Supplementary Information

Electrochemical Behavior and Voltammetric Determination of two synthetic aroyl amides opioids.

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1. Abbreviations

U-47700: 3,4-dichloro-N-[2-(dimethylammino)ciclohexyl]-N-methyl-benzammide

AH-7921: 3,4-Dicloro-N-[[1-(dimetilammino)cicloesil]metil]benzammide

CV: cyclic voltammetry

CPE: exhaustive coulometry by controlled potential electrolysis

DPV: pulsed differential voltammetry

ν: scan speed (V/s)

E_p: peak potential (V)

i_p: peak intensity (A)

RECY: recovery factor

2. Characterization of the examined compounds.

Figure S1. *The three-electrode cell used in the CPE experiments.*

3. Cyclic voltammograms and related data

Exhaustive electrolysis of U-47700 and of AH-7921

Table S1. *Results obtained from cyclic voltammetry and exhaustive electrolysis for the considered compounds. Peak potential of the studied compounds evaluated by CV, potential applied for CPE and number of electron consumed during electrolysis. CV tests were performed at a concentration of 2.5* mM*, in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine). Potentials are referred to Ag/AgCl, 3* M *NaCl. Scan speed: 50* $mV \cdot s^{-1}$*. Working electrode: glassy carbon, 2* mm *diameter. As regards the CPE, all the tests were carried out at a concentration of 5* mM *of analyte in ethanol containing 0.1* M *lithium perchlorate and 2,6-lutidine (0.10* M*).*

Compound	E_{p1}/ mV CV	E_p / mV applied for CPE	e^-/molecule CPE
U-47700	+980	+1100	1
AH-7921	+1100	+1200	1

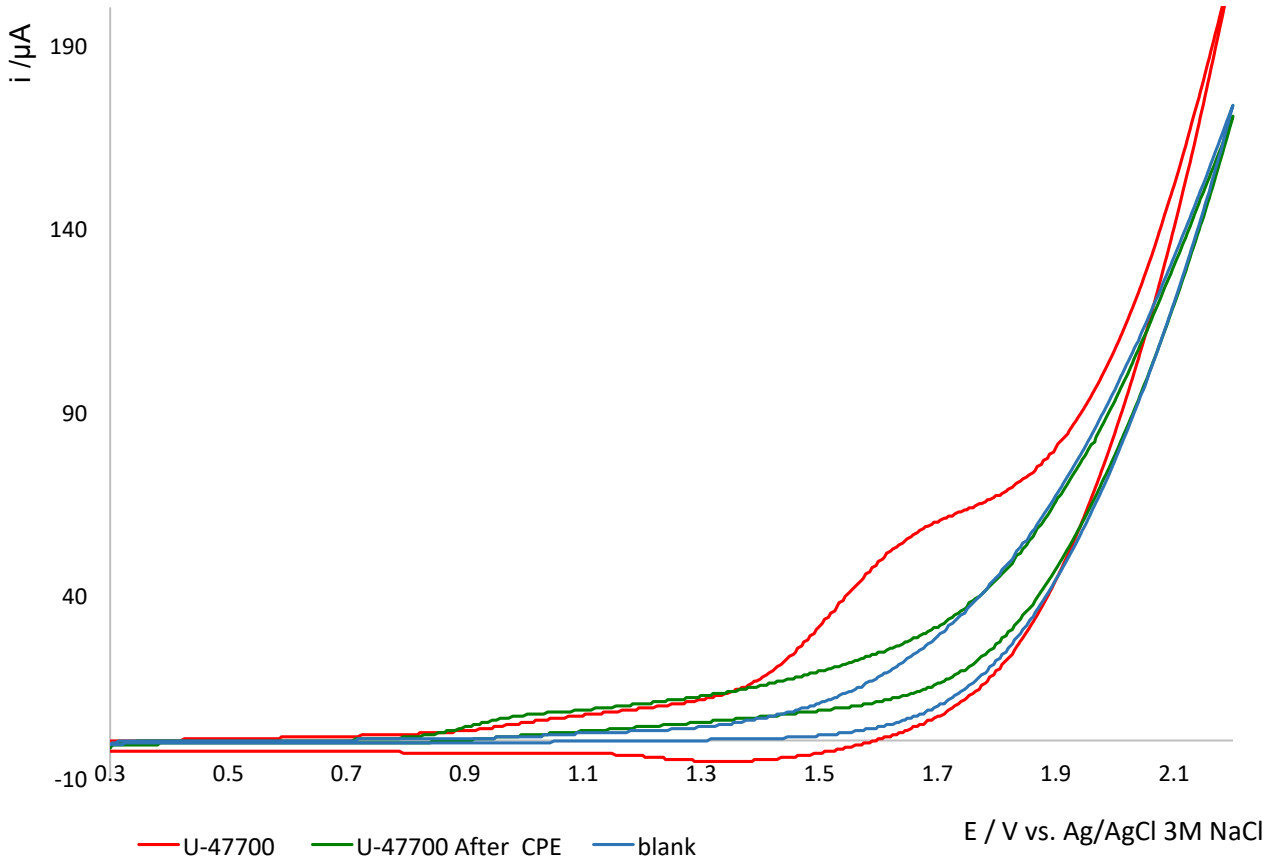


Figure S2. *Comparison between the cyclic voltammograms of U-47700 obtained before (red curve), after exhaustive electrolysis conducted at +1100* mV *(green curve). Cyclic voltammetry of U-47700 (2.5* mM, *in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine). Potentials are referred to Ag/AgCl, 3*M *NaCl. Scan speed: 50* $mV \cdot s^{-1}$. *Working electrode: glassy carbon, 2* mm *diameter. Other conditions described in the text.*

