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# Amides derived from heteroaromatic amines and selected steryl hemiesters

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ABSTRACT

The current interest of the team has been focused on investigation of novel amides with potential cytotoxicity. The presented series of compounds was synthesized from selected steryl hemiesters and heteroaromatic amines. The synthetic protocol was designed in a simple and economic way, and divided into several general methodologies applicable to the compounds synthesized. The cytotoxicity was tested on cells derived from human T-lymphoblastic leukemia, breast adenocarcinoma and cervical cancer, and compared with tests on normal human fibroblasts. Most of the lanosterol-based compounds (**3**–**5** and **7**–**10**) showed medium to good cytotoxicity, while only two derivatives of cholesterol (**18** and **19**) showed medium cytotoxicity on human T-lymphoblastic leukemia cell line. The compounds **8** and **9** displayed the reasonable cytotoxicity among this series of amides, tested on the cell lines of T-lymphoblastic leukemia [14.5 ± 0.4 μM (**8**) and 18.5 ± 3.9 μM (**9**)], breast adenocarcinoma [19.5 ± 2.1 μM (**8**) and 23.1 ± 4.0 μM (**9**)] and cervical cancer [24.8 ± 5.3 μM (**8**) and 29.1 ± 4.7 μM (**9**)]. Only the compound **8** was adequately less active on normal human fibroblasts (40.4 ± 11.1 μM).



## 1. Introduction

Recently, we have used stigmasterol, one of the most important plant sterols, to produce its conjugates with selected polyamines as potential compounds with cytotoxicity and antimicrobial activity [1]. Investigation of novel steroid conjugates with pharmacologically important activity has been the key objective of the current research. Now, cholesterol a biogenetic precursor of bile acids and steroid hormones [2], and lanosterol, a tetracyclic triterpenoid, biosynthetically derived from farnesyl pyrophosphate via squalene, were employed to produce conjugates with aromatic amines. 14-Demethylation of lanosterol by cytochrome P450 yields cholesterol [3,4]. Generally, sterols have become part of many plant and animal membranes. Mammalian cells require cholesterol for proliferation [5]. Cholesterol contributes not only to the physico-chemical properties of membranes but also to the organization of lipid rafts involved in signal transduction. Inhibition of cholesterol biosynthesis from lanosterol results in the inhibition of cell cycle progression and, in certain cell types, also in the induction of cell differentiation. Cholesterol metabolism plays a relevant role in the decision making between cell proliferation and differentiation. The relevance of these processes in cancer underscores the interest for studying the role of cholesterol in tumorigenesis and exploring the possibility of interfering with the growth of malignant cells by manipulation of cholesterol metabolism [5]. Generally, sterols bear the only polar hydroxyl function at the C(3) carbon center of the steroid ring A of the basic skeleton. The great advantage of phytosterols consists in their biodegradability, biological activity, ability to affect numerous biological processes, through which function they are considered to be biofactors [6], to pass through membranes and to bind to specific hormonal

*Abbreviations:* DMEM, Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; CEM, cells of human T-lymphoblastic leukemia; MCF7, cells of human breast adenocarcinoma; HeLa, cells of human cervical cancer; BJ, normal human fibroblasts; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; T3P, 50% solution of propylphosphonic anhydride in ethyl acetate; FMOC-Cl, 9*H*-fluoren-9-ylmethyl carbonochloridate.

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receptors (e.g., in activation of human liver X receptors) [6], and in their capability to be modified by synthetic methods [7–9].

Aminomethylpyridines and related heteroaromatic amines, including numbers of their derivatives, and the derived *N*-oxides, had already been investigated for their pharmacological activity [10–12], as markers of solid tumors [13] or agents in supramolecular self-assembly [14]. Aromatic and tertiary amine *N*-oxides were subjects of a comparative study for their potential action as DNA intercalators as bioreductive prodrugs [15]. Potential of several estrone and estradiol conjugates to form *cis*-dichloroplatinum(II) complexes have recently been reported [16].

Lipophilic derivation of aminomethylpyridines is expected to result in obtaining conjugates, bearing ester and amide bond in each molecule. Their importance consists in enabling transportation of potentially biologically active compounds through biomembrane and they often form cationic immune-stimulating complexes [17].

