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First synthesis of important secondary oxidative metabolites of morphine and codeine with the Michael addition

Research Article

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Abstract: Morphine (**1**) and codeine (**2**) are two representatives of medically important, frequently used natural opiates, therefore the exploration of their metabolic pathways and the exact characterization of the metabolites are main targets of their pharmacological studies. These morphinans also play a crucial role in drug abuse; therefore, the analysis and preparation of the metabolites for identification and quantitation in human samples are considered important aims. In order to allow the in-depth analysis of metabolites derived from the oxidative pathways through morphinone (**3**) and codeinone (**4**), synthetic procedures have been elaborated for the gram-scale preparation of glutathione and *N*-acetylcysteine adducts. Primary pharmacological studies revealed the inactive nature of these metabolites in opioid receptor binding tests.

Keywords: *Morphine • Codeine • Metabolite • Glutathione • Cysteine*

1. Introduction

The eclucidation of the exact mechanism of metabolic pathways of morphine (**1**) and related opioid analgesics is a major goal of medicinal pharmacology, as these compounds still represent the first-choice medications in the management of cancer-related and post-operative pain [1].

The metabolism of morphine (**1**) has been studied in several mammalian species, and its detoxication pathway is thought to be through 3-glucoronide formation [2]. However, it was revealed by excretion studies that most of the administered opioid was excreted as conjugates of partially known structure and quantity. Kumagi and co-workers reported [3] the presence and importance of glutathione (HSG) and cysteine (CYS) adducts of morphinone (**3**), a well-known primary metabolite of morphine (**1**). The same metabolic pathway was confirmed for codeine (**2**), a potent and established anti-tussive agent [4] throughout the formation of codeinone (**4**) [5].

In brief, morphine (**1**) and codeine (**2**) are transformed to morphinone (**3**) and codeinone (**4**) in the liver by the respective dehydrogenase enzymes. Compounds **3** and **4** are known as efficient Michael acceptors therefore they are able to form adducts **5** and **6**, respectively, with glutathione [6]. This is the general detoxication pathway for morphinone (**3**) and codeinone (**4**), which are 9- and 30-times more toxic than parent molecules **1** and **2**, respectively. The body, however, is unable to excrete glutathione adducts; instead, compounds **5** and **6** are further metabolized to *N*-acetyl-cysteine conjugates **7** and **8**.

Adducts **5-8** were isolated in small amounts by HPLC and characterized by NMR and MS, however

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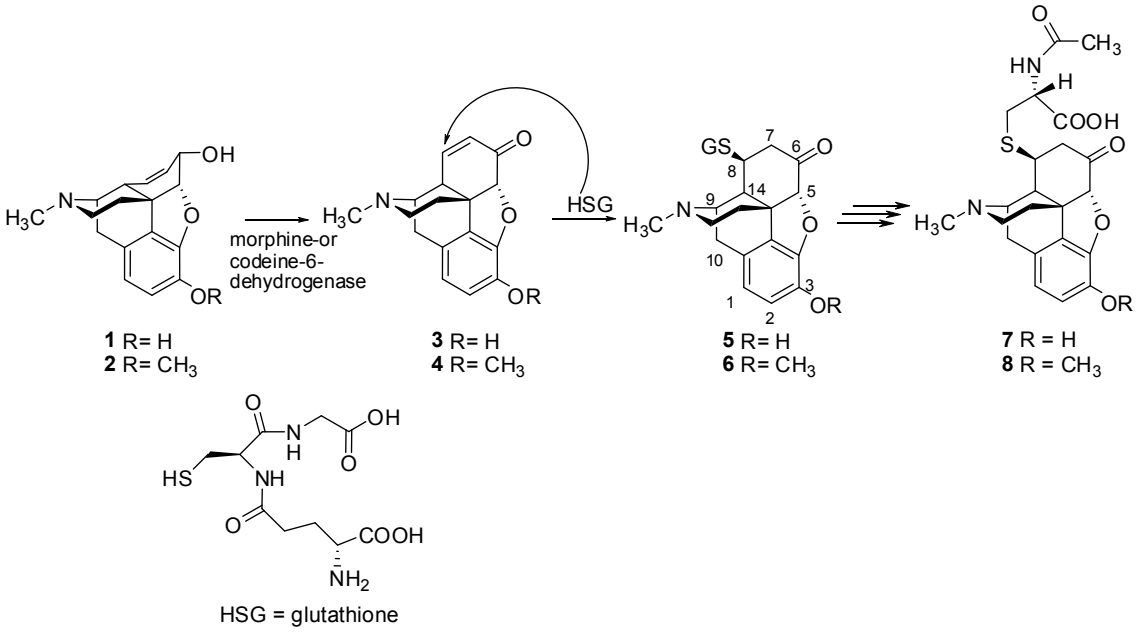


Scheme 1. The oxidative metabolic pathways of morphine (1) and codeine (2).

detailed physical and biological studies have not been possible. Accordingly, we schemed and elaborated synthetic routes for the preparation of these important metabolites in gram-scales. Besides the previously mentioned physical and biological studies and further pharmacological investigations these metabolites could be important in the fight against drug abuse based on for example the fact that direct quantitative relation was revealed between the administered amount of opiate and its oxidative *N*-acetyl-cysteine conjugate in excretion samples by Ishida *et al.* [5a]. This and similar observations may find application in the determination of the exposure time to the abused drug and the route of administration.