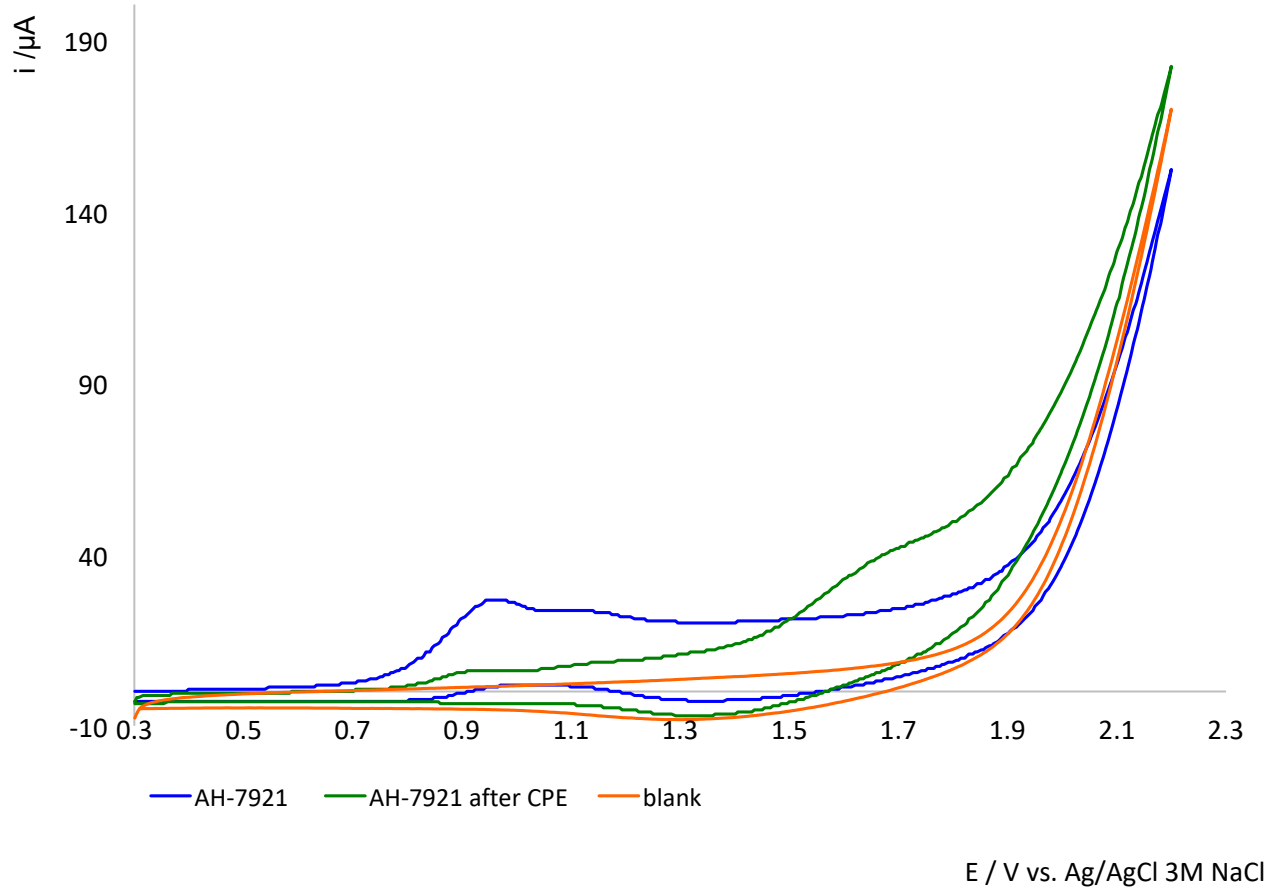


Figure S3. *Comparison between the cyclic voltammograms of AH-7921 obtained before (blue curve), after exhaustive electrolysis conducted at +1200* mV *(green curve). Cyclic voltammetry of AH-7921 (2.5* mM*, in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine). Potentials are referred to Ag/AgCl, 3* M *NaCl. Scan speed: 50* $mV \cdot s^{-1}$. *Working electrode: glassy carbon, 2* mm *diameter. Other conditions described in the text.*