Based on the above referenced facts, a clear connection exists between the selected sterols, cholesterol and lanosterol, which have been involved in the investigation focused on designing novel potential cytotoxic agents. The objectives of this investigation have consisted in (a) designing and synthesizing a series amides derived from lanosterol hemiesters and cholesterol hemiesters with α-, β- and γ-aminomethylpyridines and their *N*-oxides, and (b) performing introductory cytotoxicity tests of the target compounds.

## 2. Experimental

### 2.1. General

The $^{1}H$ NMR and the $^{13}C$ NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer at 600.13 and 150.90 MHz in $CDCl_3$ using tetramethylsilane ($\delta = 0.0$) as internal reference. $^{1}H$ NMR data are presented in the following order: chemical shift ($\delta$) expressed in ppm, number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad), coupling constants in Hertz, proton position in the molecule. For unambiguous assignment of both $^{1}H$ and $^{13}C$ signals 2D NMR H,C-gHSQC and gHMBC spectra were measured using standard parameters sets and pulse programs delivered by producer of the spectrometer. Infrared spectra were measured with a Nicolet 205 FT-IR spectrometer. Mass spectra were measured with a waters ZMD mass spectrometer in a positive ESI mode and for some cases in negative ESI mode. Analytical HPLC was carried out on a TSP (Thermoseparation Products, USA) instrument equipped with a ConstaMetric 4100 Bio pump and a SpectroMonitor 5000 UV DAD. The analyses of the products were performed on a reverse phase Nucleosil 120-5 C18 column (250 × 4 mm; Watrex, Czech Republic) using a methanol/water mixture (9:1, v/v) as mobile phase at 0.5–1.0 mL $min^{-1}$. The eluate was monitored at 220, 254, and 275 nm, and the UV spectra were run from 200 to 300 nm. ACD/Labs software, ACD/log*D* DB, version 12.01, was used for calculation of solubility, partition coefficient (log*P*) and distribution coefficient (log*D*) of the prepared compounds. Chemical names of the compounds used or prepared in this work were generated according to the rules given in the ACD/Labs software, and are based on general nomenclature rules. TLC was carried out on silica gel plates (Merck $60_{F254}$) and the visualization was performed by both, the UV detection, spraying with the methanolic solution of phosphomolybdic acid (5%) followed by heating. For column chromatography, silica gel 60 (0.063–0.200 mm) from Merck was used, and chloroform/methanol mixtures (80:1–40:1) were employed as mobile phases. The purity of the prepared compounds was checked by crystallization, if possible (melting points are presented), and by HPLC, NMR and MS analysis. All chemicals and solvents were purchased from regular commercial sources in analytical grade and the solvents were purified by general methods before use.

### 2.2. Synthetic protocol I: 4-[(3β)-lanosta-8,24-dien-3-yloxy]-4-oxobutanoic acid (**2**) and 4-[(3β)-cholest-5-en-3-yloxy]-4-oxobutanoic acid (**12**)

Succinic anhydride (14.7 mmol; 1.57 equiv) and DMAP (1.584 mmol, 0.17 equiv) was added to a solution of (3β)-lanosta-8,24-dien-3-ol (**1**) or (3β)-cholest-5-en-3-ol (**11**) (9.37 mmol) in pyridine (28 mL). The reaction mixture was stirred over 7 days at r.t. After stopping the reaction, the resulting mixture was poured onto ice, and hydrochloric acid was added to adjust pH = 7, extracted with chloroform, and dried over sodium sulfate. Evaporation of the solvent gave a solid which was purified by column chromatography, affording the products (**2**) or (**12**) in 79% or 96% yield, respectively, and with >99.9% analytical purity (Schemes 1–2). The analytical data of the products **2** and **12** are presented in the Supplementary material.

### 2.3. Synthetic protocol II: preparation of **3**–**10** and **13**–**20**

(a) 1-(Pyridin-2-yl)-, 1-(pyridin-3-yl)- and 1-(pyridin-4-yl)methanamine (**21**–**23**), their *N*-oxides (**33**–**35**) or 1-[4-(1-oxidopyridin-2-yl)phenyl]methanamine (**36**) (0.28 mmol; 1 equiv), DCC (0.34 mmol; 1.2 equiv) and DMAP (0.085 mmol; 0.3 equiv) were added to a solution of **2** or **12** (0.28 mmol) in dichloromethane (3 mL), the reaction mixture was stirred for 1–7 days at r.t., and then evaporated. The obtained residue was purified by chromatography, affording the products **3**–**5**, **7**–**10**, **13**–**15** and **17**–**20** in the yields 42–85%, and with >99.9% analytical purity (Schemes 1–2).