2. Experimental procedure

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Thin layer chromatography was performed on pre-coated Merck 5554 Kieselgel 60 F254 foils. The spots were visualized with Dragendorff's reagent. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer, chemical shifts are reported in parts per million (δ) from internal TMS and coupling constants (*J*) are measured in hertz. High resolution mass spectral measurements were performed with a Bruker micrOTOF-Q instrument in the ESI mode. Optical rotation was determined with a Perkin–Elmer Model 241 polarimeter with a water-jacketed 10 cm cell and is given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on Perkin–Elmer 16 PC FTIR spectrometer.

The MW-induced reactions were carried out in a Discover model microwave reactor manufactured by CEM Corporation. Controlled temperature, power, pressure and time settings were used for all reactions.

The coupling constant used to establish the necessary delay for the selection of the proton coupled to the carbon in the HSQC spectrum was 135 Hz, corresponding to a delay of 3.7 ms; the delay for the HMBC spectra was 70 ms corresponding to a long-range coupling constant of 7 Hz. 2D TOCSY spectrum was acquired with 70 ms spin-lock time using the standard microprogram of the XWINNMR program.

2.1. Synthesis of gluthatione adducts 6 and 5

In a pressurized glass vial equipped with a magnetic stirring bar under an atmosphere of argon codeinone•HCl (**4HCl**) or 3-OAc-morphinone (**9**) (2 mmol), gluthatione (610 mg, 2 mmol) were dissolved in 5%(w/w) $NaHCO_3$ solution (6 mL, degassed with argon before use to avoid disulfide formation). The vial was inserted into the microwave cavity of the CEM Discover microwave reactor, irradiated at the 60°C target temperature in P_{max} mode for 5 min hold time and subsequently cooled by rapid gas-jet cooling. The product mixture was allowed to cool to room temperature in the microwave cavity then removed and stirred under ambient condition for an additional 14 h. The pH of the mixture was set to 6 by 0.1 M HCl solution and extracted with EtOAc (3×15 mL). The organic layers were collected, washed

with saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to SiO_2 column chromatography. Elution with $CHCl_3$:MeOH=4:1 gave morphinans **6** and **5** as free bases, respectively.

***S*-[4,5-epoxy-3-hydroxy-17-methyl-6-oxo-morphinan-(8*S*)-yl] glutathione (5)**

Yield: 48 % (576.mg). White foam. $[\alpha]_D^{20}$ -149 (c 0.50, methanol); R_f (CH_2Cl_2/CH_3OH = 8/2) 0.17; ν_{max}(KBr disc) 3394 (OH stretch), 3054 (NH stretch), 1733 and 1729 (2 x OC=O), 1718 (C=O ring), 1653 (NC=O) cm^{-1}; HRMS (ESI) m/z (%) found: 613.6481 ($M+Na^+$, 100), calculated: 613.6472 ($M+Na^+$); ^1H and ^{13}C NMR data are in line with the high-field data presented in [4a].

***S*-[4,5-epoxy-3-methoxy-17-methyl-6-oxo-morphinan-(8*S*)-yl] glutathione (6)**

Yield: 57 % (689 mg). Pale yellow oil. $[\alpha]_D^{20}$ -162 (c 0.50, methanol); R_f (CH_2Cl_2:CH_3OH = 4:1) 0.25; ν_{max}(KBr disc) 3390 (OH stretch), 3051 (NH stretch), 1738 and 1734 (2 x OC=O), 1722 (C=O ring), 1651 (NC=O) cm^{-1}; HRMS (ESI) m/z (%) found: 627.6750 ($M+Na^+$, 100), calculated: 627.6743 ($M+Na^+$); ^1H and ^{13}C NMR data are in line with the high-field data presented in ref. 5a.

