

Efficient Synthesis of Potential Impurities in Levonadifloxacin (WCK 771)

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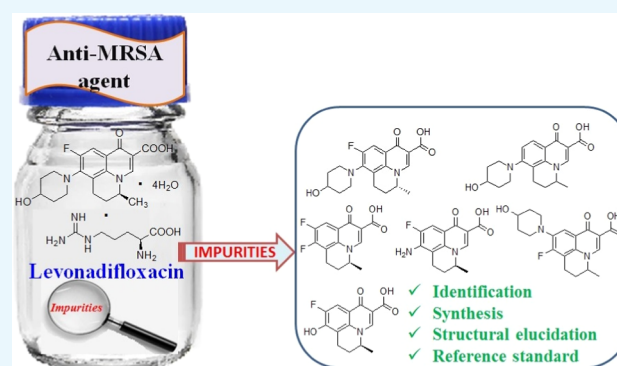


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ABSTRACT: Levonadifloxacin (WCK 771) is a novel broad-spectrum anti-methicillin-resistant *Staphylococcus aureus* (MRSA) agent recently launched in India. Five process impurities and one degradation impurity were synthesized as reference standards for their quantification by high-performance liquid chromatography (HPLC) methodology in drug substance and drug product. These compounds are not easily commercially available. The synthesis and characterization of these impurities are discussed in detail.



INTRODUCTION

Levonadifloxacin (WCK 771), with chemical structure S-(−)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[*i,j*]quinolizine-2-carboxylic acid L-(+)-arginine salt tetrahydrate, has been developed by Wockhardt.¹ It is a novel broad-spectrum anti-methicillin-resistant *Staphylococcus aureus* (MRSA)/anti-vancomycin-resistant *S. aureus* (VRSa) agent, formulated for intravenous administration.^{2,3} This drug has been recently introduced in the Indian market by Wockhardt under the brand name of EMROK as intravenous injection for skin and soft tissue infections including acute bacterial skin and skin structure infections, diabetic foot infections, MRSA infections, and concurrent bacteremia. Understanding the drug substance (DS) and drug product (DP) impurity profile is of fundamental significance for ensuring the drug's effectiveness and is receiving vital attention from regulatory authorities. The impurity identification and control is important for patient safety, since certain impurities, depending on their structural features, could be potential mutagenic impurities.⁴ The International Conference of Harmonization (ICH) has published guidelines on impurities in new drug substances and drug products.⁵

The majority of the impurities are process related and could be formed during the active pharmaceutical ingredient (API) synthesis. Moreover, few degradation impurities may be formed during storage/formulation. The impurities in new drugs are not easily accessible, mainly because they are present at a very low level, as well as their chemical properties are often very similar to those of the parent drug and therefore their separation is very difficult. It has to be pointed out that the side

products closely related to the process are convoluted in the next stages along with the main products, thus generating subsequent impurities.^{6,7}

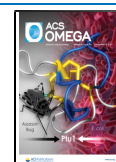
The unambiguous identification of process impurities can increase the process understanding, which in turn allows for better impurity control in the final API. Impurity profile studies are thus necessary to certify the purity, quality, safety, and efficacy of the drug product during API development. Also, purification of an API is usually the most difficult step in its production and can be facilitated by suppressing the formation of impurities.⁸ Hence, it is very important to make available these impurities during drug development for their accurate quantification in drug substance and drug product. Their reference standards are required in analytical method development, validation, and during batch release and stability studies of drug substance or product.

Previous literature or reports are not available regarding the synthesis and characterization of process-related impurities or degradation impurities of levonadifloxacin. In addition, these impurities are not commercially available.

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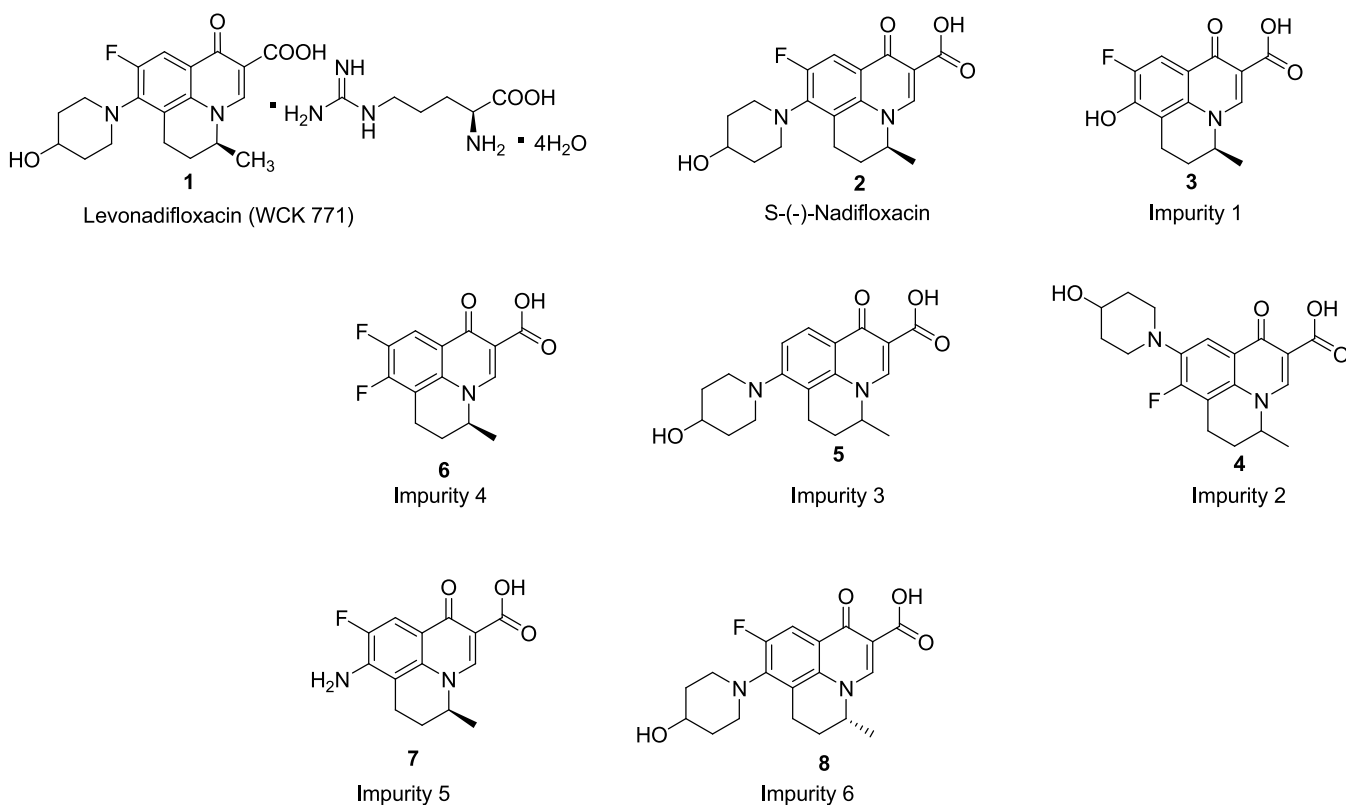


Figure 1. Levonadifloxacin (WCK 771) and its potential impurities.