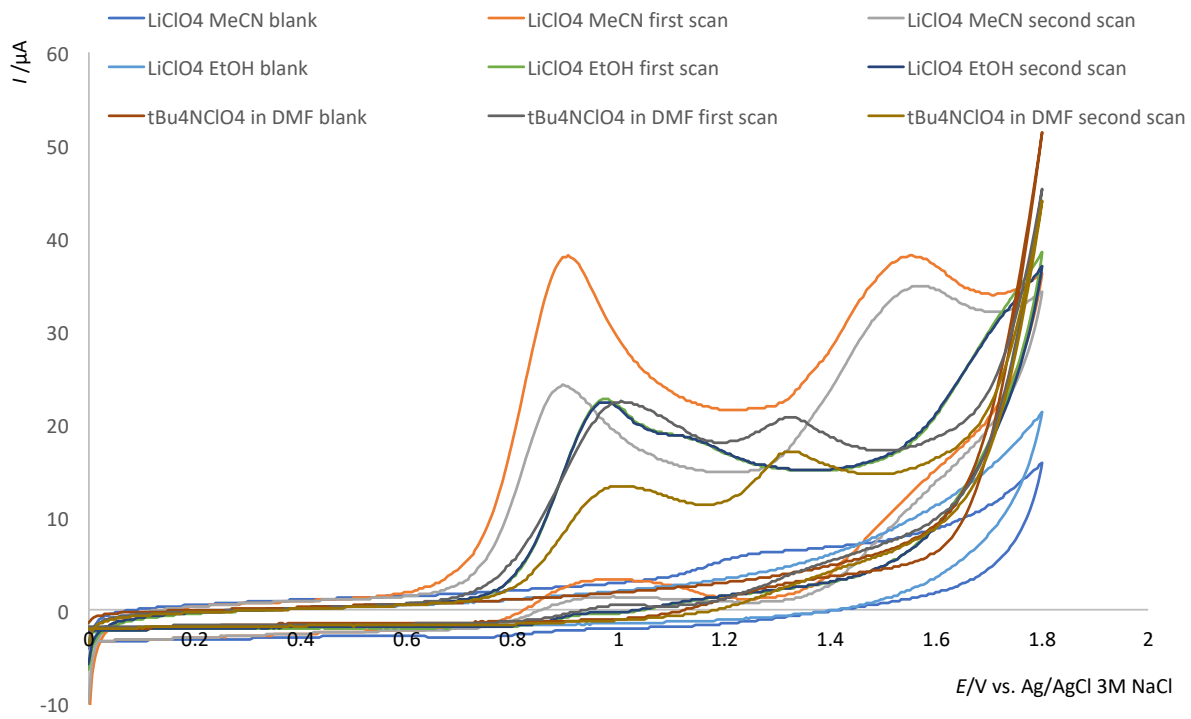


Figure S4. *Effect of different combinations of supporting electrolytes and solvents on the CV curves of AH-7921 (scan speed: 50* mV·s^{-1}*, AH-7921 concentration 2.5* mM*). Potentials are referred to Ag/AgCl, 3*M *NaCl. Working electrode: glassy carbon, 2* mm *diameter. Other conditions described in the text.*

