The DEPT spectra displayed 7 negative signals due to seven methylene groups and 17 positive peaks corresponding to six methyl groups and eleven methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic

absorptions at 3413 cm^{-1} (NH & OH stretching) and 1664 cm^{-1} (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. The assigned structure for compound **9** is clearly confirmed by the spectral data.

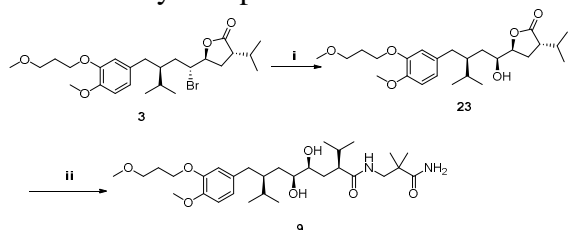


Figure 5. Synthesis of compound **9**. Reagents and conditions: (i) THF: Water, H_2O_2 , 1N HCl, 0-5°C, 75.8%; (ii) Segment-C, TEA, 85-90 °C, 87%.

N-alkylated Aliskiren **10**

N-alkylated Aliskiren **10** is a process related substance. During the lactone aminolysis of **4** with Segment-C, formation of compound **10** was observed and percentage of formation was increased with reaction time. This compound **10** was identified through the LC-MS-MS fragmentation pattern, which differs from the Aliskiren fragmentation, in which, a peak at m/z 117 is appeared for Aliskiren, which correspond to Segment-C fragment, where as for substance **10** signal appeared at m/z 215, which corresponding to dimer of Segment-C. N-alkylated **10** synthesized as depicted in the below Fig. 6., in which hydrolysis of cyano-ester **24** in presence of LiOH in MeOH: H_2O to afford acid **25** in 80% yield, which upon coupling with Segment-C using CDI in CH_3CN to afford cyano-dimer **26** in 53.5% yield. Compound **26** subjected for hydrogenation with Raney nickel in MeOH to afford segment-C dimer **27** in 95% yield. Compound **27** was subjected for lactone aminolysis with **4** in presence of 2-hydroxy pyridine and TEA to afford **27** in 80% yield.

Compound **28** was subjected for hydrogenation in presence of Pd/C in methanol and 10 % MeOH.NH₃ to afford **10** in 90% yield. The ESI mass spectrum of **10** displayed a peak at m/z 651 [(M+H)⁺] in positive ion mode and as sodium ion adduct at m/z 673 [(M+H)+Na].

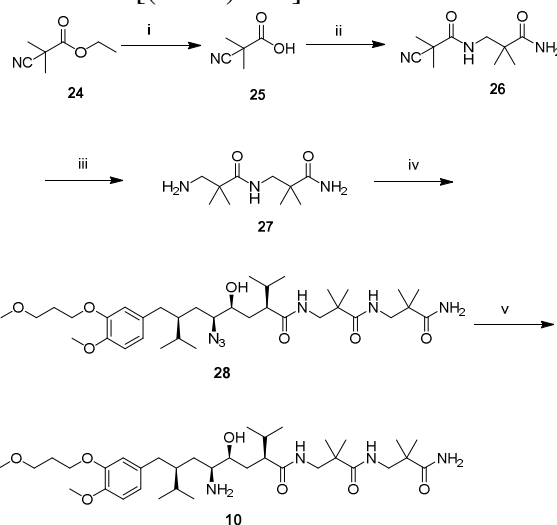


Figure 6. Synthesis of compound **10**. Reagents and conditions: (i) MeOH:Water, LiOH.H₂O, 25-35 °C, 80 %; (ii) Segment-C, Carbonyldiimidazole (CDI), acetonitrile, 0-5 to 25-35 °C, 53.5%; (iii) 100 % Raney Ni, 10 % MeOH.NH₃, MeOH, 6-7 Kg/cm² H₂, 55-60°C, 95%; (iv) **4**, 2-OH pyridine, TEA, 85-90°C, 14-16 h, 80 %, (v) 10 % Pd/C, 10 % MeOH.NH₃, MeOH, 5-6 Kg/cm² H₂, 25-35 °C, 90%.