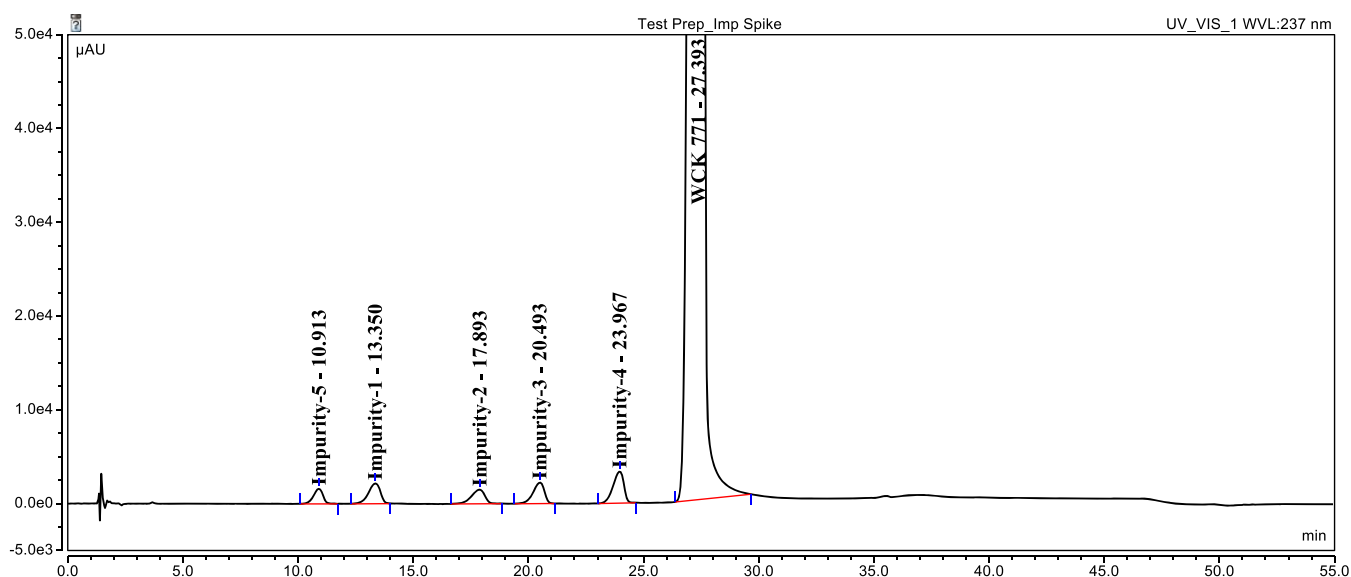


Figure 2. HPLC chromatogram of the levonadifloxacin sample spiked with five impurities.

RESULTS AND DISCUSSION

The aim of our study was to develop an efficient, straightforward, and economical route for synthesis of the identified impurities of levonadifloxacin. During drug development, impurities were observed in the bulk drug substance. The mass of the impurities was identified using liquid chromatography mass spectrometry and the probable structures were predicted. Moreover, the structure of the impurity was confirmed after isolation or by chemical synthesis and detailed spectroscopic analysis, followed by co-injection using the original high-performance liquid chromatography

(HPLC) method. The chemical structures of levonadifloxacin and identified impurities are shown in Figure 1.

A typical HPLC chromatogram of the levonadifloxacin drug substance is shown in Figure 2. The impurities are marked as impurity 1 (retention time (RT): 13.35 min), impurity 2 (RT: 17.89 min), impurity 3 (RT: 20.49 min), impurity 4 (RT: 23.96 min), and impurity 5 (RT: 10.91 min). Impurities 1–4 are process impurities formed during synthesis of the drug substance, whereas impurity 5 is a degradation impurity formed in the drug product. Impurity 6 (RT: 16.27 min) is an enantiomer of compound 2 and was analyzed by the chiral

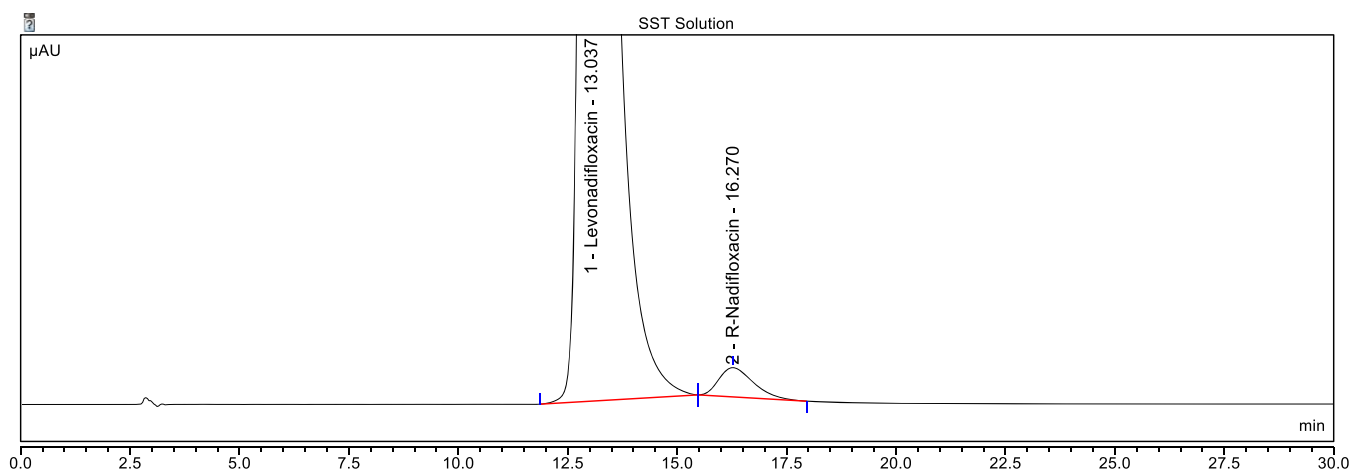
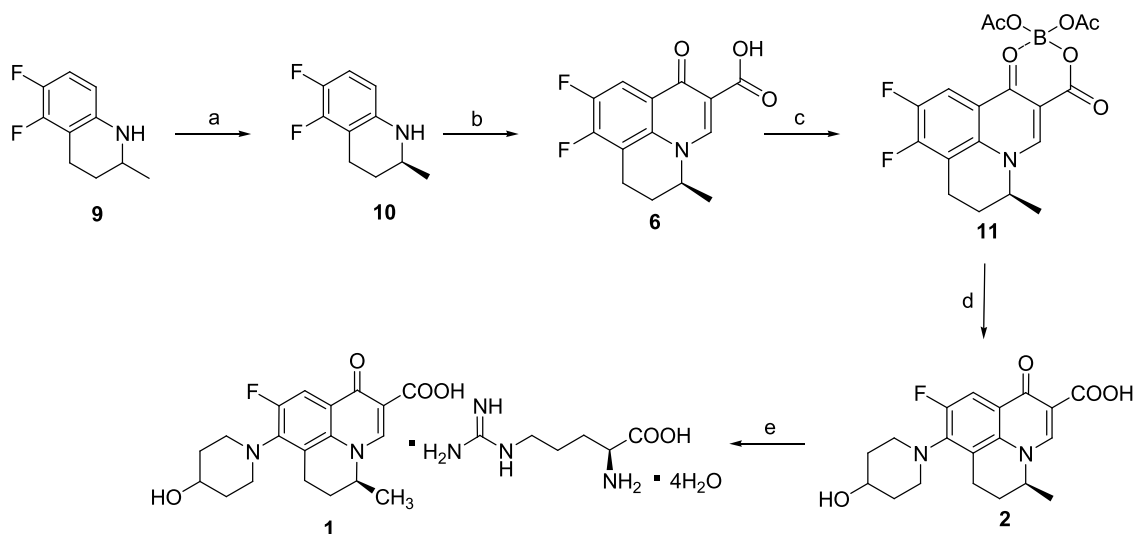


Figure 3. Chiral HPLC chromatogram of the levonadifloxacin sample spiked with R-nadifloxacin (impurity 6).

