Eur. J. Med. Chem. 36 (2001) 951–960

PII: S0223-5234(01)01288-0/SCO

Short communication

Synthesis and biological activity of *O*-acyl and *O*-alkyl chelidonine derivatives

Grzegorz Grynkiewicz[a,*], Ewa Chojecka-Koryn[a], Maria Gadzikowska[b], Anna Chodkowska[c], Ewa Jagiełło-Wójtowicz[c]

[a]Pharmaceutical Research Institute, Rydygiera 8, 01-793 Warsaw, Poland
[b]Department of Inorganic and Analytical Chemistry, Medical University, Staszica 4, 20-081 Lublin, Poland
[c]Department of Toxicology, Medical University, Chodzki 7, 20-093 Lublin, Poland

Received 17 April 2001; revised 22 October 2001; accepted 24 October 2001

Abstract – A group of 11 derivatives of chelidonine was obtained by acylations and/or alkylations of the secondary hydroxyl group with the aim of testing their biological activity. This paper focuses on the new derivatives influence on CNS in mice. The highest activity was observed for compounds **2c**, **3c** and **4**, which produced antinociceptive and antiserotoninergic effects, not recorded for the parent alkaloid **1**. © 2001 Éditions scientifiques et médicales Elsevier SAS

***O*-acyl and *O*-alkyl derivatives of chelidonine / synthesis / biological activity**

1. Introduction

Chelidonine (Stylophorin) belongs to the rare category of natural products which were isolated from different plant sources in both enantiomeric forms and also as a racemic mixture (for which separate name—Diphylline was coined) [1]. Therefore, any study aimed at the biological activity of the alkaloid or its derivatives must be preceded by clear and unanimous definition of its stereochemistry. After erroneous assignment of (+)-chelidonine configuration based on its chiroptical properties [2] the absolute configuration was firmly established by X-ray diffraction of its bromobenzoate ester [3]. Although the alkaloid sample optical rotation measurement can be now safely used for defining its chirality centers, a certain amount of confusion persists, because of various graphical representations of the molecule and inconsistent atom numbering. *figure 1* represents structural formulae of dextrorotatory isomer of chelidonine used in our experiments, in common stereochemical convention. However, in plane 180° rotation of the formulae, are customarily associated with use of completely different numbering systems. Generally, systematic name [5b*R*-(5bα,6β,12bα)]-5b,6,7,12b,13,14-hexahydro-13-methyl[1,3]benzodioxo[5,6-*c*]-1,3-dioxo[4,5-i]phenanthridin-6-ol (**1**) (corresponding to the west side of *figure 1*) is not used in chemical literature. Instead, trivial name, chelidonine; supplemented by either optical rotation sign or stereochemical descriptors for C-11, C-13 and C-14 atoms (eastern part of *figure 1*) is commonly applied.

Chelidonine is a major component in the roots of the *Chelidonium majus* L. (known also as greater celandine, a plant, which has a rich record of applications in ethnopharmacology), and also occurs in other Papaveraceae plants. This easily available benzophenantridine alkaloid, first isolated in 1839 [4], is endowed with pleiothropic pharmacological activity [5–7] but recent studies tend to concentrate on its successful application in experimental oncological practice [8].

Despite numerous hints of bioactivity from contemporary pharmacognosy–phytochemistry fields and considerable interest evoked by Ukrain®, a semisynthetic antitumor preparation derived from *C. majus* L. alkaloids [8], chelidonine itself has not been

* Correspondence and reprints:
E-mail address: gryn@stratus.ifarm.waw.pl
(G. Grynkiewicz).

studied as a pharmacophoric lead compound thus far. Therefore, we would like to present our simple rationale, along with supporting experimental evidence, that this alkaloid constitutes an interesting object from medicinal chemistry point of view.

2. Chemistry

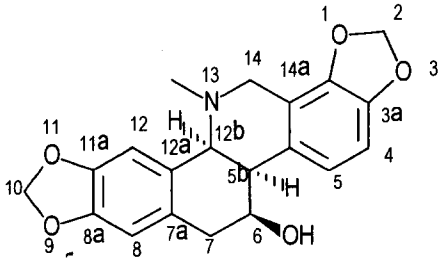
Molecular structure of chelidonine (**1**), deduced from a wealth of spectroscopic data [2] and supported by X-ray diffraction measurements [3, 9] may at first sight look too simple to be an effective ligand of a functional biomacromolecule. Indeed, from a point of view of such useful SAR generalisations as Lipinski's 'rule of five' [10], the molecule in question lacks appropriate number of hydrogen bond donors needed for optimal pharmacophoric interactions. Nevertheless, it should be pointed out that simple, biogenetically related alkaloids from benzophenantridine class can exhibit remarkably selective binding to certain macromolecular targets. Thus, cheleritrine is a potent protein kinase C inhibitor [11], protopine inhibits voltage and receptor-operated calcium channels [12] and sanguinarine was found to inhibit nuclear factor (NF-κB) involved in TNF gene expression [13].

In our plans of chemical derivatisation, we have considered **1** as a bi-functional molecule, taking into account possibility of tertiary amine quaternisation and hydroxyl group transformations. Surprisingly, we have found out that **1** is extremely recalcitrant towards *N*-alkylation under wide range of reaction conditions. Seeking an explanation for this lack of reactivity, we have encountered in chemical literature certain controversy regarding conformational mobility of the molecule [14, 15]. Re-examination of the issue with help of high field NMR spectroscopy supplemented with NOE techniques, has led to conclusion that chelidonine attains global conformational energy minimum easily, featuring strong intramolecular hydrogen bonding which locks up the molecule in pH independent manner (both, free base and its protonated form retain the same shape although polarity of the hydrogen bonding N–H–O arrangement is reversed!) [16]. Undoubtedly, the known pharmacological activity of **1** is strongly dependent on its particular topological characteristics, which does not vary from solid state to solution [16]. On the contrary, making new covalent bond on the hydroxyl group oxygen atom of the alkaloid permanently removes the bridging hydrogen and must lead to different kind of conformational dynamics, which in consequence may also be reflected in a biological activity profile. Based on such simple reasoning, we have decided to advance the question: 'how various esters and ethers of **1** compare in biological activity with the prototype structure?'. Since influence on CNS is frequently perceived as undesirable side effect in potential drug candidates, it has been decided that this particular activity of new derivatives of **1** should be examined first, with aid of standard rodent pharmacology models.

