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Synthesis and μ-Opioid Activity of the Primary Metabolites of Carfentanil

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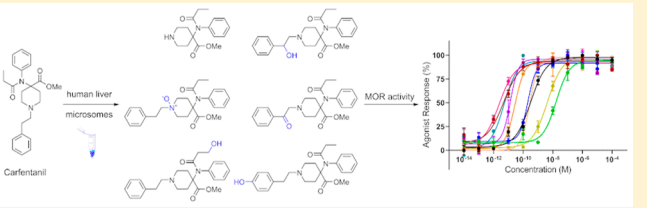
Supporting Information

ABSTRACT: Carfentanil is a synthetic opioid significantly more potent than clinically prescribed fentanyl. The primary metabolites of carfentanil, generated from human liver microsomes, were structurally confirmed through chemical synthesis. The synthesized compounds were evaluated for μ-opioid receptor (MOR) functional activity. Of the six metabolites assayed, a major metabolite showed comparable activity to the parent opioid. Three other metabolites showed significant MOR functional activity. The availability of the metabolites could aid improvements in the analysis of biomedical samples obtained from suspected human exposures to carfentanil and development of treatment protocols.



KEYWORDS: *Carfentanil, μ-opioid receptor, metabolite, chemical synthesis*

Carfentanil is a potent synthetic opioid developed by Janssen Pharmaceuticals.[1,2] It is approved for veterinarian use as a large animal sedative but is severely restricted for human sedation due to its extremely high potency and low therapeutic index.[3] Limited human *in vivo* pharmacological data has been reported.[4] Illicit carfentanil use has been increasing worldwide and many unintentional overdose deaths have been documented.[5] Furthermore, British researchers confirmed the fatal use of aerosolized carfentanil and remifentanil by Russian Defense Forces to resolve a 2002 terrorist siege.[6]

Carfentanil selectively binds to the μ-opioid receptor (MOR).[7] Treatment for exposure utilizes a nonselective, competitive opioid receptor antagonist such as naloxone.[8,9] The serum half-life of naloxone is generally less than that of known opioids. Postnaloxone renarcotization has been documented in carfentanil sedated large animals.[10−12] Nonlinear serum accumulation of the drug, documented in rats,[13] combined with the reported 5.7 h human serum half-life[4] could contribute to carfentanil's prolonged toxidromic effects along with any MOR-active metabolites.

Metabolism of carfentanil by human liver microsomes was reported in 2016.[14] The authors proposed chemical structures for the metabolites using mass spectral data and computational methods. The proposed primary metabolite structures were **M1** and **M3**−**M8** (Figure 1). The present work reports the confirmation of the primary metabolite structures through chemical synthesis in conjunction with metabolic screening. The synthesized primary metabolites were then evaluated for MOR functional activity.

Prior to the initiation of synthesis, analysis of the proposed structures and their mass spectral data was conducted. **M1** and **M3**−**M6** were considered both structurally and synthetically viable, whereas **M7** and **M8** were deemed problematic.

For **M7**, it was reasoned that the product resulting from hydroxylation alpha to the nitrogen atom was likely to be unstable due to the presence of a hemiaminal. The alternative hydroxylation site, alpha to the quaternary amino-ester carbon atom, would be sterically congested. Given these factors, **M2** was targeted for synthesis instead of **M7**. **M2** would result from a sterically more favorable hydroxylation, avoid hemiaminal formation, and would be consistent with the reported mass spectral data, except for a single fragment assigned as an unmodified phenylethyl group. **M2** is synthetically more accessible than **M7** and its synthesis has been reported.[2]

Three structural isomers of **M6** were targeted for synthesis to determine which isomer was generated by the liver microsomes.

M8 contains an oxidized amide nitrogen atom. Metabolically, this transformation is unlikely. Synthetically, selective amide oxidation would be very difficult in the presence of the piperidine nitrogen; therefore, **M8** was not targeted for synthesis.