Scheme 1. Synthesis of Levonadifloxacin (1)^a



^aReagents: (a) dibenzoyl-L-tartaric acid (L-DBTA)/EtOAc, NaOH/MeOH; (b) diethyl ethoxymethylenemalonate (EMME), polyphosphoric acid (PPA), HCl/MeOH/H₂O; (c) B(OH)₃/CH₃COOH/(CH₃CO)₂O; (d) 4-hydroxypiperidine/triethylamine (TEA)/dimethyl sulfoxide (DMSO), NaOH/H₂O/MeOH; (e) L-arginine/acetone/H₂O.

HPLC method; the representative chromatogram is presented in Figure 3.

Levonadifloxacin. Levonadifloxacin is an L-arginine salt of S-nadifloxacin (2) and is synthesized from 5,6-difluoro-1,2,3,4-tetrahydro-2-methylquinoline⁹ (9) through several steps. A schematic representation of the synthesis route of levonadifloxacin is given in Scheme 1.

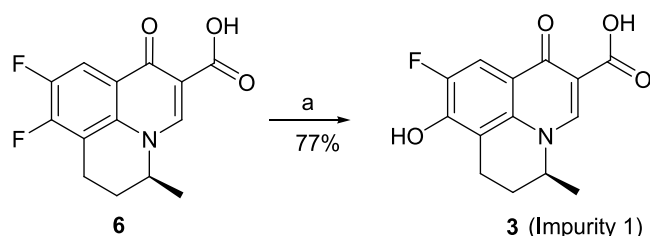
Compound 1 was fully characterized using different spectrometry analysis. The ES–MS spectrum of 1 showed a protonated molecular ion peak at m/z 361.1 ($M + H$)⁺ corresponding to the monoisotopic mass of free acid. The IR spectrum showed typical absorptions at 3550–3000 cm⁻¹ (strong band due to the N–H bending of L-arginine and O–H stretching of carboxylic acid), 2959 cm⁻¹ (C–H stretching), 1645 cm⁻¹ (N–H bending of L-arginine), 1611 cm⁻¹ (C=O stretching), 1451 cm⁻¹ (C–H bending of CH₂), and 1133 cm⁻¹ (C–F stretching). The ¹H nuclear magnetic resonance (NMR) spectrum displayed aromatic proton signals at δ 8.62 (s, 1H) and 7.88 (d, 1H), and the rest of the aliphatic signals at δ 4.64 (unresolved m, 1H), 3.79 (m, 1H), 3.49 (t, 1H), 3.40–2.85 (unresolved m, 8H), 2.25–2.04 (unresolved m, 4H),

1.92–1.60 (unresolved m, 6H), and 1.48 (d, 3H). The ¹³C NMR spectrum was assigned with the help of the DEPT experiment. Based on the above spectral data and elemental analysis, the molecular formula was confirmed as C₂₅H₄₃FN₆O₁₀ and the corresponding structure was characterized as the L-arginine salt of S-nadifloxacin, namely S-(–)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1H,5H-benzo[*i,j*]quinolizine-2-carboxylic acid L-arginine salt tetrahydrate.

Impurity 1. Impurity 1 may be formed during the transformation of compound 11 to 2. This reaction involves nucleophilic substitution by hydroxide anion, leading to formation of compound 3 as impurity 1. Aqueous sodium hydroxide was utilized to hydrolyze the boron complex (Scheme 2).

Compound 6 was treated with aqueous sodium hydroxide solution at an elevated temperature (100–110 °C) to produce compound 3.

The ES–MS spectrum of impurity 1 showed a protonated molecular ion peak at m/z 278.1 ($M + H$)⁺, indicating the molecular mass of this impurity to be 277.08, which is 83 amu

Scheme 2. Synthesis of Impurity 1^a

^aReagents: (a) aq. 1 N NaOH solution, 100 °C, 10 h.

less than that of S-nadifloxacin. The IR spectrum shows typical absorptions at 1739 cm⁻¹ (C=O acid stretching), 1626 cm⁻¹ (C=O keto stretching), 1537 cm⁻¹ (C=C aromatic stretching), and 1448 cm⁻¹ (C–H bending of CH₂). In the ¹H NMR and ¹³C NMR spectra of this compound, the protons and carbons corresponding to hydroxyl piperidine as seen in compound 1 were absent. Based on the above spectral data, the molecular formula of impurity 1 was confirmed as C₁₄H₁₂FN₂O₄ and the corresponding structure was characterized as (S)-9-fluoro-8-hydroxy-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid.

Impurity 2. Impurity 2 is a regioisomer of compound 2, which may form during the substitution reaction of compound 11 with 4-hydroxypiperidine in basic medium to give compound 4 as impurity 2 (Scheme 3).

Compound 9 was treated with EMME and cyclized using PPA followed by acid hydrolysis to afford acid derivative 12. The reaction of boron complex 11 at elevated temperature (125–130 °C) resulted in less than 1.0% formation of compound 4, which is not feasible to isolate in gram scale. Reaction of compound 12 (without the boron complex) with 4-hydroxypiperidine using TEA as the base in DMSO afforded compound 4 in ~5% yield by HPLC. Further, compound 4 was successfully prepared by direct nucleophile substitution of 12 by 4-hydroxypiperidine in hexamethylphosphoramide (HMPA) at 150 °C to give the regioisomeric mixture (~16:72%) by HPLC (Figure S37). Attempts to purify by recrystallization or column chromatography failed. Therefore, compound 4 was purified and isolated using preparative HPLC.

The ES–MS spectrum of impurity 2 showed a protonated molecular ion peak at *m/z* 361.1 (M + H)⁺, indicating the molecular mass of this impurity to be 360.15, which is a positional isomer of S-nadifloxacin. The IR spectrum shows typical absorptions at 3377 cm⁻¹ (O–H stretching), 2949 cm⁻¹ (C–H stretching), 1699 cm⁻¹ (C=O acid stretching), 1622 cm⁻¹ (C=O keto stretching), 1520 cm⁻¹ (C=C aromatic stretching), and 1466 cm⁻¹ (C–H bending of CH₂). ¹H NMR showed the characteristic hydrogen at δ

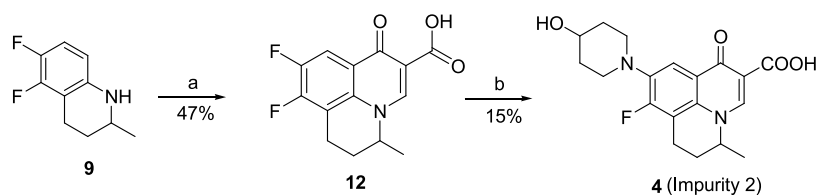
7.85–7.77 ppm as a doublet with a coupling constant of 10 Hz, indicating a long-range coupling with fluorine, whereas compound 2 showed the same hydrogen at δ 7.68–7.70 ppm as a doublet with a coupling constant of 15 Hz, indicating strong coupling with the adjacent fluorine. In the spiked HPLC spectrum, the retention time (RT) of impurity 2 is 15.78 min and that of S-nadifloxacin is 26.16 min. Based on the above data along with ¹H, ¹⁹F, 2D, and ¹³C NMR, the molecular formula of impurity 2 was confirmed as C₁₉H₂₁FN₂O₄ and the corresponding structure was characterized as (RS)-8-fluoro-6,7-dihydro-9-(4-hydroxy-1-piperidinyl)-5-methyl-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid.

Impurity 3. 3,4-Difluoroaniline is converted to 8-bromo-5,6-difluoro-2-methylquinoline,⁹ which is an intermediate for the synthesis of S-nadifloxacin.

