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Identification and Synthesis of Impurities Formed During Preparation of Azelnidipine

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Azelnidipine (**1**), dihydropyridine-based calcium antagonists (DHPs) are one of the widely used drugs for treatment of hypertension. During the laboratory optimization and later during its bulk synthesis the formation of various impurities was observed. The impurities formed were monitored and their structures were tentatively assigned on the basis of their fragmentation patterns in LC-MS. Most of the impurities were synthesized and their assigned constitutions confirmed by co-injection in HPLC. We describe herein the formation, synthesis and characterization of these impurities. Our study will be of immense help to others to obtain chemically high pure azelnidipine.

Keywords: Impurity profile, Related substances, Dihydropyridine, Azelnidipine.

INTRODUCTION

The safety of a drug product is not only dependent on the toxicological properties of the active drug substance (or active pharmaceutical ingredient, API), but also on the impurities formed during the various chemical transformations. Therefore, identification, quantification and control of impurities in the drug substance and drug product are important parts of drug development for obtaining marketing approval. It is more challenging for an organic chemist to identify the impurities which are formed in small quantities in a drug substance. Since most of the time it is very difficult to identify and control impurities within acceptable levels in the process, extra purification steps may then be necessary thereby making the process less competitive. More often the syntheses of impurities are not described in the literature which makes it even more difficult for the organic chemist who must then design a synthesis, which is time consuming. The development of a drug substance is incomplete without the identification of an impurity profile involved in the process. Furthermore, it is not mandatory to design synthetic routes for the impurities. Thus, in our study we explored the formation, identification, synthesis and characterization of impurities found in the preparation of azelnidipine. This study will be of immense help for organic chemists to understand the potential impurities in azelnidipine synthesis and thereby obtain the pure compound.

Azelnidipine (**1**) (Fig. 1), is a dihydropyridine calcium channel blocker. It is sold in Japan by Daiichi-Sankyo pharmaceuticals, Inc. Unlike nicardipine, it has a gradual onset and has a long-lasting hypotensive effect, with little increase in heart rate.

1,4-Dihydropyridine calcium antagonists, are clinically very useful antihypertensive and many other analogs are also under development. Some of them are designed so as to provide an additional action favorable for lowering the blood pressure, such as antithrombotic[1,2], α-adrenolytic[3] and organic nitrile vasodilating effects[4]. The vasodilating effects of alkyl nitrite particularly attracted our interest because its blood pressure controlling action seemed to be more direct. Therefore, the introduction of a nitroxy group into the ester side chain of the DHP ring was examined. Few 2-amino-1,4-dihydropyridine derivatives have been reported because of the difficulty in synthesizing the precursors, amidinoacetates[5,6]. The benzylideneacetoacetates, the other important synthetic intermediates, were obtained through the Knoevenagel reaction by employing acetoacetates and substituted benzaldehydes[7]. The acetoacetates were easily obtained by the reaction of the alcohols with diketene[8,9].

Azelnidipine is designated chemically as 3-(1-benzhydrylazetidin-3-yl)-5-isopropyl-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Its literature synthesis (**Scheme-I**) involves 3-nitrobenzaldehyde **5** with isopropyl acetoacetate **6**. The product of (Z)-isopropyl 2-(3-nitrobenzylidene)-3-oxobutanoate (**7a**, **b**, **c**), on treatment with piperidine and acetic acid, coupling of (**7**) and 1-benzhydrylazetidin-3-yl 3-amino-3-iminopropanoate acetate (**8**) gave azelnidipine (**1**).

