chemistry proceedings

MDPI

Proceeding Paper

Synephrine Analogues as Glucocorticoid Receptor Agonists †

Eugenia Oleynik [1,*], Ekaterina Mikhina [1], Diana Grigoreva [2], Varvara Maksimova [2], Lyubov Grebenkina [1], Andrey Matveev [1], Ekaterina Zhidkova [2], Ekaterina Lesovaya [2], Marianna Yakubovskaya [2]

[1] Department of Biotechnology and Industrial Pharmacy, Lomonosov Institute of Fine Chemical Technologies, MIREA-Russian Technological University; email1@email.com (E.M.); email2@email.com (L.G.); email3@email.com (A.M.)

[2] Federal State Budgetary Institution "N.N. Blokhin National Medical Research Center of Oncology" of the Ministry of Health of the Russian Federation; email4@email.com (D.G.); email5@email.com (V.M.); email6@email.com (E.Z.); email7@email.com (E.L.); email8@email.com (M.Y.)

* Correspondence: oleynik_evgenia@mail.ru

† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: The work carried out the synthesis of a number of new synephrine analogues by universal method. Some of synthetized compounds showed cytotoxicity on myeloid leukemia cells K562 and lymphoma cell line Granta-519. Molecular docking using glucocorticoid receptor (GR) model (PDB identifier 1P93) was performed in order to understand possible under-lying mechanism of compounds action. The simulation showed similarity of synephrine analogues' binding to the binding of dexamethasone in the GR ligand-binding domain. The synthesized analogues exhibited cytotoxicity profiles similar to those of dexamethasone.

Keywords: glucocorticoid receptor; synephrine analogues; chronic myelogenous leukemia; mantle cell lymphoma

Citation: Oleynik, E.; Mikhina, E.; Grigoreva, D.; Maksimova, V.; Grebenkina, L.; Matveev, A.; Zhidkova, E.; Lesovaya, E.; Yakubovskaya, M. Synephrine Analogues as Glucocorticoid Receptor Agonists. **2023**, *14*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2023

1. Introduction

Glucocorticoids (GC) are steroid hormones regulating many cellular and physiological functions and being best known for their anti-inflammatory properties. Recently, more evidence has emerged that glucocorticoid regulation of inflammation affects oncogenesis [1,2]. Steroid GC are being used as immunomodulators during application of main antitumor therapy methods, including chemo- and radiation therapy [3,4]. In addition, GC are widely used in therapy of leukemia and lymphoma as cytostatic drugs [5]. GC implement their biological effect via activation of glucocorticoid receptor which leads to suppression of tumor cells' growth and proliferation, inducing their apoptosis [6–8].

Synthetic GC dexamethasone (Dex) is glucocorticoid receptors' agonist commonly used as a direct chemotherapy agent in case of certain malignant neoplasms' types, however, it has a number of shortcomings. The effect of Dex outside the zone of therapeutic interests may cause wide range of complications, including systemic toxicity, local allergic reactions, changes in heart function etc., which makes the search and development of different less toxic agents especially important [9,10].

Nowadays it is known that the class of non-steroidal Dex analogues shows similar biological effects while being less overall toxic [11,12]. The design of such compounds may consist of replacing Dex sterane backbone with less rigid hydrocarbon skeleton, predominantly preserving spatial arrangement of original functional groups. We have assumed that such analogues could be obtained on the basis of synephrine molecule, which mimics the Dex backbone (Figure 1).

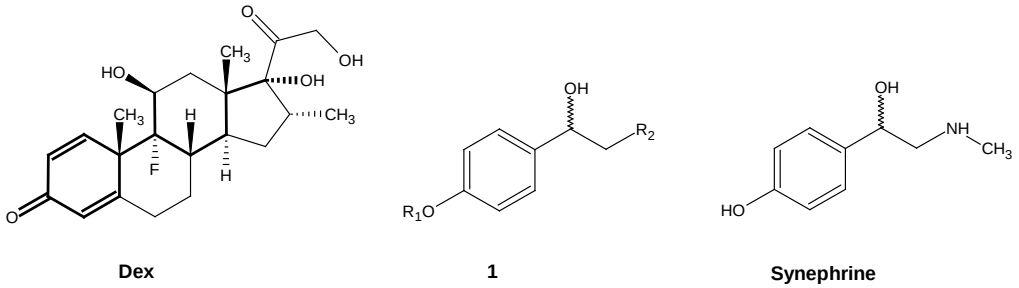

Figure 1. Structures of Dex, synephrine and its synthetic analogues **1**.

