A diastereoselective synthesis of Cebranopadol, a novel analgesic showing NOP/mu mixed agonism

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A diastereoselective synthesis of the title compound as a single \(E\) diastereomer has been efficiently accomplished by assembling the featured pyrano-indole scaffold of the spiro[cyclohexane-dihydropyrano[3,4-b]-indole]-amine framework through an oxa-Pictet-Spengler reaction, promoted by a cheap and green Zeolite catalyst. Basic pharmacological experiments demonstrate that Cebranopadol acts as a mixed nociception/orphanin FQ (NOP) and mu (MOP) opioid receptor agonist useful for treatment of chronic pain.

Nociceptin/orphanin FQ (N/OFQ), the endogenous agonist of the N/OFQ peptide receptor (NOP) regulates various biological functions\(^1\) including pain transmission\(^2\). Grüntenthal researchers have recently reported the results of structure activity studies\(^3,4\) that led to the identification of Cebranopadol (trans-6′-fluoro-4′-9′-dihydro-N,N-dimethyl-4-phenyl-spiro[cyclohexane-1,1′(3′H)-pyrano[3,4-b]indol]-4-amine) as a potent NOP and mu receptor (MOP) agonist (Fig. 1).

This compound binds with high affinity to the NOP receptors and behaves as full agonist. Rodent studies demonstrated that Cebranopadol elicits potent and efficacious antinociceptive action in several models of nociceptive and neuropathic pain. Importantly, compared to morphine, Cebranopadol displayed a favorable side effect profile and reduced tolerance liability\(^5\). Cebranopadol is under clinical development and several clinical trials are assessing its analgesic therapeutic potential in different pain conditions\(^6\).

**Results and Discussion**

Grüntenthal researchers synthetized Cebranopadol as retrosynthetically depicted in Fig. 2 based on an oxa-Pictet-Spengler reaction between the fluoro-silyl-indole \(^6\), in turn prepared by Larock indole synthesis\(^7\) of the commercially available 2-iodo-4-fluoroaniline \(^8\) and 1-silyl-1-butynol \(^9\), and the aminoketone \(^7\) bearing the structurally important 4-N,N-dimethylamino-4-phenyl-cyclohexane head by a two step approach involving a Strecker synthesis\(^8\) of monoketal protected 1,4-cyclohexanedione 1 by treatment with HNMe\(_2\) HCl/KCN (67–99%) and a Bruylants reaction\(^9\) of the resulting aminonitrile with PhMgCl in THF at 0 °C to room temperature (low overall yield). It deals with a classical approach that at the end of the synthesis requires a diastereomer separation by HPLC.

Our interest in this area led us to be contemporaneously involved in the synthesis of Cebranopadol with two important guidelines in our project developed along the same pathway: a) avoid the use of highly toxic potassium cyanide; b) identifying suitable experimental conditions to make the oxa-Pictet-Spengler reaction diastereoselective. (Fig. 3).

The approach proposed a start from the cheap and commercially available ketone 1 that undergoes a Grignard reaction with phenyl magnesium bromide in THF for 12 h yielding the corresponding alcohol 3 at 70% yield. A nucleophilic substitution of the tertiary and benzylic alcohol using the classical sodium azide/TFA in chloroform approach failed. Different methods have been tried to overcome the very low yield (5%) because of the competitive elimination reaction to an alkene. In our hands the best way to obtain the azide 5 was the reaction with trimethylsilylazide catalysed by indium tribromide\(^10\) that allowed us to obtain compound 5 in a clean and fast step with good yield (50%). Lithium aluminium hydride reduction to the primary amine 10 followed by reductive amination with formaldehyde allowed us to obtain the tertiary amine 4 in good overall yield. Deprotection of...
the ketone using hydrochloric acid in acetone produced the intermediate 7 (Fig. 4). The stage was set for the crucial oxa-Pictet-Spengler reaction which has been performed in different conditions such as bismuth triflate, hydrochloric acid, Zeolite beta-25 and 4 Å molecular sieves giving rise to a mixture of diastereomers in low yield. The cyclization reaction has been tried also using a TMSOTf at room temperature producing Cebranopadol in 90% yield but lacking stereoselectivity.