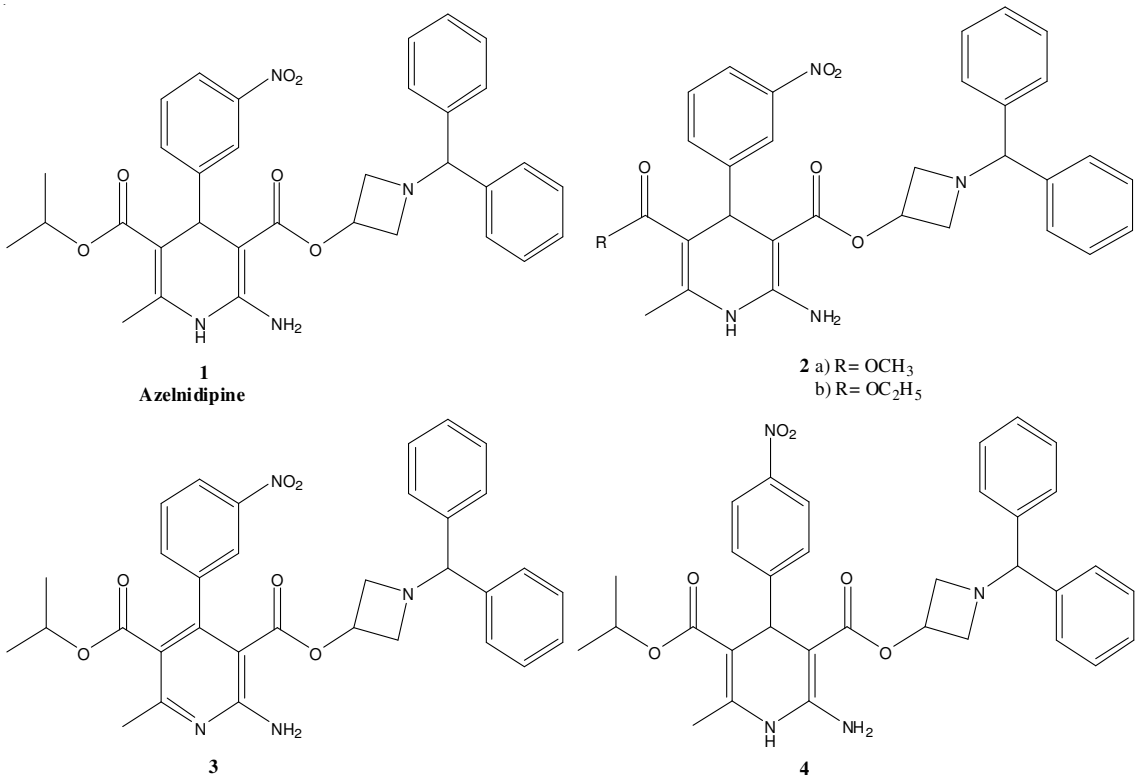
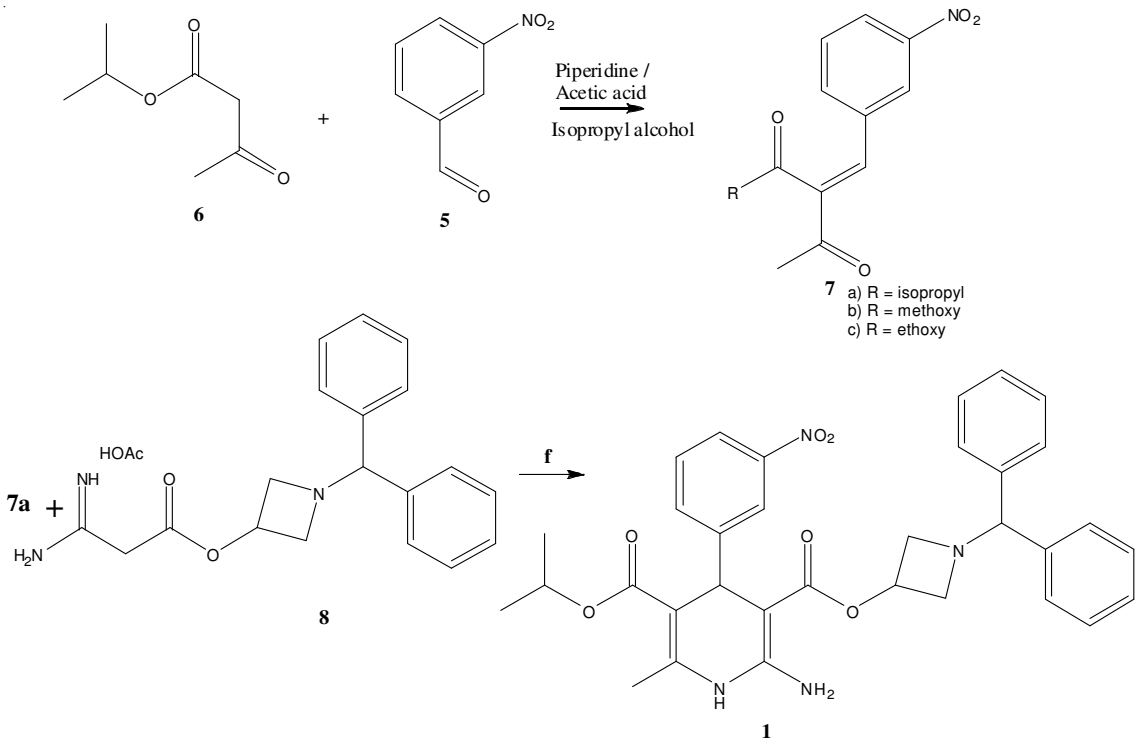


Fig. 1. Azelnidipine (**1**), process related impurities and metabolites



Scheme-I

During the laboratory optimization of azelnidipine (**1**), many process related impurities were identified. The guidelines recommended by ICH state that the acceptable levels for a known and unknown compound (impurity) in the drug should be less than 0.15 and 0.10 %, respectively[10]. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified and characterized.

