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Screening the binding affinity of bile acid derivatives for the glucocorticoid receptor ligand-binding domain

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Abstract: The necessity of anti-inflammatory drugs such as glucocorticoids has been evident during the COVID-19 pandemic. Glucocorticoids, are the standard therapy for the treatment of moderate and severe COVID-19 patients. However, serious side effects limit the use of these drugs, and anti-inflammatory drugs with better pharmacological properties are urgently required. Bile acids are of interest, because of their anti-inflammatory and immunomodulatory properties, facilitated through an unclear mechanism involving transmembrane and nuclear receptors. In this work, we screened the binding activity of a number of bile acid derivatives, for the ligand-binding domain of glucocorticoid receptor (GR-LBD), the most important receptor for anti-inflammatory processes. Tested compounds include oximes, lactones, lactams, tetrazoles, dienones, C-24 alcohols and cholic acid amides. Cholic acid oxime, deoxycholic acid dienone, 3-keto-24-cholic alcohol and cholic acid amide showed best binding affinities for GR-LBD among tested compounds. The in silico molecular docking explanation is provided. SAR analysis showed that expansion of B and C steroid rings or attachment of heterocycle to C ring is not beneficial for binding; side chain should contain hydrogen donor group; the GR-LBD tolerate well different functionalities on C-3 position. These results provide valuable information toward synthesis of the new glucocorticoids based on bile acids.

Keywords: organic synthesis; docking studies; molecular modelling.

INTRODUCTION

Glucocorticoids (GCs) are a class of steroid hormones that are among the most commonly prescribed drugs used for the treatment of allergic, inflam-

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matory, and immune disorders such as rheumatoid arthritis, asthma, brain edema, shock, and various blood cancers.[1,2] More recently, they were also the first drugs shown to reduce deaths from COVID19.[3] GCs are the most effective, cost-efficient, and necessary anti-inflammatory and immunomodulatory drugs available. However, the use of GCs is limited by serious adverse effects, such as diabetes, osteoporosis, muscle wasting, hypertension, and glaucoma. Also, certain groups of patients do not respond well to GC therapy.[4] Thus, there is an urgent medical need for new molecules with both enhanced GC therapeutic activities, and fewer or less severe side effects. GCs mediate their effects via the glucocorticoid receptor (GR), a ligand-activated transcription factor. GC activated GR performs its anti-inflammatory functions through a number of mechanisms, among which the most important are transrepression and transactivation.[5,6]

As steroid molecules, bile acids (BAs) play important roles as hormones that regulate a large number of metabolic processes, including inflammation. BAs are enzymatically synthesized in hepatocytes from cholesterol, conjugated with glycine or taurine, and stored in the gallbladder. When food is ingested, liver-synthesized primary BAs are secreted in the small intestine where they emulsify dietary lipids and lipid-soluble vitamins, enabling their absorption. A fraction of primary BAs is converted to secondary BAs by intestinal microbiota. Secreted BAs are reabsorbed and returned to the liver by a very efficient process known as enterohepatic circulation. The hormonal role of BAs is mediated through the BA--activated nuclear receptor farnesoid X receptor (FXR), which controls gene transcription in BA homeostasis and *via* G-protein coupled receptor TGR5. TGR5 is broadly expressed in humans and is involved in various physiological and pathological processes, including energy expenditure, glucose homeostasis, obesity, and bile acid homeostasis.[7]

The anti-inflammatory properties of BAs were reported by Hench in the 1930s. He observed alleviation of rheumatic symptoms in patients with the onset of severe jaundice, a side effect associated with increased BA serum concentrations.[8] BAs were first used as starting compounds for the synthesis of cortisol, which resulted in the 1950 Nobel Prize in Physiology or Medicine for Hench, Kendall and Reichstein.[9] FXR is involved in the pathophysiology of several inflammatory diseases, including non-alcoholic fatty liver diseases, inflammatory bowel disease and atherosclerosis.[10,11] Activation of TGR5 in macrophages and monocytes suppresses lipopolysaccharide-induced production of cytokines and prevents liver damage. The beneficial effects of TRG5 activation were noticed in multiple inflammatory diseases, including diet-induced obesity, atherosclerosis, colitis and steatohepatitis. More information about TGR5 and immunometabolism can be found in an excellent review by Perino and Schoonjans.[12]

Although the hormonal and anti-inflammatory activity of BAs *via* FXR and TGR5 pathways is well established, there is reasonable evidence that some of the

anti-inflammatory activities of BAs is also mediated through GRs. It was found that 5*β*-cholanic acid can bind to GR and modulate GR signaling in cell models of Parkinson's disease.[13] Taurochenodeoxycholic acid also exhibited anti-inflammatory and immunomodulatory properties by inhibiting transcription and expression of AP-1 *via* stimulation of the GR.[14] Ursodeoxycholic acid exerts immune-suppressive effects by reducing IFN-*γ* production by liver lymphocytes, such as NK and NKT cells, in a GR-dependent manner, which may be an important immunological mechanism.[15]

The promiscuity of bile acids for FXR, TGR5 and GR could be exploited in an anti-inflammatory and immunomodulatory manner. In the present work, we explored the GR binding affinity of BA derivatives as a screen for unexplored BA structural features that may be important for GR binding. This work is aimed at the development of anti-inflammatory compounds that could lead to drugs with fewer side effects than current GCs.