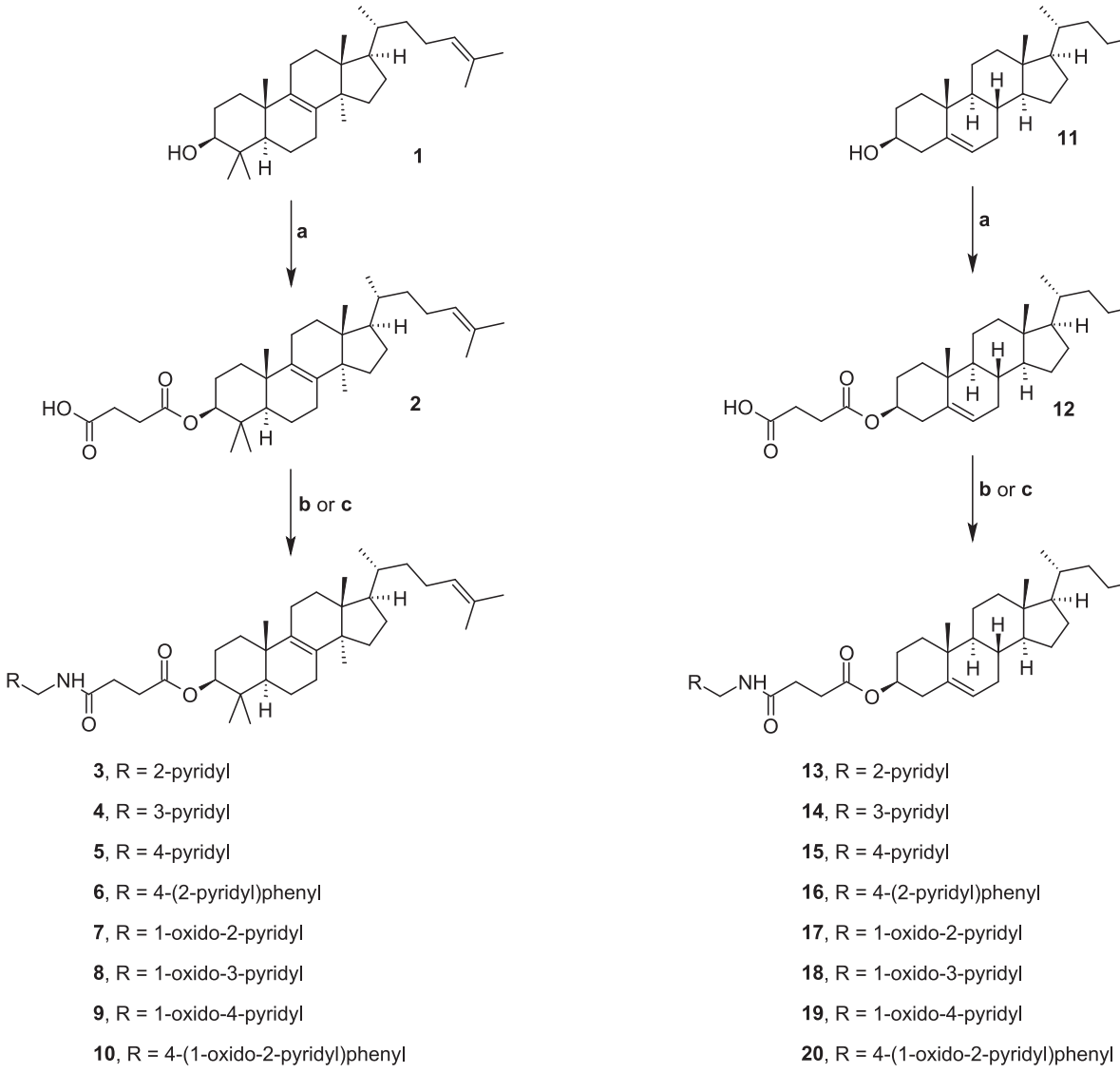
(b) T3P (0.16 mL; 0.27 mmol; 2 equiv) was added to a solution of **2** or **12** (0.13 mmol) and 1-[4-(1-pyridin-2-yl)phenyl)methanamine hydrochloride (**24**) (0.16 mmol; 1.2 equiv) in pyridine (2 mL). The reaction mixture was stirred for 3 days at r.t., then an additional amount of T3P (0.39 mmol; 3 equiv) was added, and stirring continued for an additional 3 h. The reaction mixture was washed with a saturated solution of sodium bicarbonate, extracted with chloroform and dried over sodium sulfate. Evaporation of the solvent afforded a solid which was purified by column chromatography, and gave the products **6** or **16** in the yields 87% or 63%, respectively, and with >99.9% analytical purity (Schemes 1–2). The analytical data of the products **3**–**10** and **13**–**20** are presented in the Supplementary material.