Much to our delight, the last synthetic steps involving an oxa-Pictet-Spengler reaction to install the dihydropyrano[3,4-b]indole moiety have been performed in a diastereoselective manner using an unusual and green Zeolite catalyst and catalytic p-toluenesulphonic acid in refluxing toluene that allowed us to obtain the E diastereomer exclusively as depicted in Fig. 5.

The setting up of an oxa-Pictet-Spengler reaction, allowed us to also obtain different pyrano indole scaffolds starting from commercially and non commercially available ketones. ROESY NMR experiments (see Supporting

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**Figure 1.** Cebranopadol.

**Figure 2.** Retrosynthesis of Cebranopadol.
Figure 3. Different approaches to the synthesis of Cebranopadol.

Figure 4. Synthesis of intermediate 7.

Figure 5. Unusual diastereoselective oxa-Pictet-Spengler reaction.
Information page S20)of the final compound confirms that the zeolite K-10 catalyst produced a single E diastereomer during the oxa-Pictet-Spengler reaction. On the contrary, the trimethylsilyl triflate approach allowed us to obtain a mixture of E and Z diastereomer with a good overall yield but without regioselectivity. The zeolite catalyst is easily recovered and its catalytic properties did not change up to 5 times reusing-cycle.

HR-LC-MS spectra of Cebranopadol obtained using Zeolite catalyst (panel A) and TMSOTf (panel B) are depicted in Fig. 6.

The basic pharmacological profile of Cebranopadol has been investigated by measuring calcium mobilization in cells coexpressing NOP or classical opioid receptors and chimeric G proteins as described in detail in Camarda et al.13, 14.

The results of these experiments, summarized in Table 1, indicated that Cebranopadol behaved as full agonist showing very similar potency at NOP and MOP receptors. Cebranopadol was also able to activate kappa (KOP)
Table 1. Pharmacological profile of Cebranopadol (Cebra) in cells coexpressing human recombinant NOP or classical opioid receptors and chimeric G protein.

|            | NOP          | MOP          | KOP          | DOP          |
|------------|--------------|--------------|--------------|--------------|
| pEC50±σ    | pEC50±σ      | pEC50±σ      | pEC50±σ      | pEC50±σ      |
| N/OFQ      | 9.59±1.00    | inactive     | inactive     | inactive     |
| Fentanyl   | inactive     | 8.13±1.00    | inactive     | inactive     |
| Dyn A      | inactive     | 6.67±0.82    | 8.54±1.00    | 7.73±0.99    |
| DPDPE      | inactive     | 8.15±1.00    | 8.54±1.00    | 8.02±0.78    |
| Cebra      | 7.28±0.89    | 7.20±0.99    | 5.98±0.55    | 6.31±0.81    |

Conclusions

In conclusion, we have developed a robust and easy method for the synthesis of Cebranopadol (in 15% overall yield) as a single E diastereomer using a green Zeolite catalyst in the key oxapicet-Spangler reaction. The azide approach will allow us to easily insert different substituents onto the basic amine to better understand the crucial role of tertiary amines in the interaction with Asp136 and Asp147 of the NOP and MOP receptor binding pockets. Basic pharmacological experiments demonstrate that Cebranopadol acts as a mixed NOP/MOP agonist.

Methods

Chemical Materials and Methods. All NMR spectra were analysed using Mestre Nova 6.0.2 software and FID data are available on request. Analytical thin layer chromatography (TLC) was performed on silica gel Macherey-Nagel poligram SIL/UV 254 of 0.25 mm, visualization was achieved using UV light (254) and Potassium Permanganate (Kmno4) 2% in water. Flash column chromatography was undertaken using Isolera one (Biotage Sweden). Products were dried using sodium sulfate anhydrous Carlo Erba. Proton nuclear magnetic resonance (1H NMR) and carbon nuclear magnetic resonance (13C NMR) were recorded using V ARIAN 400 MHz. Chemical shifts (δ) were quoted in ppm relative to residual solvent and coupling constants (J) were quoted in Hertz (Hz). Multiplicity was reported with the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = doublet of doublet; dt = doublet of triplet, dq = doublet of quartet; Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR Spectrum 100 using zirconium-selenium diamond as a cell. Melting points were recorded using a Buchi-Tottoli and were reported uncorrected. Molecular weights were measured with a mass spectrometer electrospray ESI MICROMASS ZMD 2000 and high resolution spectra with an Agilent ESI-Q-TOF LC/MS 6520 System. Solvents and chemicals used for TLC, chromatographic purification, crystallization and reactions were reported with the following abbreviations: Et2O for diethyl ether, THF for tetrahydrofuran, AcOEt for ethyl acetate, DCM for methylene chloride, LiAlH4 for lithium aluminium hydride.