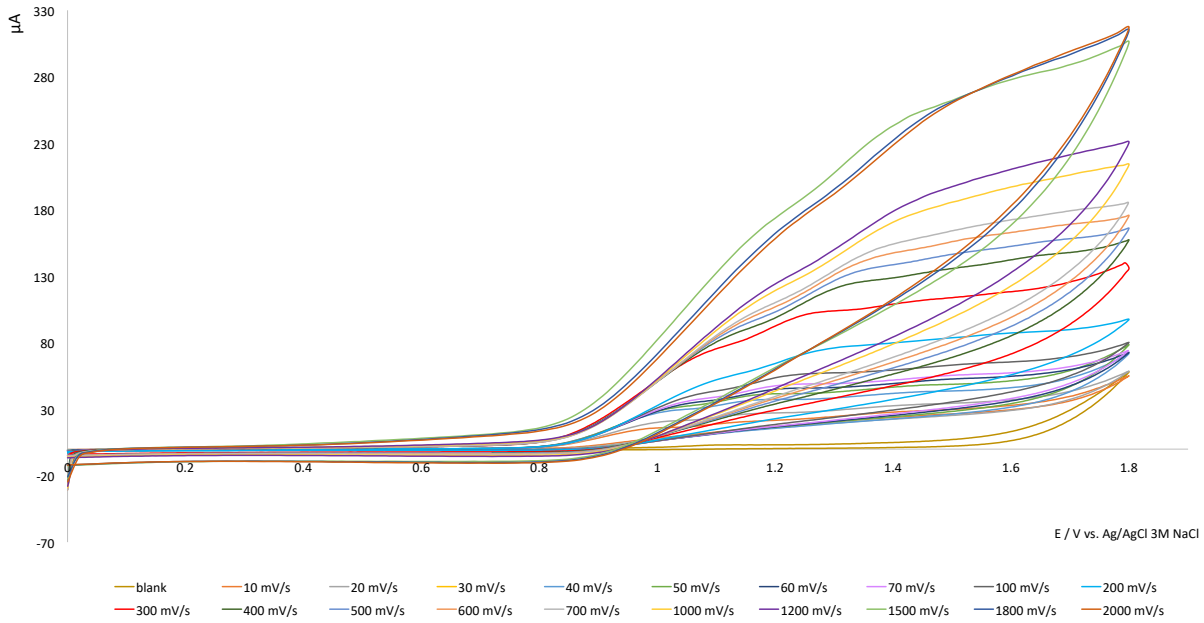


Figure S5. *Cyclic voltammetry of U-47700 (2.5* mM*, in ethanol/0.1 M lithium perchlorate and 0.10* M *2,6-lutidine) at different scan speed. Potentials are referred to Ag/AgCl, 3* M *NaCl. Working electrode: glassy carbon, 2* mm *diameter. Other conditions described in the text.*

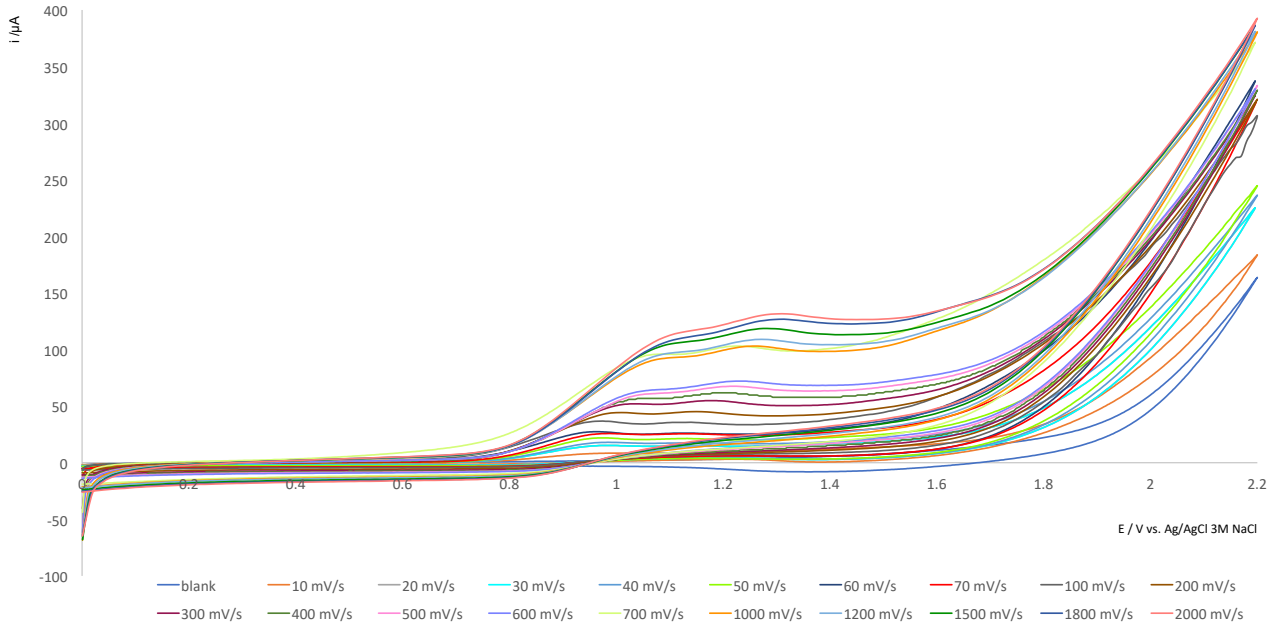


Figure S6. *Cyclic voltammetry of AH-7921 (2.5 mM, in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine) at different scan speed. Potentials are referred to Ag/AgCl, 3* M *NaCl. Working electrode: glassy carbon, 2* mm *diameter. Other conditions described in the text.*