2.2. Synthesis of heterocycle-fused codeinone analog 10

Codeinone (**4**) (297 mg, 1 mmol) and L-cysteine ethyl ester.HCl (550 mg, 3 mmol) was dissolved in 1,4-dioxane (10 mL). To the mixed solution 4 mL of 1M NaOH solution was added in one portion and stirred for 1 h at room temperature. The solution is evaporated to dryness under reduced pressure; the residue was diluted with distilled water (10 mL) and extracted with chloroform (3×8 mL). The organic layers were collected, dried over anhydrous $MgSO_4$ and evaporated to dryness. The white remaining gum was subjected to column chromatography (on SiO_2 stationary phase using $CHCl_3$:CH_3OH=4:1 as an eluent). The obtained colorless oil was crystallized from methanol yielding 130 mg (29%) of compound **10** as white crystals. M.p. 219-21°C; R_f (CH_2Cl_2:CH_3OH = 4:1)= 0.33; $[\alpha]_D^{20}$ -109 (c 0.50, chloroform); ν_{max}(KBr disc) 3111 (NH stretch), 1748 (OC=O), 1622 (C=N ring) cm^{-1}; HRMS (ESI) m/z (%) found: 379.4796 ($M+Na^+$, 100), calculated: 379.4803 ($M+Na^+$); ^1H-NMR ($CDCl_3$): δ = 1.31 (t, 3H, $CH_2C\underline{H}_3$), 1.79 (m, 1H, 15α-H), 2.08 (td, 1H, 15β-H, $J_{15\alpha,15\beta;15\alpha,16\alpha}$ 12.4, $J_{15\alpha,16\beta}$ 5.0), 2.17-2.24 (m, 1H, 10α-H), 2.31 (td, 1H, 16α-H, $J_{16\alpha,16\beta;16\alpha,15\beta}$ 12.0, $J_{16\alpha,15\beta}$ 4.1), 2.44 (s, 3H, NCH_3), 2.60 (dd, 1H, 16β-H, $J_{16\beta,16\alpha}$ 12.1, $J_{16\beta,15\beta}$ 4.6), 2.61-2.64 (m, 1H, 14-H), 2.72 (d, 2H, SCH_2, *J* 6.1), 3.01 (d, 1H, 10β-H, *J* 16.1), 3.51 (d, 1H, C<u>H</u>COOEt, *J* 5.8), 3.87 (s, 3H, OCH_3), 4.22 (m, 2H, $OC\underline{H}_2CH_3$), 4.31 (m, 1H, 9-H), 4.50 (d, 1H, 7-H, *J* 3.3), 4.81 (s, 1H, 5β-H), 6.61 (d, 1H, 1-H, $J_{1,2}$ 7.9), 6.69 (d, 1H, 2-H, $J_{1,2}$ 7.9); ^{13}C-NMR ($CDCl_3$): δ = 14.08 ($OCH_2\underline{C}H_3$), 19.78 (C-10), 27.39 (SCH_2), 35.19 (C-15), 41.14 (C-8), 42.27 (C-13), 42.95 (NCH_3), 43.69 (C-14), 46.78 (C-16), 56.02 (CH), 56.47 (C-9), 56.90 (OCH_3), 61.69 ($O\underline{C}H_2CH_3$), 88.12 (C-5), 99.53 (C-6), 114.80 (C-1), 119.24 (C-2), 126.63 (C-11), 129.10 (C-12), 142.95 (C-3), 144.15 (C-4), 152.7 (C-7), 171.49 ($\underline{C}OOCH_2CH_3$).

2.3. Synthesis of methyl esters of 8β-(N-acetylcysteine)-adducts 11 and 12

Codeinone (**4**) or 3-OAc-morphinone (**9**) (1 mmol) and 350 mg of *N*-acetyl-L-cysteine methyl ester were dissolved in 10 mL of acetonitrile. To the colourless solution 500 mg of $NaHCO_3$ (6 mmol) was given and stirred for 2.5 h at room temperature. The solution is evaporated to dryness under reduced pressure; the residue was diluted with distilled water (10 mL) and extracted with chloroform (3×8 mL). The organic layers were collected, dried over anhydrous $MgSO_4$ and evaporated to dryness. Residue **11** and **12** were found to be pure on the basis of subsequent spectral analyses.

***S*-[4,5-epoxy-3-hydroxy-17-methyl-6-oxo-morphinan-(8*S*)-yl]-*N*-acetylcysteine methyl ester (12)**

This procedure gave rise to 110 mg of compound **12** (21%) as colourless oil. R_f (CH_2Cl_2:CH_3OH = 4:1)= 0.32; $[\alpha]_D^{20}$ -111 (c 0.50, chloroform); ν_{max}(KBr disc) 3383 (OH stretch), 3051 (NH stretch), 1741 (OC=O), 1711 (C=O ring), 1663 (NC=O) cm^{-1}; HRMS (ESI) m/z (%) found: 483.5435 ($M+Na^+$, 100), calculated: 483.5432 ($M+Na^+$); ^1H-NMR ($CDCl_3$): δ = 1.82 (m, 1H, 15α-H), 2.02 (s, 3H, $COC\underline{H}_3$), 2.11-2.43 (m, 5H, 7α-H, 7β-H, 10α-H, 15β-H, 16α-H), 2.51 (s, 3H, NCH_3), 2.63-2.78 (m, 2H, 14-H, 16β-H), 2.84 (dd, 1H, 8α-H, $J_{8\alpha,14}$ 5.4, $J_{7\beta,8\alpha}$ 2.5), 2.92-3.07 (m, 2H, 10β-H, SCH_2), 3.53 (s, 3H, $COOC\underline{H}_3$), 4.11 (m, 1H, 9-H), 4.72 (s, 1H, 5β-H), 4.82 (m, 1H, CH), 6.41 (br s, 1H, NH), 6.64 (d, 1H, 1-H, $J_{1,2}$ 8.1), 6.76 (d, 1H, 2-H, $J_{1,2}$ 8.1); ^{13}C-NMR ($CDCl_3$): δ = 19.49 (C-10), 22.98 ($COCH_3$), 31.14 (SCH_2), 34.55 (C-15), 41.24 (C-8), 42.37 (NCH_3), 43.99 (C-14), 46.76 (C-13), 47.29 (C-16), 50.30 (C-7), 52.32 (CH), 52.44 ($COO\underline{C}H_3$), 57.33 (C-9), 91.02 (C-5), 118.26 (C-1), 120.69 (C-2), 123.25 (C-11), 125.96 (C-12), 139.54 (C-3), 143.79 (C-4), 170.13 ($\underline{C}OCH_3$), 170.93 ($\underline{C}OOCH_3$), 205.47 (C-6).