EXPERIMENTAL

All reactions were run under a nitrogen atmosphere unless otherwise noted. All of the final compounds synthesized were characterized by ^{1}H and ^{13}C NMR and melting point for solids.

Synthesis of 3-(1-benzhydrylazetidin-3-yl)-5-methyl-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-

3,5-dicarboxylate (2a): To stirred solution of imidinoacetate (1g, 0.0026 mmol), (Z)-methyl 2-(3-nitrobenzylidene)-3-oxobutanoate (0.65 g, 0.0026 mmol) and isopropyl alcohol (25 mL) was added sodium methoxide (140 mg, 0.0026 mmol) heated reflux for 2 h, filtered and distilled under vacuum results to get residue was treated with cyclohexane obtained then (1.2 g) yellow colored compound obtained. ^{1}H NMR (DMSO-d_6) δ: 8.03-8.08 (2H, m), 7.55-7.64 (2H, m), 4.84-4.89 (2H, m), 2.89-2.93 (1H, m), 3.43-3.48 (1H, m), 2.42 (1H, t), 3.28-4.25 (2H, m), 7.14-7.39 (10 H, m), 2.28 (3H, m), 3.52 (3H, s), 6.80 (2H, br), 8.95 (1H,s). ^{13}C NMR (DMSO-d_6) δ: 121.81, 151.48, 120.77, 129.53, 134.21, 147.28, 38.45, 102.34, 145.81, 151.93, 76.28, 166.91, 61.35, 59.42, 61.35, 77.22, 142.14, 142.24, 128.40, 127.01, 128.40, 18.66, 167.12, 50.78. IR (KBr, (ν_{max}, cm^{-1}): 3441, 3027, 2926, 2850, 1676, 1648, 1489, 1526, 1452, 1434, 1384, 1348, 1318, 1290, 1217, 1110, 1069, 1029, 745, 705. Mass *m/z* = 555 (M + H)$^+$, 553 (M-H)$^-$, 589 (M-H + HCl)$^-$, 667 (M - H + $CF_3COOH)^-$.

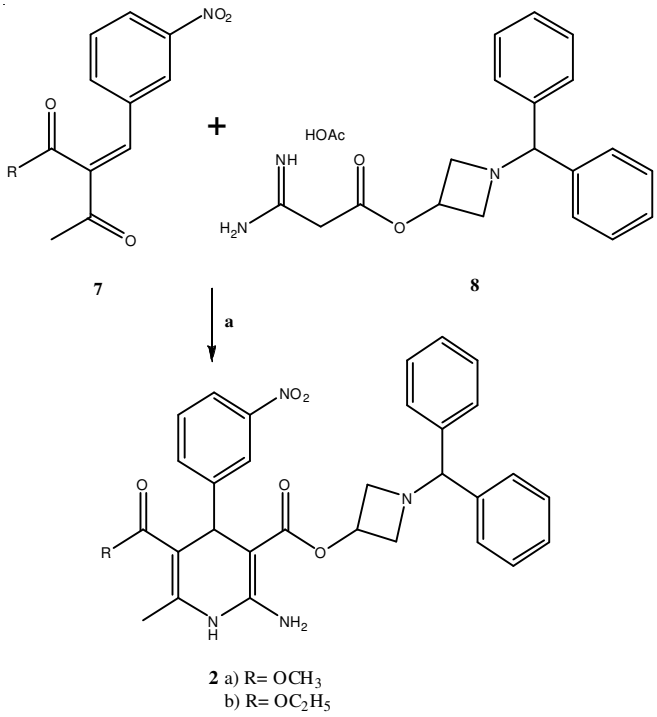
Synthesis of 3-(1-benzhydrylazetidin-3-yl)-5-ethyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (2b): To stirred solution of imidinoacetate (1 g, 0.0026 mmol), (Z)-ethyl-2-(3-nitrobenzylidene)-3-oxobutanoate (1.16 g, 0.0026 mmol) and isopropyl alcohol (25 mL) was added sodium methoxide (140 mg, 0.0026 mmol) heated reflux for 2 h, filtered and distilled under vacuum results to get residue was treated with cyclohexane then (1 g) yellow colored compound obtained. ^{1}H NMR (DMSO-d_6) δ: 8.04-8.09 (2H, m), 7.55-7.64 (2H, m), 4.84-4.88 (2H, m), 2.87-2.91 (1H, dd), 3.45 (1H, t), 2.35 (1H, t), 3.28 (1H, t), 4.20 (1H, s), 7.13-7.38 (10H, m), 2.28 (3H, s), 3.91-4.03 (2H, m), 1.11 (3H, t), 6.77 (2H, br), 8.85 (1H, s). ^{13}C NMR (DMSO-d_6) δ: 122.22, 151.63, 120.71, 129.51, 134.40, 147.08, 38.67, 102.53, 145.50, 151.80,76.30, 166.37, 61.36, 59.35, 60.11, 77.25, 142.11, 142.21, 128.39, 126.95, 127.02, 18.64, 167.14,59.20, 14.01. IR (KBr, ν_{max}, cm^{-1}): 3423, 3313, 3086, 3030, 2982, 2962, 2942, 2900, 2850, 1683, 1660, 1524, 1493, 1455, 1388, 1375, 1363, 1347, 1314, 1291, 1210, 1168, 1130, 1085, 1072, 827, 747, 704. Mass *m/z* = 569 (M + H)$^+$, 682 (M - H + $CF_3COOH)^-$.