During the palladium-catalyzed hydrogenation of 8-bromo-5,6-difluoro-2-methylquinoline, defluorination and debromination along with reduction of the pyridine ring of quinoline moiety due to the prolonged reaction time and high temperature condition lead to compound 16. This further undergoes similar reaction transformations, leading to formation of compound 5 as impurity 3 as shown in Scheme 4.

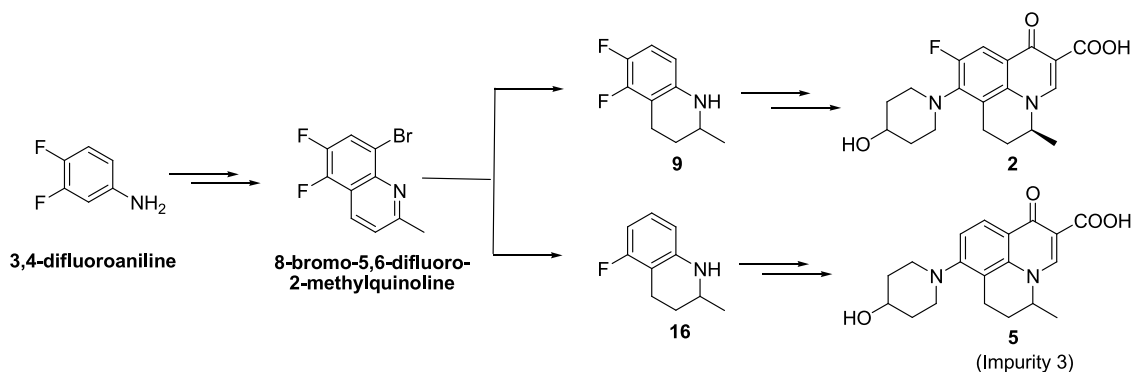
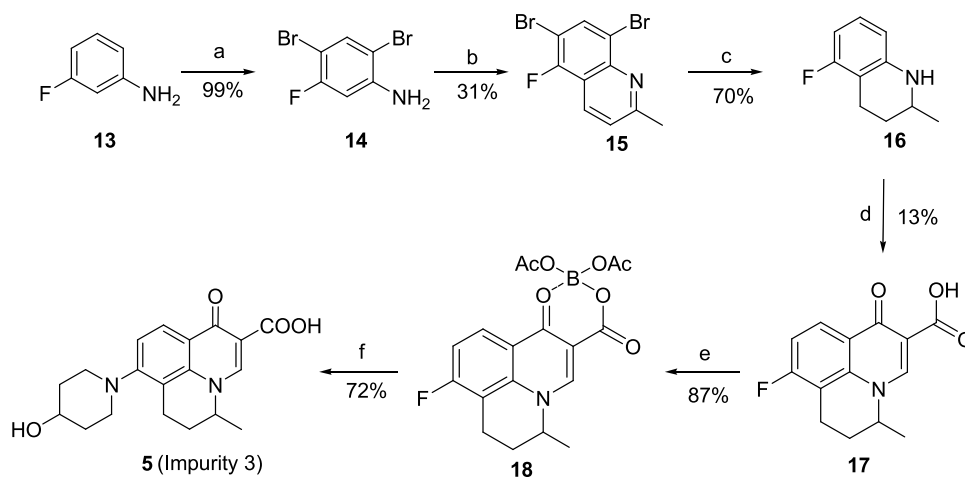
Bromination of 3-fluoroaniline (13) with liquid bromine gives compound 14. Cyclized derivative 15 is obtained by treatment with boric acid, sodium 3-nitrobenzene sulfonate, and crotonaldehyde under heating. Debromination as well as pyridine ring reduction of the quinoline derivative in a single step was achieved using 5% Pd/C under hydrogen atmosphere to furnish compound 16. Subsequent transformations were carried out using a similar procedure as utilized for synthesis of 3 via boron complex 18 to afford compound 5 (Scheme 5).

The ES–MS spectrum of impurity 3 showed a protonated molecular ion peak at *m/z* 343.2 (M + H)⁺, indicating the molecular mass of this impurity to be 342.16, which is 18 amu less than that of S-nadifloxacin, indicating the absence of fluorine atom. The IR spectrum shows typical absorptions at 3447 cm⁻¹ (O–H stretching), 2936 cm⁻¹ (C–H stretching), 1707 cm⁻¹ (C=O acid stretching), 1618 cm⁻¹ (C=O keto stretching), 1518 cm⁻¹ (C=C aromatic stretching), 1445 cm⁻¹ (C–H bending of CH₂). In the ¹H NMR spectrum of this impurity, three aromatic protons, specifically a singlet appearing at δ 8.19 ppm with one-proton integration, is evidence for the presence of hydrogen instead of fluorine, whereas S-nadifloxacin shows only two aromatic signals. The ¹⁹F NMR spectrum showed no signal, indicating the absence of fluorine atom. Based on the above data and ¹³C NMR, the molecular formula of impurity 2 was confirmed as C₁₉H₂₂N₂O₄ and the corresponding structure was characterized as (S)-9-fluoro-8-hydroxy-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid.

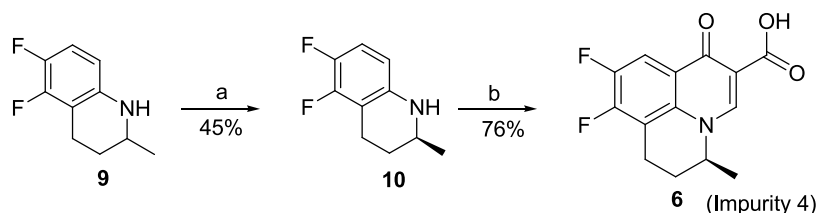
Scheme 3. Synthesis of Impurity 2^a

^aReagents: (a) EMME, 125 °C, 15 h; PPA, 125 °C, 4 h; HCl/MeOH/H₂O, 55 °C, 16 h; (b) 4-hydroxypiperidine/hexamethylphosphoramide (HMPA), 150 °C, 6 h.

Scheme 4. Proposed Pathway for Formation of Impurity 3

Scheme 5. Synthesis of Impurity 3⁴⁴

⁴⁴Reagents: (a) liq. Br₂/DCM, -10 °C, 1 h; (b) B(OH)₃/H₂O/sodium 3-nitrobenzene sulfonate/FeSO₄, 80 °C, 1 h HCl/crotonaldehyde, 95 °C, 6 h; (c) MeOH/5% Pd/C, 55 °C, 15 h; (d) EMME, 125 °C, 15 h; PPA, 125 °C, 4 h; HCl/MeOH/H₂O, 55 °C, 12 h; (e) B(OH)₃/CH₃COOH/(CH₃CO)₂O, 125 °C, 4 h then compound 17, 125 °C, 2 h; (f) 4-hydroxypiperidine/TEA/DMSO, 50 °C, 4 h; NaOH/H₂O/MeOH, 40 °C, 4 h.

Scheme 6. Synthesis of Impurity 4⁴⁴

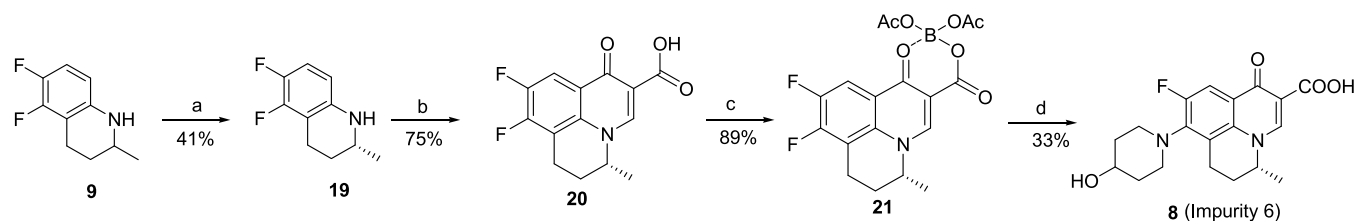
⁴⁴Reagents: (a) L-DBTA/EtOAc, 30 °C, 2 h; NaOH/MeOH, 30 °C, 1 h; (b) EMME, 125 °C, 15 h; PPA, 125 °C, 4 h; HCl/MeOH/H₂O, 55 °C, 15 h.

