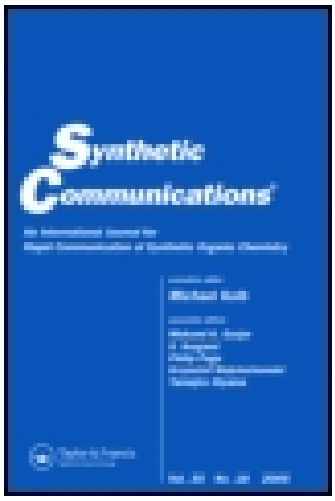

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Synthesis of Repaglinide Congeners

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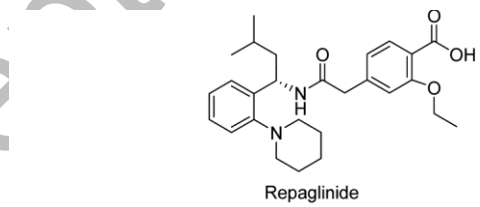
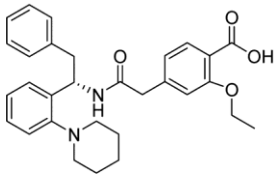
Abstract

This report describes a synthesis of two potent impurities of repaglinide, benzyl repaglinide **1** and repaglinide isomer **2** from commercially available raw materials 2-fluoro benzonitrile, (*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamine (**5**) and 3-ethoxy-[4-(ethoxycarbonyl)phenyl]acetic acid (**7**). These impurities are the crucial components in determining the quality of the drug substance, repaglinide during its manufacturing.



Repaglinide



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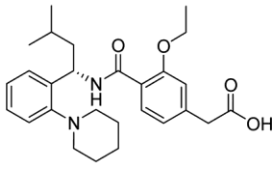

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KEYWORDS: Hypoglycemic agent, *N*-acetyl-L-glutamic acid, impurity, repaglinide isomer, benzyl repaglinide

INTRODUCTION

Repaglinide **3** is an antidiabetic drug and chemically known as (*S*)-(+)-2-ethoxy-4-(2-{3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamino}-2-oxoethyl)benzoic acid, which lowers the blood glucose levels by stimulating the release of insulin from the pancreas (Fig. 1).[1,2] It is an oral hypoglycemic agent used for treating type-2 non-insulin dependent diabetes mellitus.[3,4]

Synthesis of **3** as depicted above, involves the condensation of two key raw materials (*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamine (**5**) and 3-ethoxy-[4-(ethoxycarbonyl)phenyl]acetic acid (**7**) followed by ester hydrolysis in basic medium.[5] Most of the API's (active pharmaceutical ingredients) are prepared by the multistep chemical synthesis and during its synthesis some of the process related impurities, also called as related substances may form in trace levels and retain in the drug substance. These impurities may have a significant impact on the quality and safety of API based on their toxic behavior. Therefore, it is always recommended by ICH guidelines to study the origin and control of such impurities which are present in the API at a level ≥ 0.05% w/w.[6] Furthermore, it is equally important to identify, synthesize and characterize such impurities in a pure form to check the analytical parameters such as specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ) and relative retention factor.[7]

During the process development of **3** in our laboratory, we have anticipated the plausible formation of the potent impurities namely, (*S*)-2-ethoxy-4-[2-({2-phenyl-1-[2-(piperidin-1-yl)phenyl]ethyl}amino)-2-oxoethyl]benzoic acid (**1**, benzyl repaglinide) and 2-(4-{(*S*)-3-methyl-1-[2-(piperidin-1-yl)phenyl]butylcarbamoyl}-3-ethoxyphenyl)acetic acid (**2**, repaglinide isomer). These impurities were the expected contaminants from the key raw materials **5** and **7**. Based on their importance, **1** is described as a qualified impurity in the pharmacopieal monograph with a limit of not more than 0.1% w/w in the drug substance **3**.[8] To the best of our knowledge, the synthesis of these impurities were not reported so far. Due to the stringent impurity profiling of API from the drug regulatory authorities and their importance, we herein report the synthesis and characterization of these impurities (**1** and **2**).

