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Technical Notes

Understanding and Controlling the Formation of an Impurity during the Development of Muraglitazar, a PPAR Dual Agonist

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Abstract:

Impurity A, observed during the process research and development of muraglitazar, was isolated via preparative HPLC for structural identification using one-dimensional and two-dimensional NMR techniques. The origin of impurity A was identified as arising three steps earlier as a minor contaminant present in one of the starting materials: 4-hydroxybenzaldehyde. As a result, a series of corresponding impurities were formed in each synthetic step leading up to impurity A. These findings permitted the addition of a new specification for this starting material to eliminate the problem.

Introduction

Muraglitazar (**1**) (Figure 1) was developed within Bristol-Myers Squibb as a non-thiazolidinedione peroxisome proliferator-activated receptor (PPAR) α/γ dual agonist for the treatment of type-2 diabetes and dyslipidemia. Such dual PPAR agonists[1−5] are projected to offer both glycemic and lipid control to the patient and are a significant step toward the treatment of these diseases. Muraglitazar was the first of these non-thiazolidinedione candidates to complete clinical trials and advance to FDA registration.

Regulatory agencies have heightened their scrutiny of the safety profile and risk vs benefits of new drug entities. As a result, increased emphasis has been placed on the identification, formation, fate, and process control of impurities in starting materials, isolated intermediates, and the active pharmaceutical ingredients (API). Since the treatment of type-2 diabetes will require chronic dosing, the potential long-term exposure to any process-related impurities in the API is a significant concern. Therefore, when an unknown impurity (impurity A) was found in a batch of muraglitazar at a 0.06 HPLC area %, an investigation was initiated. While not found in all batches and below the 0.1% threshold of identification specified in ICH guideline,[6] it is our practice that impurities observed at an alert level of 0.05−0.1 AP in API batches should be identified to minimize any risk to the patient. We anticipated that our investigation would allow the structural identification of impurity A, the root cause(s) of its formation, and ultimately how to effectively control its level to ensure future deliveries of reproducibly high-quality API. This paper describes the identification of impurity A and its precursor impurities B, C, and D observed in the course of synthesis of muraglitazar. The precursor D contaminated one of the starting materials, and its identification permitted the establishment of a new purchase specification to eliminate this problem.

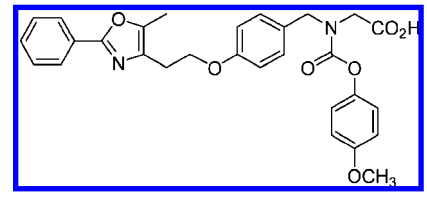

Figure 1. **Muraglitazar (1).**

Results and Discussion

The synthetic route shown in Scheme 1 has been used to manufacture several batches of the API **1**.[7] The analysis of a developmental batch of muraglitazar API indicated the presence of a new impurity at 0.06 HPLC area %, identified as 'A'. Further work demonstrated that this impurity was not easily purged out upon recrystallization and suggested that upstream control would be important. LC/MS and LC/MS/MS analysis indicated that this impurity had a molecular weight of 807 Da but provided no other useful information (Figure 2. Therefore, impurity A was isolated via preparative HPLC in order to produce material for structure elucidation using comprehensive one-dimensional (1D) and two-dimensional (2D) NMR techniques.

Structure Elucidation of Impurity A. The molecular formula of impurity A was established from the isolated sample

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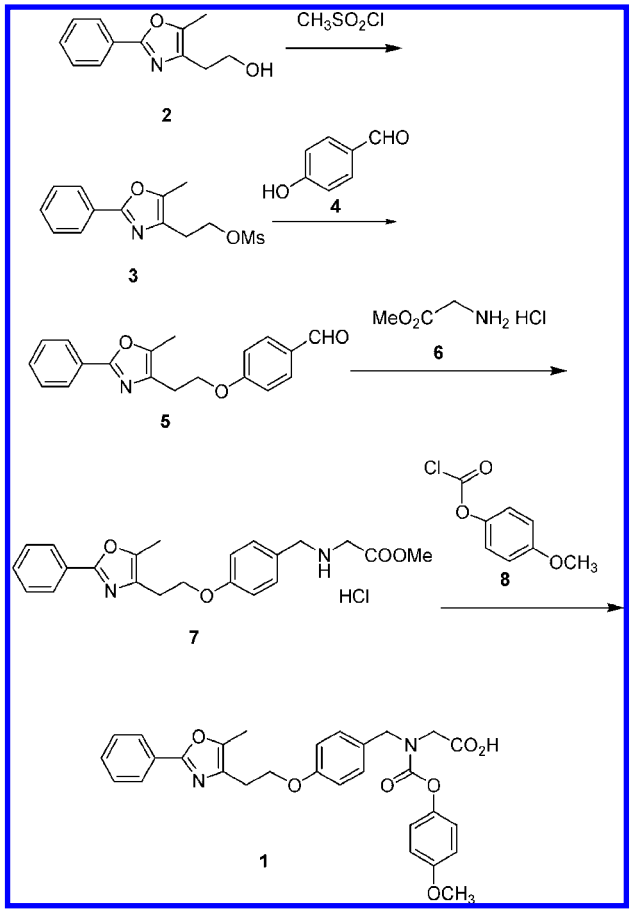
Scheme 1. **Synthetic route of muraglitazar 1**