***S*-[4,5-epoxy-17-methyl-3-methoxy-6-oxo-morphinan-(8*S*)-yl]-*N*-acetylcysteine methyl ester (11)**

This procedure gave rise to 240 mg of compound **11** (50%) as pale yellow oil. R_f (CH_2Cl_2:CH_3OH = 4:1)= 0.47; $[\alpha]_D^{20}$ -127 (c 0.50, chloroform); ν_{max}(KBr disc) 3043 (NH stretch), 1738 (OC=O), 1712 (C=O ring), 1657 (NC=O) cm^{-1}; HRMS (ESI) m/z (%) found: 497.5711 ($M+Na^+$,

100), calculated: 497.5703 (M+Na$^+$); ^{1}H-NMR ($CDCl_3$): δ = 1.91 (m, 1H, 15α-H), 2.06 (s, 3H, $COCH_3$), 2.20-2.54 (m, 8H, 7α-H, 7β-H, 10α-H, 15β-H, 16α-H, NCH_3), 2.69-2.87 (m, 2H, 14-H, 16β-H), 2.91(dd, 1H, 8α-H, $J_{8α,14}$ 5.1, $J_{7β,8α}$ 2.7), 2.96-3.14 (m, 2H, 10β-H, SCH_2), 3.54 (s, 3H, 3-OCH_3), 3.66 (s, 3H, $COOCH_3$), 3.99 (m, 1H, 9-H), 4.72 (s, 1H, 5β-H), 4.82 (m, 1H, CH), 6.38 (br s, 1H, NH), 6.63 (d, 1H, 1-H, $J_{1,2}$ 7.9), 6.80 (d, 1H, 2-H, $J_{1,2}$ 7.9); ^{13}C-NMR ($CDCl_3$): δ = 19.00 (C-10), 22.76 ($COCH_3$), 31.06 (SCH_2), 35.20 (C-15), 41.22 (C-8), 42.61 (NCH_3), 44.12 (C-14), 46.88 (C-13), 47.10 (C-16), 51.30 (C-7), 52.05 (CH), 52.27 ($COOCH_3$), 52.54 (C-3-OCH_3), 56.61 (C-9), 91.09 (C-5), 114.67 (C-1), 120.10 (C-2), 126.09 (C-11), 126.44 (C-12), 142.73 (C-3), 144.84 (C-4), 169.69 ($COCH_3$), 170.63 ($COOCH_3$), 204.61 (C-6).

2.4. Hydrolysis of esters 11 and 12 to form 8β-(N-acetylcysteine)-adducts 13 and 14

A solution of compound **11** or **12** (0.5 mmol) in CH_3OH:H_2O=3:1 (10 mL) was treated with LiOH (156 mg, 3.71 mmol). The mixture was stirred overnight at room temperature and then its pH was set to 6 by 0.1 M HCl solution and extracted with EtOAc (3x15 mL). The organic layers were collected, washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to SiO_2 column chromatography. Elution with $CHCl_3$:CH_3OH=3:2 gave morphinans **13** and **14** as free bases, respectively.

***S*-[4,5-epoxy-3-hydroxy-17-methyl-6-oxo-morphinan-(8*S*)-yl]-*N*-acetylcysteine (13)**

This procedure gave rise to 161 mg of compound **13** (72%) as colourless oil. R_f (CH_2Cl_2:CH_3OH = 4:1)= 0.16; $[α]_D^{20}$ -131 (c 0.50, methanol); v_{max}(KBr disc) 3380 (OH stretch), 3050 (NH stretch), 1753 (OC=O), 1710 (C=O ring), 1670 (NC=O) cm^{-1}; HRMS (ESI) m/z (%) found: 468.5520 (M+Na$^+$, 100), calculated: 468.5518 (M+Na$^+$); ^{1}H-NMR ($CDCl_3$): δ = 1.89 (m, 1H, 15α-H), 2.02 (s, 3H, $COCH_3$), 2.20-2.59 (m, 8H, 7α-H, 7β-H, 10α-H, 15β-H, 16α-H, NCH_3), 2.67-2.89 (m, 2H, 8α-H, 14-H, 16β-H), 2.94-3.13 (m, 2H, 10β-H, SCH_2), 4.17 (m, 1H, 9-H), 4.74 (s, 1H, 5β-H), 4.87 (m, 1H, CH), 6.58 (br s, 1H, NH), 6.69 (d, 1H, 1-H, $J_{1,2}$ 8.1), 6.79 (d, 1H, 2-H, $J_{1,2}$ 8.0); ^{13}C-NMR ($CDCl_3$): δ = 19.94 (C-10), 23.06 ($COCH_3$), 31.55 (SCH_2), 34.28 (C-15), 41.10 (C-8), 43.09 (NCH_3), 44.77 (C-14), 46.76 (C-13), 47.27 (C-16), 51.61 (C-7), 52.55 (CH), 57.69 (C-9), 91.20 (C-5), 118.44 (C-1), 121.39 (C-2), 123.64 (C-11), 125.90 (C-12), 140.12 (C-3), 144.20 (C-4), 166.93 (COOH), 170.32 ($COCH_3$), 205.70 (C-6).