RESULT AND DISCUSSION

The addition of Grignard reagent, prepared by the method of Leigh (reaction medium containing toluene with one equivalent of THF), one could expect the formation of benzyl counterpart along with the regular product.[9,10] It is evident from the literature that on continuous heating of refluxing toluene in Grignard reaction condition, benzyl substituted product gets formed in minor level (Scheme 1). Similarly, addition of isobutylmagnesium bromide on nitrile **4** in toluene/THF mixture at higher temperature results **5** as a major product with a plausible formation of **6**. The introduction of benzyl moiety is attributed from toluene which is used as a solvent during the insertion of isobutyl group in nitrile **4**. (*S*)-Benzylamine **6** which reacts in a similar way as **5** is considered as a route cause for the contamination of benzyl repaglinide **1** in **3.**

Benzyl repaglinide **1** was prepared as shown in Scheme 2, starting with nitrile **4**. The addition of Grignard reagent (benzylmagnesium chloride) over nitrile **4**, prepared in toluene/THF medium afforded imine intermediate **9.** Reduction of **9** using $NaBH_4$ gave a racemic benzylamine **10**. Since, benzyl repaglinide **1** is a chiral impurity, we have screened racemic benzylamine **10** with different optically active reagents to obtain chirally pure **1.** Finally, promising results were obtained with *N*-acetyl-L-glutamic acid. (*S*,*S*)-Glutamate salt **11** on basification yielded (*S*)-2-phenyl-1-[2-(piperidin-1-yl)phenyl]ethylamine (**6**). Coupling of **6** with the acid chloride derivative of phenylacetic acid **7** in presence of triethylamine gave amide derivative **12** which on saponification afforded **1** in good yield. The chromatographic and enantiomeric purity of **1** was found to be above 99% by HPLC. The chemical structure of **1** was confirmed by spectral analysis and in comparison with **3.**

The origin of impurity **2** is again due to the contamination of phenylacetic acid **7** containing benzoic acid **8.** The literature reports the preparation of phenylacetic acid **7** starting with O-alkylation of 2- hydroxy-4-methylbenzoic acid.[11] While preparing **7** from its penultimate under basic conditions afforded **7** as a major product along with 2-ethoxy-[4-(carboxymethyl)]benzoic acid (**13**) and 2-ethoxy-[4-(ethoxycarbonyl)methyl]benzoic acid (**8**), as an expected contaminants. Di-acid **13** is mainly responsible for the formation of repaglinide dimer, a major impurity of **3**.[12]

The benzoic acid **8** was prepared, starting with **7** as shown in Scheme 3**.** Hydrolysis of **7** under basic condition leads to the formation of **13** as a major product.[13]

Di-acid **13** on esterification using ethanol in toluene containing catalytic amount of sulfuric acid, afforded **8** as a major product along with di-ester **14** in a good yield. The compound **8** was distinguished from **7** by the chemical shift values of proton at C6 position in the aromatic ring of **8** (δ 8.13 ppm) against the proton at C5 position of **7** (δ 7.74 ppm). This difference in the chemical shift values were due to the shielding effect created by the ester group in **7**. Benzoic acid **8** was converted into its acid chloride derivative, using thionyl chloride and coupled with **5** to obtain amide intermediate **15**, which on hydrolysis under basic condition, resulted repaglinide isomer **2** in a good yield with purity more than 94% by HPLC (Scheme 4).

CONCLUSION

We have successfully demonstrated the synthesis and characterization of **1** and **2,** the potent impurities of repaglinide. This study not only helped us to establish the impurity profile of **3**, but also allowed us to understand the cause for its generation and control.