Synthesis of 8-phenyl-1,4-dioxaspiro[4.5]decan-8-ol (3). In a two neck round bottom flask, under an argon atmosphere, 1,4-cyclohexanedione monoethylene acetale (1) (1.5 g, 9.6 mmol) was dissolved in THF (50 mL). At 0 °C, phenyl magnesium bromide (20 ml, 19.22 mmol) was added and the reaction was stirred overnight at room temperature. The reaction mixture was checked by TLC (AcOEt/Petroleum ether 1:6), quenched with NH4Cl saturated solution and washed with AcOEt. The organic layers were dried with Na2SO4, filtered and the solvent was removed under vacuum. The crude product was purified by flash chromatography (AcOEt/ Petroleum ether 1:1) with an yield of 70% to give the title compound (3) as a white solid. MS (ESI): [M-OH]- = 217.27, m. p.: 98–100 °C

1H-NMR (400 MHz, Chloroform-d), δ: 7.58–7.46 (m, 1 H, Ar), 7.41–7.31 (m, 2 H, Ar), 7.30–7.22 (m, 2 H, Ar), 4.03–3.91 (m, 4 H, O-CH2-CH2-O-), 2.24–2.05 (m, 4 H, CH2 cyclohexane), 1.86–1.77 (m, 2 H CH2 cyclohexane), 1.74–1.64 (m, 3 H CH2 cyclohexane and -OH). 13C-NMR (100 MHz, Chloroform-d), δ: 143.36 (Cq-Ar), 128.81, 128.81, 127.63, 125.54 (CH-Ar), 107.89 (-O-Cq-O-), 65.90 (-O-CH2-CH2-O), 64.59 (-O-CH2-CH2-O), 36.73 (CH2 cyclohexane), 30.89 (CH2 cyclohexane).

Synthesis of 8-azido-8-phenyl-1,4-dioxaspiro[4.5]decan-8-ol (5). To a solution of LiAlH4 (437 mg, 11.53 mmol) in 15 mL of THF, was added the azide (5) (1 g, 3.83 mmol) dissolved in the same solvent at 0 °C.
Synthesis of N,N-dimethyl-8-phenyl-1,4-dioxaspiro[4,5]decan-8-amine (4). To a solution of compound (10) (1 g, 3.71 mmol) in 50 mL of MeOH, formaldehyde (1.04 mL, 37.17 mmol), sodium triacetoxyborohydride (1.57 g, 7.43 mmol) and a catalytic amount of AcOH were added at room temperature. The reaction mixture was stirred overnight at 65 °C. The reaction mixture was monitored by TLC (AcOEt/Petroleum ether 1:3). The solvent was concentrated to dryness, diluted in AcOEt and the organic layers were washed with NaOH 10% solution (2 × 20 mL) in order to obtain the title compound (4) as a yellow crystalline solid with a yield of 81%. HRMS (ESI): [M+H]+ = 252.22; 1H-NMR (400 MHz, Chloroform-d), δ: 8.21–8.14 (bs, 1 H, NH), 7.28–7.22 (m, 1 H, CH-Ar), 7.09–7.05 (m, 1 H, CH-Ar), 6.99–6.91 (m, 1 H, CH-Ar), 6.98–6.92 (m, 1 H, CH-Ar), 3.92–3.84 (m, 2 H, CH 2, C1a, C3a), 2.98 (m, 2 H, CH 2, C1a, C3a), 2.10 (s, 6 H, N(CH 3)2), 2.07 (m, 2 H, -CH 2, CH1e, CH3e). 13C-NMR (100 MHz, Chloroform-d), δ: 158.88, 156.55, 132.92, 128.04, 124.36, 113.42, 111.94, 111.75, 103.97, 63.16, 28.86, 20.6, 0.38.