***S*-[4,5-epoxy-17-methyl-3-methoxy-6-oxo-morphinan-(8*S*)-yl]-*N*-acetylcysteine (14)**

This procedure gave rise to 175 mg of compound **14** (76%) as yellow oil. R_f (CH_2Cl_2:CH_3OH = 4:1)= 0.14; $[α]_D^{20}$ -139 (c 0.50, methanol); v_{max}(KBr disc) 3397 (OH stretch), 3039 (NH stretch), 1748 (OC=O), 1709 (C=O ring), 1656 (NC=O) cm^{-1}; HRMS (ESI) m/z (%) found: 482.5799 (M+Na$^+$, 100), calculated: 482.5789 (M+Na$^+$); ^{1}H-NMR ($CDCl_3$): δ = 1.78 (m, 1H, 15α-H), 1.97 (s, 3H, $COCH_3$), 2.27-2.57 (m, 8H, 7α-H, 7β-H, 10α-H, 15β-H, 16α-H, NCH_3), 2.63-2.84 (m, 2H, 14-H, 16β-H), 2.91(dd, 1H, 8α-H, $J_{8α,14}$ 4.7, $J_{7β,8α}$ 2.6), 3.01-3.18 (m, 2H, 10β-H, SCH_2), 3.61 (s, 3H, 3-OCH_3), 3.79 (m, 1H, 9-H), 4.71 (s, 1H, 5β-H), 4.87 (m, 1H, CH), 6.35 (br s, 1H, NH), 6.68 (d, 1H, 1-H, $J_{1,2}$ 8.0), 6.78 (d, 1H, 2-H, $J_{1,2}$ 8.0); ^{13}C-NMR ($CDCl_3$): δ = 19.21 (C-10), 22.80 ($COCH_3$), 30.94 (SCH_2), 35.11 (C-15), 41.30 (C-8), 43.12 (NCH_3), 44.63 (C-14), 46.88 (C-13), 47.10 (C-16), 51.30 (C-7), 52.05 (CH), 52.54 (C-3-OCH_3), 56.61 (C-9), 91.09 (C-5), 114.67 (C-1), 120.10 (C-2), 126.09 (C-11), 126.44 (C-12), 142.73 (C-3), 144.84 (C-4), 165.61 (COOH), 169.64 ($COCH_3$), 204.70 (C-6).

2.5. Computational procedure

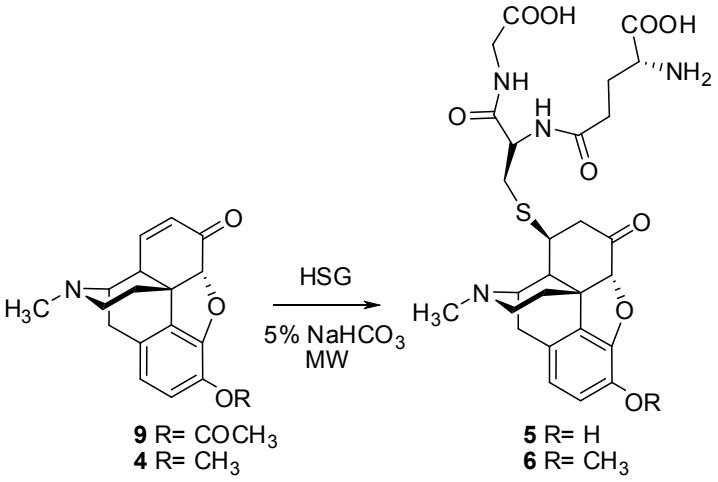
We have carried out the geometry optimization at Becke's three parameter hybrid (B3LYP) [13] levels in the DFT with the basis set 6-31G* using Gaussian 03 [14].

3. Results and discussion

3.1. Synthesis of 8β-glutathione adducts

After searching the literature it was found that only a few Michael addition of glutathione were reported on α,β-unsaturated ketones. One of the most pertinent examples was published by Evans and his co-workers [7] describing the glutathione addition to cyclopentenones under divergent conditions. Codeinone (**4**) and 3-acetylmorphinone (**9**) were chosen as starting compounds as in the case of these derivatives the phenolic 3-OH is appropriately protected [8a and 8b, respectively]. Our initial reactions were based on Evans' conditions; however it was observed that there were solubility issues in the applied polar media (Scheme 2).

Codeinone (**4**) was suspended in acetone and an equimolar amount of glutathione solution prepared with distilled water was added slowly (Table 1). The reaction was initiated with sonication/heating and microwave irradiation before stirring at room temperature. The expected product **6** was obtained in low yields, probably due to the poor solubility of the morphinan in the solvent mixture. Solvents were changed to the ethanol-buffer pH 7.4 combination, however no significant improvement was observed. The conversion of compound **4** to its hydrochloride salt form was found to be favourable, but acceptable yields were only achieved after turning



Scheme 2. Synthesis of 8β-glutathionyl-dihydromorphinone (**5**) and -dihydrocodeinone (**6**).

Table 1. Optimization of glutathione addition to compounds **4** and **9**.