EXPERIMENTAL

Melting points were determined with a Reichert Thermopan apparatus. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 300 MHz and Varian 400 and 500 MHz spectrometer using TMS as the internal standard in DMSO-d_6 or $CDCl_3$. IR spectra were recorded using a Perkin-Elmer spectrum one Fourier Transform (FT) IR spectrophotometer. High-resolution mass spectral analyses were performed using the electrospray ionization (ESI) method on Xevo G2 QTOf mass spectrometer. HPLC measurements were run on a Zorbax SB-Aq (150 mm x 4.6mm, 5µm; make: Agilent).The

flow rate was maintained at 1.5 mL / min with a column temperature of 45 ^{o}C. UV detection occurred at λ = 240 nm. All reagents were used as purchased unless otherwise stated. Reactions were performed under nitrogen atmosphere and the work-up were done in a well-ventilated fuming hood.

Preparation Of (*S*)-2-Ethoxy-4-[2-({2-Phenyl-1-[2-(Piperidin-1-Yl)Phenyl]Ethyl}Amino)-2-Oxoethyl]Benzoic Acid (1)

Sodium hydroxide (0.6 g, 15.0 mmol) was added to a stirred solution of **12** (5.0 g, 9.7 mmol) in a mixture of methanol (50 mL) and water (25 mL) at 25-30 °C. The reaction mixture was stirred for 4 h at reflux. Thereafter, it was concentrated under vacuum to remove methanol and diluted with water (50 mL) at 25-30 °C. Added CH_2Cl_2 (20 mL) and stirred for 10 min at 25-30 °C. The organic layer was discarded and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL) at pH 4.5-5.0. The solvent was evaporated under vacuum and the residue was dissolved in 2-propanol (15 mL) at reflux. The solution was cooled to 5-10 °C and stirred for 2 h. The product was filtered, washed with cold 2-propanol (5 mL) and dried under reduced pressure at 45 °C to obtain **1** as white solid. Yield 3.9 g (82.9%); Purity 99.94%; Chiral purity 99.99%; Mp132-134 °C; $[\alpha]^{20}_{D}$ +25.6° (*c*1, methanol); ^{1}H NMR(500 MHz, DMSO-d_6) δ 1.31 (t, 3H, *J* = 7.0 Hz), 1.60 (m, 6H), 2.46 (br s, 2H), 2.77 (dd, 1H, J_1 = 10.0 Hz, J_2 = 14.0 Hz), 2.88 (dd, 1H, J_1 = 5.0 Hz, J_2 = 13.5 Hz), 3.03 (br s, 2H), 3.41 (s, 2H), 3.97 (q, 2H, *J* = 6.5 Hz), 5.52 (m, 1H), 6.68 (dd, 1H, J_1 = 1.5 Hz, J_2 = 7.5 Hz), 6.87 (d, 1H, *J* = 1.5 Hz), 7.09 (m, 2H), 7.21 (m, 6H), 7.42 (dd, 1H, J_1 = 1.0 Hz, J_2 = 7.5 Hz), 7.48 (d, 1H, *J* = 8.0 Hz), 8.57 (d, 1H, *J* = 8.5 Hz, exchangeable with D_2O), 12.41 (br s, 1H, exchangeable with D_2O); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 14.5, 24.0, 26.9, 26.9, 43.1, 43.8, 52.8, 54.7, 65.8, 113.2, 116.3, 122.7, 122.7, 124.9, 126.2, 127.6, 128.0, 128.1, 129.1, 133.8, 137.2, 137.9, 143.0, 152.3, 157.4, 165.3, 168.4; HRMS (ESI, QTOF) for $C_{30}H_{34}N_2O_4$ $[M+H]^+$: *m/z* calcd: 487.2598; found: 487.2593.