Synthesis of **M4** and **M2** metabolites began with **M1** (Scheme 1). This intermediate was employed in Janssen's original synthesis of carfentanil.[2] Reported improvements to the Janssen methodology were used herein to produce **M1** as an intermediate in metabolite production.[15]

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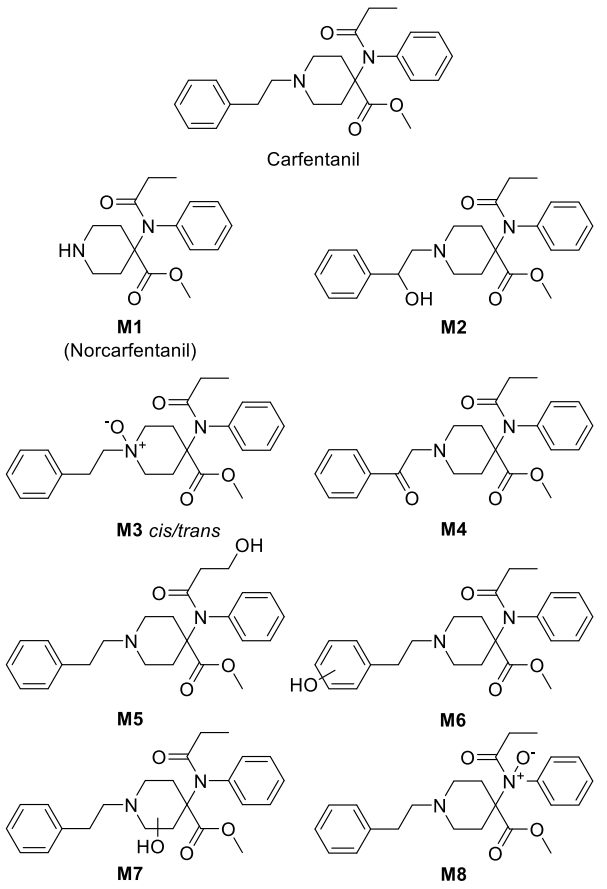
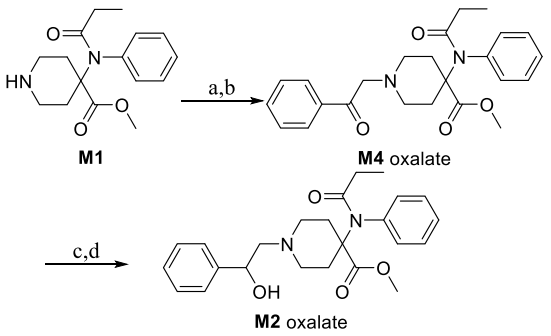


Figure 1. Structures of carfentanil and proposed primary metabolites.

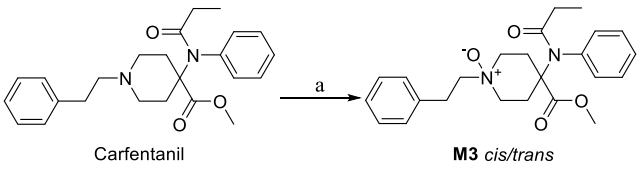
Scheme 1[a]



[a](a) Reagents and conditions: 2-bromoacetophenone, K_2CO_3, methylethyl ketone; (b) oxalic acid, isopropanol; (c) $NaBH_4$, methanol. (d) oxalic acid, isopropanol.

M1 was alkylated with 2-bromoacetophenone to provide **M4**. The ketone was reduced using sodium borohydride to generate **M2** as a racemic mixture. **M3** *cis/trans* was synthesized by oxidation of carfentanil using basic hydrogen peroxide (Scheme 2). The starting carfentanil oxalate was synthesized as reported.[15] The isomers were chromatographically separated and characterized. Unfortunately, the chromatographically separated neat isomers proved to be unstable. It is known that some amine oxides decompose on standing at room temperature.[16] Although decomposition of the **M3** isomers was not studied, usual decomposition products of such amine oxides are a secondary amine and an aldehyde. The possibility that this decomposition pathway might occur