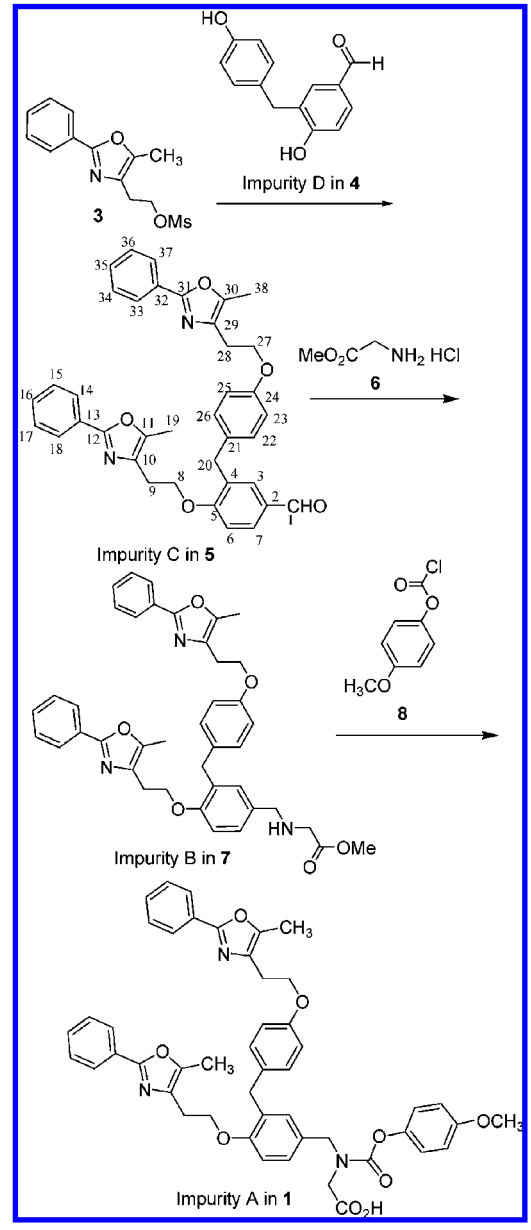


as $C_{48}H_{45}N_3O_9$ using exact mass of $[M + H]^+$ ($m/z = 808.3235$) determined by positive ESI HRMS (calcd for $C_{48}H_{46}N_3O_9$: 808.3234). Comparison of the NMR data with that of **1** indicated that impurity A contained the backbone structure of **1**, along with resonances that can be assigned to an additional side chain: 5-methyl-2-phenyl-4-(2-(*p*-tolyloxy)ethyl)oxazole (Figure 3). ^1H and ^{13}C NMR data of impurity A, in conjunction with DEPT and $^1H-^{13}C$ heteronuclear multiple quantum correlation (HMQC) data, indicated the presence of an additional methylene group leading to a proton signal at 3.84 ppm and carbon chemical shift at 34.8 ppm. This methylene group links the muraglitazar 'core' of the impurity with the additional side-chain portion of the molecule and is attached at the C-7 position, the determination of which is based on the long-range correlation observed from H-6 (6.94 ppm) to both C-23 (34.8 ppm) and C-4 (51.0 ppm) in the HMBC experiment. This information permitted an assignment of this impurity as a derivative of **1** (Figure 4), which is consistent with all data.[8]

Investigation of the Root Causes of the Formation of Impurity A. The unambiguous structural information in conjunction with the synthetic sequence for muraglitazar **1**, as shown in Scheme 1, suggested the following hypothesis. Impurity A could have originated from impurity D present as a minor contaminant in starting material, 4-hydroxybenzaldehyde **4** (Scheme 2). Impurity D, which contains two phenol groups, would undergo a bis addition of the mesylate **3** to

(8) Key ^1H and ^{13}C NMR chemical shift assignments of impurity A are shown in Table 1 in the Supporting Information.

Scheme 2. **Proposed formation of impurity a during the synthesis of 1**



produce impurity C, corresponding to **5**. Due to its similar structure, impurity C was not be purged during the isolation of **5** and was carried over into next reaction. Impurity C would then react with glycine methyl ester **6** to form impurity B during the synthesis of **7**. Similarly, impurity B in **7** would react with **8** to ultimately form impurity A during the synthesis of **1**. In order to confirm this hypothesis, we undertook efforts to identify the presence of impurities B, C, and D in the corresponding batches of penultimate **7**, prepenultimate **5**, and starting material **4**, respectively, as described below.