Preparation Of 2-(4-{(*S*)-3-Methyl-1-[2-(Piperidin-1-Yl)Phenyl]Butylcarbamoyl}-3-Ethoxyphenyl)Acetic Acid (2)

To a stirred suspension of **8** (2.0 g, 7.9 mmol) in toluene (20 mL), thionyl chloride (1.2 g, 10.1 mmol) was added at 25-30 °C. The mixture was stirred for 2 h at 25-30 °C. This acid chloride solution was added over **5** (1.6 g, 6.8 mmol) in toluene (20 mL), contains triethylamine (0.8 g, 7.9 mmol) at 25-30 °C. The reaction mixture was stirred for an hour and diluted with water (20 mL). The mixture was stirred for 15 min and the organic layer was separated. The solvent was removed under reduced pressure to afford amide intermediate **15**. The residue was dissolved in aqueous ethanol (20 mL, 50% v/v) and treated with sodium hydroxide (0.6 g, 15.0 mmol) for 3 h at 60 °C. The reaction mixture was concentrated under vacuum and diluted with water (20 mL). To the suspension CH_2Cl_2 (40 mL) was added and stirred for 10 min at 25-30 °C. The organic layer was discarded and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL) at pH 4.5-5.0. The solvent was evaporated under vacuum and the residue was purified by column chromatography (CH_2Cl_2/ hexane) to yield **2** as a pale yellow solid. Yield 1.720 g (58.3%); Mp 106-108°C; $[\alpha]^{20}_D$ +77.8° (*c* 0.5, methanol); Purity 95.44%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.95 (dd, 6H, J_1 = 6.5 Hz, J_2 = 6.0 Hz), 1.42 (m, 4H), 1.63 (m, 9H), 2.62 (br s, 2H), 3.14 (br s, 2H), 3.60 (s, 2H), 4.16 (m, 2H), 5.58 (m, 1H), 6.90 (dd, 1H, J_1

= 1.0 Hz, J_2 = 8.0 Hz), 7.05 (m, 2H), 7.18 (m, 2H), 7.32 (dd, 1H, J_1 = 2.0 Hz, J_2 = 8.0 Hz), 7.62 (d, 1H, J = 7.5 Hz), 8.37 (d, 1H, J = 8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.2, 23.1, 24.4, 25.4, 26.7, 41.2, 47.1, 47.8, 54.6, 64.7, 113.1, 120.5, 121.1, 122.2, 124.2, 125.8, 127.5, 132.5, 138.6, 139.6, 152.3, 156.9, 164.5, 175.2; HRMS (ESI, QTOF) for $C_{27}H_{36}N_2O_4[M+H]^+$: *m/z* calcd: 453.2754; found: 453.2750.

SUPPLEMENTAL MATERIAL

Full experimental detail, ^{1}H and ^{13}C NMR spectra, HPLC traces for this article can be accessed on the publisher's website.