In ^1H NMR spectrum of compound **10** showed additional signals at δ 1.03 along with 1.06 provides the information that presence of extra di-methyl groups of corresponding segment-C dimer and presence of triplet at δ 7.73 indicates the extra amide bond attached to two hydrogen containing carbon atom. The DEPT spectra displayed 8 negative signals due to eight methylene groups and 19 positive peaks corresponding to eight methyl groups and eleven methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3351

cm⁻¹ (NH & OH stretching) and 1651 cm⁻¹ (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ¹³C NMR spectrum. The assigned structure for compound **10** is clearly confirmed by the spectral data.

Schiff's base 11

Schiff's base **11** is not a process related substance and it is acetone solvent contaminated impurity. Because, Aliskiren is having high reactivity towards carbonyl compounds particularly with acetone, even traces level of acetone can generate the **11**. Schiff's base prepared by refluxing the compound **6** in acetone solvent in 89.5% yield. The ESI mass spectrum of impurity **11** displayed a peak at m/z 592 [(M+H)⁺] in positive ion mode and as sodium ion adduct at m/z 614 [(M+H)⁺Na]. In ¹H NMR spectrum of compound **11** additional signals at δ 1.10 and 1.23 indicate the presence of two methyl group in the compound. The DEPT spectra displayed 7 negative signals due to seven methylene groups and 19 positive peaks corresponding to eight methyl groups and eleven methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3439 cm⁻¹ (NH & OH stretching) and 1668 cm⁻¹ (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ¹³C NMR spectrum. The assigned structure for compound **11** is clearly confirmed by the spectral data.

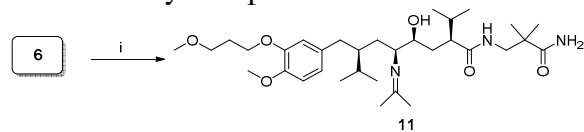


Figure 7. Synthesis of compound **11**. Reagents and conditions: (i) acetone, 35-40 °C, 89.5%.

Enantiomer 12

In order to synthesis the enantiomer of Aliskiren the following strategy is adopted.

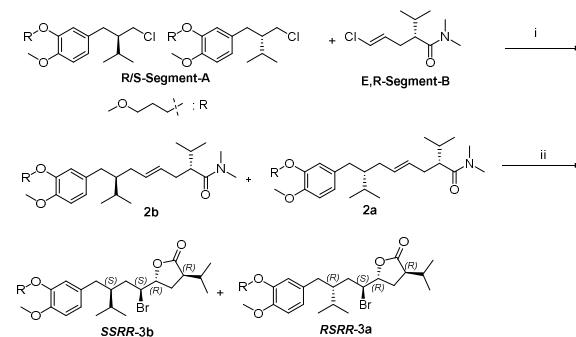


Figure 8: Synthesis of Chirally Pure bromolactonization compounds (**RSRR-3a**): (i) (a) for RMgX preparation: Mg, THF, 1,2-dibromomethane, 60-65 °C, (b) for coupling: Fe(acac)₃, NMP, 0-5 °C (c) for work up: 10% HCl solution, toluene; (ii) NBS, H₃PO₄, THF, 0-5 °C, 5% NaHSO₃, ethyl acetate, 5% NaHCO₃, DM water, sat. NaCl solution.

As shown in the **Fig. 8.**, coupled racemic mixture of (*R/S*)-Segment-A with (*E, R*)-Segment-B (enantiomer of Segment-B) to get the mixture of cross coupled product **2a**. After that the **2a** compound is subjected for bromolactonization reaction in presence of NBS and orthophosphoric acid and obtained the mixture of bromolactonization **RSRR-3a** & **SSRR-3a** compounds. This mixture subjected for silica gel column purification, but partial enrichment of diastereo selectively was observed for **RSRR-3a** & **SSRR-3a** compounds. Afterwards the partially enriched **RSRR-3a** & **SSRR-3a** mixture was used for the synthesis of Aliskiren enantiomer **12** as depicted in the below **Fig. 9**. Thereafter compound **12**, which contain mixture of *enat-1* and *dia-1* in the ratio of 77.14:14.10, was purified by reverse phase preparative HPLC. The electro spray ionization (ESI) mass spectrum of **12**

displayed signal at m/z 552.0 $[(M+H)^+]$. The DEPT spectra displayed 7 negative signals due to seven methylene groups and 17 positive peaks corresponding to six methyl groups and eleven methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3413 cm^{-1} (NH & OH stretching) and 1667 cm^{-1} (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. The assigned structure for compound **12** is clearly confirmed by the spectral data, Chiral HPLC and SOR.