Scheme 2[a]

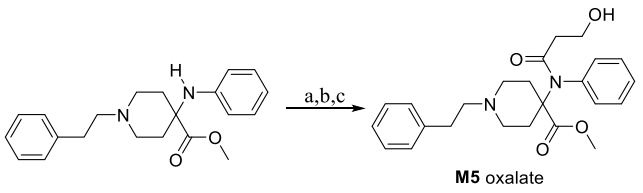


[a]Reagents and conditions: (a) K_2CO_3, H_2O_2, acetonitrile, H_2O.

in carfentanil metabolism to yield **M1** and phenylacetaldehyde cannot be discounted at the present time.

M5 was synthesized by acylation of the secondary amine with acrylolyl chloride followed by a Michael-type hydroxide addition (Scheme 3). The starting amine was prepared as reported.[17]

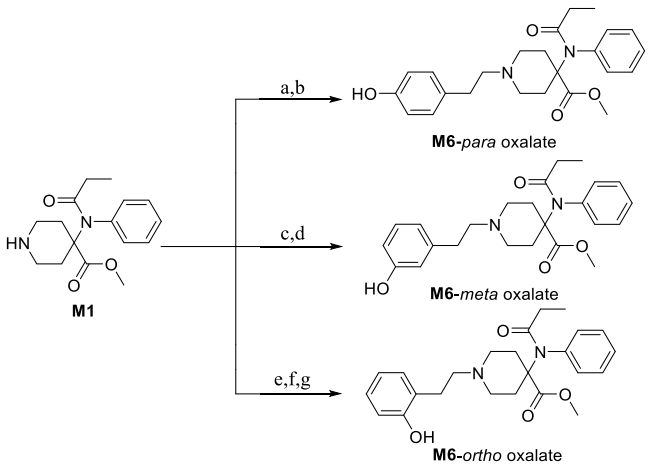
Scheme 3[a]



[a]Reagents and conditions: (a) acryolyl chloride, 1,2-dichloroethane; (b) 2 M NaOH, *t*-butanol; (c) oxalic acid, isopropanol.

Synthesis of three **M6** structural isomers is shown in Scheme 4. 2-(4-Hydroxyphenyl)ethyl bromide was commercially

Scheme 4[a]



[a]Reagents and conditions: (a) 2-(4-hydroxyphenyl)ethyl bromide, K_2CO_3, 4-methyl-2-pentanone; (b) oxalic acid, methanol; (c) 2-(3-hydroxyphenyl)ethyl bromide, K_2CO_3, 4-methyl-2-pentanone; (d) oxalic acid, isopropanol; (e) 2-(2-THPO-phenyl)ethyl bromide, K_2CO_3, 4-methyl-2-pentanone; (f) *p*-toluenesulfonic acid(H_2O), methanol; (g) oxalic acid, isopropanol.

available. 2-(3-Hydroxy-phenyl)ethyl bromide was synthesized as previously reported.[18] Both were used to alkylate **M1** without protection of the phenolic hydroxyl group. Generation of **M6**-*ortho* required the use of a phenolic protecting group during amine alkylation. Tetrahydropyranyl (THP) protected 2-(2-hydroxyphenyl)ethyl) bromide was used as the alkylating

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reagent and was synthesized from commercially available 2-(2-methoxyphenyl)ethanol.[19]

In the current study, the metabolism of carfentanil was accomplished and the structures of the primary metabolites **M1**−**M6** were confirmed by comparison with the synthesized compounds (metabolic methods and data are available in the Supporting Information). **M6**-*para* was the only phenolic isomer detected.

