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Research Article

Regulation of 11β-hydroxysteroid dehydrogenase isoforms: pharmacophore search and molecular design of prospective 11β-HSD1 inhibitors

Mikhail V. Korokin[1], Svetlana K. Kotovskaya[2], Tatyana V. Avtina[1], Ivan S. Koklin[1], Ilya I. Butorin[2], Konstantin V. Savateev[2], Grigoriy V. Urakov[2], Sergey V. Androv[2], Eduard I. Taran[1], Alim A. Kochkarov[1], Liliya V. Korokina[1], Victor V. Fedotov[2], Timofey D. Moseev[2], Oleg S. Gudyrev[1], Natalia V. Koroleva[1], Viktor V. Vasyutkin[1], Polina I. Pykhtina[3]

1 Belgorod State National Research University; 85 Pobedy St., Belgorod 308015 Russia,
2 Ural Federal University named after the First President of Russia B.N. Yeltsin,19 Mira Ave., Ekaterinburg 620062 Russia,
3 Kursk State Medical University, 3 K. Marx St., Kursk 305040 Russia.

Corresponding author: Mikhail V. Korokin (mkorokin@mail.ru)

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Abstract

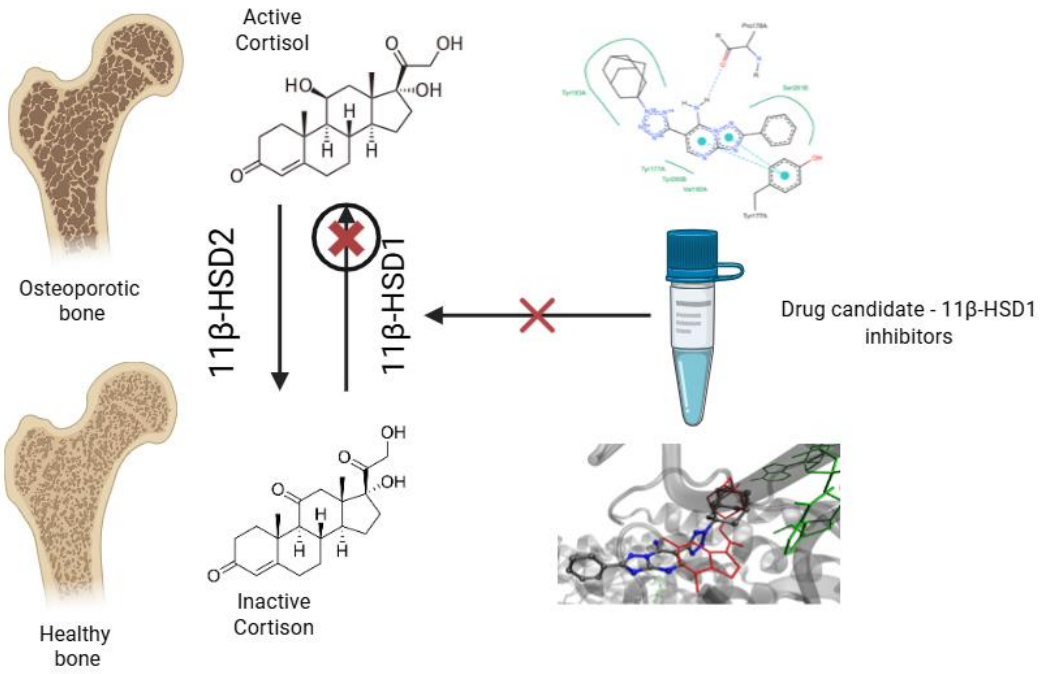
Introduction: Osteoporosis is an important medical and social public health problem in an aging or elderly society, the issue of pharmacological correction of which remains unresolved to this day.

Material and Methods: A design of prospective 11β-HSD1 inhibitors was performed on the basis of the literature data. A virtual screening of the designed compounds was performed using pharmacophore searching (DataWarrior, Flexophore), pharmacophore alignment (PhESA), and molecular docking (Jamda). 2D protein-ligand interaction maps were generated using PoseEdit, and 3D visualizations were produced in VMD. The cytotoxicity of the compounds was assessed in HEK-293 cells across a concentration range of 8–1024 μM, with IC50 values calculated in RStudio.

Results: A comprehensive virtual screening of prospective 11β-HSD1 inhibitors was performed, incorporating pharmacophore searching, PhESA alignment, and molecular docking against the 11β-HSD1 complex (PDB: 4YYZ). Several compounds (TS-897, TS-883, TS-945, and others) demonstrated Jamda Score values comparable to or exceeding that of the native ligand, indicating strong predicted binding affinity. Additional candidates were identified based on high pharmacophore similarity (>0.95). Among the 14 proposed compounds, four (IV-81, IV-91, IV-158) exhibited no cytotoxicity in HEK-293 cells (IC50 > 1024 μM). These findings support the potential of the synthesized azoloazine derivatives as novel 11β-HSD1 inhibitors for further in vitro and in vivo studies in osteoporosis therapy.