Run	Reactant	Solvent	Condition	Yield (%)[1]
1.	4	acetone+water	thermal[2]	3
2.	4	acetone+water	MW[3]	11
3.	4	ethanol+buffer pH 7.4	thermal[2]	4
4.	4.HCl	ethanol+buffer pH 7.4	thermal[2]	27
5.	4.HCl	5% $NaHCO_3$	thermal[2]	45
6.	4.HCl	5% $NaHCO_3$	MW[3]	57
7.	9	5% $NaHCO_3$	MW[3]	48

[1]Yields after isolation with column chromatography; [2]thermal conditions: sonication at 60°C for 10 min and stirring at room temperature for 14 h; [3]MW conditions: irradiation at 60°C for 5 min under nitrogen in P_{max} mode and stirring at room temperature for 14 h.

the pH of the medium into more basic by applying 5% $NaHCO_3$ solution (pH~8.5). The microwave promotion provided compound **6** in somewhat higher yield. 3-Acetylmorphinone (**9**) has higher water solubility than codeinone (**4**), therefore the conditions optimized for compound **4** were also appropriate for the synthesis of glutathione adduct **5**. Besides the expected Michael-addition the deprotection of 3-hydroxyl function also took place resulting 8β-glutathionyl-dihydromorphinone (**5**) in average yield (48%). It must be stated that the thorough investigation of the crude product after every Michael addition showed the presence of only one type of product that was isolated, analyzed and identified as the 8β-substituted morphinan. This stereoselectivity, observed for every single Michael addition to the α,β-unsaturated ketone system of ring C of morphinans, is in accordance with the observations regarding the *in vivo* formation of metabolites [5,6] and the synthesis of Michael adduct 8-substituted morphinans [9]. The reason for this is most probably the steric hindrance of the morphinan skeleton observed from the geometry of ring A. It is well-known that the Michael addition is thermodynamically controlled; therefore the occurrence of 8β-derivatized products with lower enthalpy of formation is reasonable. A particularly informative theoretical investigation was presented by Ishida et al. in this context [5a]. The NMR and MS measurements for products **5** and **6** confirmed the expected conformation. The coupling constants between H8α-H14 (~5.5 Hz) and H7β-H8α (~2.5 Hz) were particularly informative.

Evans and co-workers investigated the possibility of thiol exchange under physiological conditions [7]. The glutathione adduct was successfully converted into cysteine adduct (in 63% yield) using excess *N*-Boc-cysteine methyl ester in pH 7.4 buffer. We adapted this procedure for product **5** using *N*-acetyl-L-cysteine methyl ester, but the formation of cysteine adduct **8** was detected only in traces. The reason for the lack of significant ratio of thiol exchange even in very long reaction times (96 h) could be the steric hindrance in the proximity of ring C of the morphinan skeleton in comparison to literature example, *i.e.*, a β-substituted cyclopentanone adduct. The spatial orientation of 8β-glutathionyl-dihydrocodeinone (**6**) obtained by geometry

optimization with density functional theory at the B3LYP/6-31G* level is presented in Fig. 1 confirming the probability of steric hindrance.

3.2. Synthesis of 8β-cysteine adducts

Due to the unsuccessful thiol exchange starting from glutathione derivatives it was necessary to establish a procedure for the synthesis of cysteine adducts.

First, the reaction of codeinone (**4**) with the HCl salt of L-cysteine ethyl ester was studied (Scheme 3). In this case the highest yields were achieved in the 1,4-dioxane/1 M NaOH solvent mixture under mild conditions. Compound **4** and the cysteine ester were dissolved in dioxane and the NaOH solution was added in portions. After 1 hour of stirring at room temperature the reaction mixture was evaporated and the residue was subjected to column chromatography.

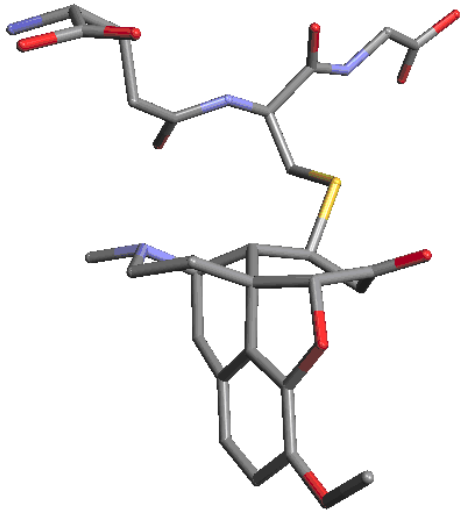


Figure 1. Optimized geometry of compound **6** obtained at the B3LYP/6-31G* level of density functional theory.

The primary characterization of the main product revealed that the formation of the expected Michael adduct was followed by an intramolecular ring closure with the 6-keto functionality. The formation of this structurally unique 1,4-thiazepine-fused morphinan **10** was particularly exciting for us as it is a fundamental objective of our research group to reveal new strategies for the synthesis of potentially opioid active heterocycle-fused morphinans [10].

Therefore several spectroscopic techniques were applied to explore the exact structure of product **10**, such as COSY, TOCSY, HSQC and ^{13}C HMBC. The double bond is positioned between C6-C7 and is not in exo-position related to the C-ring of the morphinan backbone.

The most important correlations were depicted in Fig. 2.

The chemical shifts of protons were identified based on the unambiguous assignments of H10α and H10β (Fig. 2; Panel A). The presented structure of compound **10** was proven by the coupling between H7-H8α and H8α-H14 (COSY, TOCSY in Fig. 2; Panel A); the heteronuclear coupling between C7-NH and C5-NH (Fig. 2; Panel B); the quaternary, non-carbonyl character of C6; the allyl couplings between H7-NH (COSY in Fig. 2; Panel C) and the homoallyl couplings between H5β-H8α (COSY, TOCSY in Fig. 2; Panel C).

