UDC 547:314

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Synthesis and anti-inflammatory, cytotoxic activity of heteroatomic derivatives of estafiatine

Amination reactions of guaianolide of estafiatine were investigated in this article. It is shown that secondary amines interact only with the conjugated exomethylene bond of the lactone cycle with the formation of Michael adducts. Discussed in this paper sesquiterpene lactone estafiatine (1) and its modified derivatives were studied in order to obtain new biologically active compounds and to determine of influence of structural features of the molecules of this series on their biological activity. It is found that the number of synthesized nitrogen-containing derivatives of estafiatine have anti-inflammatory and cytotoxic activity.

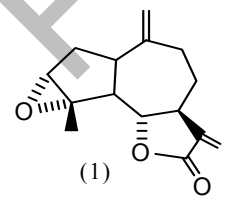
Key words: sesquiterpene lactone, guaianolide, estafiatine, amination, Michael adducts, conjugated exomethylene bond, anti-inflammatory and cytotoxic activity.

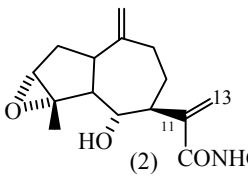
Guaianolides are a large group of evolutionary-related secondary metabolites, which are descended from a common bicyclic guaian sesquiterpene skeleton. More than thousand different guaianolides were isolated from natural sources. Most of them have expressed biological activity. These natural compounds were popular targets for total synthesis for more than three decades, and interest in them has been updated based on the latest understanding of their biological and possible pharmacological relevance [1–3].

It is known that exomethylene double bond conjugated with the carbonyl of γ-lactone cycle is one of the characteristic groups in the structure of most natural biologically active guaianolides. It is considered that the presence of this group affects the biological activity of the molecules of the compounds of this series [4, 5].

In this regard, the study of the amination reaction of guaianolide estafiatine (1), which was isolated from Noble Yarrow (Achillea nobilis L.) with method of water extraction induces interest for us.

Interaction of estafiatine (1) with monoethanolamine and methylamine in ethanol medium under reflux leads to the formation of hydroxy amides (2) and (4) with 65 % and 53 % yields, and amines (3) and (5) with 20 % and 30 % yields. Interaction of estafiatine (1) with benzylamine leads only to amine (6) with 96 % yield. ^{1}H-NMR spectrum data of obtained compounds are shown in Table 1.


(1)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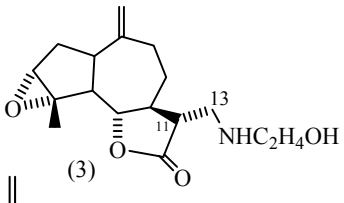
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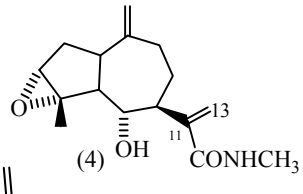
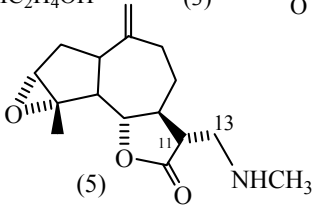
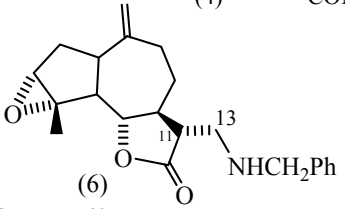

(4) $CONHCH_3$


(5) $NHCH_3$


(6) $NHCH_2Ph$

The reaction of conjugated nucleophilic addition of amines by exomethylene group of γ-lactone cycle occurs stereoselectively with formation of amines which have α-oriented C-13 carbon atom.