Conclusion: As a result of this work, original azoloazine derivatives have been developed for further biological testing aimed at regulating the activity of 11β-hydroxysteroid dehydrogenase isoforms (11β-HSDs) for pharmacological correction of bone remodeling and osteoreparation disorders.

Graphical Abstract



Keywords

osteoporosis, 11β-Hydroxysteroid Dehydrogenase, regulation, 11β-HSD inhibitors, drug targets azoloazines

Introduction

Bone is a highly dynamic and metabolically active tissue and represents one of the largest systems in the human body, performing functions such as mechanical support, muscle attachment, and storage of calcium and phosphorus (Henderson and Nair 2003; Zhou et al. 2023). Bone metabolism is a continuous process of renewal and repair within the skeletal system, driven by the coordinated interplay between bone resorption and bone formation. The dynamic equilibrium maintained between these processes constitutes bone homeostasis, which is essential for preserving bone mass, structural integrity, and normal skeletal function (Du et al. 2025). The Bone Health and Osteoporosis Foundation (BHOF), formerly the National Osteoporosis Foundation, first issued its Clinician's Guide in 1999, providing evidence-based recommendations for the prevention and treatment of osteoporosis. Since then, substantial progress has been made in diagnostic technologies and therapeutic approaches (LeBoff et al. 2022).

Despite these advances, the challenge of effective pharmacological correction of impaired bone remodeling and homeostasis remains highly relevant. The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) regulates glucocorticoid levels by converting cortisone (11-dehydrocorticosterone in rodents) into cortisol (corticosterone in rodents). Dysregulation of this enzyme or glucocorticoid excess contributes to various metabolic disorders, including abnormalities in bone remodeling (Wang et al. 1992). Previous research by an American group demonstrated that 11β-HSD1 serves as a target for a newly developed compound that suppresses osteogenesis and osteoclast functional activity (John et al. 2021).

In this study, the authors substantiate a scientific concept aimed at modulating the activity of 11β-HSD isoforms and restoring homeostasis within the 11β-HSD1/11β-HSD2 enzymatic system through the design and application of original 11β-HSD1 inhibitors as a novel strategy for pharmacological correction of impaired bone remodeling and osteoreparation (Koklin et al. 2025). To address this objective, pharmacophore searching, molecular design, synthesis of

promising 11β-HSD1 inhibitor candidates, and evaluation of their cellular toxicity were performed.

Materials and Methods

Virtual screening of the candidate compounds was performed using molecular docking tools and additional methods for assessing their affinity toward 11β-HSD types 1 and 2, which represent key therapeutic targets in osteoporosis. Preliminary pharmacophore searching enabled evaluation of the structural and interaction-profile similarity of the tested compounds to known bioactive agents. This approach is useful for the initial identification of the most probable molecular targets and for selecting likely effectors of a specific target (similarity index > 0.85). Molecular docking, in turn, provides a quantitative assessment of the affinity of the designed compounds for potential targets, depending solely on their structure and conformational properties.

Together, these complementary approaches enable independent yet mutually reinforcing selection of compounds for subsequent biological testing. Compounds predicted to be active by both methods are more likely to demonstrate biological activity in experimental assays.

Pharmacophore search

For the pharmacophore search, effectors of the target enzymes 11β-HSD types 1 and 2 were first extracted from the ChEMBL database using DataWarrior 6.0.1 (Sander et al. 2015). After preparation and curation of the initial dataset, including removal of duplicates and record standardization, as well as construction of a library of the test compounds, a search for pharmacophorically similar structures was performed using the 3D Flexophore descriptors (von Korff et al. 2008) within the same software environment.

Pharmacophore alignment

Pharmacophore alignment was performed using the PhESA tool (Wahl 2024) implemented in DataWarrior. For alignment scoring, a dataset of the investigated compounds was first prepared, and their three-dimensional conformations were generated using the MMFF94s+ force field with the self-organized algorithm. The native ligand structures used for alignment were provided in conformations corresponding to their experimentally observed poses in protein–ligand complexes. The alignment results are summarized in the corresponding table in the Results and Discussion section.

Molecular docking

The three-dimensional geometries of the investigated ligands (one conformer per ligand) were generated in DataWarrior 6.0.1 using the MMFF94s+ force field with the self-organized algorithm. The resulting set of ligand structures was saved in SDF format for subsequent analyses. In the selected protein–ligand complex, all non-protein molecules were removed except for the native ligand (inhibitor) and the cofactor. The influence of the NADP co-factor was taken into account when calculating ligand affinity and positioning within the binding site.

Docking was performed using the Jamda tool (Schellhammer and Rarey 2007; Henzler et al. 2014; Flachsenberg et al. 2020; Schöning-Stierand et al. 2022; Flachsenberg et al. 2023) against the selected protein–ligand complex under standard parameters. Validation was carried out by redocking the native ligand and evaluating the root-mean-square deviation between the corresponding computed position and the observed one. redocked and real position of the native ligand (see Table 3). Jamda Score values were used to characterize ligand affinity, with lower scores indicating stronger predicted binding (e.g., '–3' is better than '–1').