These suggestions were examined by molecular docking approach using the glucocorticoid receptor model. Simulation held has shown selective binding of synephrine analogues in the hydrophobic pocket of GR binding domain.

2. Results and Discussion

2.1. Chemistry Section

There are different synthetic approaches to obtaining synephrine analogues [13,14]. We have applied, in our opinion, the most expedient one which consists in the synthesis of intermediate epoxide and its following interaction with aliphatic primary and secondary amines (Figure 2).

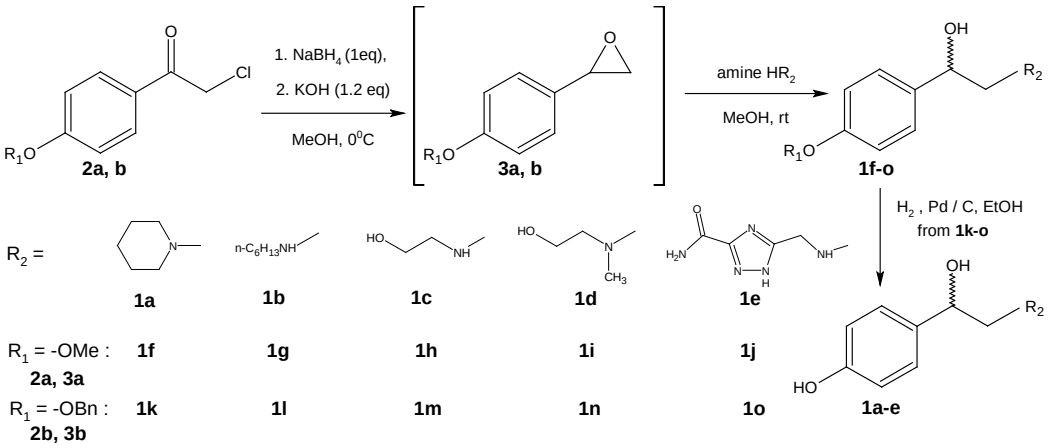

Figure 2. Synthesis of synephrine derivatives **1a-o.**

The starting compounds **2** were obtained according to known methods [15,16]. 4-Alcoxychloroacetophenones **2** was reduced with the excess of $NaBH_4$ in methanol followed by the treatment with *KOH* and addition of fivefold excess of amine without isolation of intermediate products. Target compounds **1f–o** were isolated by column chromatography on silica gel after volatile components' evaporation followed by excess amine extraction. The compounds **1a–e** were obtained by hydrogenation of **1k–o** on palladium on carbon (Figure 2).

For the initial study of biological properties compounds were synthesized as a mix of enantiomers. Further investigation suggests synthesis of individual enantiomers only in case of active compounds detection. Individual enantiomers can be obtained by stereoselective reduction of corresponding ketones **2** [17].

2.2. In Silico Studies

Molecular docking has shown a number of significant non-covalent interactions demonstrated by studied compounds in GR ligand-binding domain. Thus, π-alkyl interactions with Met604, Leu608 were identified, which are also characteristic of steroid

ligands. In addition, important hydrogen interactions which are characteristic of Dex were also found, including interactions with Gln642 и Thr739 (Figure 3). Dex location determined in the experiment coincides with literature data reported by other authors [12].