Impurity 4. Impurity 4 (Scheme 6) is an intermediate of compound 2 and is prepared using the experimental procedure described in the literature.⁹

Impurity 5. Impurity 5 is a degradation impurity that may be formed during the formulation of levonadifloxacin. During the autoclave sterilization process of levonadifloxacin, thermal decomposition of arginine may cause stoichiometric release of ammonia,¹⁰ which further undergoes S_NAr reaction with compound 2, or degradation of the piperazine ring, leading to the formation of impurity 5. Impurity 5 is prepared using the experimental procedure described in the literature.¹¹

The ES-MS spectrum of impurity 5 showed a protonated molecular ion peak at m/z 277.2 ($M + H$)⁺, indicating the molecular mass of this impurity to be 276.09, which is 84 amu

less than that of S-nadifloxacin. The IR spectrum shows typical absorptions at 3501 cm⁻¹ (N-H stretching), 3399 cm⁻¹ (O-H stretching), 1720 cm⁻¹ (C=O acid stretching), 1635 cm⁻¹ (C=O keto stretching), 1556 cm⁻¹ (C-H aromatic ring bending), and 1479 cm⁻¹ (C-H bending of CH₂). In the ¹H NMR spectrum of this impurity, the broad singlet signal appearing at δ 6.41 ppm with two-proton integration is evidence for the presence of the aromatic amine group, whereas the DEPT spectrum displayed two negative signals due to two methylene groups, which indicate the absence of the 4-hydroxypiperidine ring. Based on the above data, the molecular formula of impurity 5 was confirmed as C₁₄H₁₃FN₂O₃ and the corresponding structure was charac-

Scheme 7. Synthesis of Impurity 6^a

^aReagents: (a) D-DBTA/EtOAc, 30 °C, 2 h; NaOH/MeOH, 30 °C, 1 h; (b) EMME, 125 °C, 15 h; PPA, 125 °C, 4 h; HCl/MeOH/H₂O, 55 °C, 15 h; (c) B(OH)₃/CH₃COOH/(CH₃CO)₂O, 125 °C, 4 h, then compound 20, 125 °C, 2 h; (d) 4-hydroxypiperidine/TEA/DMSO, 50 °C, 2 h; NaOH/H₂O/MeOH, 40 °C, 4 h.

terized as 8-amino-9-fluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid.

Impurity 6. Impurity 6 is an enantiomer of compound 2, which is the penultimate intermediate for synthesis of compound 1. This impurity is formed due to traces of compound 20 being associated with compound 10, which subsequently undergoes a similar reaction transformation, leading to impurity 6 (Scheme 7).

Compound 9 was treated with (+)-dibenzoyl-D-tartaric acid (D-DBTA) to afford stereoselective R-isomer as 19 in ethyl acetate. This was further treated with ethoxymethylenemalononic acid diethyl ester (EMME) and cyclized using polyphosphoric acid (PPA) followed by acid hydrolysis to afford compound 20. Compound 20 was further treated with boric acid/acetic acid/anhydride to get the corresponding boron complex 21. Nucleophile substitution using 4-hydroxypiperidine in basic condition followed by treatment with aqueous sodium hydroxide furnished compound 8.

The ES-MS spectrum of impurity 6 showed a protonated molecular ion peak at m/z 361.2 ($M + H$)⁺, indicating the molecular mass of this impurity to be 360.15, which indicates the isomer of S-nadifloxacin. The IR spectrum shows typical absorptions at 3487 cm⁻¹ (O-H stretching), 2945 cm⁻¹ (C-H stretching), 1708 cm⁻¹ (C=O acid stretching), 1622 cm⁻¹ (C=O keto stretching), 1526 cm⁻¹ (C=C aromatic stretching), 1466 cm⁻¹ (C-H bending of CH₂), and 1070 cm⁻¹ (C-F stretching). Based on the above data along with ¹H, ¹⁹F, ¹³C NMR, and chiral HPLC (Figure S77), the molecular formula of impurity 6 was confirmed as C₁₉H₂₁FN₂O₄ and the corresponding structure was characterized as (R)-(+)-9-fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid.

CONCLUSIONS

A short and efficient synthesis of five process impurities and one degradation impurity using economical starting materials of the anti-MRSA agent drug levonadifloxacin (WCK 771) has been established. The synthesis of these impurities provided an access to the reference standards for analytical method development, batch release, and also for qualification through toxicity studies.

EXPERIMENTAL SECTION

(S)-(-)-9-fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid L-(+)-arginine salt tetrahydrate⁹ (1). ¹H NMR (CD₃OD, 500 MHz): δ (ppm) 8.62 (s, 1H), 7.89 (d, 1H), 4.64 (m, 1H), 3.79 (m, H), 3.49 (t, 1H), 3.40–2.85 (m,

8H), 2.25–1.60 (m, 10H), 1.43 (d, 3H). ¹⁹F NMR (CD₃OD, 376 MHz): δ (ppm) -123.550 (s). ¹³C NMR (CD₃OD, 125 MHz): δ (ppm) 177.15, 176.98, 172.17, 160.20, 158.22 (d, $J = 250$ Hz), 158.87, 147.70, 143.41, 143.32, 134.85, 127.63, 126.13, 115.02, 110.92, 110.73, 69.14, 58.55, 55.83, 50.40, 41.95, 36.52, 36.18, 30.51, 26.90, 25.77, 20.13, 19.93. ES-MS: $m/z = 361.1$ [$M + H$]⁺ (corresponding to free acid). IR (KBr, cm⁻¹): 3272, 2959, 1611, 1451, 1133. Elemental analysis calculated for C₂₅H₄₃FN₆O₁₀·4H₂O: C, 49.50; H, 7.22; N, 13.85. Found: C, 49.21; H, 7.14; N, 13.96.

(S)-(-)-9-fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid⁹ (2). ¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 15.30 (s, 1H), 8.97 (s, 1H), 7.88 (d, 1H, $J = 15$ Hz), 4.90 (m, 1H), 4.75 (s, 1H), 3.69 (s, 1H), 3.50–2.85 (m, 6H), 2.20–1.45 (m, 6H), 1.43 (d, 3H). ¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ (ppm) -120.575 (s). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 176.47, 166.07, 158.00, 156.01 (d, $J = 249$ Hz), 147.35, 142.76, 142.66, 133.60, 126.38, 121.89, 121.83, 108.95, 108.77, 106.47, 65.92, 57.07, 48.60, 35.41, 35.01, 24.87, 19.58, 18.54. ES-MS: $m/z = 361.1$ [$M + H$]⁺. IR (KBr, cm⁻¹): 3486, 2944, 1707, 1620, 1524, 1444, 1070. Elemental analysis calculated for C₁₉H₂₁FN₂O₄: C, 63.33; H, 5.83; N, 7.77. Found: C, 63.15; H, 7.77; N, 5.96.