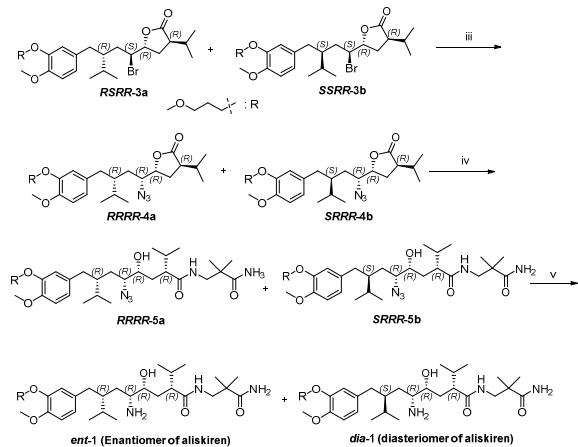
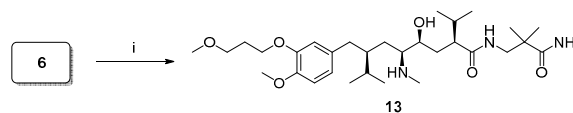


Figure 9: Synthesis of enantiomer of Aliskiren (*ent-1*): (iii) NaN_3 , TPG, DM water, $80\text{--}85\text{ }^\circ\text{C}$, dimethylpropyl amine, ethyl acetate; (iv) **Segment-C**, TEA, $85\text{--}90\text{ }^\circ\text{C}$, Ethyl acetate, Dm water, sat NaCl solution; (v) 10% Pd/C, MTBE, ethanolamine, $5\text{--}6\text{ kg/cm}^2$ H_2 gas pressure.

N-methyl Aliskiren **13**

This N-Methyl Aliskiren **13** is a process related substance. This was identified through the LC-MS-MS fragmentation pattern, which differs from the Aliskiren fragmentation, in which signal at m/z 436 appeared for Aliskiren, which correspond to lactone amine, where as for substance **13**,

signal appeared at m/z 450, which corresponding to lactone amine derivative with extra methyl group. Formation of **13** was observed during the hydrogenation of azide intermediate **4** in presence of Pd/C, MeOH.NH_3 and MeOH. Hence, reaction medium was MeOH, there may be possibility for formation of N-methyl impurity by Palladium mediated N-methylation. Compound **13** was synthesized by reaction of Aliskiren freebase **6** with benzaldehyde in presence of CH_3CN to afford imine which upon reaction with DMS afforded desired compound in quantitative yield as depicted in **Fig.10**. Crude **13** was isolated by reverse phase preparative HPLC. The electro spray ionization (ESI) mass spectrum of **29** displayed signal at m/z 565.0 $[(M+H)^+]$ in positive ion mode and as sodium adduct at m/z 588.0 $[(M+H)^+\text{Na}]$. The DEPT spectra displayed 6 negative signals due to seven methylene groups and 18 positive peaks corresponding to seven methyl groups and eleven methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3412 cm^{-1} (NH & OH stretching) and 1666 cm^{-1} (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. The assigned structure for compound **13** is clearly confirmed by the spectral data.



Scheme 10: Synthesis of N-methyl Aliskiren (**13**). Reagents and conditions: (i) PhCHO , CH_3CN , DMS, $25\text{--}35\text{ }^\circ\text{C}$ to $50\text{--}55\text{ }^\circ\text{C}$.

Metabolite-14, 15 and 16

Formation of metabolite precursors for

Metabolite-**14**, **15** and **16** were identified as ether cleavage impurities of **Segment-A**, while synthesis **Segment-A** Grignard reagent, which carry through the synthetic process to give the corresponding impurities in the final product.