The order of abundance for the primary metabolites in the previous report was as follows: **M7** > **M1** > **M4** > **M5** > **M6** > **M3** > **M8** as determined by mass spectral peak intensity utilizing human hepatocytes. Proposed structure **M7** was the most abundant metabolite after 1, 4, and 6 h. In our hands, employing human liver microsomes, the order of abundance was **M1** (40.0%), **M2** (12.0%), **M3** (2.5%), **M4** (1.9%), **M5** (<1.0%), and **M6** (<1.0%) after 2 h. The order of HPLC elution, in both studies, was **M1**, **M5**, **M6**, (**M7**/**M2**), **M4**, and (**M3**/**M8**). On the basis of mass spectral fragmentation data, relative abundance, and elution order, it is reasonable to conclude that proposed structures **M7** and **M8** correspond to structures **M2** and an isomer of **M3**, respectively. In the present work, an unconfirmed primary metabolite was observed which did not correspond to any synthetic compound. This metabolite had a relative abundance below 1%, a MW = 411.22773, and may correspond to proposed structure **M7**.

The pharmacological assay evaluated the potency of reference compound Ala^2-MePhe^4-Glyol^5-Enkephalin (DAMGO), carfentanil, **M1**−**M5**, and all **M6** isomers with respect to their ability to elicit a second messenger cyclic adenosine monophosphate (cAMP) signal via agonism of the MOR. (MOR assay methods and concentration−response curves are shown in Supporting Information.) Median effective concentration (EC_{50}) values and relative potency are shown in Table 1. The confirmed metabolites are listed in decreasing order of metabolic abundance.

Table 1. Calculated EC_{50} Values Observed Using the Lance *Ultra* cAMP Assay[a]

compound	EC_{50} (nM)	±SEM	rel. potency
fentanyl	0.51	0.041	*1/100*
carfentanil	0.0049	0.0016	1
M1	17	4.1	1/3400
M2	0.0051	0.0011	1
M3 *cis/trans*	4.4	0.73	1/900
M4	0.20	0.052	1/42
M5	0.28	0.026	1/56
M6-*ortho*	0.0024	0.00064	2
M6-*meta*	0.014	0.0027	1/3
M6-*para*	0.028	0.0061	1/6

[a]Relative potency to carfentanil is provided for reference. EC_{50} and standard error of mean (SEM) values expressed as nM.

The most abundant metabolite in this work, **M1**, showed very little MOR agonism, while **M2** showed comparable activity to carfentanil. All confirmed metabolites, except **M1** and **M3**, are more active than the clinically prescribed MOR binding fentanyl. Interestingly, **M2** was found to have a median effective dose 1.67 times less potent than carfentanil in a rat tail withdrawal test.[2] **M6**-*ortho* was the most active compound tested but was not a confirmed liver microsomal metabolite. It is speculated that the relatively similar location of the hydroxyl groups in **M2** and **M6**-*ortho* might result in a favorable hydrogen bonding interaction with the receptor that could account for their higher binding affinities.

The primary metabolites of carfentanil have been synthesized and evaluated for MOR functional activity. The synthesized metabolites could aid in the analysis of human biomedical samples from subjects with suspected carfentanil exposure and in the development of treatment protocols. While there exist a wide range of parameters which can influence biological effects of psychotropic drug metabolism,[20] the MOR data presented indicate that four of the synthesized metabolites could prolong the already potent analgesic effects of carfentanil, particularly the major active metabolite **M2**.

EXPERIMENTAL PROCEDURES

Synthetic starting materials were purchased from Aldrich Chemical Co. (Milwaukee, WI). Flash chromatography was performed on a Grace Reveleris X2 semiautomatic purification system. NMR data were obtained on a JEOL 400 MHz spectrometer, and chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane. UHPLC-HRMS was performed on a Thermo-Fisher Scientific Ultimate 3000 HPLC system coupled to an Orbitrap Fusion Tribrid mass spectrometer.