EXPERIMENTAL

General synthetic methods

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance III HD 400 (400 MHz 1H, 101 MHz 13C) apparatus using tetramethylsilane as an internal standard. HRMS spectra (TOF) were recorded on a 6210 time-of-flight LC/MS Agilent Technologies (ESI+) instrument. IR spectra were recorded on a Perkin Elmer Spectrum Two FT-IR spectrometer, and Thermo Nexus 670 FT-IR spectrometer, and melting points were determined on Stuart MP-10 apparatus. Flash chromatography was performed on silica gel 60 (0.04–0.063 mm, Merck). Synthesis of compounds **28** and **29** was done according to a procedure described in our earlier publication,[16] and compound **27** was obtained following the Iqbal and Elliott protocol.[17] For synthesis of **25**, we used a different route then Leppik,[18] and **1** was obtained by a method described by Hüttenrauch.[19]

Spectral data of the compounds are given in the Supplementary material to this paper.

*(3*EZ*, 7*Z*, 12*Z*)-3,7,12-Trioximino-5*β*-cholan-24-oic acid (**1**)*

NaOAc (1.09 g, 13.3 mmol) and $NH_2OH{\times}HCl$ (0.45 g, 7.09 mmol) were added to a suspension of dehydrocholic acid (DCA, 0.63 g, 1.6 mmol) in MeOH (20 mL). The mixture was refluxed for 40 min, and the resulting suspension filtered and washed with cold water. Compound **1** was obtained as a pure mixture of geometric isomers, as a white powder with a yield of 0.6541 g (91 %; thermal decomposition before melting started at 240 °C).

*Methyl (7*Z*,12*Z*)-3,3-dimethoxy-7,12-dioximino-5*β*-cholan-24-oate (**2**)*

NaOAc (0.80 g, 9.8 mmol) and $NH_2OH{\times}HCl$ (0.25 g, 3.9 mmol) were added to a solution of **3** (0.44 g, 1 mmol) in MeOH (20 mL). The mixture was refluxed for 1 h, and the resulting suspension filtered and washed with cold water and dried. Pure compound **2** was obtained as a white powder at a yield of 0.35 g (72 %; thermal decomposition before melting started at 240 °C).

*Methyl 3,3-dimethoxy-7,12-dioxo-5*β*-cholan-24-oate (**3**)*

TsOH (0.16 g, 0.9 mmol) and 2,2-dimethoxypropane (7 mL, 57 mmol) were added to a solution of DCA (1 g, 2.5 mmol) in MeOH (50 mL). The mixture was refluxed for 3 h, evaporated under vacuum, dissolved in EtOAc, washed with cold water (2×15 mL) and dried.

Pure compound **3** was obtained as a white powder at a yield of 1.1 g (93 %; thermal decomposition before melting started at 225 °C).

*12α-Hydroxy-3-oxo-5β-chola-4,6-dien-24-oic acid (**25**)*

Compound **27** (0.4985 g; 1.05 mmol) was dissolved in methanolic KOH (1 g; 17.8 mmol in 50 mL MeOH) and refluxed for 30 min. After reaction completion, the reaction mixture was poured into water (200 mL) and acidified with HCl (1:2) to pH 1. The resulting precipitate was filtered, washed with brine to neutral pH and dried. The raw mixture was purified by flash chromatography (CH_2Cl_2/EtOAc 94:6). Compound **25** was obtained as white needle-like crystals (after recrystallisation from acetone) at a yield of 0.3642 g (90 %); mp 251 °C, mp lit.[20] 249–252 °C.

*12α-Hydroxy-3-oxo-5β-chola-4,6-dien-24-oic acid (**25**) and ethyl 12α-hydroxy-3-oxo-5β--chola-4,6-dien-24-oate (**26**)*

Compound **27** (0.4872 g; 1.02 mmol) was dissolved in ethanolic KOH (0.19 g; 3.4 mmol in 70 mL EtOH) and refluxed for 1 h. After reaction completion, reaction mixture was poured in water (200 mL) and acidified with HCl (1:2) to pH 1. The resulting precipitate was filtered, washed with brine to neutral pH and dried. The raw mixture was purified by flash chromatography (CH_2Cl_2/EtOAc 94:6). Compound **25** was obtained at a yield of 0.1116 g (28 %) and compound **26** (mp 136 °C) at a yield of 0.2872 g (67 %).

2-(5β-chol-3-ene-7α,12α,24-triol)-N-*(1-hydroxy-2-methylpropan-2-yl)acetamide (**30**)*

Triethylamine (0.3 mL), 2-amino-2-methyl-1-propanol (0.2 mL, 1.1 mmol) and EEDQ (0.23 g, 0.9 mmol) were added to a suspension of compound **29** (0.299 g, 0.69 mmol) in EtOAc (10 mL). The reaction mixture was refluxed for 5 h. After cooling to room temperature, the reaction mixture was washed successively with 3 M HCl (2×4 mL), H_2O (4 mL), 10 % $NaHCO_3$ (2×10 mL) and then with water to neutrality (3×5 mL). The organic layer was dried and evaporated in vacuum to give an oily residue, which was further purified by flash column chromatography (CH_2Cl_2/MeOH 96:4). Pure **30** was obtained at a yield of 0.2612 g (75 %).