Table 1

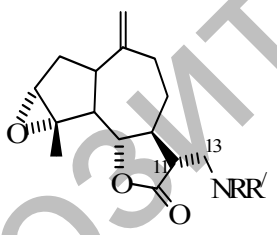
^{1}H-NMR-spectrum data of compounds (1)–(6)

Protons	Compounds					
	(1)	(2)	(3)	(4)	(5)	(6)
Me-4	1,53 s	1,56 s	1,50 s	1,53 s	1,56 s	1,53 s
H-3	3,28 brs	3,28 brs	3,75 brs	2,84 brs	2,90 brs	2,84 brs
H-6	4,01 quint (10,5;9)	4,0 t (10)	3,95 t (10)	3,34 t (10)	3,18 t (10)	3,03 brt (9)
H-13a	5,42 d (3,5)	5,40 d (2,5)	2,67 m	5,41 d (3)	2,50 d (3)	2,53 m
H-13b	6,12 d (3,5)	6,1 d (2,5)	2,67 m	6,21 d (3)	2,50 m	2,53 m
H-14a	4,78 brs	4,78 brs	4,75 brs	4,53 brs	4,56 brs	4,50 brs
H-14b	4,78 brs	4,90 brs	4,82 brd	4,45 brs	4,64 brs	4,56 brs
Other protons	-	$CONH(CH_2)_2OH$; 2,14 brt (1H), (7,5) 2,17 brs (4H)	$HN(CH_2)_2H$ 3,50 brs (1H), 2,10 brs (4H)	CONH CH_3; 2,52 brs (1H), 1,84 s (3H)	$HNCH_3$; 2,70 brs (1H), 1,90 s (3H)	NCH_2Ph -3,43 s (1H), 7,09 brs (5H)

Notes. s (singlet); brs (broad singlet); d (doublet); t (triplet); quint (quintet); m (multiplet); brt (broad triplet). ^{1}H NMR spectra of all obtained compounds were registered on a spectrometer Bruker Avance-400 (operating frequency 400,13 MHz), solvent CDCl3, internal reference TMS.

The reactions of conjugated nucleophilic addition of acyclic and cyclic amines by exomethylene group of γ-lactone cycle of (1) were investigated by us in detail.

Thus, reaction of (1) with diethanolamine, diethylamine, dimethylamine, piperidine and morpholine in ethanol medium under reflux leads to aminoadducts (7)–(11) with 95 % yields (^{1}H-NMR spectrum data are shown in Table 2).



(7) RR′= -CH_3 (10) RR′= -$(CH_2)_5$-

(8) = -C_2H_5 (11) = -C_2H_4OH

(9) = -$(CH_2)_2O(CH_2)_2$-

It is obvious that reaction of guaian (1) with secondary amines controls with range of hardness and softness of bases by Pearson concept.

The anti-inflammatory and cytotoxic activity (*in vitro)* of the synthesized nitrogen-containing derivatives of estafiatine (1) was studied by us. The nitrogen-containing derivatives of estafiatine (1) (Table 3) were evaluated for anti-inflammatory and cytotoxicity activities using two cell lines, including mouse J774.A1 macrophage cells and human THP-1Blue monocytic cells. Anti-inflammatory effect was estimated as ability of test compound to inhibit lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor (TNF) in J774.A1 cells or NF-kB-dependent production of alkaline phosphatase (AP) in transfected cells THP-1Blue. Cytokines were measured in cell supernatants using enzyme-linked immunosorbent assay (ELISA). NO production in the cell supernatants was measured using Griess reagent. AP production was measured using specific substrate QuantiBlueTM. Cytotoxicity was analyzed with a CellTiter-Glo Luminescent Cell Viability Assay Kit.

By looking at the table 3 we can see that estafiatine (1) and its dimethylamino-derivative (7) were found to have a potent anti-inflammatory activity at nontoxic concentrations. Morpholine-(9), piperidine-(10) and benzylamino-(6) derivatives were low-active or inactive at tested concentrations (<150 μM). Inspection of these compounds suggested that bulky substituents could alter ligand interaction with a biological target.

Table 2

^{1}H-NMR-spectrum data of compounds (1), (7)–(11)