Chemical Synthesis. ***M4* Oxalate.** **M1** (1.23 g, 4.24 mmol), 2-bromoacetophenone (0.93 g, 4.66 mmol), and potassium carbonate (5.86 g, 42.40 mmol) were stirred with 20 mL of methylethyl ketone. The mixture was heated at reflux for 16 h, allowed to cool to room temperature, and filtered. The filtrate was concentrated under reduced pressure. The residue was flash chromatographed eluting with 0% to 5% methanol in dichloromethane. This provided 1.90 g of the free base as a tan oil. Oxalic acid dihydrate (0.64 g. 5.08 mmol) was dissolved in a minimum of hot isopropanol and added to the free base dissolved in minimal hot isopropanol. The mixture was allowed to cool and refrigerated for 16 h. The precipitated salt was filtered and dried to provide 1.30 g of **M4** oxalate in a 65% yield. Further purification was accomplished by recrystallization from methanol. mp 206−209 °C; ^{1}H-NMR (400 MHz, methanol-d_4) δ 7.98−7.96 (m, 2H), 7.69 (m, 1H), 7.58−7.50 (m, 5H), 7.45−7.43 (m, 2H), 4.86−4.84 (m, 2H), 3.82 (s, 3H), 3.48−3.42 (m, 4H), 2.53 (br d, J = 14.2 Hz, 2H), 2.09−2.01 (m, 2H), 1.94 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.6 Hz, 3H); ^{13}C-NMR (101 MHz, methanol-d_4) δ 190.9, 175.5, 172.6, 165.1, 138.2, 134.6, 133.7, 130.3, 129.8, 129.4, 128.8, 128.0, 60.3, 51.9, 50.5, 50.4, 30.2, 28.5, 8.1; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{29}N_2O_4$ $[M + H]^+$ 409.21288, found 409.21218.

***M2* Oxalate.** **M4** oxalate (0.82 g, 1.64 mmol) was stirred with 50 mL of saturated aqueous sodium bicarbonate and 25 mL of chloroform for 1 h at room temperature. The organic layer was separated, and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried with sodium sulfate and filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in 50 mL of methanol and cooled in an ice water bath. Sodium borohydride (0.13 g, 3.28 mmol) was added in small portions. After the addition, the ice bath was removed, and the reaction mixture was stirred for 6 h. Acetic acid (0.50 mL) was added, and the mixture was stirred for 16 h, and then concentrated under reduced pressure. The residue was taken up in chloroform and saturated aqueous sodium bicarbonate solution. The organic layer was separated, and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried with sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The residue was purified using flash chromatography eluting with 0% to 4% methanol in dichloromethane. This provided 0.63 g of the free base as a tan oil. The oxalate salt was prepared as described for **M4** oxalate. The precipitate was filtered and dried to provide 0.65 g of a white solid in a 79% yield from **M1**. mp 189−191 °C; ^{1}H-NMR (400 MHz, methanol-d_4) δ 7.55−7.27 (m, 10H), 5.04 (t, J = 6.9 Hz, 1H), 3.81 (s, 3H), 3.62−3.54 (m, 2H), 3.44−3.38 (m, 2H), 3.20 (d, J = 6.9 Hz, 2H), 2.50 (td, J = 17.6, 2.9

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Hz, 2H), 2.08−1.91 (m, 4H), 0.93 (t, J = 7.3 Hz, 3H); ^{13}C-NMR (101 MHz, methanol-d_4) δ 175.5, 172.6, 165.4, 140.8, 138.2, 130.3, 130.2, 129.7, 129.4, 128.4, 128.1, 125.8, 67.4, 62.3, 60.4, 51.9, 48.7, 30.2, 30.0, 28.5, 8.1; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{31}N_2O_4$ $[M + H]^+$ 411.22783, found 411.22826.

***M3** cis/trans.* Carfentanil oxalate (0.27 g, 0.55 mmol) and potassium carbonate (0.08 g, 0.60 mmol) were dissolved in 2 mL of 1:1 v/v water/acetonitrile and stirred at room temperature for 30 min. Acetonitrile (4.5 mL) and hydrogen peroxide (1 mL, 30% aq.) were added, and the solution was heated to 60 °C for 3 h. Upon consumption of the starting material, the solution was allowed to cool to room temperature and mixed with brine and dichloromethane. The organic layer was separated, and the aqueous solution was extracted two times with dichloromethane. The combined organic extracts were dried with sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The oil was triturated with diethyl ether to afford 0.28 g of a pale yellow solid. The isomers were separated using flash chromatography eluting with 0% to 40% methanol in ethyl acetate. **M3** *trans* was eluted first as the major product, followed by **M3** *cis*. (The isolated isomers were not stable, which necessitated the use of the crude **M3** *cis/trans* mixture for receptor assays whose purity was determined to be 80% by ^{1}H NMR using *p*-xylene as an internal standard.)