Metabolite-14

This metabolite-**14** is a process related substance. Metabolite-14 was prepared starting with Aliskiren freebase **6** according to **Fig. 11**. Selective ether cleavage of **6** using 1M BBr solution in CH₂Cl₂ yielded metabolite-**14** in quantitative yield, which was isolated by reverse phase preparative HPLC. The ESI mass spectrum exhibited a molecular ion at *m/z*, 480 and 502 corresponding to the adduct ions [(M+H)⁺] and [(M+H)⁺+Na], respectively in positive ion mode, indicating molecular weight of 479, which was lesser by 72 amu than that of Aliskiren Hemifumarate. The ES-MS-MS spectrum displayed daughter ions *m/z* at 462, 117, 364, 346, and 137. The fragmentation pattern is similar to the fragmentation pattern of Aliskiren Hemifumarate, which is indicating that the structure of metabolite 1 is similar to the structure of Aliskiren Hemifumarate. The ¹H NMR spectrum showed an absence of signal at δ 3.94, 3.48, 3.24, 1.95, ppm indicating the absence of methoxy propyl side chain in the metabolite. The ¹³C NMR also supported the absence of carbon peaks at δ 65.3, 29.1, 68.6, 52.9 ppm. The DEPT spectra displayed 4 negative signals due to four methylene groups and 16 positive peaks corresponding to seven methyl groups and nine methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3362 cm⁻¹ (NH & OH stretching) and 1673 cm⁻¹ (amide C=O stretching), the C=O stretching

being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ¹³C NMR spectrum. The assigned structure for compound **14** is clearly confirmed by the spectral data.

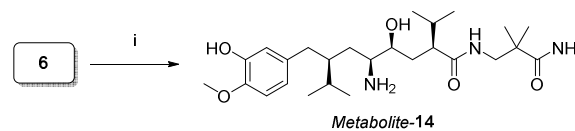


Figure 11: synthesis of metabolite **14**: (i) CH₂Cl₂, 1M BBr solution, -10 to -5°C. 10% Methanolic NH₃ solution, 5% Na₂CO₃ solution, ethyl acetate 31.0% yield, (purity by HPLC before preparative 30.42%, after preparative 87.68 %).

Metabolite-15

This related substance **15** is a process related substance. Selective ether cleavage of **6** using AlCl₃, ethanethiol in CH₂Cl₂ yielded metabolite-**15** in quantitative yield, which was isolated by reverse phase preparative HPLC. The ESI mass spectrum exhibited a molecular ion at *m/z*, 538 and 560 corresponding to the adduct ions [(M+H)⁺], [(M+H)⁺+Na] respectively in positive ion mode, indicating molecular weight of 537, which was lesser by 14 amu than that of Aliskiren Hemifumarate. The ES-MS-MS spectrum displayed daughter ions *m/z* at 520, 117, 422, 404 and 195. The fragmentation pattern is similar to the fragmentation pattern of Aliskiren Hemifumarate (Fig. 5), which is indicating that the structure of metabolite 2 is similar to the structure of Aliskiren Hemifumarate. The ¹H NMR spectrum (DMSO-*d*₆) showed an absence of signal at δ 3.24, ppm indicating the absence of methoxy group in the metabolite. The ¹³C NMR also supported the absence of carbon peaks at δ 68.6 ppm. The DEPT spectra

displayed 7 negative signals due to seven methylene groups and 16 positive peaks corresponding to seven methyl groups and nine methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3414 cm^{-1} (NH & OH stretching) and 1669 cm^{-1} (amide C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. The assigned structure for compound **15** is clearly confirmed by the spectral data.

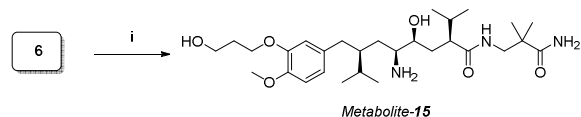


Figure 12: synthesis of metabolite **15**: (i) AlCl_3 , CH_2Cl_2 , ethanethiol, 0-5°C. 10% Na_2CO_3 solution, ethyl acetate, yield 25.0%, purity by HPLC 77.06%.