Two-dimensional maps of noncovalent interactions were generated using PoseEdit (Diedrich et al. 2023) from the proteins.plus platform, and three-dimensional visualizations of the docking results were prepared in VMD 1.9.2 (Humphrey et al. 1996).

Screening of cytotoxicity in cultured cell lines

Cytotoxicity studies were performed using human embryonic kidney cells (HEK-293, ATCC CRL-1573) (Graham et al. 1977), obtained from the Cell Culture Collection of Vertebrates (Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia). Cells were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum at 37 °C, 5% CO_2, and 98% humidity. Subculturing with 0.25% trypsin solution was performed once cultures reached ≥90% confluency.

Cell viability was assessed as follows. Compounds dissolved in DMSO were diluted with DMEM/F-12 medium containing 10% fetal bovine serum to obtain the target concentrations: 8, 16, 32, 64, 128, 256, 512, and 1024 μM. The final DMSO concentration did not exceed 1%.

Eleven compounds were provided for testing; however, only four achieved adequate solubility under the experimental conditions. Compounds KC2545, TS883, TS888, TS889, IV106, NTV51, and EAH-02 could not be evaluated due to insufficient solubility.

Cells were seeded in 96-well plates at a density of 4×10^3 cells per well. After 24 h, test compounds were added at the specified concentrations. Following a 72-h incubation, 20 µL of MTT solution (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; 5 mg/mL) was added to each well. After 2.5 h, the medium was removed and replaced with 200 µL of a 1:1 mixture of DMSO and isopropanol. Optical density was measured at 570 nm using a plate spectrophotometer.

The effects of the compounds on cell viability were assessed via the MTT assay, and IC_{50} values were calculated (Table 5; Figs 19 and 20).

Statistical analysis

Statistical analysis was performed in RStudio (Version 2023.09.1) using R (version 4.3.2). Dose–response curves and cytotoxicity indices (IC_{50}) were computed with the drc package (Ritz et al. 2015).

Results and Discussion

Pharmacophore search

The pharmacophore search was conducted to identify the most likely target for the designed compounds between 11β-HSD types 1 and 2, based on the degree of pharmacophore similarity between the tested molecules and the most active inhibitors of each enzyme. The search was performed using a pre-compiled dataset of 11β-HSD1 and 11β-HSD2 inhibitors (see Materials and Methods) in DataWarrior 6.0.1, employing the three-dimensional Flexophore pharmacophore fingerprints.

For most of the investigated compounds, highly active pharmacophore-similar inhibitors of 11β-HSD1 (similarity index > 0.85) were identified. In addition, several known inhibitors with analogous pyrazolopyrimidine scaffolds were found in the database (Fig. 1).

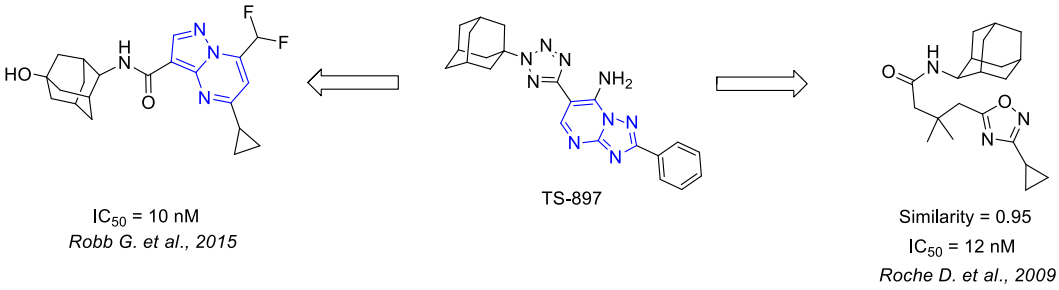


Figure 1. Example of pharmacophore-based search results showing the identification of a known inhibitor with a similar heterocyclic scaffold.

The structures of the compounds involved in the calculations are presented in Table 1. The complete results of the pharmacophore search are provided in Table 2.

Since most of the similar hits corresponded to inhibitors of 11β-HSD1 as pharmacophore analogs, this enzyme type was chosen as the primary target for subsequent calculations.

Pharmacophore alignment using PhESA

When performing molecular docking, an important criterion for selecting a complex of the chosen target with a known effector is the pharmacophore alignment score. This metric allows assessing the similarity between the pharmacophore profiles of the investigated compounds and the native ligand.

In this study, pharmacophore alignment was carried out using the PhESA tool within the DataWarrior software among several complexes of 11β-HSD1 with various known inhibitors – native ligands (Fig. 2).

The results showed that the best alignment was achieved with an azolopyrimidine-type inhibitor (PDB ID: 4yyz), which allows selecting this complex of 11β-HSD1 with the corresponding pyrazolopyrimidine-type native ligand as the most optimal for further molecular modeling.