Synthesis of 3-(1-benzhydrylazetidin-3-yl) 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (3): To stirred solution of 3-(1-benzhy-drylazetidin-3-yl)-5-isopropyl-2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (1 g, 0.0017 mmol) with dichloromethane (15 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.52 g) at ambient temperature for 5 h then extracted with 10 mL water, washed the organic layer with 5 % sodium carbonate solution then residue was treated with cyclohexane then 800 mg of orange colored compound obtained. ^{1}H NMR (DMSO-d_6) δ: 8.08 (1H, t), 8.43-8.46 (1H, dd), 7.80 (1H, t), 7.67 (1H, d), 4.8-4.87 (1H, m), 2.08 (1H, t), 3.14-3.23 (2H, m), 2.2 (1H, t), 3.99 (1H, s), 7.15-7.29 (10H, m), 2.36 (3H, s), 4.66-4.74 (1H, m), 0.84,0.91 (6H, dd), 7.15-7.29 (2H, m). ^{13}C NMR (DMSO-d_6) δ: 122.79, 147.77, 122.72, 129.78, 134.82, 140.55, 147.24, 118.63, 158.30, 158.16, 103.04, 165.65, 63.41, 58.36, 58.71, 76.99, 141.84, 128.45, 126.86, 127.13, 22.94, 166.37, 68.27, 20.82. IR (KBr, ν_{max}, cm^{-1}): 3503, 3381, 3054, 3030, 2982, 2964, 2845, 1716, 1694, 1590, 1557, 1522, 1450, 1350, 1306, 1241, 1122, 1103, 1073, 741, 704. Mass *m/z* = 581 (M + H) +, 580 (M - H)$^-$.

Synthesis of 3-(1-benzhydrylazetidin-3-yl)-5-isopropyl-2-amino-6-methyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (4): To stirred solution of imidinoacetate (1g, 0.0026 mmol), (Z)-isopropyl 2-(4-nitrobenzylidene)-3-oxobutanoate (0.65 g, 0.0026 mmol) and isopropyl alcohol (25 mL) was added sodium methoxide (140 mg, 0.0026 mmol) heated reflux for 2 h, filtered and distilled under vacuum results to get residue was treated with cyclohexane then 1.1 g of yellow colored compound obtained. ^{1}H NMR (DMSO-d_6) δ: 8.18 (2H, d), 7.45 (2H, d), 4.75-4.83 (2H, m), 2.91 (1H, t), 3.43 (1H, t), 2.40 (1H, br), 3.26-3.33 (1H, m), 4.27 (1H, s), 7.14-7.40 (10H, m), 2.26 (3H, s), 4.75-4.83 (1H, m), 1.03,1.18 (6H, d,d), 6.78 (2H, br), 8.86 (1H, s), 8.86 (1H, s). ^{13}C NMR (DMSO-d_6) δ: 129, 123.09, 145.44, 157.22, 38.67, 102.59, 145.23, 151.87, 75.99, 165.92, 61.68, 59.32, 59.88, 76.93, 142.20, 142.30, 128.40, 127.01, 126.96, 18.66, 167.23, 66.41, 21.53, 21.82. IR (KBr, ν_{max}, cm^{-1}): 3437, 3027, 2978, 2927, 2850, 1677, 1646, 1516, 1490, 1452, 1385, 1373, 1345, 1287, 1215, 1106, 1067, 828, 746, 705. Mass *m/z* = 583 (M + H) +, 581 (M - H)-, 617 (M - H + HCl)$^-$, 695 (M - H + $CF_3COOH)^-$.