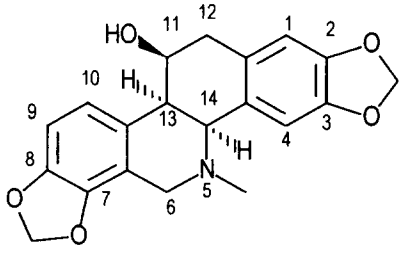
Our choice of functional groups accommodates two distinct types of derivatisation (*figure 2*):

1. alkylation, leading to a stable, ether type derivatives;
2. acylation, affording esters potentially susceptible to biodegradation of a newly formed bond.

In both cases some variation of substituents (alkyl, aryl, substituted alkyls and aryls, etc.) was exercised



1. (+) - 5bR,6S,12bS



1. (+) - 11S,13R,14S

Figure 1. Chelidonine numbering systems in current use.

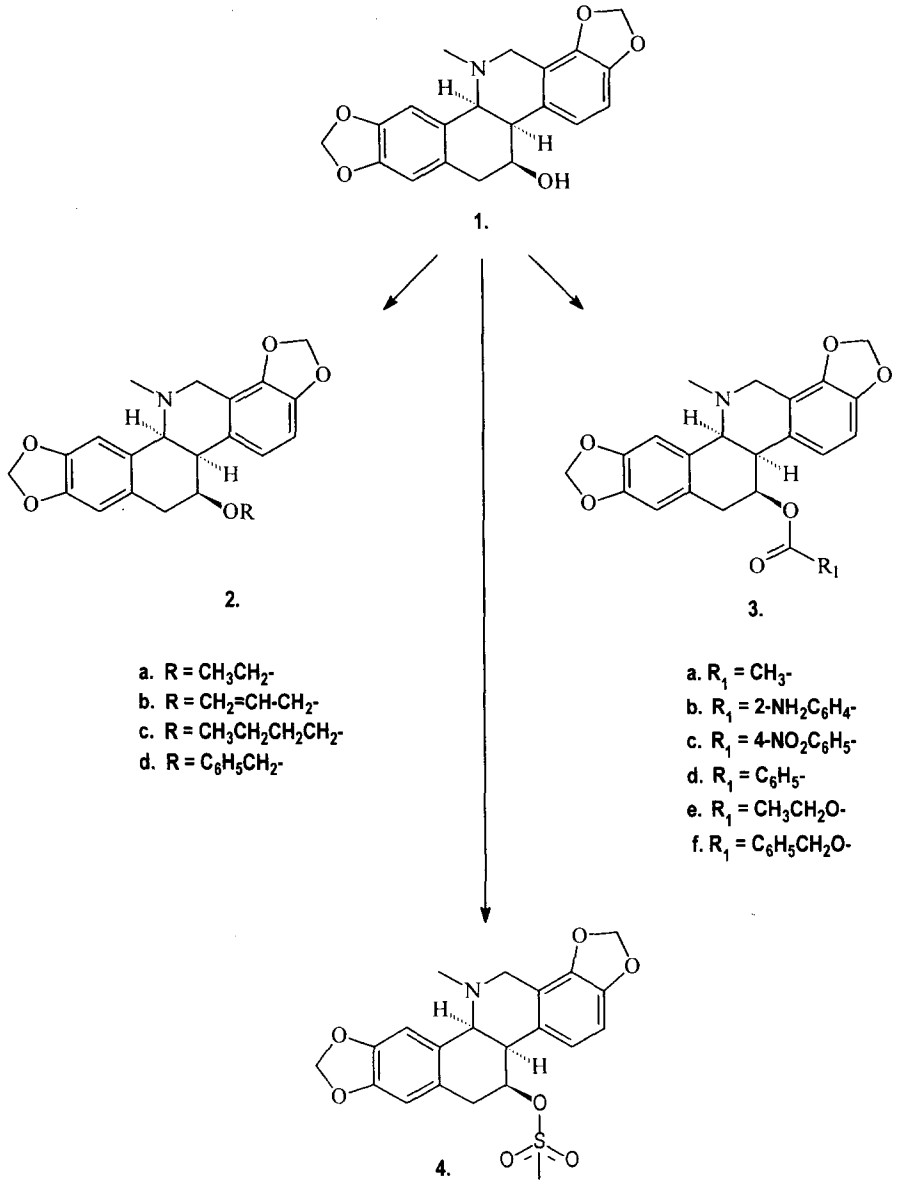


Figure 2. Chelidonine and its derivatives tested for CNS activity.

in order to reveal possible distinct trends connected with introduced structural features. Chemical methods used for preparation of new derivatives constitute simple variants of standard esterification (action of an acyl chloride in the presence of a base) and etherification (modified Williamson method) procedures recommended for secondary alcohols by manuals of organic synthesis [17].

3. Results and discussion

A group of 11 derivatives of **1** has been synthesised (among which *O*-acetyl and *O*-benzoyl derivatives were already known [18–22]) and their influence on CNS in mice has been examined following intraperitoneal (i.p.) administration. The first sub-set of four *O*-alkyl derivatives containing 2–7 carbon atoms (**2**)

consist of chemically (and probably also metabolically) inert derivatives in terms of C–O bond cleavage, although compounds **2b** and **2d** offer a chance of further transformations of the allyl chain or the aromatic ring. The second sub-set represents wider structural variety, comprising alkyl and aryl carboxylates, mixed carbonates and a sulphonate. It should be remembered that all these compound have relatively labile C–O–acyl bond, which is susceptible to cleavage by ubiquitous enzymes, like lipases, acyltransferases or hydrolases. Each of such enzymatic transformation would result in conversion of an examined compound into starting chelidonine. The three benzoates **3b**, **3c** and **3d** present in this group were prepared in an attempt to find out if electron density of the acyl moiety can significantly influence observed biological effects. It turned out that most of the newly synthesised compounds exert statistically significant effects on CNS activity in mice.

Presented results have shown that the newly investigated *O*-acyl and *O*-alkyl chelidonine derivatives exert depressive action on the CNS in mice. Of the 11 compounds tested, only *O*-ethyl (**2a**) and *O*-benzyl (**2b**) derivatives of chelidonine were practically devoid of pharmacological activity.