Table 1. Structures of the compounds involved in the calculations

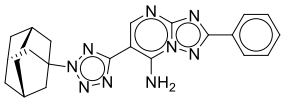
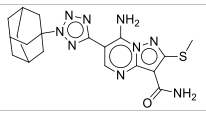
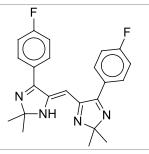
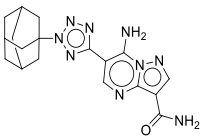
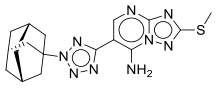
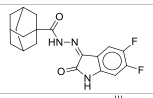
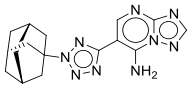
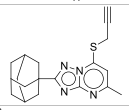
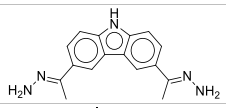
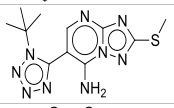
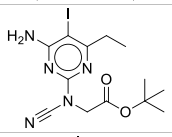
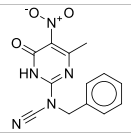
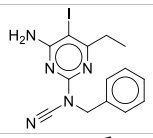
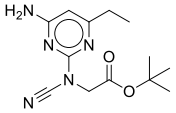
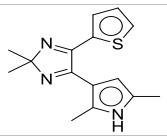
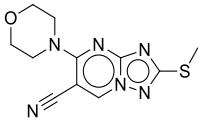
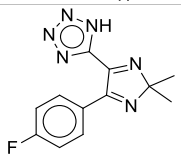
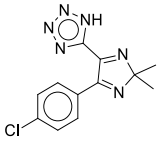
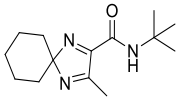
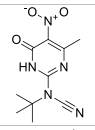
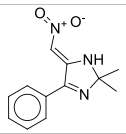
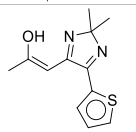
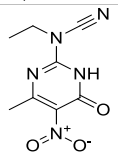
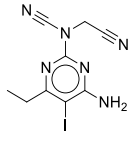
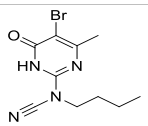
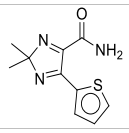
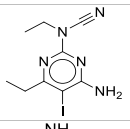
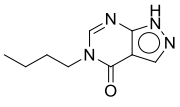
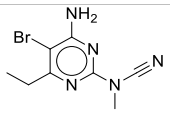
№	ID	Structures	№	ID	Structures
1	**TS-897**		2	**TS-889**	
3			4	**TS-945**	
5	**TS-888**		6	**TD-470**	
7	**TS-883**		8	**KC-2545**	
9	**NTV-51**		10		
11			12	**IV-91**	
13	**FV-1646**		14		
15			16		
17			18		
19			20	**IV-81**	
21			22	**EAH-O2**	
23	**IV-106**		24		
25	**IV-158**		26		
27			28		
29					