Fluorescent assay in yeast

A yeast-based fluorescent screen was applied for testing the relative binding affinities of BA derivatives **1**, **2**, **4**–**23** and **25**–**31** for the GR ligand binding domain (LBD). Assays were conducted following our previously published procedure; optimized for identification of steroidal ligands of the GR.[21,13] *Saccharomyces cerevisiae* FY250 strain (MATα, ura3-52, his32Δ00, leu2Δ1, trp1Δ6) and expression vector pRF4-6-rGR LBD-EYFP were kindly provided by Dr. Blake Peterson from The University of Kansas.[22] Yeast cells were transformed with plasmid DNA by treatment with lithium acetate and polyethylene glycol, to improve the efficiency of exogenous DNA uptake, following the procedure of Gietz *et al.*[23] Transformed yeast cells were then incubated at 30 °C until the appearance of transformant colonies on agar plates. Selection medium supplemented with 2 % raffinose was then inoculated with recombinant yeast cells grown to saturation in a Biosan orbital shaker-incubator ES-20/60. Saturated yeast cells were diluted in fresh medium and grown to mid-logarithmic phase ($OD_{600\ nm} \approx$ 0.5), monitored using a Nicolet Evolution 100 UV–Vis spectrophotometer. Protein expression was induced by addition of galactose to a final concentration of 2 %. Bile acid derivatives, prednisolone or estradiol (high- and low-affinity GR ligands) were added to a final concentration of 100 μM. Stock solutions of all tested compounds were freshly prepared in DMSO. Incubation was continued for 15 h at 25 °C. Resulting fluorescence intensity was detected by

fluorimeter (Fluoroskan Ascent FL) and fluorescence microscopy (Olympus BX51) using a FITC filter. For fluorimetry, 150 μL of cell suspension was added to a microplate in triplicate and fluorescence was recorded using an excitation and emission filter set of 485 and 538 nm. Growth medium without cells served as a blank. To normalize fluorescence intensity to cell number, the optical density of yeast cells was measured at 600 nm using a Thermo Lab systems Multiscan EX spectrophotometer. Ligand binding affinity was calculated as the fold fluorescence change between cells exposed to test compounds and those treated with negative control ligand, estradiol. Histograms showing the relative binding affinity of bile acid derivatives and control ligands for GR-LBD were created in Origin Pro 8 with included error bars, obtained by propagation of standard error of the mean. Additionally, the fluorescence intensity distribution of recombinant yeast cells expressing GR LBD-YFP treated with bile acid derivatives or control compounds, was visualized by fluorescence microscopy.

Molecular docking

Coordinates for the GR-LBD receptor were converted to PDBQT format in the program VegaZZ using the available "receptor.c" script.[24] Structural coordinates for compounds **1**, **25**, **28** and **31** were created in the program Avogadro 1.0.3;[25] Avogadro: an open-source molecular builder and visualization tool based on the structure of dexamethasone, taken from PDB 1M2Z.[26] Ligand geometries were optimized in Avogadro 1.0.3 using an MMFF94 force field and 500 steps of conjugate gradient minimization with a convergence setting of 10e-7. Non-polar hydrogens were merged and gasteiger partial charges added in VEGAZZ 3.1.0,[27] using the "ligand.c" script, and resulting ligand coordinates were converted to PDBQT format for Autodock. Grid maps for atoms present in the tested compounds were created using the program AutoGrid, with a grid center of 31.04, 7.76, 12.52, grid spacing of 0.0375 nm and dielectric of –0.1465. Docking simulations in Autodock 4.2,[29] were conducted using the Lamarckian genetic algorithm with the following parameters: GA population size 150. GA num evaluations 250000. GA num generations 27000. AutoGrid calculations and Autodock simulations were conducted using the PyRx virtual screening tool (version 0.8).[27] Results were visualized in the program PyMol (v0.99).[28]

RESULTS AND DISCUSSION

Chemistry

Compounds used in the present study were chosen to examine the influence of easy-to-achieve modifications of the BA molecule on GR binding affinity. Structures of compounds investigated in the present work can be categorized into roughly seven categories: oximes, lactones, lactams, tetrazoles, dienones, C-24 alcohols and cholic acid amides.

Structures of oxime derivatives are shown in Fig. 1.

Synthesis of oximes **1** and **2** is shown in Scheme 1. The reaction of dehydrocholic acid (DCA) with hydroxylamine hydrochloride afforded a mixture of geometric isomers **1**, at the C-3 oxime group. The mixture of isomers **1** was obtained with a yield of 91 % and individual isomers were not separated. The isomer ratio of **1** could be speculated to be 3:2 based on the intensity of C-3 signals (156.87 and 156.67 ppm) present in ^{13}C-NMR spectra. Compound **2** was obtained in a two-step sequence. First, the carbonyl group at C-3 was regioselectively trans-

formed into acetal, while the carboxyl group was esterified which afforded **3** in excellent yield (93 %). This reaction represents a very good way for regioselective protection of the C-3 bile acid carbonyl group. Next, C-7 and C-12 carbonyl groups were converted into oxime to obtain dioxime **2**.