It was found that the compounds **2a**, **2b** and **3b**, **3c**, **3d**, **3e**, **3f** significantly decreased spontaneous locomotor activity (*tables I–IV*). In contrast, compounds **2c**, **2d**, **3a**, **3c** and **4** (in doses 130 mg kg^{-1} i.p.) did not exhibit such effect. Five of examined compounds: **2a**, **3a**, **3e**, **3f** and **4** potentiated the action of hypnotic drug (*tables V and VI*). Only *O*-(*n*-butyl) chelidonine (**2c**) significantly shortened the time sleep induced by hexobarbital (*table V*).

The results presented in *tables VII and VIIII* indicate pronounced antinociceptive properties of the derivatives **2b**, **2c**, **3b**, **3c**, **3d**, **3e**, **3f** and **4**, which are absent in the parent alkaloid. Only compounds **2a**, **2d**,

Table I. Physico-chemical data of *O*-alkyl chelidonine derivatives.

Compound	Molecular formula	Molecular weight	M.p. (°C)	Log *P*	NMR [δ (ppm)] [a]
2a	$C_{22}H_{23}NO_5$	381	145–147	3.10	3.17 (m, 2H, CH_2CH_3) 1.34 (t, J 7.1 Hz, 3H, CH_2CH_3)
2b	$C_{23}H_{23}NO_5$	393	157–158	3.50	6.06 (m, 1H, –CH=) 5.45–5.19 (m, 2H, =CH_2) 4.21 (m, 2H, OCH_2)
2c	$C_{24}H_{27}NO_3$	409	120	3.97	3.84–3.40 (m, 2H, OCH_2) 1.68 (m, 2H, CH_2) 1.48 (m, 2H, CH_2) 0.98 (t, J 7.3 Hz, 3H, CH_3)
2d	$C_{27}H_{25}NO_5$	443	125–127	4.54	7.45–7.20 (m, 5H, ArH) 4.75 (2H, CH_2Ph))

[a] Side chain proton signals.

Table II. Physico-chemical data of *O*-acyl chelidonine derivatives.

Compound	Molecular formula	Molecular weight	M.p.(°C)	Log *P*	NMR [δ (ppm)] [a]
3a	$C_{22}H_{21}NO_6$	395	198–199	2.61	2.56 (s, 3H, CH_3)
3b	$C_{27}H_{24}N_2O_6$	472	107–111	3.74	7.92–6.62 (m, 4H, ArH) 5.80 (m, 2H, $ArNH_2$)
3c	$C_{27}H_{22}N_2O_8$	502	216–218	4.48	8.14–7.38 (m, 4H, ArH)
3d	$C_{27}H_{23}NO_6$	457	221–223	4.53	8.11–7.21 (m, 5H, ArH)
3e	$C_{23}H_{23}NO_7$	425	83–85	3.58	4.19 (q, $J = 7.2$ Hz, 2H, CH_2CH_3) 1.30 (t, $J = 7.2$ Hz, 3H, CH_2CH_3)
3f	$C_{28}H_{25}NO_7$	487	82–83	5.01	7.47–7.19 (m, 5H, ArH) 5.23 (s, 2H, CH_2Ph)
4	$C_{21}H_{21}N2O_7S$	431	Foam [b]	2.53	3.08 (s, 3H, SO_2CH_3)

[a] Side chain proton signals.
[b] 123 °C, darkening; 155–163 dec.

Table III. The effects of *O*-alkyl chelidonine on locomotor activity of mice.

Compound	Dose (mg kg^{-1} i.p.)	Activity (counts per 60 min)
Control	–	5366.1 ± 333.8
1	65.0	3735.1 ± 799.8*
	130.0	1773.0 ± 372.4*
2a	32.5	4510.0 ± 494.8
	65.0	3025.4 ± 537.9*
	130.0	2389.5 ± 465.3*
2b	32.5	4313.5 ± 854.7
	65.0	3159.7 ± 729.9*
	130.0	2194.6 ± 402.6*
Control	–	5134.1 ± 181.5
2c	130.0	4897.4 ± 360.7 ^
2d	130.0	4674.0 ± 966.8 ^

O-alkyl chelidonine derivatives were given 30 min before the test. The data represent means ± S.E.M. (Mann–Whitney test); * ^ $P<0.001$, *, vs. the control group; ^, vs. compound **1**.

Table IV. The effect of *O*-acyl chelidonine derivatives on locomotor activity mice.

Compound	Dose (mg kg^{-1} i.p.)	Activity (counts per 60 min)
Control	–	4992.2 ± 531.2
1	65.0	3467.3 ± 462.1*
	130.0	1592.7 ± 298.6*
3a	130.0	4943.6 ± 683.5 ^
3e	32.5	5371.2 ± 304.5
	65.0	3480.0 ± 493.5*
	130.0	2041.5 ± 788.7*
4	130.0	5879.4 ± 808.4 ^
Control	–	4464.1 ± 468.7
3b	32.5	4485.0 ± 521.7
	65.0	2967.7 ± 383.4*
	130.0	2130.4 ± 357.6*
3c	130.0	3319.2 ± 509.1* ^
3d	32.5	3985.7 ± 522.0
	65.0	2973.5 ± 470.3*
	130.0	2331.7 ± 161.6* ^
Control	–	4358.7 ± 756.2
3f	32.5	4839.2 ± 453.4
	65.0	2032.9 ± 581.1* ^
	130.0	2540.0 ± 1089.7*

O-acyl chelidonine derivatives were given 30 min before the test. The data represent means ± S.E.M. (Mann–Whitney test); * ^ $P<0.001$, *, vs. the control group; ^, vs. compound **1**.

3a did not affect the reactivity of the mice in the writhing syndrome test. Of the 11 examined chelidonine derivatives two of *O*-alkyl (**2b**, **2c**) and three of *O*-acyl chelidonine derivatives (**3c**, **3e**, **4**) were found to decrease the number of head twitches induced by 5-HTP (*tables IX and X*), while the reference compound **1** does not exhibit any antiserotoninergic activity. In the remaining pharmacological tests none of the investigated derivatives did not produce statistically significant effects.

In conclusion, we have found that from the examined group compounds **2c**, **3c** and **4** have a potent antinociceptive and antiserotoninergic properties, which deserve further investigations of the molecular mechanism of their action in rodents.

4. Conclusions

We have demonstrated that simple derivatisation of the secondary hydroxyl group in chelidonine can result in appearance of new pharmaceutical traits, which are absent in the parent alkaloid. Thus ether **2c**

Table V. The effect of *O*-alkyl chelidonine derivatives on the duration of hexobarbital-induced sleep in mice.