Table 2. Results of pharmacophore search among 11β-HSD type 1 inhibitors

№	Tested/Proposed compound	Pharmaco-phore similarity index	Known inhibitor	Activity parameter	Activity value	Unit of measur e-ment	Reference
1	Nc([n]1nc(-c2ccccc2)nc1nc1)c1-c1n[n](C2(C[C@H](C3)C4)C[C@H]4C[C@H]3C2)nn1	0.95	CC(C)(CC(NC1C2CC(C3)CC1CC3C2)=O)Cc1nc(C2CC2)n[o]1	IC50	12	nM	*Roche D. et al., 2009*
2	CSc(c(C(N)=O)c1nc2)n[n]1c(N)c2-c1n[n](C2(CC(C3)C4)CC4CC3C2)nn1	0.95	Cc(cccc1)c1C(Nc(cc1)cc2c1nc(SCC(NCc1ccccc1)=O)[s]2)=O	IC50	4580	nM	*Yang H. et al., 2008*
3	CC(C)(N/C1=C\C2=NC(C)(C)N=C2c(cc2)ccc2F)N=C/1c(cc1)ccc1F						
4	NC(c1c2ncc(-c3n[n](C4(CC(C5)C6)CC6CC5C4)nn3)c(N)[n]2nc1)=O	0.89	C(C1CC1)c1n[o]c(C(CC2)(CC3)CCC23c2nnc3[n]2CCCCCC3)n1	Inhibition	61	%	*Gu X. et al., 2005*
5	CSc(nc1nc2)n[n]1c(N)c2-c1n[n](C2(C[C@H](C3)C4)C[C@H]4C[C@H]3C2)nn1	0.86	C[n]1c(cccc2)c2c(CC2)c1CCN2C(c1n[n](cc(cn2)Br)c2c1)=O	IC50	10000	nM	
6	O=C(C1(CC(C2)C3)CC3CC2C1)NN=C(c(c(N1)c2)cc(F)c2F)C1=O	0.98	Cc(cccc1)c1C(N(CCC1)CC1c1nc(-c2ccccc2)n[o]1)=O	IC50	435	nM	*Xia G. et al., 2013*
7	Nc([n]1ncnc1nc1)c1-c1n[n](C2(C[C@H](C3)C4)C[C@H]4C[C@H]3C2)nn1	0.95	CC(C)(C)c(cc1)ccc1S(Nc(ccc(F)c1)c1N)(=O)=O	Activity	26	%	*Vuorinen A. et al., 2017*
8	Cc1nc2nc(C3(CC(C4)C5)CC5CC4C3)n[n]2c(SCC#C)c1	0.99	CCCSc([n](C)nc1)c1C(NC1CCCCC1)=O	IC50	18	nM	*Scott J.S. et al., 2012*
9	C/C(\c(cc1)cc2c1[nH]c(cc1)c2cc1/C(\C)=N/N)=N\N	0.85	CCN(C1CCCCC1)C(CN1CCc2n[o]cc2CC1)=O	Ki	15	nM	*Cheng H. et al., 2010*
10	CC(C)(C)[n]1nnnc1-c(cnc1n2)c(N)[n]1nc2SC						
11	CCc1nc(N(CC(OC(C)(C)C)=O)C#N)nc(N)c1I						
12	CC(N=C(NC1=O)N(Cc2ccccc2)C#N)=C1[N+]([O-])=O	0.95	CC(C)([C@H]1CC2)[C@@]2(C)C(N(C)N2Cc3cc(-c4c(C)cccc4)ccc3)=C1C2=O	IC50	15	nM	*Gillespie P. et al., 2014*
13	CCc1nc(N(Cc2ccccc2)C#N)nc(N)c1I						
14	CCc1nc(N(CC(OC(C)(C)C)=O)C#N)nc(N)c1						
15	CC1(C)N=C(c2ccc[s]2)C(c2c(C)[nH]c(C)c2)=N1						
16	CSc1n[n](cc(c(N2CCOCC2)n2)C#N)c2n1						
17	CC(C)(N=C1c2nnn[nH]2)N=C1c(cc1)ccc1F						
18	CC(C)(N=C1c2nnn[nH]2)N=C1c(cc1)ccc1Cl						
19	CC(C)(C)NC(C1=NC2(CCCCC2)N=C1C)=O						
20	CC(C)(C)N(C(NC1=O)=NC(C)=C1[N+]([O-])=O)C#N	0.96	CC(C)([C@H]1CC2)[C@@]2(C)C(N(C)N2c3ccc(C)cc3)=C1C2=O	IC50	89	nM	*Gillespie P. et al., 2014*
21	CC(C)(N/C1=C\[N+]([O-])=O)N=C/1c1ccccc1						
22	CC(C)(N=C1/C=C(/C)\O)N=C1c1ccc[s]1	0.93	OC(CC1(C2CC(C3)CC1CC3C2)c1ccccc1)=O	Ratio IC50	5		*Ye X.-Y. et al., 2011*
23	CCN(C(NC1=O)=NC(C)=C1[N+]([O-])=O)C#N	0.97	CC(C)([C@H]1CC2)[C@@]2(C)C(N(C)N2c3ccc(C)cc3)=C1C2=O	IC50	89	nM	*Gillespie P. et al., 2014*
24	CCc1nc(N(CC#N)C#N)nc(N)c1I						
25	CCCCN(C(NC1=O)=NC(C)=C1Br)C#N	0.96	O=C(c1nc(N2CCCCC2)ccc1)NC1CCCCC1	IC50	58	nM	*Ryu J.H. et al., 2015*
26	CC1(C)N=C(c2ccc[s]2)C(C(N)=O)=N1						
27	CCc1nc(N(CC)C#N)nc(N)c1I						
28	CCCCN(C=Nc1c2cn[nH]1)C2=O						
29	CCc1nc(N(C)C#N)nc(N)c1Br						