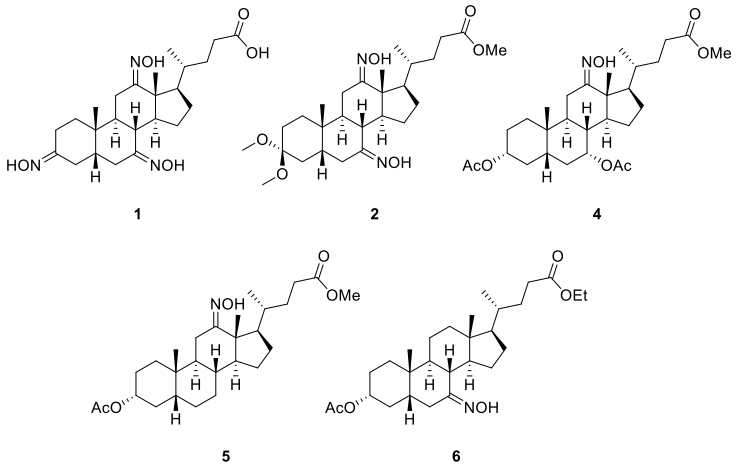
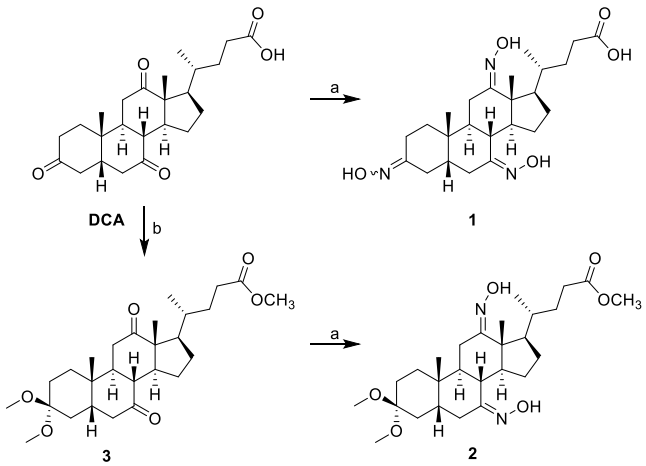


Fig. 1. Structures of oximes.



Scheme 1. Reagents and conditions: a) $NH_2OH \times HCl$, NaOAc, MeOH, 40 min, reflux; b) MeOH, 2,2-dimethoxypropane, TsOH, 3 h, reflux.

Unlike **1**, compound **2** was obtained as a stereochemically pure compound. The stereochemistry of oxime groups at C-7 and C-12 was determined as *Z*-based on the absence of cross-peaks in NOESY NMR spectra that originate from, the through space, interaction of oxime hydrogens with any steroid skeleton hydrogens. Only the *Z* configuration for the oxime groups could provide enough dis-

tance to explain the lack of NOE interactions. Stereochemistry could be explained by the larger volume available for hydroxyl groups if the oximes have a *Z* configuration (Fig. 2). Oximes **4**,[29] **5**[30] and **6**[31] were prepared by procedures found in the corresponding literature.

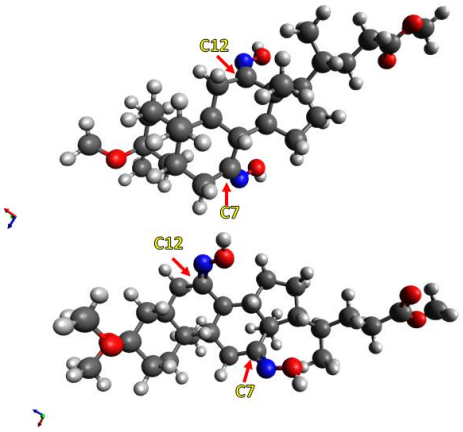


Fig. 2. Different perspectives of **2** show the available space for Z-oriented oxime groups at C-7 and C-12.

Bile acid derivatives with a lactone or lactam moiety in B or C steroid ring (Fig. 3) **7**–**14** were synthesized according to published procedures,[31–33] while the stereochemistry and physicochemical properties of these compounds were described by Poša *et al.*[34]

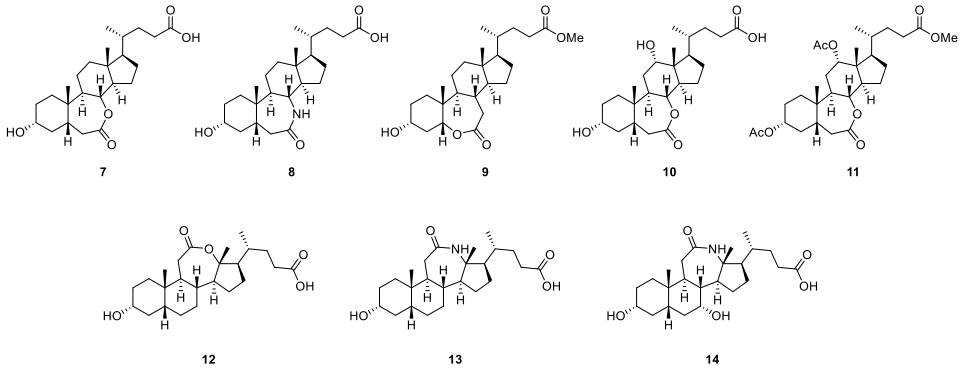


Fig. 3. Structures of bile acid lactone and lactam derivatives **7**–**14**.

The structures of tetrazoles **15**–**23** used in the present work are shown in Fig. 4. Tetrazole rings are fused on B and C steroid rings.