Compound	Dose (mg kg^{-1} i.p.)	Sleeping time (s)
Control	–	21.0 ± 6.2
1	65.0	63.0 ± 15.4*
	130.0	110.2 ± 7.9*
2a	65.0	92.0 ± 10.4* ^
2b	65.0	38.8 ± 13.8
2c	130.0	14.0 ± 5.7 ^
2d	130.0	36.6 ± 13.7 ^

O-alkyl chelidonine derivatives were given 30 min before the test. The data represent means ± S.E.M. (Student's *t*-test); * ^ $P<0.001$; *, vs. the control group; ^, vs. compound **1**.

Table VI. The effect of *O*-acyl chelidonine derivatives on the duration of hexobarbital-induced sleep in mice.

Compound	Dose (mg kg^{-1} i.p.)	Sleeping time (s)
Control	–	23.1 ± 1.8
1	65.0	63.0 ± 15.4*
	130.0	110.2 ± 7.9*
3a	130.0	74.7 ± 13.2* ^
3b	65.0	33.4 ± 14.4 ^
3c	130.0	27.7 ± 8.9 ^
3d	65.0	29.5 ± 10.3 ^
3e	130.0	102.5 ± 8.1*
4	130.0	83.7 ± 17.8*
Control	–	21.0 ± 6.2
3f	65.0	111.6 ± 4.3* ^

O-acyl chelidonine derivatives were given 30 min before the test. The data represent means ± S.E.M. (Student's *t*-test); * ^ $P<0.001$; *, vs. the control group; ^, vs. compound **1**.

Table VII. Antinociceptive activity of *O*-alkyl chelidonine derivatives in the 'writhing syndrome' test in mice.

Compound	Dose (mg kg^{-1} i.p.)	Mean writhing number	Inhibition (%)
Control	–	15.6 ± 2.5	–
1	130.0	13.8 ± 1.5	16
2a	130.0	14.5 ± 3.2	7
2b	32.5	13.1 ± 3.3	16
	65.0	5.7 ± 1.1*	63*
	130.0	4.0 ± 1.5* ^	74* ^
2c	32.5	11.7 ± 2.1	25
	65.0	7.7 ± 1.2*	50*
	130.0	4.2 ± 1.1* ^	73* ^
2d	130.0	13.4 ± 6.7	14

O-alkyl chelidonine derivatives were given 30 min before the test. Results are expressed as mean ± S.E.M. (Student's *t*-test). % of inhibition obtained by comparison with control groups. * ^ $P<0.001$; *, vs. the control group; ^, vs. compound **1**.

Table VIII. Antinociceptive activity of *O*-acyl chelidonine derivatives in the 'writhing syndrome' test in mice.

Compound	Dose (mg kg^{-1} i.p.)	Mean writhing number	Inhibition (%)
Control	–	15.6 ± 2.5	–
1	130.0	13.8 ± 1.5	16
3a	130.0	14.6 ± 4.0	6
3b	65.0	13.7 ± 2.0	12
3e	32.5	14.2 ± 3.7	9
	65.0	9.2 ± 1.9*	41*
	130.0	4.9 ± 2.4* ^	68* ^
4	32.5	12.7 ± 2.0	18
	65.0	8.9 ± 2.5*	43*
	130.0	3.7 ± 2.8* ^	76* ^
Control	–	15.0 ± 2.9	
3c	32.5	12.1 ± 2.3	22
	65.0	8.5 ± 1.0*	45*
	130.0	2.2 ± 1.1* ^	85* ^
3d	65.0	15.2 ± 2.2	4
	130.0	7.5 ± 2.0* ^	52* ^
3f	32.5	13.2 ± 2.5	15
	65.0	7.7 ± 1.2*	50*
	130.0	7.7 ± 2.5* ^	50* ^

O-acyl chelidonine derivatives were given 30 min before the test. Results are expressed as mean ± S.E.M. (Student's *t*-test). % of inhibition obtained by comparison with control groups. * ^ $P<0.001$; *, vs. the control group; ^, vs. compound **1**.

and esters **3c** and **4** produced antinociceptive and antiserotoninergic effects in mice, interestingly, without modifying the locomotor activity. Since close analogs of the three compounds do not seem to follow this promising activity trend, we have concluded that very subtle structural effects must be responsible, warranting further investigation of new compounds, as well as their possible molecular targets.

Table IX. The effect of *O*-alkyl chelidonine derivatives on the head twitch responses induced by 5-hydroxytryptophan in mice.

Compound	Dose (mg kg^{-1} i.p.)	Number of head twitch responses
Control	–	15.1 ± 3.2
1	65.0	9.8 ± 2.1
	130.0	6.3 ± 1.8*
2a	65.0	13.4 ± 2.9
2b	65.0	20.9 ± 4.5* ^
	130.0	4.0 ± 1.4*
2c	32.5	9.6 ± 1.2
	65.0	1.6 ± 0.7* ^
	130.0	0.0* ^
2d	130.0	11.7 ± 3.7

O-alkyl chelidonine derivatives were given 30 min before the test. The data represent mean ± S.E.M. (Student's *t*-test); * ^ $P<0.001$; *, vs. the control group; ^, vs. compound **1**.

Table X. The effect of O-acyl chelidonine derivatives on the head twitch responses induced by 5-hydroxytryptophan in mice.

Compound	Dose (mg kg^{-1} i.p.)	Number of head twitch responses
Control	–	14.6 ± 1.4
1	65.0	9.8 ± 2.1*
	130.0	6.3 ± 1.8*
3a	130.0	9.7 ± 3.4
3b	130.0	11.7 ± 2.2 ^
3c	32.5	11.2 ± 1.8
	65.0	1.0 ± 0.4* ^
	130.0	0.0* ^
3d	65.0	14.2 ± 2.5 ^
3e	65.0	13.6 ± 3.5
	130.0	4.8 ± 1.1*
3f	130.0	8.9 ± 3.1*
4	65.0	16.2 ± 2.3 ^
	130.0	4.6 ± 1.5*

O-acyl chelidonine derivatives were given 30 min before the test. The data represent mean ± S.E.M. (Student's *t*-test); * ^ $P<0.001$; *, vs. the control group; ^, vs. compound **1**.

