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Biopharmaceutical characterization of some new papaverine decomposition products

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Received September 23, 2010, accepted October 14, 2010

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Pharmazie 66: 165–167 (2011) *doi: 10.1691/ph.2011.0276*

2,3,9,10-Tetramethoxy-12-oxo-12-*H*-indolo[2,1-*a*]isoquinolinium chloride **1** (compound X) and 13-(3,4-dimethoxyphenyl)-2,3,8,9-tetramethoxy-6*a*,12*a*-diazadibenzo[*a,g*] fluorenylium chloride **2** (compound NF) are new papaverine oxidation products. A solution of compound **1** bleaches on addition of sodium hydroxide solution. A new entity, 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt **3** (compound WP), is formed. The physico-chemical properties of compounds 1–3, such as solubility in water and lipophilicity, have been measured. The IC_{50} for compounds **1** and **3** was also assessed.

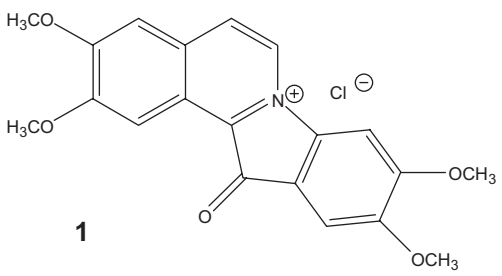
1. Introduction

Papaverine hydrochloride is used as a spasmolytic agent in the treatment of acute renal colic (Snir et al. 2008). It is an unstable drug and can be oxidized easily to papaverinol and papaveraldine (Machovičová and Parrák 1955; Piotrowska 2006). Some time ago, the new derivatives 2,3,9,10-tetramethoxy-12-oxo-12-*H*-indolo[2,1-*a*]isoquinolinium chloride **1** (compound X) and 13-(3,4-dimethoxyphenyl)-2,3,8,9-tetramethoxy-6*a*,12*a*-diazadibenzo[*a*,*g*]fluorenylium chloride **2** (compound NF) were identified as new papaverine decomposition products. Their structures have been elucidated recently (Hermann et al. 2002, Girreser et al. 2003).

On the addition of suitable sodium hydroxide aqueous solution to a brown solution of compound X, a bleaching effect can be observed as well as a change in its UV-Vis spectrum. This phenomenon was observed and reported a few years ago (Girreser et al. 2003). A new entity is formed which was found to be 2-(2-carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt **3** (compound WP) (Girreser et al. 2009; Wyrzykiewicz et al. 2009).

Compounds X and NF possess *in vitro* cytotoxic activity toward tumor cells such as: BM (malignant melanoma), A-594 (lung adrenocarcinoma), Hep-2 (laryngeal cancer), EPG-86 (gastric cancer) and MCF-7 (breast cancer) (Mądry et al. 2006).

Biopharmaceutical characterization of potential new drugs is a crucial tool for predicting their biological properties. In this context it is necessary to characterize these substances with respect to their hydrophobicity/lipophilicity, since water solubility and lipophilicity constant (log P) are important parameters. This article reports the water solubility and lipophilicity of the above new compounds and some of their salts. Moreover, the cytotoxicities of the new compounds WP and X were also determined.



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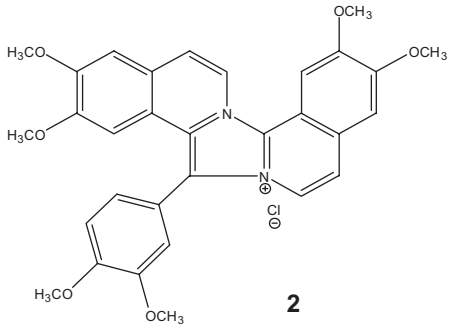

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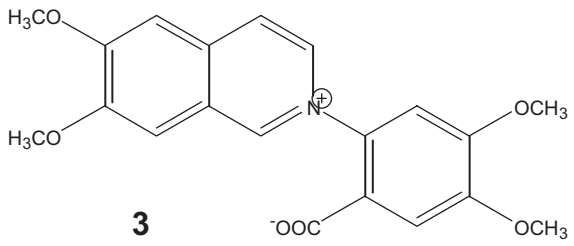

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2. Investigations, results and discussion

At 25 °C X chloride showed the best solubility of the derivatives investigated (Table 1). The least soluble moiety was X hexafluorophosphate, both at 25 °C and 37 °C.