M3 *trans* ^{1}H-NMR (400 MHz, chloroform-d) δ 7.34−7.39 (m, 3H), 7.20−7.28 (m, 4H), 7.13−7.16 (m, 3H), 3.73 (s, 3H), 3.47 (t, J = 11.4 Hz, 2H), 3.28−3.32 (m, 3H), 3.10−3.17 (m, 4H), 2.50 (td, J = 13.6, 3.9 Hz, 2H), 2.14 (d, J = 13.6 Hz, 2H), 1.86 (q, J = 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C-NMR (101 MHz, chloroform-d) δ 174.36, 173.45, 138.60, 137.43, 130.12, 129.74, 129.09, 128.90, 128.79, 126.75, 72.56, 61.83, 60.66, 52.57, 49.92, 28.85, 27.88, 9.10; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{31}N_2O_4$ $[M + H]^+$ 411.22783, found 411.22840.

M3 *cis* ^{1}H-NMR (400 MHz, chloroform-d) δ 7.49 (s, 5H), 7.28−7.31 (m, 2H), 7.21−7.25 (m, 1H), 7.18 (d, J = 7.1 Hz, 2H), 3.84 (s, 3H), 3.29−3.33 (m, 2H), 3.15−3.25 (m, 4H), 2.97−3.09 (m, 4H), 2.13 (d, J = 11.0 Hz, 2H), 1.92 (q, J = 7.4 Hz, 2H), 0.95 (t, J = 7.4 Hz, 3H); ^{13}C-NMR (101 MHz, chloroform-d) δ 175.25, 173.70, 139.62, 137.34, 130.52, 129.93, 129.40, 128.95, 128.92, 126.94, 61.69, 61.54, 53.02, 29.20, 28.82, 27.85, 9.06; HRMS (ESI-Orbitrap m/z calculated for $C_{24}H_{31}N_2O_4$ $[M + H]$ 411.22783, found 411.22830.

***M5** Oxalate.* Synthesized amine (0.77 g, 2.29 mmol) and acryloyl chloride (1.03 g, 11.43 mmol) were dissolved in 15 mL of 1,2-dichloroethane in a pressure tube. The vessel was heated in an oil bath at 85 °C for 16 h, then allowed to cool. The mixture was concentrated under reduced pressure. The residue was taken up in saturated aqueous sodium bicarbonate and chloroform. The organic layer was separated, and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried with sodium sulfate. The mixture was filtered through Celite with a layer of silica gel underneath. The filtrate was concentrated under reduced pressure. In a pressure tube, the residue was dissolved in 27 mL of *t*-butanol, and 13.50 mL of 2 M aqueous sodium hydroxide was added. The reaction vessel was heated in an oil bath at 105 °C for 48 h then allowed to cool to room temperature. The solution was filtered through Celite, which was then rinsed with ethanol. The filtrate was concentrated under reduced pressure. The residue was taken up in saturated brine and chloroform. The organic layer was separated, and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried with sodium sulfate. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with 50% to 0% hexanes in dichloromethane then 0% to 75% acetone in dichloromethane to provide 0.33 g of the free base as a tan oil. The oxalate salt was prepared as described for **M4** oxalate. The precipitate was filtered, and 0.37 g of a white solid were obtained in a 32% yield from amine. mp 168−171 °C; ^{1}H-NMR (400 MHz, methanol-d_4) δ 7.53−7.37 (m, 5H), 7.30−7.19 (m, 5H), 3.80 (s, 3H), 3.65 (t, J = 6.4 Hz, 2H), 3.49−3.48 (m, 2H), 3.35−3.24 (m, 4H), 2.99−2.95 (m, 2H), 2.49 (d, J = 14.4 Hz, 2H), 2.13 (t, J = 6.3 Hz, 2H), 1.98−1.96 (m, 2H); ^{13}C-NMR (101 MHz, methanol-d_4) δ 172.7, 172.6, 165.1, 138.1, 136.3, 130.3, 129.8, 129.4, 128.6, 128.5, 126.9, 60.4, 57.5, 57.4, 51.9, 49.5, 38.0, 30.3, 30.1; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{31}N_2O_4$ $[M + H]$ 411.22783, found 411.22837.