(S)-8,9-difluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid⁹ (6). ¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 14.99 (s, 1H), 9.07 (s, 1H), 8.13 (t, 1H), 4.97 (m, 1H), 3.20–2.95 (m, 2H), 2.25–2.05 (m, 2H), 1.40 (d, 3H). ¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ (ppm) -137.941 (d, $J = 25$ MHz), -130.352 (d, $J = 25$ Hz). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 176.53, 165.66, 151.28, 151.16, 149.28, 149.16 (dd, $J = 250$ Hz, 15 Hz), 148.45, 148.21, 149.07, 148.96, 147.08 (dd, $J = 250$ Hz, 15 Hz), 146.97, 133.46, 133.40, 122.43, 122.38, 118.18, 118.02, 110.34, 110.19, 110.16, 110.01, 107.53, 57.04, 24.02, 19.66, 15.05. ES-MS: $m/z = 279.9$ [$M + H$]⁺. IR (KBr, cm⁻¹): 2964, 1728, 1623, 1474, 1067. Elemental analysis calculated for C₁₄H₁₁F₂N₃O₃: C, 60.22; H, 3.97; N, 5.02. Found: C, 60.18; H, 4.00; N, 4.88.

8-Amino-9-fluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid¹¹ (7). ¹H NMR (DMSO-*d*₆, 400 MHz): δ (ppm) 15.79 (s, 1H), 8.81 (s, 1H), 7.74 (d, 1H), 6.41 (s, 1H), 4.84 (m, 1H), 2.81–2.58 (m, 2H), 2.20–2.0 (m, 2H), 1.33 (d, 3H). ¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ (ppm) -131.837 (s). ¹³C NMR (DMSO-*d*₆, 100 MHz): δ (ppm) 176.14, 176.11, 166.62, 150.46, 148.03 (d, $J = 243$ Hz), 146.27, 140.33, 140.19, 134.09, 114.57, 114.49, 108.77, 108.73, 107.21, 107.01, 105.82, 56.50, 24.44, 19.40, 17.06. ES-MS: $m/z = 277.2$ [$M + H$]⁺. IR (KBr, cm⁻¹): 3501, 3399, 1720, 1635, 1556, 1479. Elemental analysis calculated for

C₁₄H₁₃FN₂O₃: C, 60.87; H, 4.76; N, 10.14. Found: C, 60.50; H, 4.76; N, 9.92.

(S)-9-fluoro-8-hydroxy-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (3). The mixture of compound 6 (30 g, 0.11 mol) and aqueous 1 N sodium hydroxide solution was stirred at 100 °C for 10 h. After completion of the reaction, the mixture was cooled to 10 °C and the pH was adjusted to ~5 using conc. HCl (~200 mL) to obtain a solid. The resulting solid was filtered, washed with water (250 mL), and dried at 60 °C for 6 h to afford an off-white solid (30 g). The obtained solid was suspended in dichloromethane (50 mL), filtered, and dried at 55 °C to afford compound 3 as an off-white solid (23 g, 77.2%).

¹H NMR (DMSO-*d*₆, 400 MHz): δ (ppm) 15.46 (s, 1H), 11.29 (s, 1H), 8.94 (s, 1H), 7.91 (d, 1H), 4.90 (m, 1H), 3.15–2.70 (m, 2H), 2.20–2.00 (m, 2H), 1.36 (d, 3H). ¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ (ppm) –132.969 (s). ¹³C NMR (DMSO-*d*₆, 100 MHz): δ (ppm) 176.59, 176.56, 166.28, 151.38, 148.95 (d, *J* = 243 Hz), 147.64, 147.48, 147.13, 133.96, 118.12, 118.05, 115.87, 115.84, 108.37, 108.17, 106.43, 56.84, 24.47, 19.65, 16.13. ES–MS: *m/z* = 277.9 [M + H]⁺. IR (KBr, cm⁻¹): 1739, 1626, 1537, 1448. Elemental analysis calculated for C₁₄H₁₂FNO₄: C, 60.65; H, 4.36; N, 5.05. Found: C, 54.08; H, 4.03; N, 4.35.

(RS)-8,9-difluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (12). Compound 9 (110 g, 0.60 mol) and EMME (155.6 g, 0.72 mol) were stirred at 125 °C for 15 h. After completion of the reaction, PPA (80 g) was added and stirred at 125 °C for 4 h. After completion of the reaction (checked by ES–MS–RM1), the mixture was cooled to 50 °C. Water (220 mL) and methanol (440 mL) were added sequentially into the reaction mass to obtain a solid. Then, conc. HCl (110 mL) was added and the solution was stirred at 55 °C for 16 h. After completion of the reaction, the mixture was cooled to 30 °C and water (770 mL) was added. After 1 h, the resulting solid was filtered and washed with water (550 mL) to obtain a light-brown solid (220 g). The obtained solid (220 g) and methanol (550 mL) were suspended at 45 °C for 1 h, then cooled to 25 °C, and stirred for another 1 h at the same temperature. The resulting solid was filtered, washed with methanol (100 mL), and dried at 60 °C for 6 h to obtain compound 12 as an off-white solid (56 g, 47.1% yield). ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 14.74 (s, 1H), 8.77 (s, 1H), 8.19 (t, 1H), 4.63 (m, 1H), 3.30–3.26 (m, 1H), 3.08–3.02 (m, 1H), 2.3–2.27 (m, 2H), 1.56–1.55 (d, 3H). ES–MS: *m/z* = 280.1 (M + H)⁺; HPLC: 96.87%.

(RS)-8-fluoro-6,7-dihydro-9-(4-hydroxy-1-piperidin-yl)-5-methyl-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (4). To a solution of compound 12 (50 g, 0.18 mol) in hexamethylphosphoramide (HMPA, 200 mL) was added 4-hydroxy-piperidine (77.8 g, 0.77 mol) under stirring at 25–35 °C. Then, the reaction mixture was stirred at 150 °C for 6 h. The reaction progress was monitored by HPLC (RM1). After completion of the reaction, the mixture was cooled to 30 °C and water (600 mL) was added to obtain a solid. Conc. HCl solution (~50 mL) was added slowly until pH ~3–4 and extracted with ethyl acetate (3 × 600 mL). The combined organic phases were washed with water (500 mL) and evaporated under reduced pressure to obtain a crude residue (57 g). This was further purified using preparative HPLC to afford compound 4 as an off-white solid (10.0 g, 15.4% yield).

¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 15.46 (s, 1H), 8.94 (s, 1H), 7.70–7.68 (d, 1H, *J* = 10 Hz), 4.90 (m, 1H), 4.75 (d, 1H), 3.68 (m, 1H), 3.67–3.34 (m, 2H), 3.14–2.82 (m, 4H), 2.22–2.04 (m, 2H), 1.96–1.50 (m, 4H), 1.39 (d, 3H). ¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ (ppm) –114.540 (s). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 176.44, 166.22, 156.40, 154.39 (d, *J* = 251 Hz), 146.30, 139.48, 139.39, 131.67, 131.60, 122.19, 115.44, 111.27, 107.03, 65.37, 56.66, 48.14, 48.06, 34.17, 24.38, 19.68, 14.86. ES–MS: *m/z* = 361.2 [M + H]⁺. IR (KBr, cm⁻¹): 3377, 2949, 1699, 1622, 1520, 1466. Elemental analysis calculated for C₁₉H₂₁FN₂O₄: C, 63.32; H, 5.87; N, 7.77. Found: C, 63.19; H, 5.90; N, 7.61.