ACKNOWLEDGMENTS

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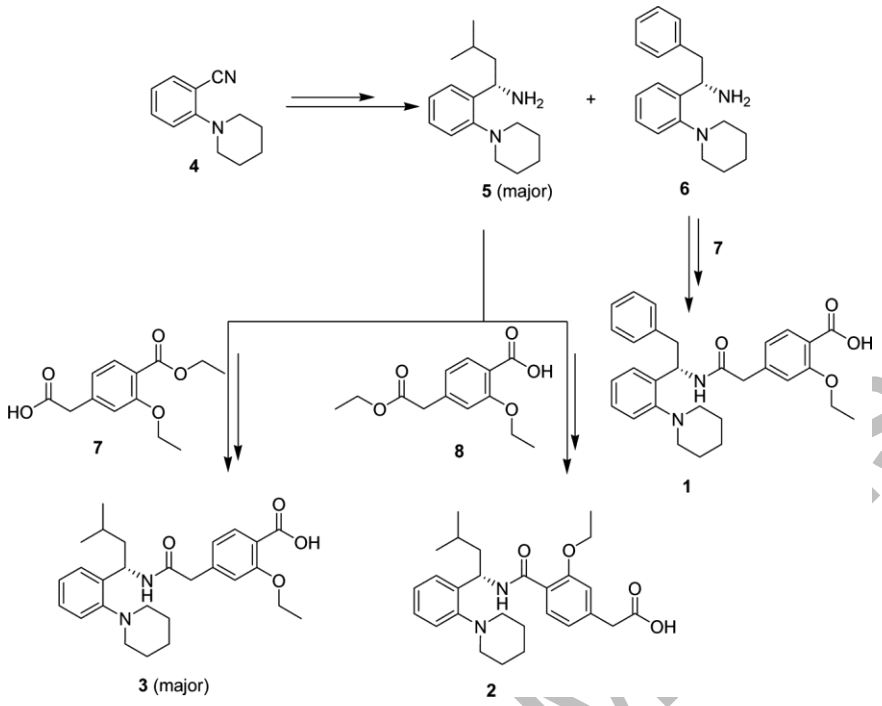
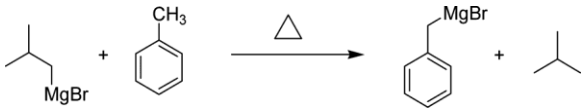
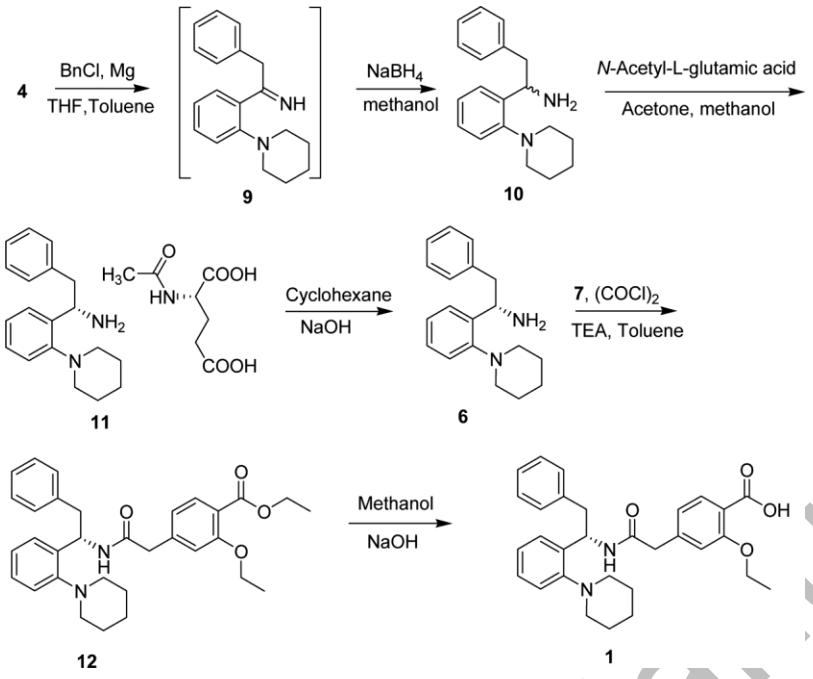
Figure 1. Repaglinide (**3**) along with its related substances, benzyl repaglinide **1** and repaglinide isomer **2**.



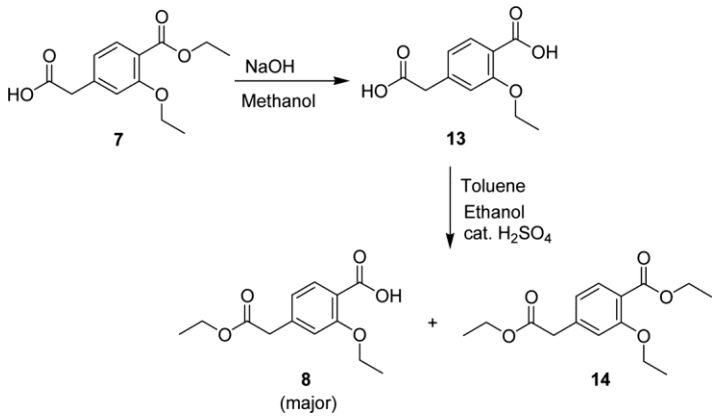
Scheme 1. Expected formation of benzylmagnesium bromide.



Scheme 2. Synthesis of benzyl repaglinide **1**.



Scheme 3. Synthesis of benzoic acid **8**.



Scheme 4. Synthesis of repaglinide isomer **2**.