# Synthesis of conjugated metabolites of morphine derivatives

PhD thesis

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# Introduction

Giving relief to pain accompanying illnesses and injuries has always been the integral part of medical therapy. Opioid alkaloids and their semi-synthetic analogues are found among the most effective analgesics used in the medicinal practice.

The most known entity of the opioid group is morphine. It was first isolated as the most abundant alkaloid in opium, later identified as an endogenous substance in the human body. Despite the fact that more than 200 years have passed since its isolation, morphine is still the most widely used analgesic drug against severe pain. Besides the potent analgesic effect, morphine also possesses numerous unwanted side-effects (euphoria, respiratory depression, constipation, nausea, vomiting, tolerance, dependence) that limit its use. Furthermore, some morphine derivatives are widely used as illicit drugs, and are under control.

Extensive research has been carried out in the recent decades in order to better understand the cellular mechanisms underlying the analgesic effect of opioids. Sufficient knowledge could lead to the rational development of novel synthetic and semi-synthetic, selective and effective opioids devoid of side-effects.

An important part of pharmacological action and the crucial element of pharmacokinetics is the metabolic transformation of drugs. Metabolism also plays an important role in the case of morphine-like substances; therefore I reviewed the relevant publications in this field. In pharmaceutical chemistry metabolism means the biochemical transformations of exogenous substances which result in the increase of polarity and water solubility, enabling the gradual excretion of drug molecules primarily through the kidneys.

Animal and human studies showed that morphine is metabolized in different routes by enzymatic transformation on three heteroatoms:

(a) conjugated metabolites are formed: morphine-3-*O*-glucuronide, morphine-6-*O*-glucuronide, morphine-3-*O*-sulfate, morphine-3-*O*-glucoside and / or morphine-6-*O*-glucoside.

(b) Normorphine is formed in *N*-demethylation reaction, which itself undergoes conjugation and oxidation.

(c) Dihydromorphinone is formed in reactions of unknown mechanism.

It was discovered in the early seventies, that morphine-6-*O*-glucuronide possesses analgesic properties. When given peripherally, morphine-3-

*O*-sulfate and morphine-3-*O*-glucuronide exhibit minimal analgesic effect, whereas in strong contrast to this morphine-6-*O*-glucuronide is up to 670-fold more potent than its parent compound, morphine. The synthetic metabolite analogue morphine-6-O-sulfate is also more active than morphine even when administered peripherally.

There is a need for the development of highly polar, permanently charged morphine derivatives that could effectively counter inflammatory or other severe pain without penetration into the brain. Morphine metabolites and their chemically modified analogues are again in the center of attention both from chemical and pharmacological point of view. Based on existing knowledge our group contributes to this field by the synthesis of known and novel, potentially highly effective synthetic metabolite analogues of morphine derivatives. Spectroscopic data of known compounds was revised using modern spectroscopic methods.

## Objectives

Based on our extensive review of publications in the field of morphine metabolites our aims were to find suitable methods for the preparation of

naturally occurring sulfate ester and glucoside metabolites of morphine and codeine derivatives, and by using or modifying these methods we aimed to synthesize known natural and novel synthetic sulfate ester and glucoside metabolites. Known compounds (with a few exceptions) lack satisfactory spectral data (NMR, MS, CD, UV/VIS), justifying the repeated synthesis and detailed analysis of these compounds. However, the major part of our preparative work is focused around novel molecules previously not reported in the literature.

Literature data point out that morphine derivatives containing an alkoxy group in position C-14 possess exceptional analgesic activity. We therefore aimed to synthesize the novel 14-methoxymorphine and 14-methoxycodeine and their sulfate esters. Due to their zwitterionic structure sulfate esters are apparently incapable of blood-brain-barrier penetration; they may play a role as peripheral analgesics in future clinical practice.

The purity of the synthesized compounds was checked by chromatography (TLC and HPLC) and spectroscopy. We planned to carry out detailed structural analyses by NMR and HRMS, including

assignation of signals in $^{1}$H-NMR and $^{13}$C-NMR spectra. We aimed to study chiral properties by optical spectrophotometry (CD and ORD). Spectral data and the structural information within may facilitate the identification of morphine metabolites and metabolite analogues even in biological matrices, and provide useful points of reference for further studies involving these compounds. Since most of the planned substances are potentially effective analgesics, we planned to carry out detailed pharmacological profiling in cooperation with the Department of Pharmacology and Pharmacotherapy, Semmelweis University.

## Methods

Opiates used in syntheses were purchased from Alkaloida Vegyészeti Gyár Zrt. (Tiszavasvári, Hungary). Reagents were purchased from Sigma-Aldrich (Steinheim, Germany) and Alfa Aesar (Ward Hill, MA, USA). Solvents were purchased from Molar Chemicals (Budapest, Hungary) and Merck (Darmstadt, Germany). Deuterated solvents for NMR spectroscopy: >99.5 % isotopic purity DMSO-$d_6$ and >99,5 % isotopic purity chloroform-$d_1$. Deuterated solvents were purchased from Sigma-Aldrich and Merck.