5. Experimental

5.1. Chemistry

Melting points (m.p.) were determined in capillary tubes and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Gemini 2000 spectrometer (200 MHz) in $CDCl_3$, chemical shifts are reported relative to internal TMS in the following format: chemical shift δ (ppm), number of protons, multiplicity. IR (KBr) spectra were recorded on a Perkin–Elmer FTIR 1725X spectrometer. Chromatographic separations were performed using flash chromatography on a silica gel column (Kieselgel 60, Merck). Thin-layer chromatography was carried out on Merck silica gel plates. Chelidonine **1** was used as a free base (m.p. 135 °C; $[\alpha]_D$ +116 °C = 1.0; $CHCl_3$) for chemical experiments and as equimolar amount of hydrochloride in biological activity tests. The alkaloid hydrochloride was obtained, as certified industrial material, batch No. 010597, from manufacturer: Herbapol S.A., Wroclaw, Poland. All chelidonine derivatives have been checked by HPLC (Waters™ 486, equipped with Discovery C-18 column; isocratic mode, methanol–water and acetonitrile–water mobile phases) and only compounds above 97% purity were qualified for biological testing. Log *P* values were calculated using CHEMPLUS™ extension for HYPERCHEM™ program, Hypercude Inc., Waterloo, Ont., Canada 1997.

5.1.1. General procedure for the O-alkyl chelidonine preparation

Excess of sodium hydride (NaH, ca. 1.5–3 equiv.) was added to a solution of 1 equiv. chelidonine (or chelidonine hydrochloride suspension) in anhydrous dimethylformamide (DMF). After stirring for 1 h, alkyl halide (1.3–1.6 equiv.) was added, and the reaction mixture was stirred for additional 5–16 h at room temperature (r.t. or at 60 °C). The reaction mixture was then cooled, poured into cold water and extracted with ethyl acetate. The combined extracts were washed with water, dried with anhydrous magnesium sulfate ($MgSO_4$) and evaporated. The residue was purified by flash column chromatography on silica gel.

5.1.1.1. O-Ethyl chelidonine, $R = CH_3CH_2$– (**2a**)

NaH (0.45 g, 80%, 15 mmol) and ethyl bromide (1 mL, 13.06 mmol) were added to a solution of chelidonine (2.86 g, 8.1 mmol) and treated as described above. Crude product was purified by flash column chromatography (5% CH_2Cl_2 in hexane–ethyl acetate 17:3 v/v) yielded 1.00 g (32.4%) of the pure product: m.p. 145–147 °C; $[\alpha]_D^{25}$ +168.6° (0.977 g per 100 mL, $CHCl_3$); IR (cm^{-1}) *v*, 2925.48, 1478.03, 1376.43, 1320.93, 1227.43, 1042.49, 842.68, 803.17, 517.92; ^{1}H-NMR δ, 7.52 (1H, dd, $J = 8.2$, 0.9 Hz, ArH), 7.25 (1H, s, ArH), 6.66 (1H, d, $J = 8.2$ Hz, ArH), 6.39 (1H, s, ArH), 5.83 (4H, m, –OCH_2O–), 4.04 (1H, m), 3.88–3.36 (6H, m), 2.74 (2H, m), 2.63 (2H, s, NCH_3), 1.34 (3H, t, CH_2CH_3).

5.1.1.2. O-Allyl chelidonine, $R = CH_2$=CH–CH_2– (**2b**)

Chelidonine hydrochloride (3 g, 7.71 mmol) was suspended in DMF (40 mL), 700 mg (80%, 23.3 mmole) and allyl bromide (1.0 mL, 11.55 mmol) were added following above described procedure. Mixture was stirred for 5 h at r.t. and for 5 h at 60 °C, crude product was chromatographed and recrystallised from ethyl acetate and hexane mixture (2.05 g, 67.66%): m.p. 157–158 °C; $[\alpha]_D^{25}$ +154.8° (0.980 g per 100 mL, $CHCl_3$); IR (cm^{-1}) *v*: 2885.03, 1476.19, 1223.40, 1129.92, 1038.36, 938.68, 836.68; ^{1}H-NMR δ: 7.62 (1H, d, $J = 8.2$ Hz, ArH), 7.25 (1H, s, ArH), 6.66 (1H, d, $J = 8.2$ Hz, ArH), 6.39 (s, 1H, ArH), 6.06 (1H, m, –CH=), 5.83 (4H, m, –OCH_2O–), 5.81 (1H, m)m 5.45–5.19 (2H, m, $=CH_2$), 4.21 (2H, m), 4.05 (1H, m), 3.36 (8H, m), 2.78 (2H, m), 2.63 (3H, s, NCH_3).

5.1.1.3. O-(n-Butyl) chelidonine, $R = CH_3(CH_2)_3$– (**2c**)

To 5.2 g (14.7 mmol) of chelidonine dissolved in 60 mL of DMF, 600 mg (80%, 20 mmol) of NaH and 2 mL (17.55 mmol) of *n*-butyl iodide were added, and the mixture was heated at 60 °C for 10 h. The oily residue obtained by using described general procedure was purified by chromatography (hexane–ethyl acetate–methylene chloride, 17:3:2, v/v/v), and recrystallised from *iso*-propanol, to give 2.2 g (36.5%) as a white solid: m.p. 120 °C; $[\alpha]_D^{25}$ +152.5° (1.0 g per 100 mL, $CHCl_3$); IR (cm^{-1}) *v*: 2888.75, 1477.14, 1377.97, 1250.45, 1040.10; ^{1}H-NMR δ: 7.5 (1H, d, $J = 8$ Hz, ArH), 7.28 (1H, s, ArH), 6.63 (1H, d, $J = 8$ Hz, ArH), 6.4 (1H, s, ArH), 5.78–5.94 (4H, m, O–CH_2–O), 4.08 (1H, m, C(OR)H, $w_{1/2} = 4$ Hz), 3.40–3.84 (6H, m), 2.83 (2H, m, CH_2), 2.64 (3H, s, NCH_3), 1.68 (2H, m, CH_2), 1.48 (2H, m, CH_2), 0.98 (3H, t, $J = 7.3$ Hz, CH_3).