The general synthesis of tetrazole compounds is shown in Scheme 2, using the synthesis of **16** as an example. Bile acid derivative **24**, with C-3 acetoxy, C-24 ethoxycarbonyl groups, and a carbonyl group at C-12, was subjected to Schmidt reaction condition (Scheme 2) with trimethylsilyl azide as an azide source and tri-

methylsilyl trifluoromethanesulfonate as an amide intermediate activator. Details of the synthesis of the tetrazole compounds used in the present study have been published by our group.[35]

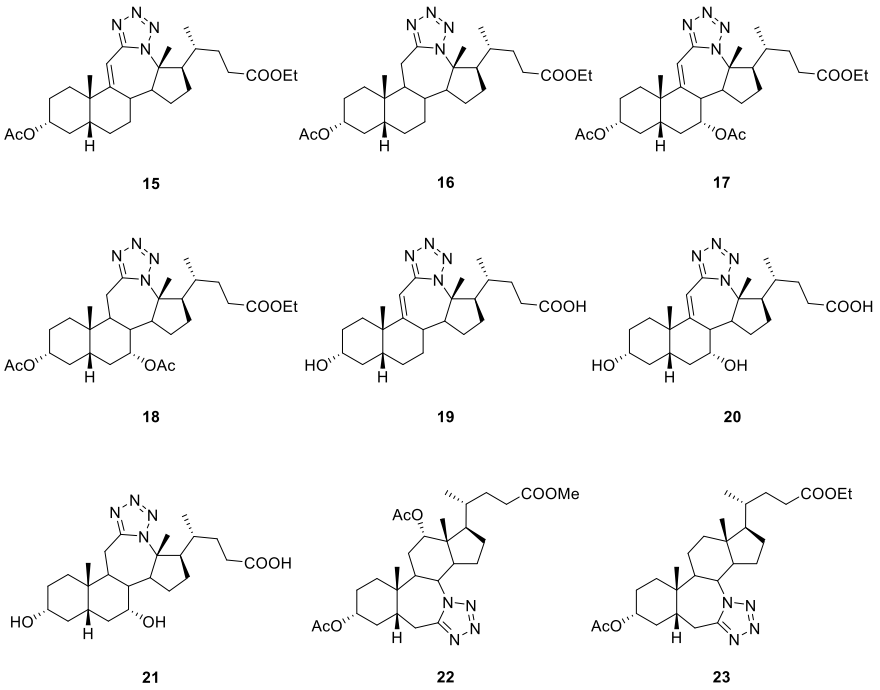
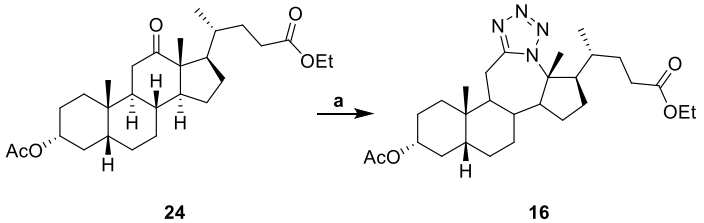


Fig. 4. Structures of the bile acid tetrazole derivatives **15**–**23**.



Scheme 2. The general synthesis of tetrazole compounds **15**–**23** is illustrated using the synthesis of **16** as an example. Reagents and conditions: $TMSN_3$, TMSOTf, ACN, rt, 3 h (a).

Structures of dienones **25** and **26**, alcohols **28** and **29** and amides **30** and **31** are shown in Fig. 5.

Dienones **25** and **26** were obtained by the following procedures: when compound **27** (Scheme 3) was refluxed in methanolic potassium hydroxide solution, elimination and hydrolysis occurred to afford **25** at a yield of 90 % (78 % reported by Leppik *et. al.*[18]). Reaction samples were collected every 5 min and since

only one product was detectable by TLC, it appears that both elimination and hydrolysis reactions occur simultaneously. The same result was obtained upon treatment of **27** with methanolic KOH solution for five days at room temperature, or 2 M NaOH solution for five hours at room temperature, or by reflux with HCl solution (1:3) in water/acetone 5/7 for five hours. When ethanol was used instead of methanol, in addition to **25** (28 %), transesterification occurs to afford ethyl ester **26** (67 %). Signals corresponding to vinylic protons in **25** are easily visible by ^{1}H-NMR as overlapping singlet peaks of H-4 and H-6 at 6.16 ppm, and a singlet from H-7 at 5.61 ppm. An additional quartet at 4.03 and a triplet at 1.16 in the ^{1}H-NMR of **26** suggests the presence of an esterified carboxyl group.

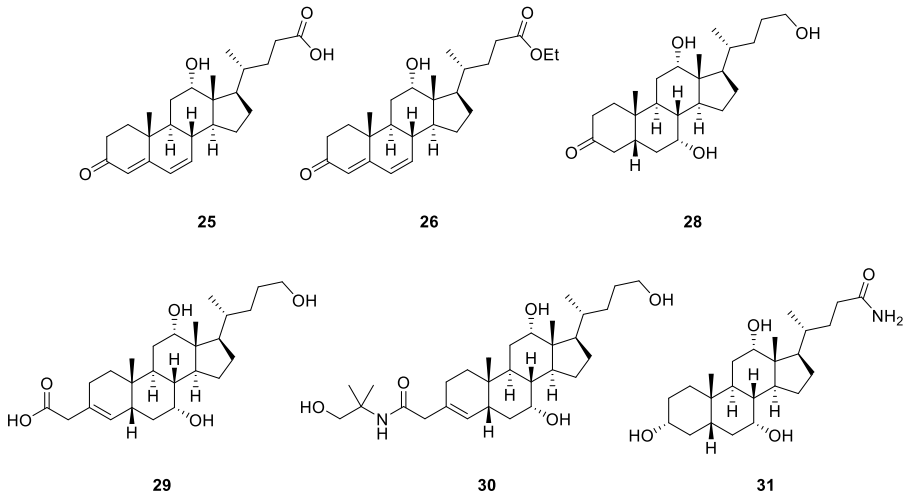
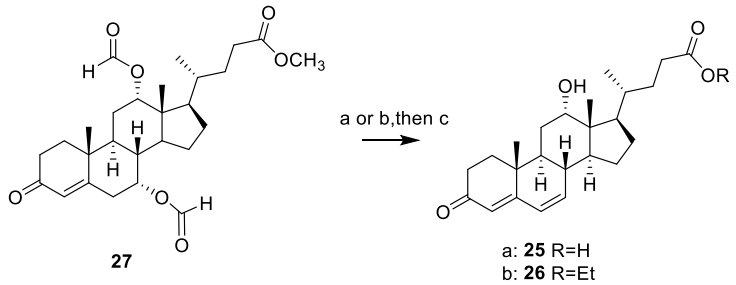