***M6**-para Oxalate.* **M1** (1.23 g, 4.25 mmol), 2-(4-hydroxyphenyl)-ethyl bromide (0.95 g, 4.70 mmol), and potassium carbonate (1.76 g, 12.75 mmol) were mixed with 40 mL of 4-methyl-2-pentanone and heated at reflux for 20 h. The mixture was allowed to cool to room temperature. The solids were filtered, and the filtrate was concentrated under reduced pressure. The residue was taken up in chloroform and water. The organic layer was separated, and the aqueous solution was washed two times with chloroform. The combined organic layers were washed with brine. The organic solution was dried with sodium sulfate, filtered, and the volatiles were evaporated to provide a brown oil. The residue was purified using flash chromatography eluting with 0−6% methanol in dichloromethane to provide 1.18 g of the free base as a tan oil. Oxalic acid dihydrate (0.17 g. 1.36 mmol) was dissolved in a minimum of hot methanol and added to the free base (0.51 g, 1.24 mmol) dissolved in minimal hot methanol. The mixture was allowed to cool, and the solvent removed under reduced pressure to a volume of approximately 1.5 mL. The mixture was allowed to cool and refrigerated for 16 h. The precipitate was filtered and dried. The collected salt was recrystallized from methanol/isopropanol followed by heated vacuum drying to provide 0.56 g of a white solid in a 61% yield from **M1**. mp 194−196 °C; ^{1}H-NMR (400 MHz, methanol-d_4) δ 7.51−7.35 (m, 5H), 7.03 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 1H), 3.80 (s, 3H), 3.46−3.17 (m, 6H), 2.89−2.85 (m, 2H), 2.47 (br d, J = 14.2 Hz, 2H), 1.98−1.89 (m, 4H), 0.92 (t, J = 7.6 Hz, 3H); ^{13}C-NMR (101 MHz, methanol-d_4) δ 175.4, 172.6, 165.3, 156.4, 138.2, 130.2, 129.8, 129.5, 129.4, 126.7, 115.3, 60.4, 57.9, 51.9, 49.4, 30.3, 29.3, 28.5, 8.1; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{31}N_2O_4$ $[M + H]$ 411.22783, found 411.22836.

***M6**-meta Oxalate.* **M1** (1.19 g, 4.10 mmol), 2-(3-hydroxyphenyl)-ethyl bromide (0.95 g, 4.51 mmol), and potassium carbonate (1.70 g, 12.30 mmol) were mixed with 40 mL of 4-methyl-2-pentanone and heated at reflux for 20 h. The mixture was allowed to cool to room temperature. The solids were filtered, and the filtrate was concentrated under reduced pressure. The residue was taken up in chloroform and water. The organic layer was separated, and the aqueous solution was washed two times with chloroform. The combined organic layers were washed with brine. The organic solution was dried with sodium sulfate and filtered, and the volatiles were evaporated to provide a brown oil. The residue was flash chromatographed eluting with 0% to 6% methanol in dichloromethane. The process was repeated to improve purity, and 0.91 g of the free base was obtained as a tan oil. The oxalate salt was prepared as described for **M4** oxalate. The precipitate was filtered and dried to provide 0.48 g of a white solid in a 47% yield from **M1**. Further purification was accomplished by recrystallization from water/methanol followed by heated vacuum drying. mp 99−101 °C; ^{1}H-NMR (400 MHz, methanol-d_4) δ 7.52−7.35 (m, 5H), 7.11−7.07 (m, 1H), 6.68−6.63 (m, 3H), 3.80 (s, 3H), 3.48−3.22 (m, 6H), 2.91−2.87 (m, 2H), 2.48 (d, J = 14.4 Hz, 2H), 1.94−1.89 (m, 4H), 0.92 (t, J = 7.4 Hz, 3H); ^{13}C-NMR (101 MHz, methanol-d_4) δ 175.4, 172.5, 165.1, 157.7, 138.2, 137.6, 130.2, 129.8, 129.6, 129.4, 119.4, 115.3, 113.8, 60.4, 57.5, 51.9, 49.5, 30.3, 30.0, 28.5, 8.1; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{31}N_2O_4$ $[M + H]$ 411.22783, found 411.22849.