Table 1: Solubility (g/l) of new papaverine decomposition products in water at 25 °C and 37 °C

Substance	Solubility ± SD	
	25 °C	37 °C
X hexafluorophosphate	0.0713 ± 0.0037	0.1075 ± 0.0024
X iodide	2.1049 ± 0.0967	2.3579 ± 0.1906
X chloride	4.3343 ± 0.1817	4.5762 ± 0.2136
Compound WP	0.3156 ± 0.0113	0.4803 ± 0.0269
Compound NF	1.6409 ± 0.1276	1.9717 ± 0.0942

Table 2: R_{m0} values for new papaverine decomposition products

Compound	R_{m0}
WP	0.6917
X	2.3359
NF	2.7405

An increase of solubility was observed with the change in temperature from 25 °C to 37 °C. At 37 °C the most soluble was X chloride.

According to the European Pharmacopoeia VI, X hexafluorophosphate at 25 °C can be classified as a practically insoluble compound. At 37 °C the above compound can be classified as very slightly soluble. X chloride and iodide as well as NF chloride can be classified as slightly soluble materials at both 25 °C and 37 °C.

Lipophilicity is considered to be the most important physicochemical parameter to explain differences in biological activity between compounds of similar structure. Boyce and Milborrow suggested using the R_{m0} coefficient to determine lipophilicity (Table 2). It enables us to avoid the difficulties when the lipophilicity is determined by the flask shaking method (Biagi et al. 1994).

The lipophilicities of the compounds investigated were determined by $TLC_{[T_1]}$ Eq. (2). NF and X chlorides are more lipophillic compared with compound WP. Their log P values were 3.85, 3.28 and 0.97, respectively (Table 3).

Compounds X and NF are characterized by an aromatic ring structure. Besides that, compound WP has the lowest 'b' value in Eq. (2), showing that it has the lowest lipophilicity of the derivatives investigated. The ring-opening structure of compound WP results in the formation of the hydrophilic carboxylate function. The difference in structure between compounds X and WP also affects their *in vitro* biological activities. The cytotoxicity assay confirms the cytotoxic activity of compounds X and NF (Mądry et al. 2006). Compound NF is characterized by an IC_{50} of 14.16 μM at 24 h and 1.158 μM at 72 h elapsed (Rubiś et al. 2009). Compound X significantly decreases cell viability. Its IC_{50} is 21.00 μM at 24 h and 0.414 μM at 72 h. The new derivative, i.e. compound WP, is characterized by a higher IC_{50} than the former compounds (Table 4). Its IC_{50} value implies that it possesses no cytotoxic activity. The low cytotoxic activity may also be associated with its low value of log P (0.97) as a result of a remarkable change in its chemical structure. Compounds NF and X are more lipophilic than WP and may be considered as potential cytotoxic agents.

Table 3: Log P (±SD) values of new papaverine decomposition products calculated by a TLC method at room temperature (25 °C)

Substance	Log P ± SD
WP	0.97 ± 0.08
X	3.28 ± 0.16
NF	3.85 ± 0.21

Table 4: IC_{50} values for new papaverine decomposition products on 24 and 72 h elapsed

Compound	IC_{50}	
	24 h	72 h
X	21.00 μM	0.414 μM
WP	63.08 mM [c)]	40.78 mM[c)]
NF	14.16 μM[d)]	1,158 μM[d)]

IC50 values assessed by MTT method and calculated using CalcuSyn software. [c)]extrapolated value – outside range of concentrations studied, [d)] (Rubiś et al. 2009).

3. Experimental

3.1. Synthesis of the compounds

3.1.1. 2,3,9,10-Tetramethoxy-12-oxo-12-H-indolo[2,1-a] isoquinolinium chloride (1, compound X)

2,3,9,10-Tetramethoxy-12-oxo-12-*H*-indolo[2,1-*a*]isoquinolinium chloride is obtained by photooxidation of a 0.3% (m/v) chloroform solution of papaverinol. The solution was exposed to UV_{254} light for 4.5 h. After this time the chloroform was evaporated off and the crude material was dissolved in hot methanol and left to crystallize. The crystallized compound X was washed with methanol and dried in a desiccator over calcium chloride (anh.) (Girreser et al. 2003).

3.1.2. 2,3,9,10-Tetramethoxy-12-oxo-12-H-indolo[2,1-a] isoquinolinium iodide (X iodide)

In order to obtain 2,3,9,10-tetramethoxy-12-oxo-12-*H*-indolo[2,1-*a*]isoquinolinium iodide, methyl iodide is added to crude compound X chloride dissolved in hot methanol methyl iodide and boiled under a reflux condenser. The crystallized X iodide is filtered, washed with methanol and dried in a desiccator over calcium chloride (anh.) (Girreser et al. 2003).

3.1.3. 2,3,9,10-Tetramethoxy-12-oxo-12-H-indolo[2,1-a] isoquinolinium hexaflourophosphate (X PF_6)

Compound X was dissolved in water and added to an aqueous solution of ammonium hexafluorophosphate. The precipitate was washed with methanol and dried in a desiccator over calcium chloride (anh.).