2,4-Dibromo-5-fluoroaniline (14). To a stirred solution of 3-fluoroaniline (compound 13) (100 g, 0.90 mol) in DCM (1000 mL) was added potassium carbonate (261 g, 1.89 mol), and the reaction mixture was cooled to –10 °C. The liquid bromine (302 g, 1.89 mol) was added slowly under stirring at –10 to –5 °C, and stirring was continued for 1 h at the same temperature. After completion of the starting material, water (1000 mL) was added and stirred for 0.5 h. The organic layer was separated and evaporated under reduced pressure to obtain compound 14 as a light-brown liquid (240.0 g, 99.1% yield). ES–MS: *m/z* = 267.7 (M – H)⁻.

6,8-Dibromo-5-fluoro-2-methylquinoline (15). A mixture of boric acid (185.0 g, 2.99 mol) and water (818 mL) under stirring was heated to 80 °C. Sodium 3-nitrobenzenesulfonate (148.0 g, 2.26 mol) and iron sulfate heptahydrate (13.1 g, 0.005 mol) were added and stirred for 1 h at 80 °C. The reaction mixture was further heated to 90 °C and compound 14 (240 g, 0.89 mol) was slowly added followed by conc. HCl (720 mL). Crotonaldehyde (84.0 g, 1.18 mol) was added dropwise to the mixture at the same temperature and stirring was continued for 6 h at 95 °C. After completion of the reaction, the mixture was cooled and water (720 mL) was added at 35 °C. The insoluble matter was filtered and the pH was adjusted to ~7 using aq. 25% NaOH solution (~1000 mL). The resultant precipitates were collected by filtration; the obtained wet solid was dissolved in DCM (300 mL), dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure and recrystallized from *n*-hexanes to give compound 15 as an off-white solid (90.0 g, 31.6% yield). ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 8.30–8.28 (m, 1H), 8.13 (d, 1H), 7.43 (d, 1H), 2.80 (s, 3H); ES–MS: *m/z* = 339.9 (M + H)⁺.

5-Fluoro-1,2,3,4-tetrahydro-2-methylquinoline (16). A mixture of compound 15 (40 g, 0.13 mol) and 5% Pd/C (4.0 g, 50% wet) in methanol (400 mL) was hydrogenated at 55 °C under a hydrogen pressure of 5–6 atm for 15 h. After completion of the reaction, the catalyst was filtered and the filtrate was concentrated at a reduced pressure to afford a brownish residue. Hundred milliliters of water was poured and the residue was extracted with ethyl acetate (2 × 100 mL). The combined organic layers were washed with saturated aqueous NaHCO₃ solution (100 mL) and evaporated under reduced pressure to afford compound 16 as a brown liquid (14.5 g, 69.9% yield). ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 6.89–6.86 (m, 1H), 6.34–6.30 (m, 1H), 6.25–6.23 (m, 1H), 3.80–3.6 (bs, 1H), 3.37–3.35 (m, 1H), 2.86–2.82 (m, 1H), 2.66–2.63 (m, 1H), 1.97–1.95 (m, 1H), 1.55–1.53 (m, 1H), 1.19–1.22 (m, 3H); ES–MS: *m/z* = 165.8 (M + H)⁺.

(RS)-8-fluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (17). The mixture of compound 16 (32 g, 0.19 mol) and EMME (54

g, 0.25 mol) was stirred at 125 °C for 15 h. After completion of the reaction (checked by ES–MS–RM1, 336.0 M + H), PPA (19 g) was added and stirred at 125 °C for further 4 h. After complete consumption of the starting material (checked by ES–MS–RM2, 290.0, M + H), the mixture was cooled to 50 °C. Water (70 mL) and methanol (140 mL) were added sequentially into the reaction mixture to obtain a suspension. Then, conc. HCl (35 mL) was added and the solution was stirred at 55 °C for 12 h. After completion of the reaction, water (230 mL) was added at 25 °C and stirred for 1 h at same temperature. The resulting solid was filtered and washed with water (100 mL) to yield compound 17 as a light-brown solid (12 g). Then the obtained solid (12 g) was suspended in methanol (60 mL) at 55 °C for 1 h and cooled to 25 °C and stirred for another 1 h. The resulting solid was filtered, washed with methanol (15 mL), and dried at 60 °C for 6 h to obtain compound 17 as an off-white solid (8.5 g, 13.5% yield). ¹H NMR (DMSO-*d*₆, 400 MHz): δ (ppm) 15.19 (bs, 1H), 9.05 (s, 1H), 8.30–8.26 (m, 1H), 7.54–7.50 (m, 1H), 4.96–4.93 (m, 1H), 3.09–2.90 (m, 2H), 2.16–2.14 (m, 2H), 1.40 (d, 3H); ES–MS: *m/z* = 261.9 (M + H)⁺.

(RS)-(O-B)-diacetoxy-(8-fluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxy)borane (18). The mixture of acetic acid (6.6 g, 0.11 mol), acetic anhydride (21 g, 0.70 mol), and boric acid (2.1 g, 0.033 mol) was stirred at 125 °C for 4 h. Then, compound 17 (8.5 g, 0.032 mol) was added and stirred for another 2 h at 125 °C. After completion of the reaction, water (85 mL) was added at 35 °C to obtain a suspension. After 1 h, the resulting solid was filtered, washed with water (40 mL), and dried at 60 °C for 6 h to obtain compound 18 as a light-brown solid (11 g, 86.8% yield). ES–MS: *m/z* = 388.1 (M – H)[–].

(RS)-8-(4-hydroxy-1-piperidinyl)-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (5). Part A: To a solution of compound 18 (11 g, 0.029 mol) in DMSO (22 mL) was added TEA (3.2 g, 0.031 mol) under stirring at 25 °C. Then 4-hydroxy-piperidine (3.2 g, 0.031 mol) was added and the reaction mass was stirred at 50 °C for 4 h. After completion of the reaction (checked by ES–MS–RM1; 469.3 (M – H)[–]), water (176 mL) was added at 30 °C. After 1 h, the resulting solid was filtered and washed with water (55 mL) to obtain the boron complex of compound 5 as a light-brown solid (15 g). Part B: The mixture of obtained solid (15 g), 5% aq. NaOH solution (84 mL), and methanol (36 mL) was heated to 40 °C to get a clear solution and stirred for 4 h. After completion of the reaction, water (24 mL) was added and the resulting suspension was cooled to 30 °C. Conc. HCl solution (~11 mL) was added slowly until pH ~5–6 and stirred for 1 h at the same temperature. The resulting solid was filtered and washed with water (33 mL) to obtain compound 5 as an off-white solid (12 g). The obtained solid (12 g) and methanol (48 mL) were stirred at 55 °C for 1 h. Then, the clear solution was slowly cooled to 30 °C and stirred for 1 h at the same temperature. The resulting solid was filtered, washed with methanol (15 mL), and dried at 60 °C for 6 h to obtain compound 5 as an off-white solid (7 g, 72.3% yield). ¹H NMR (DMSO-*d*₆, 400 MHz): δ (ppm) 15.57 (s, 1H), 8.93 (s, 1H), 8.19 (d, 1H), 7.38 (d, 1H), 4.86 (m, 1H), 4.75 (d, 1H), 3.70 (m, 1H), 3.42–2.60 (m, 6H), 2.18–1.49 (m, 6H), 1.45 (d, 3H). ¹³C NMR (DMSO-*d*₆, 100 MHz): δ (ppm) 177.31, 166.37, 155.34, 147.66, 136.93, 124.27, 120.06, 119.61, 118.09, 106.41, 65.60, 56.91, 49.05, 48.55, 34.76, 34.57, 24.98, 19.64, 17.92. ES–MS: *m/z* = 343.3 [M + H]⁺. IR (KBr, cm^{–1}):

3433, 2945, 1711, 1615, 1445. Elemental analysis calculated for C₁₉H₂₂N₂O₄: C, 66.65; H, 6.48; N, 8.18. Found: C, 66.69; H, 6.43; N, 8.14.