5.1.1.4. O-Benzyl chelidonine, $R = C_6H_5CH_2$– (**2d**)

Chelidonine hydrochloride (2.5 g, 6.43 mmol) was treated with 0.468 g (80%, 15 mmol) NaH and benzyl bromide (1.1 mL, 9.26 mmol) in DMF and stirred for 10

h at r.t. Residue obtained by standard work up procedure was chromatographed (5% CH_2Cl_2 in hexane–ethyl acetate 17:3, v/v) to give 1.28 g (44.9%) of alkylated product: m.p. 125–127 °C; $[\alpha]_D^{25}$ +130.4° (1.073 g per 100 mL, $CHCl_3$); IR (cm^{-1}) ν: 2890.95, 1478.31, 1253.17, 1040.01, 932.03, 812.65, 739.75, 698.01; ^{1}H-NMR δ: 7.59 (1H, d, ArH), 7.35 (6H, m, ArH), 6.65 (1H, d, $J = 8.2$, ArH), 6.4 (1H, s, ArH), 5.84 (4H, m, O–CH_2–O), 4.75 (2H, m), 4.05 (1H, m), 3.93 (1H, m), 3.39–3.84 (3H, m), 2.81 (2H, d, $J = 7.7$ Hz), 2.64 (3H, s, NCH_3).

5.1.2. General procedure for the O-acyl chelidonine preparation

Chelidonine was dissolved in anhydrous methylene chloride (CH_2Cl_2) or chloroform and pyridine (1.5–2 equiv.), and carboxylic acid chloride (1.2–1.5 equiv.) were added. The mixture was stirred at r.t., until completion of esterification (TLC control) then diluted with methylene chloride, washed with water, and dried with $MgSO_4$. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography.

*5.1.2.1. O-(p-Nitrobenzoyl) chelidonine, $R_1 = 4\text{-}NO_2C_6H_4$– (**3c**)*

To chelidonine (2.6 g, 7.365 mmol) dissolved in 50 mL CH_2Cl_2, pyridine (1.1 mL, 13.6 mmol) and 4-nitrobenzoyl chloride (2 g, 10.77 mmol) were added, and upon completion of the reaction, worked up as described above. Flash chromatography (5% CH_2Cl_2 in a mixture hexane–ethyl acetate 17:3, v/v) gave the desired product which was recrystallised from chloroform–methanol (1.7 g, 46%) obtained as a yellow solid.: m.p. 216–218 °C; $[\alpha]_D^{25}$ +56.8° (1.0 g per 100 mL, $CHCl_3$); IR (cm^{-1}) ν: 2884.19, 1719.62, 1605.85, 1476.64, 1273.80, 1099.36; ^{1}H-NMR δ: 8.14–8.38 (4H, m, ArH), 7.17 (1H, s, ArH), 7.12 (1H, d, $J = 8.2$ Hz, ArH), 6.66 (1H, d, $J = 8.2$ Hz, ArH), 6.49 (1H, s, ArH), 5.84–5.98 (4H, m, O–CH_2–O), 5.56–5.68 (1H, m, C(OR)H), 4.08 (1H, d, $J = 4.5$ Hz), 2.37 (3H, s, NCH_3).

*5.1.2.2. O-Benzoyl chelidonine, $R_1 = C_6H_5$– (**3d**)*

Benzoyl chloride (1.4 mL, 12.06 mmol) was reacted for 3 h with chelidonine (2.5 g, 7.08 mmol) dissolved in methylene chloride and pyridine (1.4 mL, 17.3 mmol) according to general procedure for *O*-acyl chelidonine preparation, to give after isolation and purification 2.3 g (71.1%) of a solid: m.p. 221–223 °C; $[\alpha]_D^{25}$ +75.5° (1.017 g per 100 mL, $CHCl_3$); IR (cm^{-1}) ν: 2891.16, 1708.07, 1478.05, 1275.31, 1108.25, 1039.01, 931.84, 713.85; ^{1}H-NMR δ: 8.11 (2H, m, ArH), 7.66–7.21 (5H, m, ArH), 6.69 (1H, d, $J = 8.2$ Hz, ArH), 6.45 (1H, s, ArH), 5. 88 (4H, m, –OCH_2O–), 5.57 (1H, m), 4.19 (1H, d, $J = 5$ Hz), 3.88–3.40 (3H, m), 3.15–2.84 (2H, m), 2.58 (3H, s, NCH_3).

5.1.3. General procedure for the O-alkyloxycarbonylo and O-aryloxycarbonylo chelidonine preparation

A solution of chelidonine or chelidonine hydrochloride, pyridine (1.3–3 equiv.) and alkyl(aryl) chloroformate (1.1–1.4 equiv.) in anhydrous methylene chloride or chloroform was stirred at r.t. for 5–10 h, checking for substrate disappearance by TLC. The mixture was then diluted with methylene chloride, washed with aq. sodium hydrogen carbonate ($NaHCO_3$), dried over $MgSO_4$, and concentrated at reduced pressure. The residue was purified by flash column chromatography.

*5.1.3.1. O-Ethoxycarbonyl chelidonine, $R_1 = CH_3CH_2O$– (**3e**)*

Chelidonine (2.4 g, 6.8 mmol), pyridine (0.8 mL, 9.89 mmol), ethyl chloroformate (0.85 mL, 8.84 mmol) were stirred for 5 h in methylene chloride (50 mL), and worked up as described above. The residue was chromatographed (5% CH_2Cl_2 in hexane–ethyl acetate 9:1 v/v) to give product (2.05 g. 70.5%): m.p. 83–85 °C; $[\alpha]_D^{25}$ +110.9° (0.573 g per 100 mL, $CHCl_3$); IR (cm^{-1}) ν: 2889.34, 1741.83, 1479.75, 1377.76, 1246.91, 1122.88, 1040.46, 937.54, 790.88; ^{1}H-NMR δ: 7.18 (2H, m, ArH), 6.62 (1H, d, $J = 8.2$, ArH), 6.33 (1H, s, ArH), 5.74–5.85 (4H, m, O–CH_2–O), 5.02–5.14 (1H, m, C(OR)H), 4.19 (2H, q, $J = 7.2$, OCH_2CH_3), 4.05 (1H, m), 3,35–3.75 (3H, m), 2.65-2.85 (2H, m), 2.5 (3H, s, NCH_3), 1.3 (3H, t, $J = 7.2$, OCH_2CH_3).