Synthesis of 1,1′-pyrano[3,4-b]indol-1′-yloxy-N,N-dimethyl-4-phenyl-4′,9′-dihydro-3′H-spiro[cyclohexane-1,1′]-pyrano[3,4-b]indol-4-amine (Cebranopadol). Method A. The ketone (7) (125 mg, 0.66 mmol), was solved in 15 mL of toluene with a catalytic amount of p-toluensulfonylic acid; to the solution were added compound (6) (149 mg, 0.66 mmol) and Zeolite K-10 (300 mg). The solution was heated under reflux with a Dean-Stark apparatus for 4 hours. The solvent was removed under vacuum and NaOH 2 N (20 mL) was added to the reaction mixture. The residue was filtered over a celite pad and dissolved in AcOEt (20 mL). The organic layers were dried, filtered and concentrated to give a crude product that was purified by flash chromatography (AcOEt/Petroleum ether 2:1) with a yield of 50% as a yellow solid that crystallized in MeOH.

Method B. In a two neck round bottom flask, under an argon atmosphere, compound (6) (84 mg, 0.336 mmol) was dissolved in DCM (10 mL). Compound (7) (61 mg, 0.28 mmol), and trimethylsilyltrimethylethyl ether (65 mL, 0.28 mmol) were added whilst stirring at minus 78 °C for 30 minutes and then was washed with water (2 × 20 mL). The organic layers were dried, filtered and concentrated under vacuum to obtain a yellow solid that was crystallized in methanol to yield Cebranopadol as a diastereomeric mixture with 90% yield. MS (ESI): [M+H]+ = 379.21; HRMS (ESI): [M+H]+ = 379.210818; [M+H]+ Found = 379.21809; [M–N(CH3)2]+ = 334.16032; m.p. = 220 °C with decomposition. 1H-NMR (400 MHz, Chloroform-d), δ: 8.54 (s, 1 H, NH), 7.37 (m, 5 H, CH-Ar), 7.28 (m, 1 H, CH-Ar), 7.13 (dd, J = 9.6, 2.5 Hz, 1H, CH18), 6.89 (ddd, J = 9.4, 8.8, 2.5 Hz, 1H, CH16), 3.96 (t, J = 5.4 Hz, 2H, -CH2), 2.75 (t, J = 5.4 Hz, 2H, -CH2), 2.53 (d, J = 13.7 Hz, 2H, CH=, C6e, C4e), 2.21 (d, J = 12.8 Hz, 2H, CH=, C1a, C3a), 2.10 (s, 6 H, N(CH3)2), 2.07 (m, 2H, -CH2, CH4a, CH6a), 1.93 (m, 2H, -CH2, CH1e, CH3e). 13C-NMR (100 MHz, Chloroform-d), δ: 159.09 (C-17-Ar), 156.76 (Cq-13), 141.34 (Cq-N-7), 139.06 (Cq-21), 132.24 (Cq-N-13), 127.57, 127.05, 126.82 (CH-Ar), 125.57 (Cq-indol, 14), 111.57 (CH, 15), 109.74 (CH, 16), 107.31 (CH, 8), 103.44 (CH, 18), 72.20 (Cq Spiro, 2), 59.81 (CH3, 10), 58.79 (Cq, 5), 38.34 (N-CH3, 22, 23), 30.93 (CH2, 1, 3), 28.35 (CH2, 4, 6), 22.67 (-CH2, 9). 19F-NMR: δ: -125.60.
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Author Contributions

A.F., S.B. and C.T., designed research; A.F., S.B., R.G., S.P., and C.T. synthesized and purified the compounds; M.C.C. and G.C. performed pharmacological studies; G.C., R.G., S.S. and C.T. analyzed data; S.P., A.F., S.B., G.C. and C.T. wrote the paper.

Additional Information

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