(R)-5, 6-difluoro-1,2,3,4-tetrahydro-2-methylquinoline (19). To a stirred solution of compound 9 (200 g, 1.09 mol) in ethyl acetate (800 mL) was added (+)-dibenzoyl-D-tartaric acid (215 g, D-DBTA) at 30 °C. The reaction mixture was stirred for 2 h and the resulting solid was filtered to obtain a diastereomeric salt of R-enantiomer as an off-white solid (300 g). The obtained wet solid (300 g) and methanol (1200 mL) were heated to 60 °C to get a clear solution. Water (800 mL) was slowly added to the reaction mass at 55–60 °C, and the resultant suspension was stirred for 1 h at the same temperature. The reaction mass was slowly cooled to 25–35 °C and stirred for 1 h. The resulting solid was filtered to afford a pure diastereomeric salt of R-enantiomer as an off-white solid (280 g). This solid (280 g) was suspended in water (1000 mL) at 30 °C and 20% aq. NaOH solution (~200 mL) was added slowly until pH ~9 and stirred for 1 h. The reaction mixture was extracted with DCM (2 × 600 mL). The combined organic layer was evaporated under reduced pressure to obtain compound 19 as a light-brown liquid (82 g, 41% yield). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 6.78–6.73 (m, 1H), 6.15–6.12 (m, 1H), 3.65 (bs, 1H), 3.33–3.32 (m, 1H), 2.87–2.84 (m, 1H), 2.67–2.65 (m, 1H), 1.97–1.93 (m, 1H), 1.52–1.50 (m, 1H), 1.21–1.19 (m, 3H); ES–MS: *m/z* = 184.0 (M + H)⁺; SOR [α]_D²⁰: + 60.5 (c 1%, MeOH).

(R)-8,9-difluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (20). Compound 19 (100 g, 0.55 mol) and EMME (153 g, 0.71 mol) were stirred at 125 °C for 15 h. After completion of the reaction, PPA (60 g) was added and the solution was stirred at 125 °C for 4 h. After complete consumption of the starting material (checked by ES–MS–RM1, 354.1 (M + H)⁺), the mixture was cooled to 50 °C. Water (200 mL) and methanol (400 mL) were added to the reaction mass to obtain a suspension. Then conc. HCl (100 mL) was added and the clear solution was stirred at 55 °C for 15 h. After completion of the reaction (checked by ES–MS–RM2, 308.0 (M + H)⁺), the mixture was cooled to 30 °C and water (700 mL) was added to get a solid suspension. The resulting solid was filtered after 1 h to obtain crude compound 13 as a light-brown solid (195 g). The crude compound 20 (195 g) was suspended in methanol (500 mL) at 55 °C for 1 h. After completion, the mixture was cooled to 30 °C and stirred for 1 h. The resulting solid was filtered, washed with methanol (100 mL), and dried at 60 °C for 6 h to obtain compound 20 as an off-white solid (115 g, 75.4% yield). ¹H NMR (CDCl₃, 400 MHz): δ (ppm) 14.71 (s, 1H), 8.75 (s, 1H), 8.17 (t, 1H), 4.63–4.60 (m, 1H), 3.28–3.24 (m, 1H), 3.06–3.00 (m, 1H), 2.28–2.25 (m, 2H), 1.54–1.53 (d, 3H); ES–MS: *m/z* = 280.1 (M + H)⁺.

(R)-(O-B)-diacetoxy-(8,9-difluoro-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxy)borane (21). To a stirred solution of acetic acid (84 g, 1.41 mol) and acetic anhydride (258 g, 2.53 mol) was added boric acid (26.5 g, 0.43 mol), and the mixture was heated to 125 °C for 4 h. Compound 20 (115 g, 0.42 mol) was added and stirred for 2 h at 125 °C. After completion of the reaction, the mixture was slowly cooled to 35 °C and water (1150 mL) was added to obtain a solid. The reaction mass was stirred for 1 h at 30 °C. The resulting solid was filtered, washed with water (575 mL), and dried at 60 °C for 6 h to obtain compound 21 as a light-brown solid (150 g, 89.4% yield). ¹H NMR (CDCl₃, 400

MHz): δ (ppm) 9.23 (s, 1H), 8.25–8.21 (m, 1H), 5.03–5.00 (m, 1H), 3.36–3.32 (m, 1H), 3.15–3.09 (m, 1H), 2.36–2.34 (m, 2H), 2.06 (s, 3H), 2.00 (s, 3H), 1.64–1.63 (d, 3H); ES–MS: $m/z = 406.2$ (M – H)[–].

(R)-(+)-9-fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-6,7-dihydro-1-oxo-1H,5H-benzo[*i,j*]-quinolizine-2-carboxylic acid (8). Part A: To a solution of compound **21** (120 g, 0.29 mol) in DMSO (240 mL) was added TEA (32.5 g, 0.32 mol) under stirring at 25 °C. The 4-hydroxy-piperidine (32.5 g, 0.32 mol) was added and the reaction mixture was stirred at 50 °C for 2 h. After completion of the reaction, the mixture was cooled to 30 °C and water (1920 mL) was added to obtain a solid. After 1 h, the resulting solid was filtered to obtain a boron complex of compound **8** as a light-brown solid (150 g). Part B: The solid obtained in the previous part (150 g), 5% aq. NaOH solution (840 mL), and methanol (360 mL) were heated to 40 °C under stirring to get a clear solution and stirred for 4 h. After completion of the reaction, water (240 mL) was added and cooled to 30 °C. Conc. HCl solution (~100 mL) was added slowly until pH ~5–6 and resultant suspension was stirred for 1 h at 25 °C. The resulting solid was filtered and washed with water (480 mL) to obtain an off-white solid (180 g). The obtained solid (180 g) and methanol (480 mL) were stirred at 65 °C for 1 h to get clear solution. Then clear solution was slowly cooled to 15 °C and stirred for 1 h. The resulting solid was filtered, washed with chilled methanol (120 mL), and dried at 60 °C for 6 h to obtain compound **8** as an off-white solid (35 g, 32.9% yield). ¹H NMR (DMSO-*d*₆, 500 MHz): δ (ppm) 15.29 (s, 1H), 8.95 (s, 1H), 7.83 (d, 1H), 4.89 (m, 1H), 4.74 (d, 1H), 3.69 (s, 1H), 3.45–2.85 (m, 6H), 2.20–1.45 (m, 6H), 1.42 (d, 3H). ¹⁹F NMR (DMSO-*d*₆, 376 MHz): δ (ppm) –120.581 (s). ¹³C NMR (DMSO-*d*₆, 125 MHz): δ (ppm) 176.50, 176.48, 166.07, 158.01, 156.02 (d, *J* = 249 Hz), 147.48, 147.22, 142.77, 142.66, 133.61, 126.38, 121.89, 121.83, 108.99, 108.91, 108.80, 108.72, 106.47, 66.10, 57.07, 48.58, 35.42, 35.00, 24.87, 19.57, 18.54. ES–MS: $m/z = 361.1$ [M + H]⁺. IR (KBr, cm^{–1}): 3487, 2969, 1707, 1620, 1524, 1444, 1070. Elemental analysis calculated for C₁₉H₂₁FN₂O₄: C, 63.33; H, 5.83; N, 7.77. Found: C, 63.74; H, 5.92; N, 7.80.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c04639>.

¹H NMR, ¹³C NMR, ¹⁹F NMR, IR and ES–MS spectra; HPLC chromatograms of intermediates and the final products (PDF)

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The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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