*5.1.3.2. O-Benzyloxycarbonyl chelidonine, $R_1 = C_6H_5CH_2O$– (**3f**)*

Product was prepared as described above: to 2.3 g (6.51 mmol) of chelidonine in chloroform (50 mL) 1.2 mL (14.8 mmol) of pyridine and 1 mL (7 mmol) of benzyl chloroformate were added. Flash chromatography yielded 2.3 g (77.3%) of desired product as white crystals: m.p. 82–83 °C; $[\alpha]_D^{25}$ +89.5° (0.390 g per 100 mL, $CHCl_3$); IR (cm^{-1}) ν: 2887.48, 1741.29, 1479.27, 1362.17, 1326.75, 1245.22, 1122.96, 1039.98, 937.90, 697.86, 515.93; ^{1}H-NMR δ: 7.47–7.19 (7H, m, ArH), 6.68 (1H, d, $J = 8.2$ Hz, ArH), 6.41 (1H, s, ArH), 5.88 (4H, m, –OCH_2O–), 5.23 (2H, s, CH_2), 5.18 (1H, m), 4.15 (1H, d, $J = 4.5$ Hz), 3.84–3.38 (3H, m), 3.06–2.75 (2H, m), 2.59 (3H, s, NCH_3).

*5.1.4. O-Acetyl chelidonine, $R_1 = CH_3$– (**3a**)*

Chelidonine hydrochloride (4 g, 10.26 mmol) was suspended in dry chloroform (40 mL) and pyridine (2 mL, 24.73 mmol) was added, followed by acetic anhydride (1.1 mL, 11.66 mmol). The solution was stirred at r.t. for 5 h and then poured into water. The layers were separated, and the aqueous layer was extracted with chloroform (3×30 mL). The combined organic extracts were washed with water, dried with $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue was purified by recrystallisation from ethyl acetate to provide product (3.1 g, 78.88%) as a white crystals: m.p. 198–199 °C, $[\alpha]_D^{25}$ +116.0° (0.950 g per 100 mL, $CHCl_3$); IR (cm^{-1}) *v*: 2882.12, 1725.19, 1478.80, 1238.64, 1026.49, 941.18, 769.58, 516.57, 431.99; ^{1}H-NMR *δ*: 7.26 (1H, m, ArH), 6.68 (1H, m, ArH), 6.42 (2H, s, ArH), 5.88 (4H, m, O–CH_2–O), 5.53 (1H, m), 4.14 (1H, d, *J* = 5 Hz), 3.84–3.37 (3H, m), 2.98–2.64 (2H, m), 2.56 (3H, s, NCH_3), 2.16 (3H, s, C(=O)CH_3) [18, 19].

*5.1.5. O-(2-Aminobenzoyl) chelidonine, $R_1 = 2\text{-}NH_2C_6H_4$– (**3b**)*

An amount of 1 g (2.83 mmol) chelidonine was dissolved in anhydrous DMF (20 mL) under argon, then isatoic anhydride (0.5 g, 3.06 mmol) was added, followed by 4-dimethylaminopyridine (80 mg). The reaction mixture was stirred for 7 h at 60 °C then cooled, poured into ice water, and extracted three times with ethyl acetate. Combined organic layers were washed with water and dried over $MgSO_4$. The solvent was removed in vacuo. Product was purified by column chromatography (10% CH_2Cl_2 in hexane–ethyl acetate 9:1 → 4:1 v/v) giving 1.27 g (95.1%) of crystalline solid: m.p. 107–111 °C; $[\alpha]_D^{25}$ +69.4° (0.916 g per 100 mL, $CHCl_3$); IR (cm^{-1}) *v*: 3482.52, 3371.71, 2887.55, 1686.94, 1616.37, 1479.54, 1040.23, 937.81, 751.97, 527.98; ^{1}H-NMR *δ*: 7.92 (1H, dd, ArH), 7.32–7.18 (3H, m, ArH), 6.73–6.62 (3H, m, ArH), 6.45 (1H, s, ArH), 5.88 (4H, m, O–CH_2–O), 5.8 (2H, m), 5.53 (1H, m), 4.12 (1H, d), 3.88–3.39 (4H, m), 3.14–2.84 (2H, m), 2.56 (3H, s, NCH_3).

*5.1.6. O-(Methanesulfonyl) chelidonine **4***

A suspension of 3.5 g (9 mmol) chelidonine hydrochloride in anhydrous chloroform was treated with pyridine (1.6 mL, 19.8 mmol) and methanesulfonyl chloride (1.0 mL, 12.9 mmol) and stirred at r.t. for 8 h. The reaction mixture was then poured into aq. $NaHCO_3$, organic layer was separated and aqqeous extracted with chloroform. The combined organic phases were dried and evaporated to leave the crude mesylate. Product was chromatographed (hexane–ethyl acetate 9:1, v/v), affording pure mesylate (0.97 g, 25%).: IR (cm^{-1}) *v*: 2890.46, 1733.48, 1481.11, 1353.60, 1255.62, 1171.90, 1039.36, 936.10; ^{1}H-NMR *δ*: 7.25 (2H, m, ArH), 6.72 (1H, d, *J* = 8.3 Hz, ArH), 6.21 (1H, s, ArH), 5.95 (4H, m, O–CH_2–O), 5.18 (1H, m, C(OR)H), 4.16 (1H, m), 3.38–3.82 (3H, m), 3.08 (3H, s, SO_2CH_3), 2.95 (1H, m), 2.53 (3H, s, NCH_3).

5.2. Pharmacological part

The experiments were carried out on male Albino-Swiss mice (20–24 g). Chelidonine hydrochloride (compound **1** in tables) and the investigated new derivatives of *O*-acyl chelidonine: **3a**, **3b**, **3c**, **3d**, **3e**, **3f** and **4**, as well as of *O*-alkyl chelidonine: **2a**, **2b**, **2c** and **2d** were administered intraperitoneally (i.p.) as suspensions in 3% Tween 80 in a constant volume of 10 cm^3 kg^{-1}. Control groups were given appropriate amounts of the solvent. The investigated compounds were administered in doses of 130; 65 or 32.5 mg kg^{-1} i.p. (i.e. equivalent to 0.1; 0.05 or 0.025 of LD_{50} calculated for chelidonine hydrochloride) [6, 23]. Each experimental groups consisted of ten animals. The experiments were performed in accordance with ethical requirements.

5.2.1. Motor coordination

Motor coordination was measured according to the method of Gross and Tripod [24]. The compounds were given in a dose of 130 mg kg^{-1} i.p. and the effect was evaluated 15, 30, 45, 60, 90, 120 and 180 min after administration.