$^{1}$H-NMR and $^{13}$C-NMR spectra were taken on a Varian 600 MHz VNMRS (Palo Alto, CA, USA) spectrometer. Chemical shift (δ) values are given in reference to tetramethylsylane (TMS). Spectra were analyzed using Varian VnmrJ and MestReNova (Santiago de Compostela, Spain) softwares. Mass spectra (sulfate esters) were recorded on an Agilent 6410 Triple Quad (Santa Clara, CA, USA) instrument using electronspray ionization (ESI) and negative polarization mode. HRMS (glucosides) were taken on an Agilent 6230 time-of-flight (TOF) mass spectrometer using ESI and positive polarization mode. Analyte samples were injected into the MS by an Agilent 1260 Infinity liquid chromatograph. Mass spectra were analyzed by Agilent MassHunter B.02.00 software. CD, UV/VIS and ORD spectra were registered on Jasco J-720 (Tokyo, Japan) and Jasco J-810 spectropolarimeters. The composition of the liquid chromatograph used for HPLC analysis of morphine glucosides: Jasco PU-980 Intelligent HPLC pump, Rheodyne 7725i injector and Jasco UV-975 Intelligent UV/VIS detector. Stationary phase: Hypercarb (100 x 4.6 mm, 5 μm) column (Thermo Fischer Scientific, Waltham, MA, USA). Chromatograms were evaluated by Borwin

software (v. 1.21). Thin-layer chromatography: we used Merck Silica Gel 60 F254 plates as stationary phase.

## Results

We synthesized sulfate esters by the reaction of the appropriate morphine derivative and pyridine-$SO_3$ complex in dry pyridine stirred at 60 °C for 3.5 hours. Morphine and dihydromorphine have two hydroxyl groups (C-3 phenolic and C-6) that can be readily sulfated. The reactivity difference of the two hydroxyl groups is not enough, however, for direct regioselective sulfation to produce the monoesters. In order to obtain the sulfate esters, we used protecting groups at the C-3 phenolic or C-6 hydroxyl groups. We used acetyl protecting groups for facile synthesis, removal and ease of access.

We synthesized numerous 3-*O*- and 6-*O*-sulfate esters of morphine, codeine, opiate antagonists and some of their *N*-methyl quaternary derivatives. We synthesized 14-methoxycodeine, 14-methoxymorphine and their sulfate esters. Sulfate monoesters were synthesized by pyridine-$SO_3$ complex, whereas diesters were prepared by concentrated sulfuric acid in DMF using *N*,*N*'-dicyclohexylcarbodiimide as the coupling agent.

For the synthesis of morphine glucosides we used acetobromo-α-D-glucose. *O*-Glucosylation at C-6 was carried out by the Koenigs-Knorr method, whereas the 3-*O*-glucoside of morphine was synthesized directly upon stirring morphine with acetobromo-α-D-glucose and aqueous sodium hydroxide in acetone.

The crude acetylated glucopyranosyl derivatives were purified using column chromatography. The pure product fractions were collected and crystallized from ethanol or diethyl ether. Removal of the acetyl protecting groups was achieved upon stirring the tetraacetyl derivatives with a small excess of aqueous lithium hydroxide in methanol at room temperature. The C-3 phenolic hydroxyl group of morphine and dihydromorphine was selectively acetylated prior to the glycosylation step to ensure regioselectivity.

Detailed spectroscopic analyses were carried out for the synthesized substances (NMR, MS, CD, UV/VIS). We report the efficient separation of glucoside and glucuronide conjugates of morphine and codeine and their parent compounds using HPLC with PGC stationary phase. Selected compounds were pharmacologically tested at the Department of Pharmacology and

Pharmacotherapy, Semmelweis University. 14-Methoxymorphine-6-*O*-sulfate was found to possess analgesic activity of more than 2000-fold compared to that of morphine. The compound has a longer duration of action and effective when injected peripherally. 14-Methoxymorphine-6-*O*-sulfate could become a clinically used analgesic in the future.

## Conclusions

- The ever-increasing knowledge of the structure and cellular mechanisms of the opioid system sets up new ways for medicinal chemistry to find novel solutions to pain management. The development of a semi-synthetic or synthetic opioid of equal analgesic strength to that of morphine but lacking dangerous side-effects is still not accomplished.
- Based on pharmacological and analytical results metabolite-like substances acting on the opioid system are in the center of attention worldwide. Our research group contributes to this field by the synthesis of potentially highly effective novel and synthetic metabolite analogues of morphine derivatives. During my doctoral work I reviewed the synthetic methods for the synthesis of sulfate esters and glucosides. I applied these, or their

modified versions, to synthesize sulfate and glucoside conjugates of morphine derivatives.