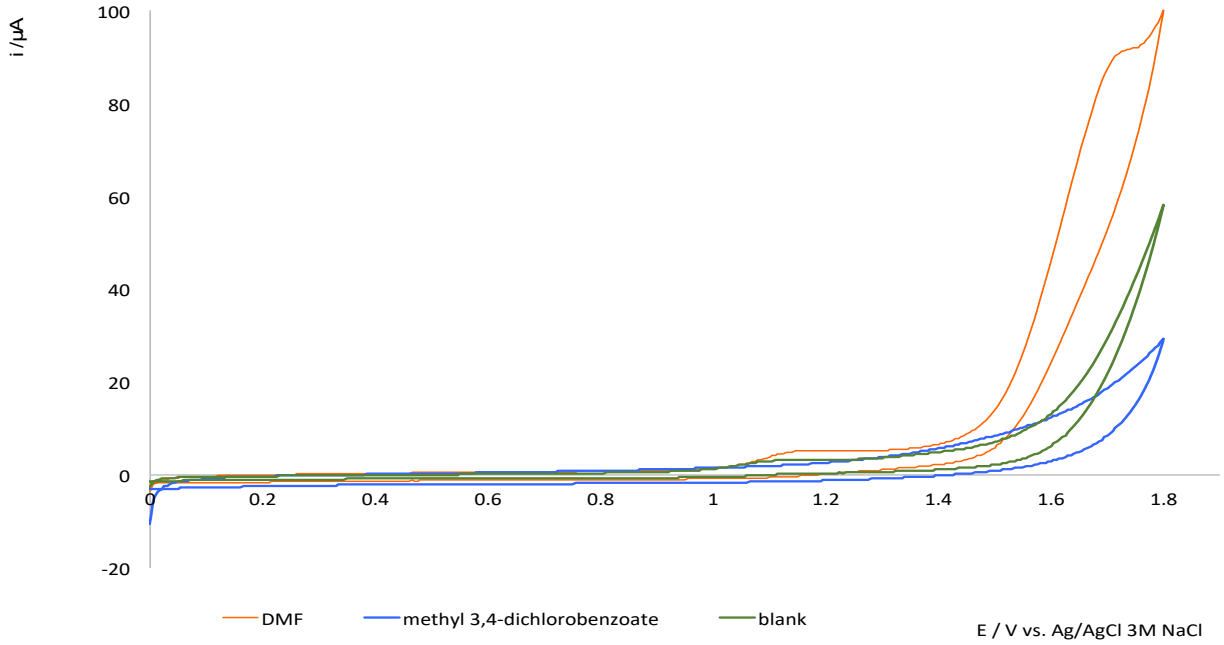
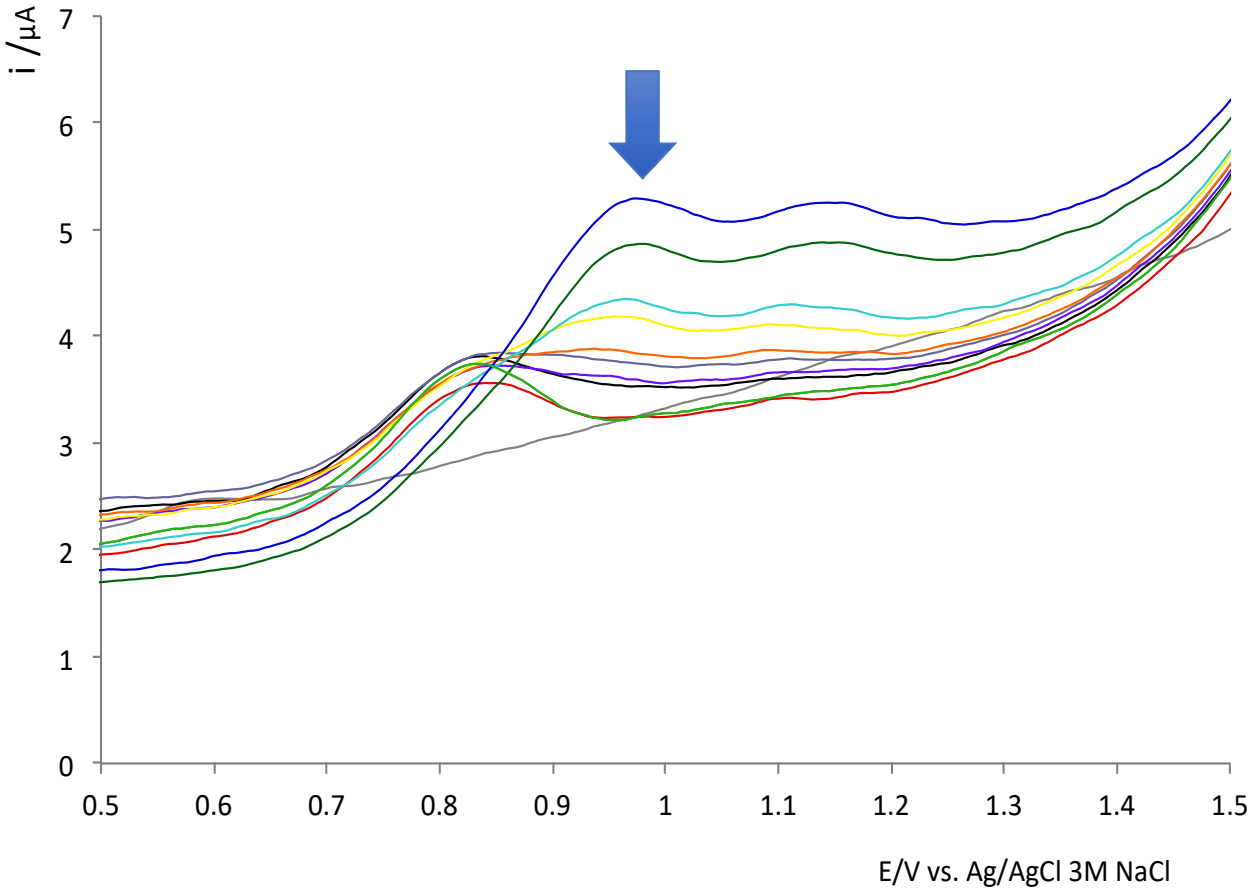