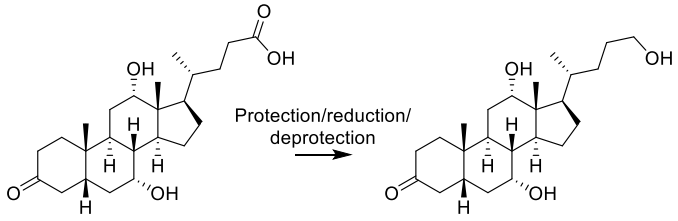
Fig. 5. Structures of dienone, alcohol and amide derivatives **25**, **26**, **28**–**31**.



Scheme 3. Reagents and conditions: a) KOH, MeOH, 0.5 h, reflux; b) KOH, EtOH, 1 h, reflux; c) HCl, H_2O.

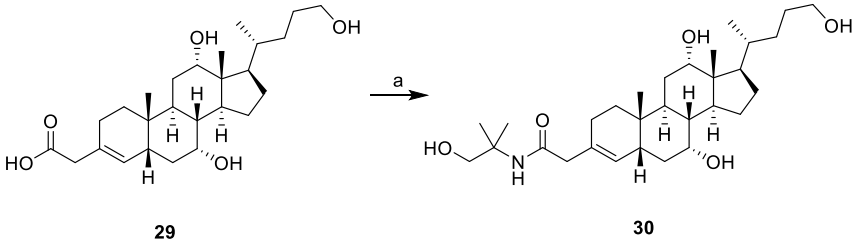
Easily obtainable and well-known 3-dehydrocholic acid (3DCA) was transformed into the bile alcohol **28** using acetal protection as seen in **3**, and subsequent reduction (Scheme 4). The same bile alcohol **28** was transformed into **29**

using a Wittig–Horner–Emmons reaction. Details concerning the synthesis of **28** and **29** are available in our previous publication.[16]



Scheme 4. Synthesis of bile alcohol **28**.

Compound **30** was obtained in the good yield (75 %) by reaction of **29** with 2-amino-2-methyl-1-propanol using EEDQ as a coupling agent (Scheme 5).



Scheme 5. Reagents and conditions: 2-Amino-2-methyl-1-propanol, EEDQ, TEA, EtOAc, 5 h, reflux (a).

The cholic acid amide **31** (Fig. 5) was synthesized according to a procedure reported by Miljković *et al.*[36]

Fluorescent assay in yeast

In order to test the relative binding affinities of the synthesized BA derivatives, we used a yeast-based fluorescent screen that is optimized for identification of steroidal ligands of the glucocorticoid receptor, as previously described.[13,21] Briefly, the fluorescence intensity of yeast cells expressing the ligand-binding domain (LBD) of the glucocorticoid receptor (GR) fused to a yellow fluorescent protein (YFP) has been shown to respond in a dose-dependent manner to treatment with GR agonists such as prednisolone.[13,21] Because the assay is measured in yeast, the concentration of ligand required to elicit a response may not necessarily be the same concentration that would affect GR activity *in vivo*. During optimization of the assay, we measured dose response for a positive control ligand (prednisolone, Fig. S-1 of the Supplementary material). As assay sensitivity was highest at a final prednisolone concentration of 100 μM, bile acid derivatives were tested at 100 μM. In the present study, bile acid derivatives **1**, **2**, **4**–**23** and **25**–**31** were tested against GR-LBD, by mea-

suring fold fluorescence changes between cells exposed to test compounds compared with those treated with a negative control ligand, E2 (estradiol), or a positive control GR agonist (prednisolone). It can be seen in Fig. 6, that bile acid derivatives **1**, **25**, **28** and **31** displayed fold fluorescence changes indicative of moderate binding affinity for GR-LBD compared with prednisolone or estradiol.

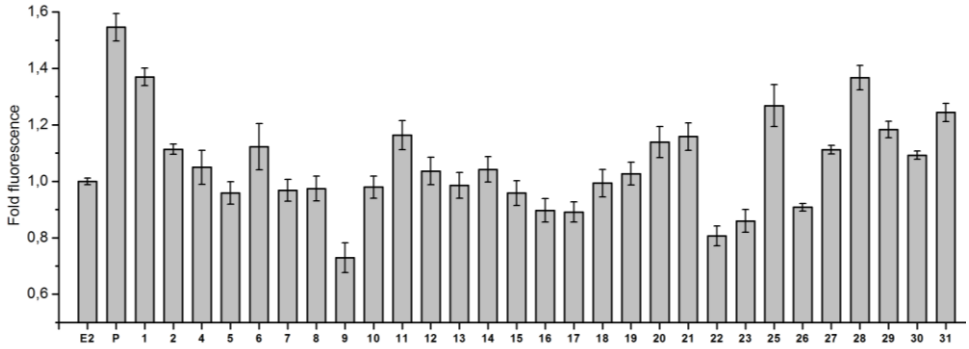


Fig. 6. Bile acid derivatives **1**, **25**, **28**, and **31** showed fold fluorescence changes consistent with moderate binding affinity for GR-LBD-YFP based on a fluorescent screen in yeast following 15 h exposure at a final concentration of 100 μM. Ligand binding affinity was calculated as the fold fluorescence change between cells exposed to test compounds compared with those treated with negative control ligand, E2 (estradiol), measured by fluorimetry. Prednisolone (P) was tested as a positive control GR agonist.