Metabolite-16

This related substance **16** is a process related substance. Selective ether cleavage of **6** using NaI in $\text{CH}_3\text{CN}:\text{CH}_2\text{Cl}_2$ yielded metabolite-**16** in quantitative yield, which was isolated by reverse phase preparative HPLC. The ESI mass spectrum exhibited a molecular ion at m/z , 538 and 560 corresponding to the adduct ions $[(\text{M}+\text{H})^+]$, $[(\text{M}+\text{H})^++\text{Na}]$ respectively in positive ion mode, indicating molecular weight of 537, which was lesser by 14 amu than that of Aliskiren Hemifumarate. The ES-MS-MS spectrum displayed daughter ions m/z at 520, 117, 422, 404 and 195. The fragmentation pattern is similar to the fragmentation pattern of Aliskiren Hemifumarate (Fig. 5), which is indicating that the structure of metabolite 3 is similar to the structure of Aliskiren Hemifumarate. The ^1H NMR spectrum ($\text{DMSO}-d_6$)

showed an absence of signal at δ 3.71 ppm indicating the absence of methoxy group in the metabolite. The ^{13}C NMR also supported the absence of carbon peaks at δ 55.5 ppm. The DEPT spectra displayed 7 negative signals due to seven methylene groups and 16 positive peaks corresponding to seven methyl groups and nine methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3359 cm^{-1} (NH & OH stretching) and 1660 cm^{-1} (amide C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. The assigned structure for compound **16** is clearly confirmed by the spectral data.

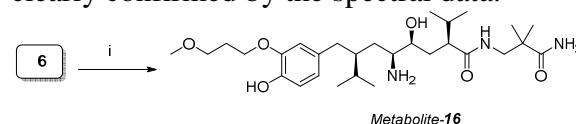


Figure 13: synthesis of metabolite **16**: (i) NaI, $\text{CH}_3\text{CN}:\text{CH}_2\text{Cl}_2$ (1:1), SiCl_4 25-35°C, 10% Na_2CO_3 solution, ethyl acetate 30.0% yield, purity by HPLC before preparative 29.81% and after preparative 94.02%.

Metabolite-17

This related substance **17**, a carryover related substance, is a result of presence of **4** in the intermediate **5** and carry through the synthetic process to give this impurity in the final product. **17** was prepared starting with **4** according to Fig. 14. Catalytic hydrogenation of **4** with 5% Pd/C in THF, TEA afforded **17** in 88% yield. The ESI mass spectrum exhibited a molecular ion at m/z 436 $[(\text{M}+\text{H})^+]$ in positive ion mode, indicating molecular weight of 435, which was lesser by 116 amu than that of Aliskiren Hemifumarate. The ES-MS-MS spectrum displayed daughter ions m/z at 416 and 209. The fragmentation

pattern is similar to the fragmentation pattern of Aliskiren Hemifumarate (Fig. 5), which is indicating that the structure of metabolite 4 is similar to the structure of Aliskiren Hemifumarate. The ^1H NMR spectrum clearly shows the absence of δ 1.04 and 1.04 ppm indicating the absence of dimethyl groups of synthon-C moiety. The ^{13}C NMR also supported the absence of carbon peaks at δ 23.4 and 23.5 ppm. The DEPT spectra displayed 6 negative signals due to six methylene groups and 17 positive peaks corresponding to six methyl groups and ten methine groups (three in aromatic and rest in aliphatic region). IR spectrum displayed characteristic absorptions at 3388 cm^{-1} (NH & OH stretching) and 1766 cm^{-1} (lactone C=O stretching), the C=O stretching being supported by the appearance of quaternary carbon signals characteristic of a carbonyl carbon in ^{13}C NMR spectrum. The assigned structure for compound **17** is clearly confirmed by the spectral data.

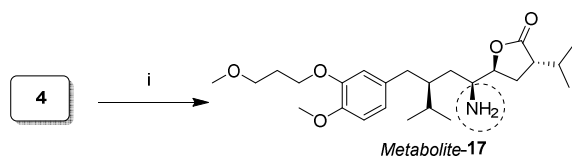


Figure 14: synthesis of metabolite 17: (i) THF, triethyl amine, 5% Pd-C 25-35°C, 5-6 kg/cm² H₂ gas pressure, yield 88%, purity by HPLC 90 %.

Conclusion

Information about the different possible related substances and their synthetic routes is a prerequisite for a thorough understanding of the impurity formation pathway of the antihypertensive drug

Aliskiren Hemifumarate. Keeping in view this regulatory importance of Aliskiren Hemifumarate related substances, the process-related related substances and metabolites in synthesis of Aliskiren Hemifumarate were identified synthesized and characterized using mass, HRMS, IR, and NMR techniques.

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Supplementary Material

Experimental procedures and compound characterization data are described in supplementary material.

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