Protons	Compounds					
	(1)	(7)	(8)	(9)	(10)	(11)
Me-4	1,53 s	1,56 s	1,56 s	1,59 s	1,56 s	1,20 d
H-3	3,28 brs	2,09 brs	3,31 brs	2,89 brs	2,87 brs	-
H-6	4,01 quint (10,5;9)	3,12 t (10)	4,0 brt (10)	3,12 t (10)	3,12 t (10)	3,18 t (10)
H-13a	5,42 d (3,5)	2,37 dd (8;9)	2,43 m	2,01 m	2,50 brd (2,5)	3,87 brs
H-13b	6,12 d (3,5)	2,65 dd (8;9)	2,43 m	2,01 m	2,65 brd (2,5)	3,87 brs
H-14a	4,78 brs	4,53 d (3)	4,87 brs	4,39 d (2,5)	4,53 d (2,5)	4,65 d (2,5)
H-14b	4,78 brs	4,53 d (3)	4,87 brs	4,39 d (2,5)	4,53 d (2,5)	4,65 d (2,5)
Other protons	–	$N(CH_3)_2$; 1,89 s (6H),	$N(CH_2CH_3)_2$; 2,18 m (4H), 0,93 t (6H, 9)	$N(CH_2)_2O(CH_2)_2$; 3,37 brt (8H, 4)	$-N(CH_2)_5$; 2,78 brs (10H)	$N(CH_2CH_2OH)_2$; –3,51 brs (4H)

Notes. s (singlet); brs (broad singlet); d (doublet); dd (doublet of doublets); t (triplet); quint (quintet); brd (broad doublet); m (multiplet); brt (broad triplet). ^{1}H NMR spectra of all obtained compounds were registered on a spectrometer Bruker Avance-400 (operating frequency 400,13 MHz), solvent $CDCl_3$, internal reference TMS.

Table 3

Ability of sesquiterpene lactones of estafiatine (1) and its derivatives to inhibit lipopolysaccharide (LPS)-induced production of nitric oxide (NO), cytokines and alkaline phosphatase (AP) in mouse and human cell lines, and cytotoxicity evaluation

Compounds	J774.A1 cells				THP-1Blue cells	
	TNF	IL-6	NO	Cytotoxicity	AP	Cytotoxicity
	IC_{50}, μM			IC_{25}, μM	IC_{50}, μM	IC_{25}, μM
Estafiatine (1)	11.1±1.3	6.0±0.7	4.9 ± 0.6	~145	7.7 ± 0.8	64.8 ± 6.7
(10)	N.A.	N.A.	12.2 ± 1.7	N.T	39.5 ± 2.8	N.T
(6)	N.A.	71.6± 5.4	31.2 ± 2.6	N.T	124.4±16.9	N.T
(9)	N.A.	N.A.	20.5 ± 2.2	N.T	89.2±5.1	N.T
(7)	6.3±0.9	3.3± 0.41	2.1 ± 0.4	40.2± 3.6	5.8 ± 0.4	42.6 ± 5.2

Notes. N.A., no inhibition at concentrations <150 μM; N.T., nontoxic at concentrations <150 μM.

Thus, in the course of the research it was found that nitrogen-containing derivatives of estafiatine (1) had expressed anti-inflammatory and cytotoxic activity at non-toxic concentrations.

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Эстафиатиннің гетероатомқұрамды туындыларының синтезі және қабынуға қарсы, цитотоксикалық белсенділігі

Мақалада гваянолид эстафиатиннің аминдеу реакциялары зерттелді. Екіншілік аминдер лактонды циклдің қосарланған экзометиленді байланысымен әрекеттесіп, нәтижесінде Михаэль аддуктары түзілетіні анықталды. Зерттеу барысында алынған сесквитерпенді лактон эстафиатин (1) және оның модификацияланған туындылары жаңа биологиялық белсенді заттарды алу және алынған заттар құрылыстарының оның биологиялық белсенділігіне әсерін анықтау үшін белгіленді. Эстафиатиннің синтезделіп алынған азотқұрамды туындылары қабынуға қарсы және цитотоксикалық белсенділік көрсететіні анықталды.

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Синтез и противовоспалительная, цитотоксическая активность гетероатомсодержащих производных эстафиатина

В статье изучены реакции аминирования гваянолида эстафиатина. Показано, что вторичные амины взаимодействуют только с сопряженной экзометиленовой связью лактонного цикла с образованием аддуктов Михаэля. Обсуждаемый сесквитерпеновый лактон эстафиатин (1) и его модифицированные производные были изучены в плане получения новых биологически активных веществ и определения влияния структурных особенностей молекул данного ряда на их биологическую активность. Установлено, что ряд синтезированных азотсодержащих производных эстафиатина обладают противовоспалительной и цитотоксической активностью.

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