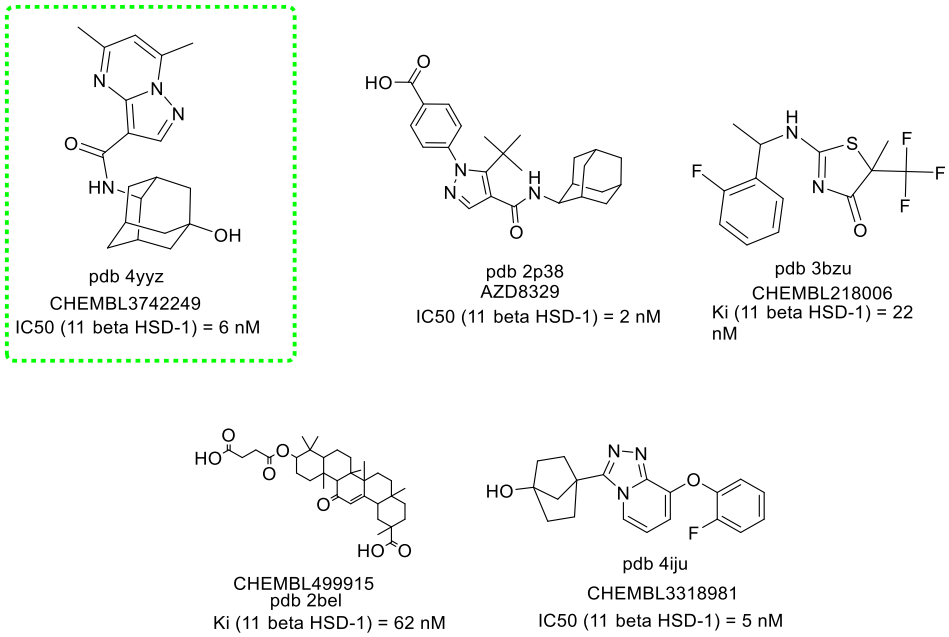


Figure 2. Native ligands of the studied complexes.

Molecular docking and compound selection criteria

Molecular docking of the studied compounds was performed using the Jamda service with the selected protein–ligand complex 4YYZ. The docking results, along with earlier screening data, are shown in Table 3.

Table 3. Results of docking, pharmacophore search, and PhESA alignment

Compounds ID	№	Jamda Score	Alignment PheSA	Highest pharmacophore similarity index
CHEMBL3742249	Native ligand	-2.58 (0.53 Å)*	-	-
TS-897	1	-2.96	0.46	0.95
TS-889	2	-2.28	0.48	0.95
	3	-1.48	0.41	
TS-945	4	-2.53	0.51	0.89
TS-888	5	-2.53	0.49	0.86
TD-470	6	-2.22	0.56	0.98
TS-883	7	-2.44	0.54	0.95
KC-2545	8	-2.25	0.55	0.99
NTV-51	9	-2.35	0.51	0.85
	10	-1.66	0.40	
	11	-1.16	0.48	
IV-91	12	-2.07	0.44	0.95
FV-1646	13	-2.28	0.53	
	14	-1.21	0.49	
	15	-1.82	0.39	
	16	-2.03	0.41	
	17	-1.89	0.39	
	18	-1.84	0.40	
	19	-1.89	0.47	
IV-81	20	-1.75	0.47	0.96
	21	-1.73	0.34	
EAH-O2	22	-1.72	0.32	0.93
IV-106	23	-1.59	0.42	0.97
	24	-1.76	0.37	
IV-158	25	-1.98	0.42	0.96
	26	-1.70	0.45	
	27	-1.74	0.41	
	28	-1.70	0.55	
	29	-1.59	0.36	

Note: * – RMSD Value for the native ligand; objects with a Jamda score < -2 are highlighted in color.

As a result of docking, for several compounds the calculated affinity (based on the Jamda score) is comparable to that of the native ligand. In one case, the proposed hit compound shows higher affinity than the native ligand according to the docking data (Fig. 3).

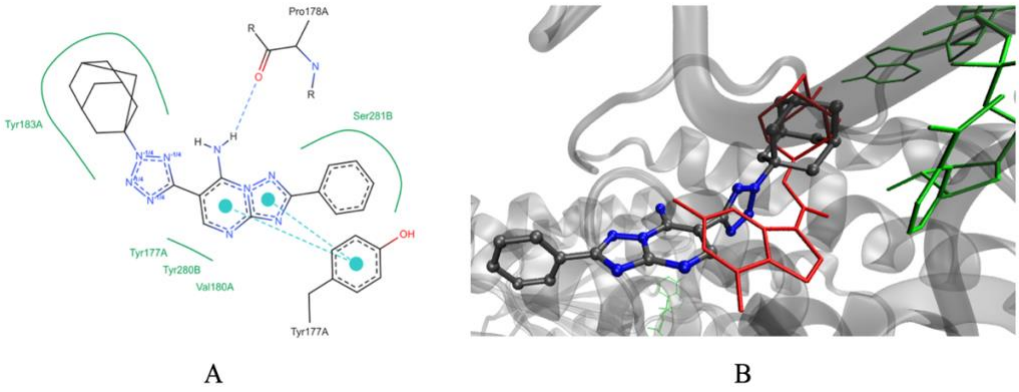


Figure 3. Docking results for lead compound 1 (TS-897): map of non-covalent interactions (**A**) and binding position within the protein (gray, semi-transparent) from docking data relative to the native ligand CHEMBL3742249 (red) and cofactor (green) (**B**).

The calculated affinity score (Jamda score) was used as the main criterion for selecting compounds for biological testing (Jamda score < -2). In addition, several compounds with lower affinity scores were also selected based on their high pharmacophore similarity to known 11β-HSD1 inhibitors (Table 3).

Thus, a total of 14 new compounds were proposed: KC-2545, TS-883, TS-888, TS-889, TS-897, TS-945, IV-81, IV-91, IV-106, IV-158, FV-1646, NTV-51, EAH-02, and TD-470.

Results of the toxicity screening study of the developed compounds on cell lines are presented in Table 4 and Figure 4.