### 2.4. Synthetic protocol III: FMOC-protected aminomethylaromates **25**–**28**

A solution of 9*H*-fluoren-9-ylmethyl carbonochloridate (FMOC-Cl; 9.24 mmol; 1 equiv) in 1,4-dioxane (23.9 mL) was slowly added to a stirred and ice cooled solution of **21**–**24** (9.25 mmol) in 1,4-dioxane (12 mL) and 10% solution of sodium carbonate (25 mL), and stirred overnight. The reaction mixture was washed with water, extracted with chloroform, dried over sodium sulfate and the solvent was evaporated. The obtained residue was purified by chromatography, affording the products **25**–**28** in the yields 79%, 76%, 58% and 78%, respectively (Scheme 3). The analytical data of the products **25**–**28** are presented in the Supplementary material.

### 2.5. Synthetic protocol IV: FMOC-protected aminomethylaromate *N*-oxides **29**–**32**

A solution of peracetic acid (39%, 0.808 mmol, 3 equiv) in acetic acid was added to a solution of **25**–**28** (0.272 mmol) in acetic acid (2 mL). The mixture was heated to 80 °C for 2.5–4 h, and then




**Scheme 1.** Synthesis of lanosterol-based amides. (a) Succinic anhydride, DMAP, pyridine; (b) **21**–**23** or **33**–**36**, DCC, DMAP, dichloromethane; (c) **24**, T3P, pyridine.

**Scheme 2.** Synthesis of cholesterol-based amides. (a) Succinic anhydride, DMAP, pyridine; (b) **21**–**23** or **33**–**36**, DCC, DMAP, dichloromethane; (c) **24**, T3P, pyridine.

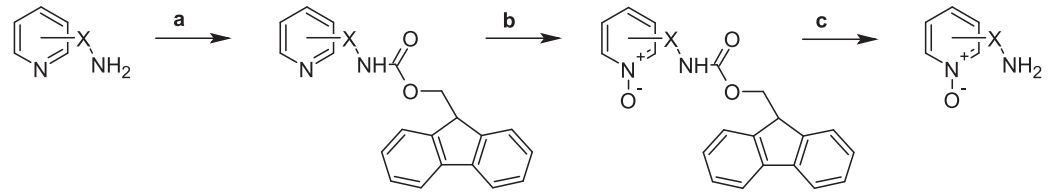
evaporated at r.t. The obtained residue was purified by column chromatography, affording the products **29**–**32** in the yields 71%, 95%, 81% or 64%, respectively (Scheme 3). The analytical data of the products **29**–**32** are presented in the Supplementary material.

### 2.6. Synthetic protocol V: Aminomethylaromate N-oxides *33*–*36*

Piperazine polymer bound (1 g; 1.5 mmol; 1.3 equiv) was added to a solution of **29**–**32** (1.15 mmol) in a mixture of triethylamine/chloroform (1:1; 64 mL), the mixture was heated to boiling point for 5 h, then filtered and evaporated. The obtained residue was purified by column chromatography, affording the products **33**–**36** in the yields 75%, 80%, 89% or 93%, respectively (Scheme 3). These products were used without analysis for the subsequent reactions.