5.2.2. Spontaneous locomotor activity

Locomotor activity of mice was measured by automatic photo resistor actometers (DIGISCAN Optical Animal Activity Monitoring System, Omnitech electronics, Inc.) 30 min after administration of investigated compounds in doses of 130; 65 or 32.5 mg kg^{-1}, the animals were placed in the actometers for 60 min. Locomotor activity was recorded automatically.

5.2.3. Hexobarbital sleeping time

Hexobarbital (75 mg kg^{-1} i.p.) was given 30 min after the administration of compounds in doses of 130 and 65 mg kg^{-1} i.p. The sleeping time of mice (from disappearance to return of the righting reflex) was measured.

5.2.4. Maximal electric shock

Electroconvulsions were induced by means of alternating current (50 Hz, 150 mA and 0.2 s) with the use of ear clip electrodes [25]. The criterion of the convulsive response was the tonic extension of hind limbs. The investigated compounds were given in a dose of 130 mg kg^{-1} i.p. 30 min before the electroshock.

5.2.5. 'Four plate' test

Anxiolytic activity was assessed by the 'four plate' test in mice [26]. Thirty min after injection of new derivatives in a dose of 130 mg kg^{-1} i.p. and the number of punished crossings was counted for 1 min.

5.2.6. Forced swimming test

Mice were individually placed and forced to swim in a glass cylinder (27×16 cm) containing 15 cm of water (25 °C). A mouse was considered immobile when it floated in the water, in an upright position, and made only small movements to keep its head above water. The total immobility time of mice was measured during the last 4 min of the 6-min test [27].

In other experiments, the investigated compounds were administered 30 min before the test.

5.2.7. Pain reactivity

Antinociceptive effects of compounds were measured by the 'writhing syndrome test' [28]. The test was performed in mice by the i.p. injection of a 3% solution of acetic acid 30 min after the administration of new derivatives in doses 130; 65 or 32.5 mg kg^{-1} i.p. The number of writhing episodes was counted for 30 min after the injection of 3% acetic acid.

5.2.8. Head twitches

The investigated compounds were given in doses of 130; 65 or 32.5 mg kg^{-1} i.p. 30 min before 5-hydroxytryptophan (5-HTP, 220 mg kg^{-1} i.p.). The number of head twitch episodes of mice was counted during 60 min after the injection of 5-HTP.

5.2.9. Statistics

The data were statistically analysed by the Student's *t*-test or the Mann–Whitney test.

Acknowledgements

This study was supported by the State Committee for Scientific Research, Warsaw, Poland, grant No 4 PO5F 015 17.

References

[1] Merck Index 12th Edition, pp. 340–341.
[2] Snatzke G., Hrbek J., Hruban L., Horeau A., Santavy F., Tetrahedron 26 (1970) 5013–5028.
[3] Takao N., Bessho N., Kamigauchi M., Iwasa K., Tomita K., Fujiwara T., Fujii S., Tetrahedron Lett. 6 (1979) 495–496.
[4] Probst J.M., Ann. Pharm. 29 (1839) 113–135.
[5] Colombo M.L., Bosisio E., Pharmacol. Res. 33 (1996) 127–134.
[6] Jagiełło-Wójtowicz E., Jusiak L., Szponar J., Kleinrok Z., Pol. J. Pharmacol. Pharm. 41 (1989) 125–131.
[7] Kleinrok Z., Szponar J., Matuszek B., Jagiełło-Wójtowicz E., Pol. J. Pharmacol. Pharm. 42 (1990) 417–424.
[8] Nowicky J.W., Manolakis G., Meijer D., Vatanasapt V., Brzosko W.J., Drugs Exp. Clin. Res. XVIII (1992) 1–4.
[9] Kamigauchi M., Miyamoto Y., Iwasa K., Sugiura M., Nishijo Z., Helv. Chim. Acta 73 (1990) 2171–2178.
[10] Lipinski C.A., Lombardo F., Dominy B.W., Feeney P.J., Adv. Drug Delivery Rev. 23 (1997) 3–25.
[11] Eckly-Michel A.E., Le Bec A., Lugnier C., Eur. J. Pharmacol. 324 (1997) 85–88.
[12] Shiomoto H., Matsuda H., Kubo M., Chem. Pharm. Bull. 39 (1991) 474–477.
[13] Chaturvedi M.M., Kumar A., Darney B.G., Chainy G.B.N., Agrawal S., Aggarwal B.B., J. Biol. Chem. 272 (1997) 30129–30134.
[14] Cushman M., Choong T.C., Heterocycles 14 (1980) 1935–1938.
[15] Sugiura M., Iwasa K., Takao N., Fujiwara H., Beierbeck H., Kotowych G., J. Chem. Soc., Perkin Trans. II (1986) 175–181.
[16] Krajewski P., Kozerski L., Grynkiewicz G., Bednarek E., Sitkowski J., Magn. Reson. Chem. 38 (2000) 757–764.
[17] Hajos Z.G., Duncan G.R., Can. J. Chem. 53 (1975) 2971–2974.
[18] Chen C.-Y., MacLean D.B., Can. J. Chem. 45 (1967) 3001–3004.
[19] Naruto S., Arakawa S., Kaneko H., Tetrahedron Lett. 14 (1968) 1705–1709.
[20] Kamigauchi M., Noda Y., Takao N., Ishida T., Inoue M., Helv. Chim. Acta 69 (1986) 1418–1423.
[21] Seoane E., An. Real Soc. Esp. Fit. Quim. Ser. B 61 (1965) 755-770; CA 63 (1965) 16395d.
[22] Wintgen, Arch. Pharm (Weinheim Ger.) 239 (1901) 442; Beilstein Database.
[23] Kleinrok Z., Jagiełło-Wójtowicz E., Matuszek B., Chodkowska A., Pol. J. Pharm. 44 (1992) 227–239.
[24] Gross F., Tripod J., Schweiz. Med. Wschr. 85 (1955) 305–309.
[25] Swinyard E.A., Brown W., Goodman L.G., J. Pharmacol. Exp. Ther. 106 (1952) 106–319.
[26] Aron C., Simon P., Larousse C., Boissier J.R., Neuropharmacology 10 (1971) 459–469.
[27] Porsolt R.D., Bertin A., Jalfre M., Arch. Int. Pharmacodyn. Ther. 229 (1977) 327–336.
[28] Witkin L., Heubner G., Galdi F., O'Keefe E., Spitaletta F., Plumer A., J. Pharmacol. Exp. Ther. 132 (1951) 400–408.