To validate these results, yeast cells treated with the same compounds were also visualized by fluorescence microscopy. As can be seen in Fig. 7, treatment of recombinant yeast expressing GR-LBD-YFP with the GR agonist prednisolone resulted in a strong increase and relocalization of fluorescence intensity, while treatment with compounds **1**, **25**, **28** and **31** resulted in a more moderate increase in overall fluorescence intensity compared to cells treated with negative control estradiol.

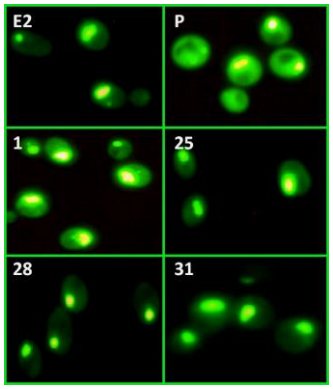


Fig. 7. Recombinant yeast cells expressing GR-LBD-YFP treated with 100 μM estradiol (E2, negative control GR ligand), prednisolone (P, positive control GR ligand) or test BA derivatives **1, 25**, **28** and **31** for 15 h, visualized using a fluorescence microscope. The intensity of cell fluorescence is proportional to GR ligand binding affinity.

Molecular docking

Based on fluorescent screening in yeast, compounds **1**, **25**, **28** and **31** have moderate binding affinity for GR-LBD compared with prednisolone, a strong GR agonist. To visualize these results in a molecular framework, binding poses and binding energies were predicted for compounds **1**, **25**, **28** and **31** by molecular docking in the program Autodock.[37] Because the structure of GR in complex with prednisolone has not been published, coordinates for GR-LBD in complex with another GR agonist, dexamethasone (PDB 1M2Z), were used as 'receptor' for docking.[26] For all docking simulations, the receptor was kept rigid and the ligand was allowed to rotate around flexible bonds. To validate the Autodock protocol for our system, control redocking simulations were conducted using dexamethasone as a positive control "ligand". Autodock correctly reproduced the X-ray structure with an *RMSD* of < 0.06 nm^2 and a strong binding energy of –57.20 kJ/mol. Based on the X-ray structure, dexamethasone (DEX) is in part held oriented in the GR ligand binding site via polar contacts with arginine 611, glutamine 570 and asparagine 564. As can be seen in Fig. 8, very similar binding poses and intermolecular contacts are formed by test compounds **28**, **25** and **31**.

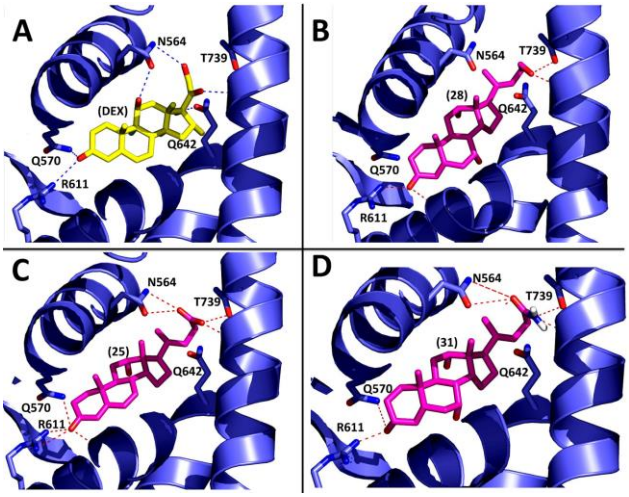


Fig. 8. Molecular docking poses and predicted binding affinities for test compounds **25**, **28** and **31** compared with dexamethasone (DEX), a positive control GR ligand. The X-ray structure of GR ligand binding domain in complex with dexamethasone (PDB 1M2Z) was used as receptor for Autodock simulations. Top ranking poses for the following compounds are shown: A) dexamethasone, B) **28**, C) **25** and D) **31**.

All three compounds bind in the same orientation, forming interactions with Asn564, Thr739 and Gln642 from the D-ring side, and Arg611 and Gln570 from the A-ring side of these BA derivatives. Consistent with experimental results, moderate binding energies of <−41.84 kJ/mol were predicted for each of these

test compounds: for compound **25**, −46.36 kJ/mol; compound **28** −45.94 kJ/mol, and compound **31** −42.16 kJ/mol. None of the test compounds had a predicted binding affinity greater than prednisolone, as expected. Interestingly, although compounds **1** displayed moderate affinity for GR-LBD in fluorescence experiments, docking of C-3 *Z*-isomer of **1** failed to predict binding to GR under the simulation conditions used. In general, Autodock is capable of correctly predicting the binding affinity and geometry for a set of compounds if ligand binding is not associated with significant backbone and side chain conformational changes.[37] Thus, binding by compound **1** likely induces conformational changes to the GR active site that are different from the GR conformation present in complex with dexamethasone, preventing Autodock from correctly estimating the binding pose and affinity for compound **1**. For compounds **25**, **28** and **31**, molecular docking results could be used in future studies as a starting point to design molecules with increased binding affinity, and/or specificity for GR-LBD.