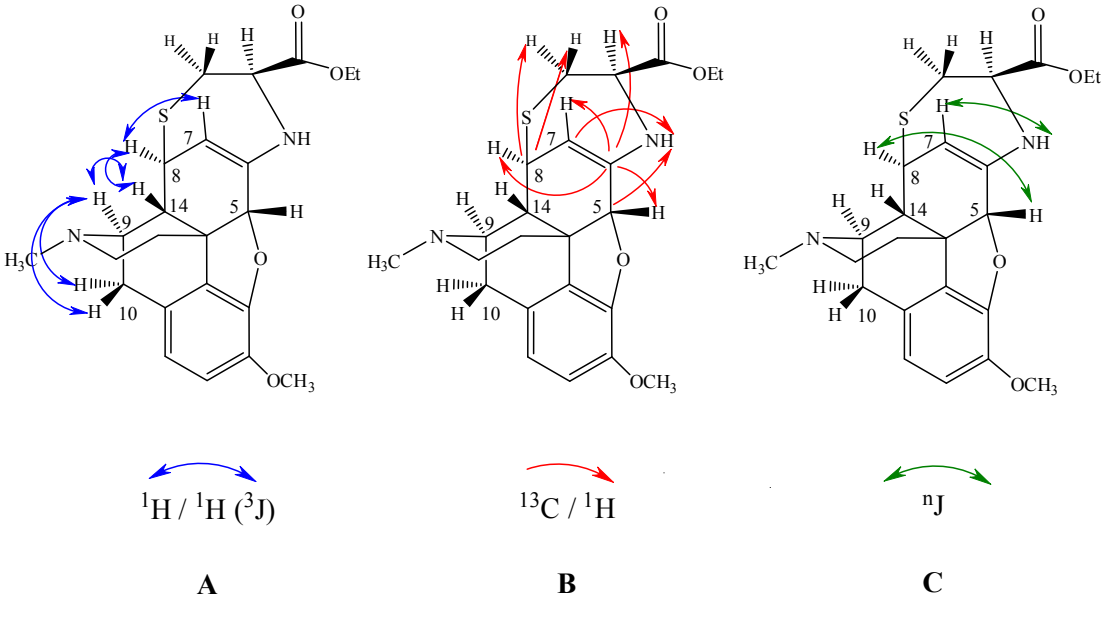
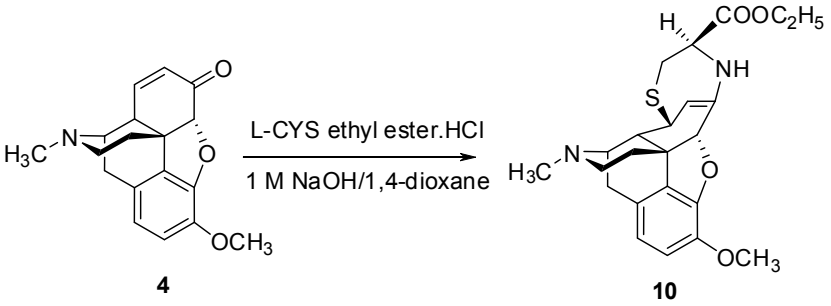
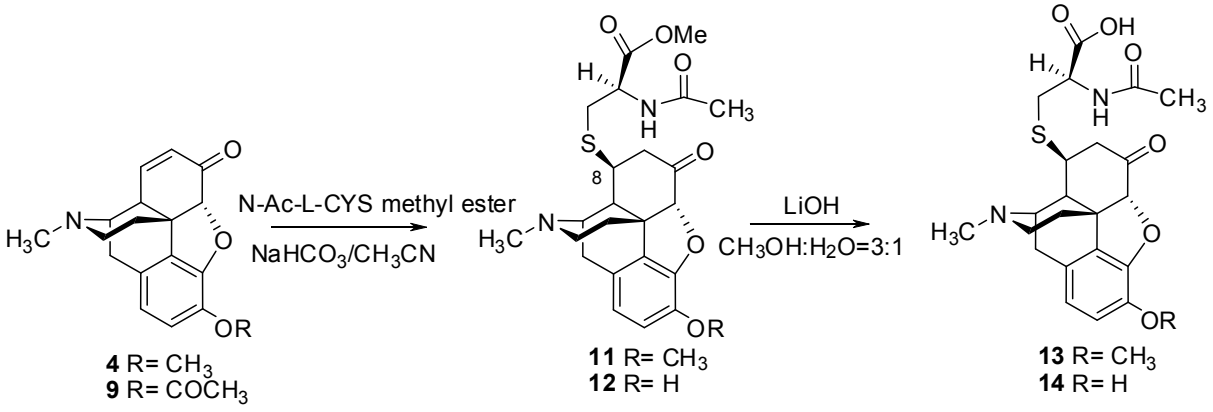


Figure 2. The most important 2D NMR correlations of compound **10**.



Scheme 3. Reaction of codeinone (**4**) and L-cysteine ethyl ester.



Scheme 4. Synthesis of *N*-acetyl-cysteine adduct of codeinone (**13**) and 3-acetylmorphinone (**14**).

After failing to synthesize the aimed 8β-cysteinyl-morphinan derivative from codeinone (**4**), the next step was to change the reagent to *N*-acetyl-L-cysteine methyl ester. In a few optimization reactions we found the acetonitrile solution of $NaHCO_3$ as an efficient reactant affording the *N*-acetyl-L-cysteine methyl ester adduct of dihydrocodeinone **11** in acceptable yield (50%) (Scheme 4).