***M6**-ortho Oxalate.* **M1** (0.99 g, 3.40 mmol), 2-(2-THPO-phenyl)ethyl bromide (0.97 g, 3.40 mmol), and potassium carbonate (1.70 g, 12.30 mmol) were mixed with 40 mL of 4-methyl-2-pentanone and heated at reflux for 48 h. The mixture was allowed to cool to room temperature. The solids were filtered, and the filtrate was concentrated under reduced pressure. The residue was taken up in chloroform and water. The organic layer was separated, and the aqueous solution was washed two times with chloroform. The combined organic layers were washed with brine. The organic solution was dried with sodium sulfate and filtered, and the volatiles were evaporated to provide a brown oil. The oil was dissolved in 40 mL of methanol, and *p*-toluenesulfonic acid monohydrate (1.00 g,

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5.10 mmol) was added. The solution was heated for 16 h. The solution was allowed to cool and the solvents removed under reduced pressure. The residue was taken up in chloroform and saturated aqueous sodium bicarbonate solution. The organic layer was separated, and the aqueous solution was extracted two times with chloroform. The combined organic extracts were dried with sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The residue purified using flash chromatography eluting with 0% to 6% methanol in dichloromethane and 0.84 g of the free base obtained as a tan oil. The oxalate salt was prepared as described for **M4** oxalate. The precipitate was filtered and recrystallized from ethanol. After heated vacuum drying, 0.54 g of a white solid was obtained in a 54% yield from **M1**. Further purification was accomplished by recrystallization from methanol/ethanol followed by heated vacuum drying. mp 210−212 °C; ^{1}H-NMR (400 MHz, methanol-d_4) δ 7.50−7.35 (m, 5H), 7.07−7.02 (m, 2H), 6.75−6.71 (m, 2H), 3.79 (s, 3H), 3.47−3.44 (m, 2H), 3.29−3.16 (m, 4H), 2.96−2.92 (m, 2H), 2.46 (br d, J = 14.4 Hz, 2H), 1.99−1.88 (m, 4H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C-NMR (101 MHz, methanol-d_4) δ 175.4, 172.7, 166.3, 155.3, 138.3, 130.3, 130.2, 129.7, 129.3, 128.2, 122.7, 119.5, 114.8, 60.6, 56.3, 51.9, 49.3, 30.4, 28.5, 25.6, 8.1; HRMS (ESI-Orbitrap), m/z calculated for $C_{24}H_{31}N_2O_4$ [M + H] 411.22783, found 411.22853.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00404.

Spectral data on the synthesized compounds, metabolism methods, UHPLC conditions, metabolite mass spectral data, and MOR assay methods and data (PDF)

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Notes

The authors declare no competing financial interest.

■ DEDICATION

This work is dedicated to Dr. Fu-Lian Hsu on the occasion of his happy retirement after 36 years of US government service.

■ ABBREVIATIONS

MOR μ-opioid receptor; THP tetrahydropyranyl; DAMGO Ala^2-MePhe^4-Glyol^5-Enkephalin; cAMP cyclic adenosine monophosphate; EC_{50} median effective concentration; SEM standard error of mean

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