Identification of Impurity B. A related batch of penultimate **7** that produced the batch of **1** containing impurity A was analyzed using LC/UV/MS, to identify the presence of any impurity peak with a MW of 671 Da that would correspond to impurity B. A minor peak (0.04 HPLC area %) with the expected MW of 671 Da was observed. LC/MS/MS analysis of the peak revealed a key fragment of *m*/*z* 292 that corresponds

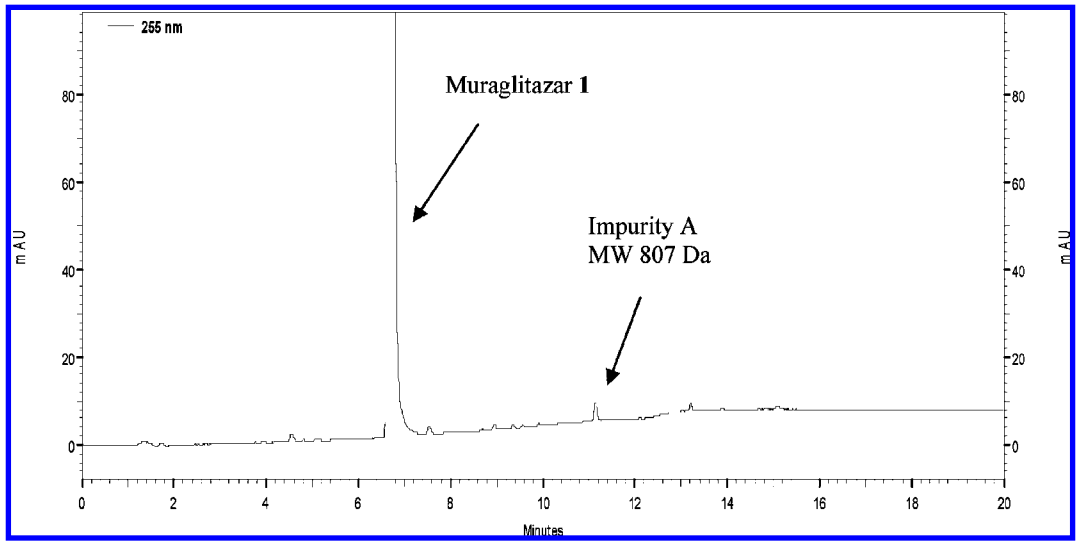


Figure 2. **Analytical HPLC chromatogram of muraglitazar 1 containing impurity A.**

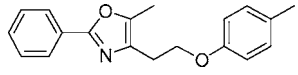


Figure 3. **5-Methyl-2-phenyl-4-(2-(*p*-tolyloxy)ethyl)oxazole.**

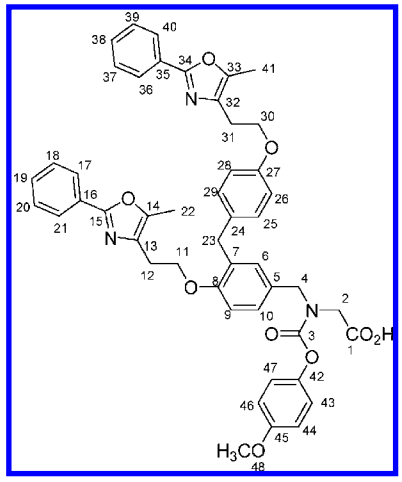


Figure 4. **Structure of impurity A.**

to the presence of an additional side chain when compared to the penultimate **7**. Thus, this minor peak was assigned as impurity B.

Identification of Impurity C. A relevant batch of prepenultimate **5** was analyzed by LC/UV/MS to detect a peak at 0.07 HPLC area % with a MW of 598 Da consistent with impurity C. This impurity peak was isolated via preparative HPLC for thorough NMR structure elucidation. The molecular formula of the isolate was established as $C_{38}H_{34}N_2O_5$ using exact mass of $[M + H]^+$ (m/z = 599.2552) determined by positive ESI HRMS (calcd for $C_{38}H_{35}N_2O_5$: 599.2546). Comparison of the ^{1}H NMR data of the isolate with that of impurity A indicated the presence of the same structural backbone except for the lack of the substituted glycine moiety in impurity C. Observation of a sharp singlet at 9.79 ppm in the ^{1}H NMR spectrum of the isolate was consistent with the presence of an aldehyde moiety. The presence of the aldehyde moiety was further supported by its one bond correlation to a carbon at 191.1 ppm observed in the HMQC experiment. Long-range HMBC correlations from both H-3 and H-7 to C-1 and from both H-3 and H-22 to C-20 further supported the assignment of the isolate as impurity C. Overall, the structure of impurity C is consistent with 1D ^{1}H and ^{13}C and 2D COSY, HMQC, and HMBC NMR and HRMS data.[9]