Starting from 3-acetylmorphinone (**9**) the desired methyl ester of the 8β-(*N*-acetylcysteine)-adduct **12** was obtained in 21% yield under similar conditions. However, in this step the Michael addition was followed by the hydrolysis of the 3-acetyl protective group giving rise to 8β-substituted dihydromorphinone **12**. The methyl ester moiety of the cysteine side-chain was found to be stable, which conforms to the observation that the esters of α-amino acids are usually more stable than a common ester due to the presence of some stabilizing H-bonding.

The deprotection of the C-terminal of compounds **11** and **12** was achieved under alkaline conditions using LiOH in diluted methanolic solution [11]. The aimed 8β-(*N*-acetylcysteine)-adducts **13** and **14** were obtained in 76 and 72% yields, respectively. In all cases the conformation of C8 was confirmed by the analysis of ^{1}H-NMR spectra focusing on H8α-H14 and H7β-H8α couplings as it was mentioned for compounds **5** and **6**.

4. Conclusions

We have presented synthetic procedures for the preparation of toxicologically and pharmacologically important metabolites of medically important opiates morphine (**1**) and codeine (**2**). The successful synthesis of secondary and tertiary metabolite gluthatione and *N*-acetylcysteine adducts (**5-6** and **13-14**, respectively) allowed us to study the opioid receptor activities of these physiologically crucial derivatives in co-operation with our pharmacological partners. These tests confirmed that these compounds are practically inactive (K_i >1,000 nM) in agreement with the conclusions of several opioid activity studies carried out by Kotick *et al*. [12] stating that spatially challenging substituents are not well tolerated in position 8 of the morphinan backbone. The complex toxicological studies of these metabolites are in progress.

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References

[1] (a) J.V. Aldrich, S.C. Vigil-Cruz, Narcotic Analgesics. In: Burger's Medicinal Chemistry and Drug Discovery (John Wiley & Sons, New York, 2003) 329; (b) M.H. Levy, New Engl. J. Med. 335, 1124 (1996)

[2] L.A. Woods, J. Pharm. Exp. Therap. 112, 158 (1954)

[3] Y. Kumagi, T. Tokdaka, S. Toki, J. Pharm. Exp. Therap. 255, 504 (1990)

[4] B.J. Canning, Pulmon. Pharmacol. Ther. 22, 75 (2009)

[5] (a) T. Ishida, Y. Kumagai, Y. Ikeda, K. Ito, M. Yano, S. Toki, K. Mihasni, T. Fujioka, Y. Iwase, S. Hachiyama, Drug Metabol. Dispos. 17, 77 (1989); (b) M. Jairaj, D.G. Watson, M.H. Grant, G.G. Skellern, Chem.-Biol. Interactions 146, 121 (2003)

[6] (a) T. Ishida, M. Yano, S. Toki, J. Anal. Toxicol. 22, 567 (1998); (b) T. Ishida, Y. Kumagai, Y. Ikeda, K. Ito, S. Yano, S. Hachiyama, Org. Mass Spectroscopy 24, 286 (1989); (c) S. Yamano, E. Kageura, T. Ishida, S. Toki, J. Biol. Chem. 260, 5259 (1985); (d) S. Yamano, A. Takahashi, T. Todaka, S. Toki, Xenobiotica 27, 645 (1997)

[7] J.F. Bickley, A. Ciucci, P. Evans, S.M. Roberts, N. Rossa, M.G. Santoro, Bioorg. Med. Chem. 12, 3221 (2004)

[8] (a) S.P. Findlay, L.F. Small, J. Am. Chem. Soc. 72, 3247 (1950); (b) H. Rapoport, D.R. Baker, H.N. Reist, J. Org. Chem. 22, 1489 (1957)

[9] T. Sagara, M. Okamura, Y. Shimohigashi, M. Ohno, K. Kanematsu, Bioorg. Med. Chem. Lett. 14, 1609 (1995)

[10] (a) A. Sipos, L. Girán, H. Mittendorfer, H. Schmidhammer, S. Berényi, Tetrahedron 64, 1023 (2008); (b) M. Tóth, Zs. Gyulai, S. Berényi, A. Sipos, Lett. Org. Chem. 4, 539 (2007); (c) A. Sipos, S. Berényi, Monatsh. Chemie 140, 387 (2009); (d) L. Girán, S. Berényi, A. Sipos, Tetrahedron 64, 10388 (2008)

[11] E.J. Corey, I. Szekely, C.S. Shiner, Tetrahedron Lett. 18, 3529 (1977)

[12] (a) J.O. Polazzi, R.N. Schut, M.P. Kotick, J. Med. Chem. 23, 174 (1980); (b) M.P. Kotick, D.L. Leland, J.O. Polazzi, R.N. Schut, J. Med. Chem. 23, 166 (1980)

[13] (a) A.D. Becke, J. Chem. Phys. 98, 5648 (1993); (b) A.D. Becke Physical Reviews. A 38, 3098 (1988); (c) C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37, 785 (1988); (d) S.H. Vosko, L. Wilk, M. Nusair, Can. J. Phys. 58, 1200 (1980)

[14] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery, Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople Gaussian 03, Revision C.02, (Gaussian, Inc., Wallingford CT, 2004)