Table 4 shows that compounds IV-91, IV-81, IV-158 do not exhibit toxic effects on human embryonic kidney cells. Compound IV-81 do not reduce the viability of the HEK-293 culture even at maximum concentrations (Fig. 4), while IV-91 and IV-158 cause only a slight decrease in the number of living cells (IC_{50} > 1024 μM) (Fig. 4).

Table 4. Cytotoxicity index (IC_{50} ± SE) of the studied compounds on human embryonic kidney cells (HEK-293), μM.

№	Compound	Cell line HEK-293
1	**IV-91**	> 1024
2	**IV-81**	> 1024
3	**IV-158**	> 1024

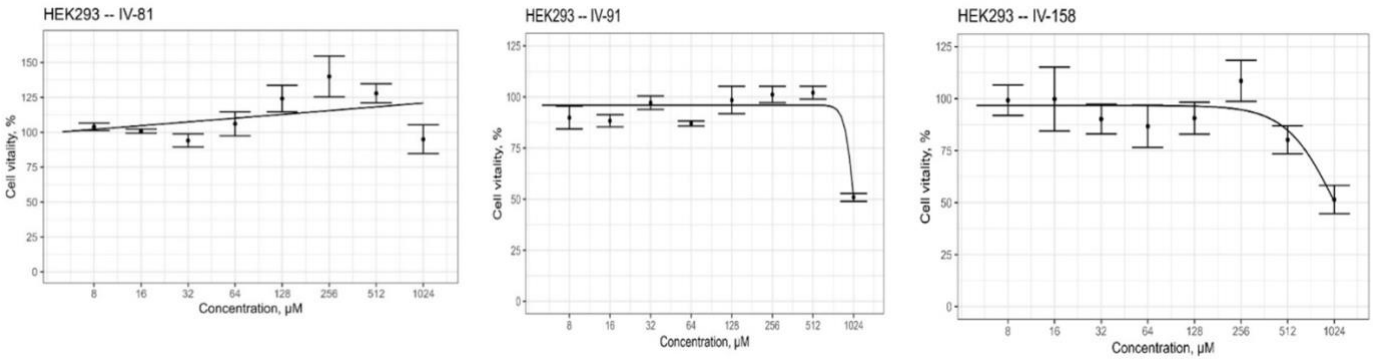


Figure 4. Dose-response curves for compounds IV-91, IV-81 and IV-158.

Conclusion

The regulation of 11β-HSD enzymes sheds light on the emerging biology of intracrine control of steroid hormones. Modulating this homeostasis opens new therapeutic opportunities for treating various diseases associated with steroid hormone metabolism disorders. A pharmacotherapeutic approach aimed at restoring the balance of activity between 11β-hydroxysteroid dehydrogenase types 1 and 2 enzymes can be considered one of the most relevant strategies for preventing and correcting the pathogenetic mechanisms underlying osteoporosis development. Both 11β-HSD1 and 11β-HSD2 enzymes are well-justified pharmacotherapeutic targets for the development of new drugs to treat osteoporosis and bone remodeling disorders.
In this study, the authors developed a series of compounds acting as inhibitors of 11β-HSD1. Virtual screening of the proposed compounds' activity was performed using pharmacophore-based search tools, molecular docking, and affinity evaluation against known 11β-HSD type 1 and 2 inhibitors, which are the main targets in osteoporosis therapy.

Preliminary pharmacophore searches allowed assessment of the compounds' similarity to known biologically active agents in terms of their possible non-covalent interaction profiles. Molecular docking results demonstrated that for several compounds, the calculated affinity (based on the Jamda score) was comparable to that of the native ligand.

Synthetic schemes were developed to obtain adamantane-containing azolopyrimidines, pyrimidocyanamide derivatives, and their salt (water-soluble) forms, as well as isatin, carbazole, and imidazole derivatives, as potential inhibitors of 11β-hydroxysteroid dehydrogenase type 1. The structure and purity of the obtained samples were confirmed by a range of modern physicochemical analytical methods.

A representative series of compounds was prepared for biological testing in both *in vitro* and *in vivo* experiments. The effect of several synthesized compounds on the viability of cultured human cells was studied using the MTT assay. Cytotoxic properties of selected compounds were evaluated on human embryonic kidney cells (HEK-293, ATCC CRL 1573). It was shown that compounds IV-91, IV-81, IV-158 exhibit no toxic effects on HEK-293 cells (IC_{50} > 1024 μM).
As a result of this work, original azoheterocycles derivatives have been developed for further biological testing aimed at regulating the activity of 11β-hydroxysteroid dehydrogenase isoforms (11β-HSDs) for pharmacological correction of bone remodeling and osteoreparation disorders.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Author Contributors