### 2.7. Cytotoxicity tests

Cell culture: stock solutions of the tested compounds (10 mmol $L^{-1}$) were prepared by dissolving an appropriate quantity of each substance in DMSO. Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), L-glutamine, penicillin and streptomycin were purchased from Sigma (MO, USA). Calcein AM was obtained from Molecular Probes (Invitrogen Corporation, CA, USA). The screening cell lines (T-lymphoblastic leukemia cell line CEM, breast carcinoma cell line MCF7, cervical carcinoma cell line HeLa, and human fibroblasts BJ) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in DMEM medium (Sigma, MO, USA), supplemented with 10% heat-inactivated foetal bovine serum, L-glutamine (2 mmol $L^{-1}$), penicillin (10 000 U) and streptomycin (10 mg $mL^{-1}$). The cell lines were maintained under standard cell culture conditions at 37 °C and 5% $CO_2$ in a humid environment. Cells were subcultured twice or three times a week using the standard trypsinization procedure. Calcein AM assay: suspensions of tested cell lines (ca. $1.0 \times 10^5$ cells $mL^{-1}$) were placed in 96-well microtiter plates and after 24 h of stabilization (time zero) the tested compounds were added (in four 20 μL aliquots) in serially diluted concentrations in dimethylsulfoxide (DMSO). Control cultures were treated with DMSO alone, and the final concentration of DMSO in the incubation mixtures never exceeded 0.6%. The test compounds were typically evaluated at six three-fold dilutions and the highest final concentration was generally 50 μM. After 72 h incubation, Calcein AM solution (100 μL, Molecular Probes,




**Scheme 3.** Synthesis of substituted pyridine-*N*-oxides. (a) FMOC-Cl, 1,4-dioxane, $Na_2CO_3$; (b) $CH_3COOOH$, $CH_3COOH$; (c) piperazine polymer bound, triethylamine/chloroform (1:1).

Invitrogen, CA, USA) was added, and incubation was continued for a further hour. The fluorescence of viable cells was then quantified using a Fluoroskan Ascent instrument (Labsystems, Finland). The percentage of surviving cells in each well was calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from control wells and multiplying by 100. These ratios were then used to construct dose–response curves from which $IC_{50}$ values, the concentrations of the respective compounds that were lethal to 50% of the tumor cells, were calculated. The results obtained for selected compounds are shown in Table 1.

## 3. Results and discussion

### 3.1. Chemistry

4-[(3β)-Lanosta-8,24-dien-3-yloxy]-4-oxobutanoic acid (**2**) and 4-[(3β)-cholest-5-en-3-yloxy]-4-oxobutanoic acid (**12**) were prepared by esterification of the respective (3β)-lanosta-8,24-dien-3-ol (**1**) or (3β)-cholest-5-en-3-ol (**11**) with succinic anhydride in pyridine under the presence of DMAP as reaction promotor [18]. Both products **2** and **12** were obtained in high yields as pure crystalline compounds. Most of the target compounds were prepared by formation of the amide bond by the condensation under the presence of DCC in dichloromethane, using DMAP as the reaction promotor [19]. However, when using 1-[4-(pyridin-2-yl)phenyl]-methanamine hydrochloride (**24**), this procedure was ineffective, and was substituted by the protocol employing T3P as the condensation agent in pyridine [20]. The products **6** and **16** were received in acceptable yields. In addition, the T3P protocol was also used to improve the yield of **13**; compared with the condensation with DCC (yield 52%), the other protocol allowed to get the product **13** in 96% yield (Schemes 1–2).

1-(Pyridin-2-yl)-, 1-(pyridin-3-yl)- and 1-(pyridin-4-yl)methanamines (**21**–**23**) are commercially available and 1-[4-(pyridin-2-yl)phenyl]methanamine hydrochloride (**24**) was synthesized in our laboratory for another purpose [21,22]. These four compounds were converted into their *N*-oxides by a sequence of reactions: (a) protecting of the amino group [23] was made by the reaction of FMOC-Cl with the compounds **21**–**24**, affording the protected derivatives **25**–**28**. Subsequently, heteroaromatic nitrogen was converted into the *N*-oxide by oxidation with peracetic acid, affording the compounds **29**–**32**. A removal of the fluorenylmethoxycarbonyl protecting group [24] was achieved by the action of polymer bound piperazine in a mixture of triethylamine/chloroform (1:1), yielding the key intermediates **33**–**36** (Scheme 3).