3.1.4. 13-(3,4-Dimethoxyphenyl)-2,3,8,9-tetramethoxy-6a,12a-diaza-dibenzo[a,g]fluorenylium chloride (compound NF)

The synthesis and isolation of compound NF has been described elsewhere (Hermann et al. 2002).

3.1.5. 2-(2-Carboxy-4,5-dimethoxyphenyl)-6,7-dimethoxyisoquinolinium inner salt (compound WP)

The synthesis and isolation of compound WP has also been described elsewhere (Girreser et al. 2009).

3.2. Solubility

The solubilities of the compound X salts (i.e. chloride, iodide and hexafluorophosphate), and compounds NF and WP were measured in water at temperatures of 25 °C and 37 °C after 24 h elapsed. The compounds 60 mg were weighed into screw-cap tubes and 2 ml of water added. The tubes were placed in a shaking water bath at 25 °C and 37 °C. The tubes were centrifuged at 1500 rpm for 15 minutes. A suitable volume of the supernatant was withdrawn, and diluted with a suitable amount of water. The absorbances were recorded at λ_{max} of the given compound against a water blank. The concentrations of the above compounds were calculated from the Beer's law equation. The solubilities were calculated in g/l from an average of 6 experiments completed for each compound (Table 1).

3.3. Partition coefficient

The partition coefficient was determined by thin layer chromatography (TLC). TLC was performed on TLC glass HPTLC plates 10 x 10 cm RP-18 F_{254s} (Merck, Darmstadt, Germany). A mixture of acetonitrile-water (v/v) was used as the mobile phase. The content of acetonitrile (POCH, Gliwice, Poland) varied in 5% increments from 45% to 80%.
Solutions (1% m/v) of the investigated compounds in methanol (POCH, Gliwice, Poland) were applied at the start line with a Hamilton syringe (10 μl). The chromatograms were developed at a distance of 8 cm at 22 ± 1 °C. After development and drying, the spots were visualized with UV_{254} light. Their R_f values were used to calculate the parameter R_m according to Eq. (1):

$$R_m = \log\left(\frac{1 - R_f}{R_f}\right) \quad (1)$$

The R_m values were extrapolated to an acetonitrile concentration of zero (R_{m0}) using Eq. (2):

$$R_m = R_{m0} + bC \quad (2)$$

where C is the concentration of acetonitrile (in %, v/v) in the mobile phase and b is the change in R_m value due to a 1% increase of acetonitrile content in the mobile phase.
The lipophilicity of the investigated compounds, expressed as the partition coefficient (log P), was calculated from Eq. (3):

$$R_{m0} = A + b_1 \log P \quad (3)$$

where R_{m0} is the R_{m0} value for a substance with known log P. The regression equation was y = 0,7093 x + 0,0070, r = 0,987.
The compounds with known lipophilicity were isatin (Fluka, Germany), *N*-(2,6-dichlorophenyl)-acetamide m.p.190-191 °C (Medical University of Lublin, Poland), *N*-(2,4-dichlorophenyl)-acetamide (Medical University of Lublin, Poland) 141–142 °C, 3,4-dichloroaniline (Aldrich, UK), 2,6-dichloroaniline (Aldrich, UK), p-nitrophenol (POCH, Gliwice, Poland) (Hansch and Leo 1995).
The log P values for the isoquinoline derivatives investigated were calculated from Eq. (3):

$$\log P = \frac{R_{m0} - A}{b_1} \quad (4)$$

where R_{m0} for the above isoquinoline derivative is calculated from Eq. (1). The R_{m0} values are listed in Table 2.

3.4. IC_{50} Determination

Estrogen-dependent human breast cancer cells (MCF-7) were cultured in RPMI1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine and 50 μg/ml gentamicin. Before reaching confluence, the cells were counted and passaged into titration plates (5000 cells per well). They were subsequently cultured for 24 or 72 h with or without ligand and 5 % MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan) was added. After 4 h of incubation a solubilization agent was added (10% SDS in 0,15 mM HCl) in order to visualize the metabolized MTT. The absorbance intensity was read by a Labsystems Multiscan RC, at λ=570 and 690 nm after 16 h. Cytotoxicity rate was expressed at the absorbance of a sample compared with control cells treated with acetone (1%) and finally the IC_{50} values were calculated using CalcuSyn software (Rubiś et al. 2009).

Acknowledgment: Prof. Dariusz Matosiuk from the Medical University of Lublin (Poland) is acknowledged for providing the samples of *N*-(2,4-dichlorophenyl)-acetamide, and *N*-(2,6-dichlorophenyl)-acetamide.

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