• In my doctoral thesis I report the synthesis of a series of sulfate esters and glucosides of morphine congeners, among which 20 new sulfate esters and 4 new glucosides are found. Selective sulfation and glucosylation reactions were achieved by using appropriate protecting groups. The synthesized substances are described by detailed structural and chiroptical spectroscopic data.

• We synthesized numerous 3-*O*- and 6-*O*-sulfate esters of morphine and codeine derivatives. Sulfate monoesters were synthesized by pyridine-$SO_3$ complex, whereas the diesters morphine-3,6-*O*-disulfate and oxymorphone-3,14-*O*-disulfate were prepared by concentrated sulfuric acid in DMF using *N*,*N*'-dicyclohexylcarbodiimide as the coupling agent.

• Opioid antagonists containing quaternary nitrogen atom are unable to pass the blood-brain-barrier due to their permanent charge, these are therefore used in the clinical practice to reduce the peripheral side-effects of opiate treatment. Based on these substances we synthesized the sulfate esters of naloxone and naltrexone, also carrying permanent charges. The C-14 sulfate esters of the

antagonist opiates are supposedly unable to absorb from the gastrointestinal tract, therefore, given orally, they may be useful for the reversal of constipation caused by morphine.

- We studied the regioselectivity of esterification by pyridine-$SO_3$ complex in morphine derivatives containing a hydroxyl group on C-14. We found that the complex reacts readily with tertiary alcohols and is suitable for the esterification of the tertiary C-14 hydroxyl group. C-6 sulfate esters of 14-hydroxy-derivatives could only be synthesized after protection of the C-14 hydroxyl.
- The synthesized sulfate esters were described in detail by different spectroscopic methods. All NMR signals in the $^{1}H$ and $^{13}C$ spectra of sixteen synthesized derivatives were completely assigned based on one- and two-dimensional homo- and heteronuclear NMR experiments. The effect of the sulfate ester groups on the chemical shifts of several atoms due to changes in charge distribution, steric interactions and ring conformation was studied.
- Detailed $^{1}H$- and $^{13}C$-NMR and CD/ORD analyses are presented that can serve as a basis for further physicochemical investigations or as a tool for the identification of various sulfate esters in

biological samples.

• Most of the synthesized sulfate esters have the potential of selective peripheral analgesic activity, supposedly with minimal central effect owing to their zwitterionic nature. Selected compounds were tested *in vitro* and *in vivo* at the Department of Pharmacology and Pharmacotherapy, Semmelweis University.

• We synthesized 14-methoxycodeine, 14-methoxymorphine and their sulfate esters. 14-Methoxymorphine-6-*O*-sulfate was found to possess 3 magnitudes higher analgesic potency compared to that of morphine. The compound is exceptionally effective when injected peripherally. Further studies are required to profile its potential side-effects; hopefully 14-methoxymorphine-6-*O*-sulfate could become a clinically important peripheral analgesic in the future.

• Six glucosides of morphine congeners including new chemical entities were systematically designed, synthesized and identified, their properties were characterized by spectroscopic techniques, in order to obtain compounds that are water soluble without the pharmacokinetically unfavorable zwitterionic character, and may

therefore be prodrug candidates in analgesic therapy.

• We developed an efficient separation of glucoside and glucuronide conjugates of morphine and codeine and their parent compounds using HPLC with porous graphitized carbon stationary phase. The method may be suitable for the quick isolation of glucosides from biological samples.

## Candidate's publications

**Publications related to the theme of the thesis:**


1. Váradi A, Gergely A, Béni Sz, Jankovics P, Noszál B, Hosztafi S, (2011) Sulfate esters of morphine derivatives: Synthesis and characterization. Eur J Pharm Sci, 42 (1-2): 65-72.
2. Lackó E, Váradi A, Rapavi R, Zádor F, Riba P, Benyhe S, Borsodi A, Hosztafi S, Tímar J, Noszál B, Fürst S, Al-Khrasani M, (2012) A novel μ-opioid receptor ligand with high in vitro and in vivo agonist efficacy. Curr Med Chem, 19 (27): 4699-4707.
3. Váradi A, Lévai D, Tóth G, Horváth P, Noszál B, Hosztafi S, (2012) Glucosides of morphine

derivatives: synthesis and characterization. Monatsh Chem, DOI: 10.1007/s00706-012-0868-4.

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1. Takács M, Bubenyák M, Váradi A, Blazics B, Horváth P, Kökösi J, (2011) Synthesis of novel ceramide-like penetration enhancers. Tetrahedron Lett, 52 (16): 1863-1865.
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3. Kökösi J, Váradi A, Bubenyák M, Rácz Á, Takács M, Horváth P, Noszál B, Szász Gy, Hermecz I, (2011) Bioizoszter alkaloid hibridek szintézise. Magyar Kémikusok Lapja, 56: 263-264.

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