### 3.2. Cytotoxicity tests

The tested derivatives were screened against several different tumor cell lines. Important difference has been observed between the cytotoxicity of the lanosterol- and cholesterol-based derivatives. Most of the former series of compounds (**3**–**5** and **7**–**10**) were effective on relatively broad spectrum of tested cell lines, while only two derivatives of cholesterol (**18** and **19**) showed medium cytotoxicity on cells of human T-lymphoblastic leukemia. The compound **8** was evaluated as the most active compound of the series investigated in this work. In turn, compound **9** showed comparable cytotoxicity on all three lines of tumor cells and on the normal human fibroblasts. The third positional isomer of this series of *N*-oxides (**7**) showed only moderate cytotoxicity on the cells of human T-lymphoblastic leukemia. The parent heteroaromatic derivatives **3**–**5** showed different ranking: **3** displayed medium cytotoxicity on CEM and HeLa cell lines, **5** medium cytotoxicity on CEM cell lines, and **4** was practically inactive. Selective medium effect of **10** on MCF7 cell lines was observed, while its parent compound **6** was inactive. From the latter series of new compounds, only **19** and **18** showed medium cytotoxicity on the CEM cell lines. All other compounds not mentioned above were found inactive (Table 1).

### 3.3. Physico-chemical characteristics

To support rational design of the target compounds investigated in this work, their physico-chemical characteristics have been calculated. Most of the compounds presented herein form triads, being derived from the respective 1-(pyridin-2-yl)-, 1-(pyridin-3-yl)- and 1-(pyridin-4-yl)methanamines. Therefore, physico-chemical characteristics calculated for the triads of the target compounds are identical, and were compared with the Lipinski [25] rule of five and with the Ghose [26] rules. The rules describe molecular properties important for a small molecule drug pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (known as ADME parameters). However, the rules do not predict whether a compound will display pharmacological activity. Lipinski [25] rule of five considers partition coefficient ($\log P$, range −0.4 to +5.6), molar refractivity (range 40–130), molecular weight (range 180–500), number of atoms in the molecule (20–70) and polar surface area (up to 14 nm). The data for comparison are partly presented in Table 1, partly in the Supplementary material. Many exceptions are already known, where pharmacologically active compounds do not correspond to all rules.

In this investigation, attention has been mainly paid to the partition coefficient ($\log P$) and the distribution coefficient ($\log D$). These two values represent the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium. In chemical and pharmaceutical sciences, both, $\log P$ and $\log D$ are measures of hydrophilicity or hydrophobicity of the studied compound, and are useful for estimating distribution of a drug within the body, where $\log D$ shows the dependence on the pH of the matrix. Hydrophobic drugs are then preferentially distributed to

**Table 1**
Calcein AM assays of **3**–**10** and **13**–**20** ($IC_{50}$ values, μM) and calculated physico-chemical characteristics.

| Compound or reference number | MW | Calcein AM assays ($IC_{50}$ values, μM) | | | | Calculated physico-chemical characteristics | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CEM | MCF7 | HeLa | BJ | log*P* | log*D* (*pH = 7.4*) | H-bond acceptor | H-bond donor | No. of rotable bonds |
| **3** | 616.91 | 33.9 ± 3.9 | >50 | 33.3 ± 7.5 | >50 | 11.31 | 9.13 | 5 | 1 | 11 |
| **4** | 616.91 | 49.7 ± 0.5 | >50 | >50 | >50 | 11.31 | 9.13 | 5 | 1 | 11 |
| **5** | 616.91 | 29.9 ± 0.6 | >50 | 47.8 ± 3.2 | >50 | 11.31 | 9.13 | 5 | 1 | 11 |
| **6** | 693.01 | >50 | >50 | >50 | >50 | 13.22 | 10.92 | 5 | 1 | 12 |
| **7** | 632.91 | 28.4 ± 6.0 | >50 | >50 | >50 | 9.38 | 8.80 | 6 | 1 | 11 |
| **8** | 632.91 | 14.5 ± 0.4 | 19.5 ± 2.1 | 24.8 ± 5.3 | 40.4 ± 11.1 | 9.38 | 8.80 | 6 | 1 | 11 |
| **9** | 632.91 | 18.5 ± 3.9 | 23.1 ± 4.0 | 29.1 ± 4.7 | 22.9 ± 0.1 | 9.38 | 8.80 | 6 | 1 | 11 |
| **10** | 709.01 | >50 | 23.4 ± 7.1 | >50 | >50 | 11.14 | 10.29 | 6 | 1 | 12 |
| **13** | 576.85 | >50 | >50 | >50 | >50 | 10.05 | 8.58 | 5 | 1 | 12 |
| **14** | 576.85 | >50 | >50 | >50 | >50 | 10.05 | 8.58 | 5 | 1 | 12 |
| **15** | 576.85 | >50 | >50 | >50 | >50 | 10.05 | 8.58 | 5 | 1 | 12 |
| **16** | 652.95 | >50 | >50 | >50 | >50 | 11.95 | 10.25 | 5 | 1 | 13 |
| **17** | 592.85 | >50 | >50 | >50 | >50 | 8.12 | 8.00 | 6 | 1 | 12 |
| **18** | 592.85 | 32.7 ± 0.2 | >50 | >50 | >50 | 8.12 | 8.00 | 6 | 1 | 12 |
| **19** | 592.85 | 23.6 ± 1.7 | >50 | >50 | >50 | 8.12 | 8.00 | 6 | 1 | 12 |
| **20** | 668.95 | >50 | >50 | >50 | >50 | 9.88 | 9.71 | 6 | 1 | 13 |
| Ref. [25] | Max. 500 | – | – | – | – | Max. 5.0 | – | Max. 10 | Max. 5 | – |
| Ref. [26] | Max. 500 | – | – | – | – | Max. 5.6 | – | – | – | – |