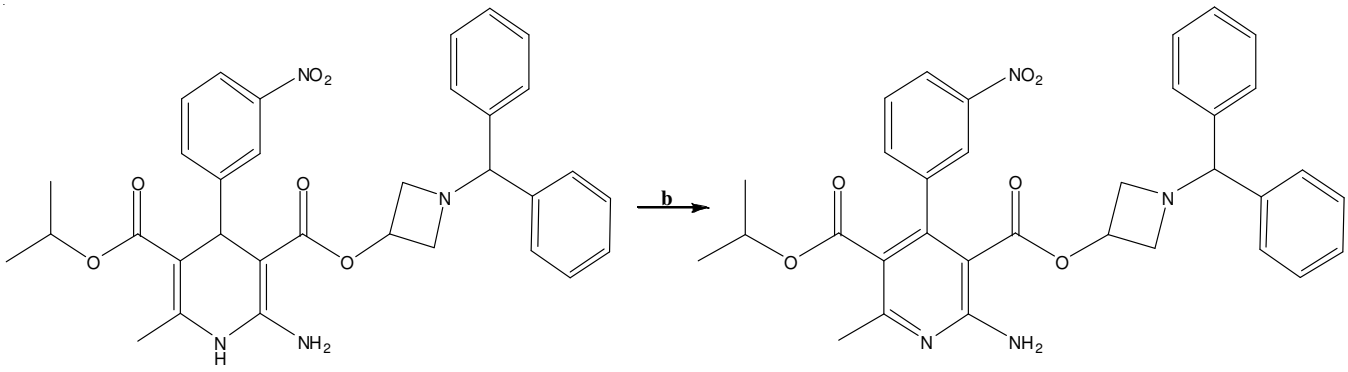
RESULTS AND DISCUSSION

During the condensation of product **1**, formation of 0.5-1 % of the two impurities **2a** and **2b** are observed, the level is reduced to less than 0.1 % during its isolation and purification. It is difficult to remove the impurity **2a** and **2b** from azelnidipine (**1**). The **2a** and **2b** was prepared by condensation of **7(b)** and **7(c)** with 1-benzhydrylazetidin-3-yl-3-amino-3-iminopropanoate acetate (**8**) in the presence of base (**Scheme-II**). The impurity **3** was prepared by oxidizing of **1** and the pyridine impurity **3** formed (**Scheme-III**). The impurity *para* nitro impurity **4** was prepared by the condensation of (Z)-isopropyl-2-(4-nitrobenzylidene)-3-oxobutanoate with **8** in the presence of base (**Scheme-IV**).



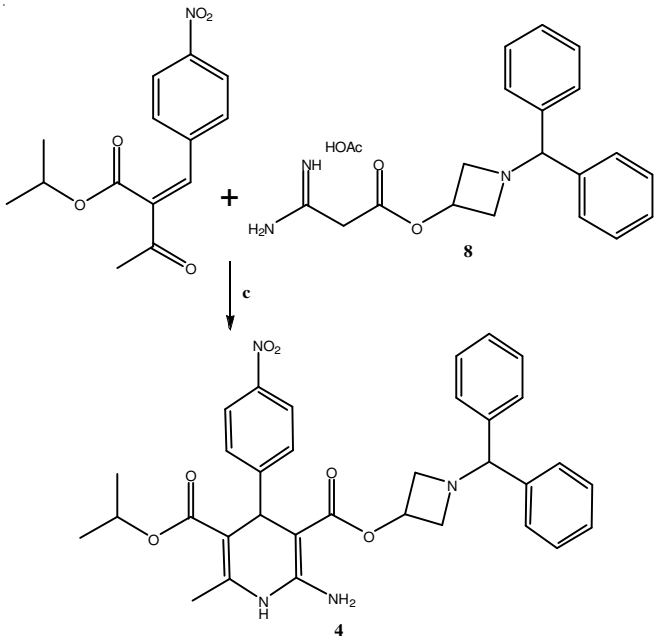
2 a) R= OCH_3
b) R= OC_2H_5

Scheme-II: Reagents, conditions, (a) sodium methoxide, isopropyl alcohol at 70 °C



1 **3**

Scheme-III: Reagents, conditions, (b) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2 room temperature



Scheme-IV: Reagents, conditions, (c) sodium methoxide, isopropyl alcohol at 70 °C

Conclusion

For the better understanding of the synthetic pathway of an active pharmaceutical ingredient (API) it is necessary to identify all the impurities formed/anticipated. In this regard we have synthesized and characterized different potential process-related impurities of azelnidipine.

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