Table 1. **Correlation between Impurity D and Impurity A**

batch no.	impurity D (HPLC area %) in starting material **4**	impurity A (HPLC area %) in API **1**
1	0.05	0.06
2	0.07	0.04
3	nondetected	nondetected
4	nondetected	nondetected

Identification of Impurity D. A relevant batch of starting material 4-hydroxybenzaldehyde **4** displayed a weak mass spectral response with a m/z of 229 ($[M + H]^+$) for a minor peak, consistent with the expected MW of impurity D. Some of this minor impurity peak was isolated via preparative HPLC. Analysis of ^{1}H NMR and DEPT in combination of HMQC NMR data clearly indicated the presence of a methylene group (two-proton singlet at 3.82 ppm and a methylene carbon at 34.9 ppm). The minor impurity was assigned as impurity D.[10]

Correlation of Impurity D with Impurity A. The isolated sample of impurity D was used as an HPLC marker to analyze four other batches of 4-hydroxybenzaldehyde **4** that were used to produce batches of muraglitazar API. When batches of 4-hydroxybenzaldehyde **4** containing trace levels of impurity D were used in the process, they consistently led to the production of API batches that were contaminated by a trace level of impurity A unlike clean lots of 4-hydroxybenzaldehyde A, as depicted in Table 1. Batches 1 and 2 illustrated that the level of impurity A in API **1** arising from impurity D in starting material **4** can fluctuate but cannot be totally purged out under current processes. Thus, a direct correlation was made between the presence of impurity D in the starting material, 4-hydroxybenzaldehyde **4**, and the presence of impurity A in the API produced three steps later.

(9) Key ^{1}H and ^{13}C NMR chemical shift assignments of impurity C are shown in Table 2 in the Supporting Information.
(10) ^{1}H and ^{13}C and DEPT NMR spectra of impurity D are shown in the Supporting Information.

Conclusion

An unknown impurity A detected during the development of muraglitazar was isolated via preparative HPLC and identified by NMR and HRMS. The structural knowledge of this impurity led to the identification of its root cause of formation that was attributed to a minor contaminant (impurity D) present in one of the starting materials. The source for the formation of the impurity A was further confirmed by a direct correlation between impurity D and impurity A. On the basis of these findings, impurity A in the future production lots of muraglitazar can be properly controlled when impurity D in starting material **4**[11] is controlled at <0.1% level to ensure the high quality of API.

Experimental Section

HPLC Conditions. Analytical HPLC was performed on a Shimadzu LC-10AD separation module equipped with a Shimadzu SPD-M10A photodiode array UV detector using Waters YMC Pro C18 (150 mm × 4.6 mm, 3 μm) column. For the isolation of impurities A, C, and D, a Shimadzu preparative HPLC equipped with an LC-8A pump, an SCL-8A system controller, an SPD-10A UV−vis detector, a FRC-10A fraction collector, and an SIL-10A autoinjector was used. A Phenomenex Luna C18 column (150 mm × 21.2 mm, 5 μm particle size) was employed for isolation of impurities A, C, and D. The mobile phase consisted of A (water containing 0.05% TFA) and B (acetonitrile containing 0.05% TFA). The flow rate was set at 21 mL/min and detection was carried out at 220 nm. A linear gradient from 55% B to 100% B over 10 min was used to isolate impurity A at R_T 11.9 min while a linear gradient from 45% B to 100% B over 10 min was used to isolate impurity C at R_T 13.5 min, and a linear gradient from 10% B to 90% B over 15 min was used to isolate impurity D at R_T 8.7 min. Fractions containing each individual impurity were pooled and concentrated under reduced pressure and freeze-dried to obtain pure isolates for spectral characterization.

NMR Spectroscopy. All the NMR spectra were collected on a Bruker DRX 400 MHz instrument equipped with a 3 mm Nalorac inverse probe with the exception of ^{13}C and DEPT-135 spectra which were collected with a 3 mm Nalorac dual probe. All spectra were collected in $CDCl_3$ for impurities A and C, and DMSO-d_6 for impurity D.

(11) Starting material, 4-hydroxybenzaldehyde, was supplied by multiple vendors. Some of the batches were free of the minor contaminant, impurity D. Therefore, we believe if we set an appropriate specification on impurity D and engage the vendors to meet the specification, the impurity A in API can be effectively controlled.

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Supporting Information Available

Tables of ^1H and ^{13}C NMR assignments for impurities A and C; ^1H, ^{13}C and DEPT-135 NMR spectra of impurity D. This material is available free of charge via the Internet at http://pubs.acs.org.

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