Note: $IC_{50}$ (μM) values were obtained from the Calcein AM assays with the tested cancer and normal cell lines. The cells were treated for 72 h with serial concentrations of the compounds. Means ± SD were obtained from three independent experiments performed in triplicate. The physico-chemical characteristics were calculated using the ACD/labs software and databases [27].

hydrophobic compartments (e.g., lipid bilayer of cells), while hydrophilic drugs are preferentially distributed to hydrophilic compartments (e.g., blood serum). The distribution coefficient is a pH dependent value, and, therefore, the value at pH = 7.4 (the physiological pH value of blood serum) is of particular importance (see also Table 1). Thus, log*P* expresses a ratio of concentrations of non-ionized compound between two phases, non-polar (octanol) and polar (water), while log*D* expresses the ratio of the sum of the concentrations of all forms of the compound (ionized and non-ionized) in each of the two phases. In pharmacology, log*P* and log*D* indicate how easily the drug can reach its intended target in the body, how strong its effect will be once it reaches its target and how long it will remain in the body in an active form.

The characteristics of the prepared compounds are in accordance with only several of the Lipinski and Ghose rules [25,26], were obtained by using ACD/Labs software and databases [27] for calculation. However, based on the experimental data, the cytotoxicity values show differences within the triads of compounds, in some cases (4-[(3β)-lanosta-8,24-dien-3-yloxy]-4-oxobutanoic acid based amides) quite important. In addition to the log*P* and log*D* values (Table 1), several other ADME parameters were obtained for the target compounds **3**–**10** and **13**–**20** using ACD/Labs software and databases [27]. The oral bioavailability of these compounds is lower than 30%, which means that they are likely not suitable for oral administration. In turn, log BB for blood–brain barrier remain within the standard range (−3.0 to +1.2) [28] for all compounds, except for **17**–**19**. For **8** and **9**, log BB = 1.26, which is close to the standard range. Even in the compounds **3**–**10** and **13**–**20** show more than one violation from the Lipinski rule of five [25], cytotoxicity data presented in the Table 1 clearly show moderate activity of several such compounds. Every time a new class of compound is being investigated, no available experimental screening may be intentionally omitted (Table 1).

## 4. Conclusion

This series of amides derived from 4-[(3β)-lanosta-8,24-dien-3-yloxy]-4-oxobutanoic acid (**2**) and 4-[(3β)-cholest-5-en-3-yloxy]-4-oxobutanoic acid (**12**) displayed only moderate cytotoxicity. However, this investigation of isomeric triads of the target compounds brought a clear message on importance of combination of experimental and theoretical data, which are not always in full agreement. The lanosterol based amides showed higher cytotoxicity than those derived from cholesterol. Moreover, the results of the cytotoxicity tests showed that despite possible worse penetration of aromatic *N*-oxide bearing compounds through the cell wall in comparison with their parent heterocycle bearing compounds, the *N*-oxides **8** and **9** were found to be the compounds with the highest cytotoxicity among this series of compounds.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2013.10.003.

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