CONCLUSION

Compounds used in the present work were designed to examine the influence of specific functionality modifications of BAs on their GR binding properties. Bile acids have a suitably functionalized steroid skeleton for modification in strategic regions for GR interactions. Our glucocorticoid receptor binding assay provided insight into structural features important for binding of the tested molecules to GR. The results of the SAR analysis are shown in Fig. 9.

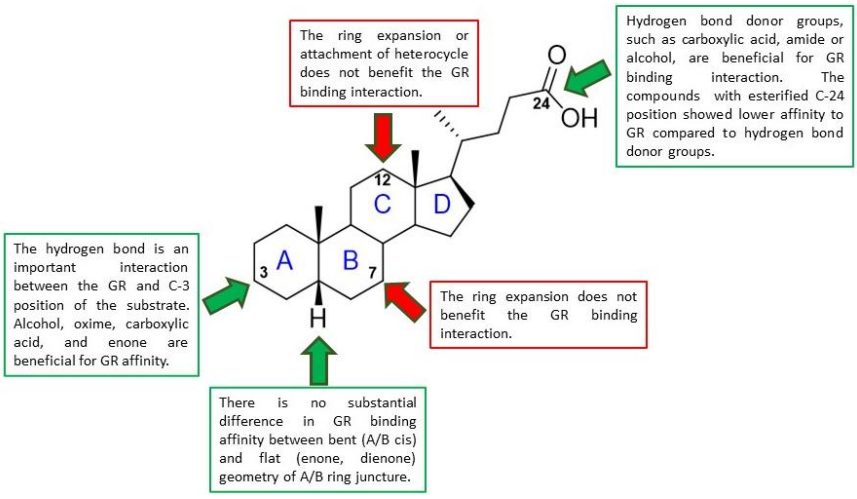


Fig. 9. Results of SAR analysis.

Four differently functionalized compounds **1**, **25**, **28** and **31** showed moderate binding affinity for GR-LBD compared with prednisolone or estradiol. The oxime geometric isomers in **1** showed the best relative binding affinity, not just among

tested oximes but among all tested compounds. Although we were not able to separate the geometric isomers in mixture **1** and molecular docking did not enable prediction of possible interactions with the enzyme, binding affinity could be attributed to the C-24 carboxylic group and oxime groups. The hydroxyl group at C-11 with β orientation (11β-OH) has been previously shown to be very important for GR-glucocorticoid interactions,[38] and the relative binding affinities measured for **1** suggest that the C-12 oxime group could be exploited as an alternative hydrogen bond donor to the 11β-OH group. Lactone, lactam, and tetrazole derivatives did not show any significant binding affinity. It seems that the B or C steroid ring expansion or attachment of heterocycle at the C ring is not beneficial for binding affinities. Compounds **28**, **25** and **26** have a keto or enone moiety, which is known to be involved in anchoring steroids in the receptor pocket.[39] However, it seems that the hydrogen bond donating group at C-24 is very important for binding to GR. This was observed for all of the tested compounds. Ethyl ester **26** has a much smaller GR binding affinity compared to free acid **25**. Compound **28** with the C-3 keto and C-24 hydroxyl group has a GR binding affinity similar to **1**, among the best of the tested compounds. There is a degree of plasticity in the GR-LBD for the steroid A-ring and C-3 functional groups, since compounds **1** with C-3 oxime *E* and *Z* groups; **29**, with carboxymethyl group at C-3, and even compound **30** with the corresponding amide at C-3 have moderate or less than moderate GR-LBD binding affinity. This work gives important insight into structural modification of bile acid steroid skeleton that could be used for development of new small molecule drug for treatment of inflammatory diseases.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12062, or from the corresponding author on request.

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И З В О Д

ИСПИТИВАЊЕ АФИНИТЕТА ДЕРИВАТА ЖУЧНИХ КИСЕЛИНА ЗА ВЕЗИВАЊЕ ЗА ЛИГАНД-ВЕЗУЈУЋИ ДОМЕН ГЛУКОКОРТИКОИДНОГ РЕЦЕПТОРА

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Антиинфламаторни лекови као што су глукокортикоиди били су неопходни током пандемије COVID-19 за лечење пацијената са умереним и тешким облицима COVID-19. Међутим, озбиљни нежељени ефекти ограничавају употребу ових лекова и хитно су пот-

ребни антиинфламаторни лекови са бољим фармаколошким својствима. Жучне киселине привлаче све више интереса, због својих антиинфламаторних и имуномодулаторних својстава, испољених засад нејасним механизмом који укључује трансмембранске и нуклеарне рецепторе. У овом раду смо испитали афинитет везивања низа деривата жучних киселина за лиганд-везујући домен глукокортикоидног рецептора (GR-LVD), најважнијег рецептора за антиинфламаторне процесе. Испитивана једињења укључују оксиме, лактоне, лактаме, тетразоле, диеноне, C-24 алкохоле и амид холне киселине. Међу тестираним једињењима: оксим холне киселине, диенон деоксихолне киселине, 3-кето-24-алкохол и амид холне киселине показали су најбоље афинитете везивања за GR-LVD. Објашњење везивања поменутих деривата помогли су *in silico* доковање. Анализа односа структуре и активности је показала да експанзија B и C стероидних прстенова или везивање хетероцикла за C прстен није од користи за везивање; бочни ланац треба да садржи водоник-донорску групу; и GR-LVD добро толеришу различите функционалности на позицији C-3. Ови резултати пружају вредне информације за прављење нових антиинфламаторних молекула базираних на жучним киселинама.

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