Figure S7. *Cyclic voltammetry of DMF and methyl 3,4-dichlorobenzoate (2.5* mM*, in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine). Potentials are referred to Ag/AgCl, 3* M *NaCl. Scan speed: 50* $mV \cdot s^{-1}$. *Working electrode: glassy carbon, 2* mm *diameter. Other conditions described in the text.*

4. Electrochemical determination of aroyl amides

Figure S8. *DPV of AH-7921 in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine and corresponding calibration curve; concentration range: from 5* $\mu g \cdot mL^{-1}$ *to 150* $\mu g \cdot mL^{-1}$*, in presence of 50* $\mu g \cdot mL^{-1}$ *of U-47700 (light green curve) and corresponding calibration curve. Potentials are referred to Ag/AgCl, 3*M *NaCl. Scan speed: 50* $mV \cdot s^{1}$*. Working electrode: glassy carbon, 2* mm *diameter. Each point of the calibration curve corresponds to the voltammetric curve of the same colour.*



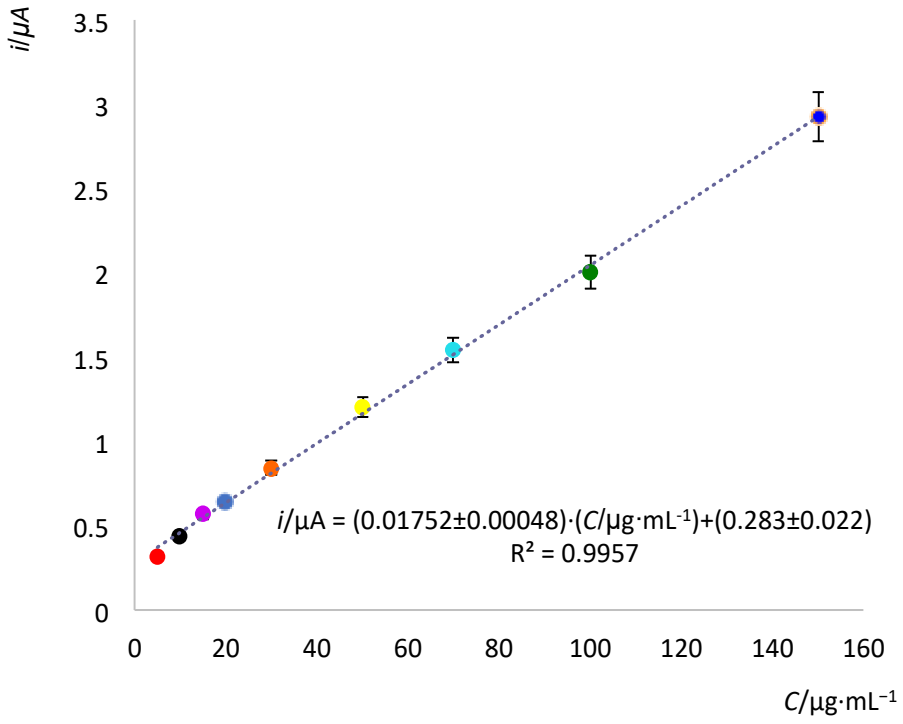
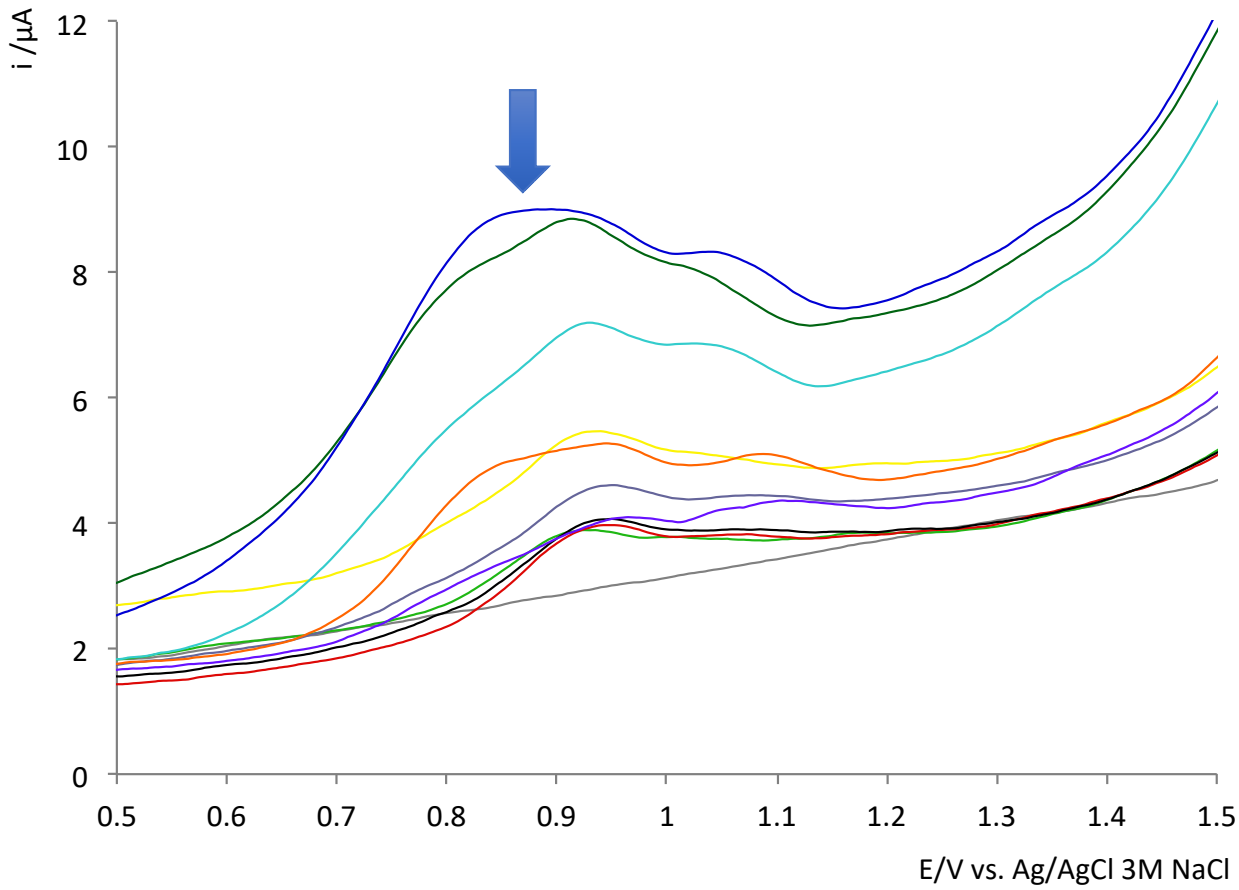
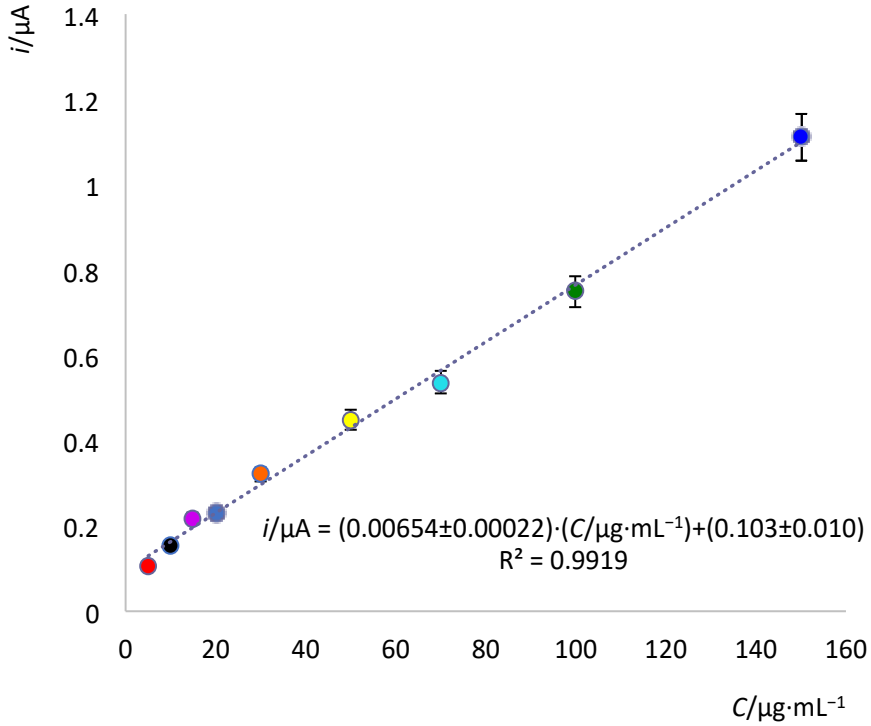