- **Mikhail V. Korokin**, Doct. Sci. (Medicine), Professor at the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: mkorokin@mail.ru; **ORCID ID:** https://orcid.org/0000-0001-5402-0697. Developing a research design, processing the results obtained, and writing the article.
- **Svetlana K. Kotovskaya**, Cand. Sci. (Chemistry), Senior Researcher of the Laboratory for the Synthesis of Biologically Active Compounds, Head of the Laboratory Pharmaceutical Substances Standardization of Innovative Centre for Chemical & Pharmaceutical Technologies, Ural Federal University Named after the First President of Russia B.N. Yeltsin, Ekaterinburg, Russia; e-mail: s.k.kotovskaya@urfu.ru; **ORCID ID:** https://orcid.org/0000-0002-3213-9838. Design of the chemical compounds and editing the text of the article.
- **Tatyana V. Avtina**, PhD in Pharmaceutical Sciences, Associate Professor of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: tatyanavtina@yandex.ru; **ORCID ID:** https://orcid.org/0000-0003-0509-5996. Developing a research design, processing the results obtained, and writing the article.
- **Ivan S. Koklin**, Cand. Sci. (Medicine), Researcher at the Research Institute of Pharmacology of Living Systems, Belgorod State National Research University, Belgorod, Russia; e-mail: ikoklin@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-1560-4195. Developing a research design, processing the results obtained, and writing the article.
- **Ilya I. Butorin**, Engineer at the Department of Organic and Biomolecular Chemistry, Ural Federal University Named after the First President of Russia B.N. Yeltsin, Ekaterinburg, Russia; e-mail: iibutorin@urfu.ru; **ORCID ID:** https://orcid.org/0000-0003-3403-5079. Investigation and data curation.
- **Konstantin V. Savateev**, Cand. Sci. (Chemistry), Associate professor of Innovative Centre for Chemical & Pharmaceutical Technologies, Ural Federal University Named after the First President of Russia B.N. Yeltsin, Ekaterinburg, Russia; e-mail: i-krafttt@yandex.ru; **ORCID ID:** https://orcid.org/0000-0001-6040-5155. Design of the chemical compounds.
- **Grigoriy V. Urakov**, postgraduate student, Research engineer at the Educational and Innovative Center of Chemical and Pharmaceutical Technologies, Chemical Technology Institute, Ural Federal University named after the first President of Russia B.N.Yeltsin, Yekaterinburg, Russia; e-mail: urakov01@bk.ru; **ORCID ID:** https://orcid.org/0009-0004-6877-7411.Synthesis of the chemical compounds
- **Sergey V. Androv**, master student, Research assistant at the Educational and Innovative Center of Chemical and Pharmaceutical Technologies, Chemical Technology Institute, Ural Federal University named after the first President of Russia B.N.Yeltsin, Yekaterinburg, Russia; e-mail: sergioandrov@gmail.com; **ORCID ID:** https://orcid.org/0009-0002-3713-8372. Synthesis of the chemical compounds
- **Eduard I. Taran**, Researcher at the Research Institute of Pharmacology of Living Systems, Belgorod State National Research University, Belgorod, Russia; e-mail: mdtaraneduard@gmail.com; **ORCID ID:** https://orcid.org/0000-0001-7880-6686. Developing a research design, processing the results obtained, and writing the article.
- **Alim A. Kochkarov**, Researcher at the Research Institute of Pharmacology of Living Systems, Belgorod State National Research University, Belgorod, Russia; e-mail: kochkarova@bsu.edu.ru; **ORCID ID:** https://orcid.org/0000-0003-4972-7262. Developing a research design, processing the results obtained, and writing the article.
- **Liliya V. Korokina**, Doct. Sci. (Medicine), Professor at the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: korokina@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-4115-1564. Developing a research design, processing the results obtained, and writing the article.

- **Victor V. Fedotov**, Cand. Sci. (Chemistry), Associate Professor of Innovative Centre for Chemical & Pharmaceutical Technologies, Ural Federal University Named after the First President of Russia B.N. Yeltsin, Ekaterinburg, Russia; e-mail: vicww4@gmail.com; **ORCID ID:** https://orcid.org/0000-0002-4558-6848. Developing a research design, synthesis.
- **Timofey D. Moseev**, Cand. Sci. (Chemistry), Assistant Professor at the Department of Organic and Biomolecular Chemistry, Ural Federal University Named after the First President of Russia B.N. Yeltsin, Ekaterinburg, Russia; e-mail: timofey.moseev@urfu.ru; **ORCID ID:** https://orcid.org/0000-0003-3341-5838. Investigation and methodology.
- **Oleg S. Gudyrev**, Cand. Sci. (Medicine), Associate Professor at the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: gudyrev@mail.ru; **ORCID ID:** https://orcid.org/0000-0003-0097-000X. Developing a research design, processing the results obtained, and writing the article.
- **Natalia V. Koroleva**, Research Associate of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: nkoroleva@gmail.com; **ORCID ID:** https://orcid.org/0009-0006-3660-3438. Investigation and methodology.
- **Viktor V. Vasyutkin**, Research Associate of the Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia; e-mail: vivasyutkin@yandex.ru. Investigation and methodology.
- **Polina I. Pykhtina**, 6 year student, Kursk State Medical University, Kursk, Russia; e-mail: sunpolli46@mail.ru; **ORCID ID:** https://orcid.org/0009-0003-7841-1037. Investigation and methodology.