Figure S9. *DPV of U-47700 in ethanol/0.1* M *lithium perchlorate and 0.10* M *2,6-lutidine and corresponding calibration curve; concentration range: from 5* µg·mL^{-1} *to 150* µg·mL^{-1}*, in presence of 50* µg·mL^{-1} *of AH-7921(light green curve) and corresponding calibration curve. Potentials are referred to Ag/AgCl, 3*M *NaCl. Scan speed: 50* mV·s^{1}*. Working electrode: glassy carbon, 2* mm *diameter. Each point of the calibration curve corresponds to the voltammetric curve of the same colour.*


i /µA
12
10
8
6
4
2
0
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
E/V vs. Ag/AgCl 3M NaCl


i/µA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
160
C/µg·mL⁻¹
i/µA = (0.00654±0.00022)·(C/µg·mL⁻¹)+(0.103±0.010)
R² = 0.9919

5. Synthetic urine composition

Human urine is mainly composed of water (95%), the rest being urea (2%), creatinine (0.1%), uric acid (0.03%), chloride, sodium, potassium, sulfate, ammonium, phosphate and other ions and molecules in smaller quantities.

The MP-AU24 protocol was followed for the formulation of synthetic urine [Sarigul, N.; Korkmaz, F.; Kurultak, İ. A New Artificial Urine Protocol to Better Imitate Human Urine. Sci. Rep. 2019, 9 (1), 20159. https://doi.org/10.1038/s41598-019-56693-4.], by dissolving the proper amount of the reported substance in 100 mL of distilled water; before use, the solution is filtered using a 0.45 µm Nylon filter.

Component	Formula	Amount (g/100 mL)
Sodium sulphate	Na_2SO_4	0.1700
Uric acid	$C_5H_4N_4O_3$	0.0250
Sodium citrate tribasic dihydrate	$Na_3C_6H_5O_7 \cdot 2\ H_2O$	0.0720
Creatinine	$C_4H_7N_3O$	0.0881
Urea	CH_4N_2O	1.5000
Potassium chloride	KCl	0.2308
Sodium chloride	$NaCl$	0.1756
Calcium chloride	$CaCl_2$	0.0185
Ammonium chloride	NH_4Cl	0.1266
Sodium oxalate dibasic monohydrate	$Na_2C_2O_4 \cdot H_2O$	0.0035
Magnesium sulphate heptahydrate	$MgSO_4 \cdot 7\ H_2O$	0.1082
Sodium dihydrogenphosphate dihydrate	$NaH_2PO_4 \cdot 2\ H_2O$	0.2912
Disodium monohydrogenphosphate dihydrate	$Na_2HPO_4 \cdot 2\ H_2O$	0.0831

Figure S10. *DPV scans of U-47700 (100* µg·mL^{-1}*) in presence of the various interfering substances: dextrose (5000* µg·mL^{-1}*), galactose (*5000 µg·mL^{-1}*), lactose (*5000 µg·mL^{-1}*), mannitol, (*5000 µg·mL^{-1}*), glucose (*5000 µg·mL^{-1}*), EDTA (50* µg·mL^{-1}*), amphetamine (50* µg·mL^{-1}*), dextrine (70* µg· mL^{-1}*). In all cases, a variation in peak intensity <10% was found.*

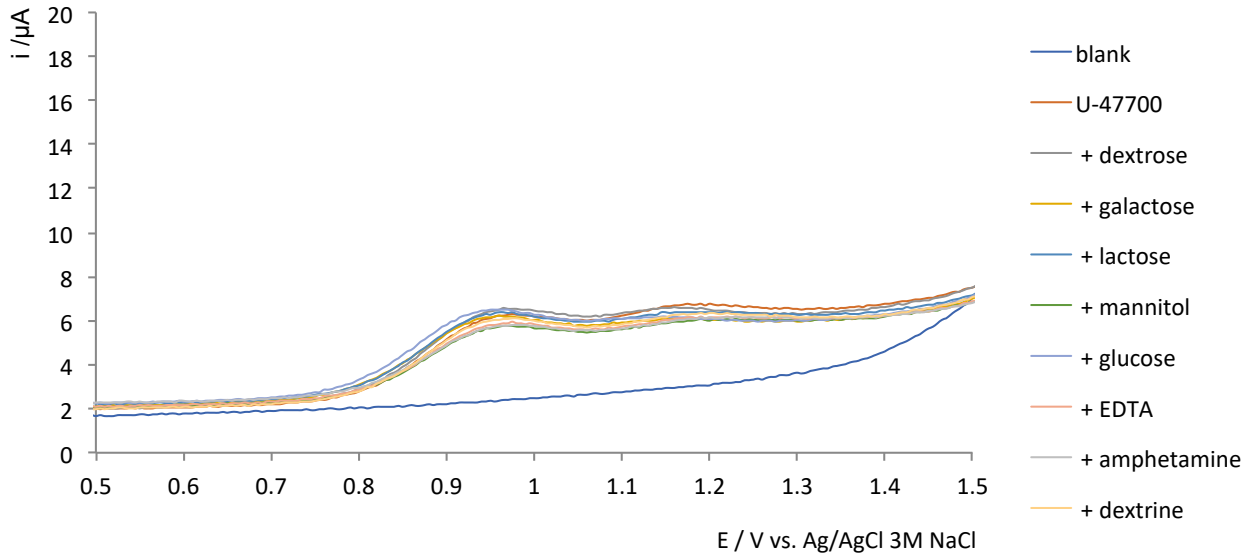


Figure S11. *DPV scans of AH-7920 (100* µg·mL^{-1}*) in presence of the various interfering substances: dextrose (5000* µg·mL^{-1}*), galactose (5000* µg·mL^{-1}*), lactose (5000* µg·mL^{-1}*), mannitol, (5000* µg·mL^{-1}*), glucose (5000* µg·mL^{-1}*), EDTA (50* µg·mL^{-1}*), amphetamine (50* µg·mL^{-1}*), dextrine (70* µg· mL^{-1}*). In all cases, a variation in peak intensity <10% was found.*