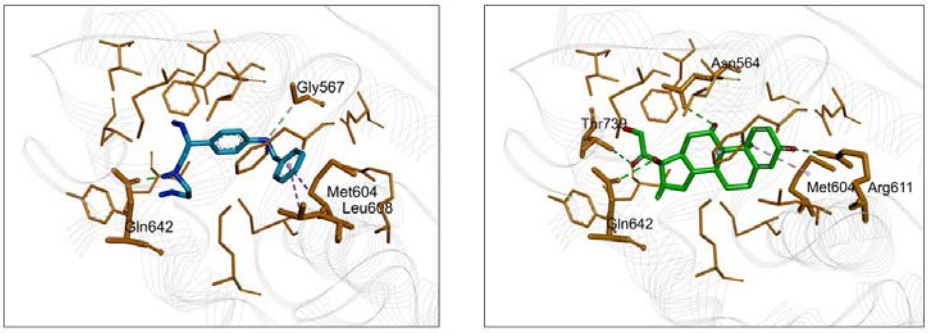

Figure 3. Structure of the GR active site with analogue of synephrine **1m** in comparison with Dex (shown in blue and green, respectively).

2.3. Biology Section

In vitro experiments were held to estimate cytotoxicity of new compounds on model of the hematopoietic system malignant neoplasms. Cells proliferation was evaluated using myeloid leukemia cells K562 and lymphoma cell line Granta-519.

Table 1. The proportion of live cells compared to the vehicle control.

Cell Line	Time, h	Compound No.				
		1a (C = 50μM)	**1g** (C = 50μM)	**1k** (C = 100μM)	**1m** (C = 100μM)	**Dex** (C = 25μM)
K562	24	0.65 ± 0.08	0.80 ± 0.08	0.75 ± 0.06	0.53 ± 0.05	0.80 ± 0.05
	48	0.52 ± 0.06	0.84 ± 0.07	0.60 ± 0.05	0.28 ± 0.09	0.65 ± 0.04
	72	0.37 ± 0.12	0.53 ± 0.08	0.20 ± 0.05	0.18 ± 0.07	0.44 ± 0.10
Granta-519	24	0.61 ± 0.11	0.93 ± 0.09	0.95 ± 0.04	0.95 ± 0.05	0.90 ± 0.04
	48	0.54 ± 0.08	0.73 ± 0.07	0.48 ± 0.08	0.64 ± 0.08	0.53 ± 0.05
	72	0.84 ± 0.09	1.00–0.07	0.70 ± 0.12	0.85 ± 0.06	0.64 ± 0.07

Dex showed cytostatic on 48 and 72 depending on cell line. Synthesized compounds demonstrated effect similar to that of Dex, which suggests that the mechanism of their action is similar to the mechanism of Dex. In case of the cell line **K**562, studied compounds demonstrated inhibitory effect on cell proliferation in 48 h (**1k–m**) and 72 h (**1g**), which is completely similar to the action of reference drug. For the Granta-519 cell line, such pattern could also be traced for **1a**,**g**,**k**,**m**, but this cells did not maintain a long-term effect, unlike Dex.

3. Conclusions

New synephrine analogues modified at the phenolic hydroxyl were synthesized. They showed a comparable to Dex effect on the myeloid leukemia cell line K562 and lymphoma cell line Granta-519. The in silico modeling data is suggested that these analogues may compete with Dex to bind to the GR active site. These results encourage further investigation of synephrine analogues as GR agonists.

4. Materials and Methods

4.1. Materials

All the chemicals were obtained from commercial sources (Merck KGaA, Darmstadt, Germany) and were used without further purification. Deuterated solvents were

purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Silica gel 60 (Merck KGaA, Darmstadt, Germany) was used for column chromatography. Analytical TLC was performed on Silufol UV-254 plates.

^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra recorded at 75 MHz on Bruker DPX-300 spectrometer. High-resolution mass spectra (HRMS) were recorded on the Agilent 6224 using electron spray ionization (ESI). HPLC measurements were carried out on the Agilent 1200 Series.

4.2. Synthesis

Synephrine analogues 1f–o (general synthesis procedure)

To a solution of 4-alcoxychloroacetophenone **2** (1.5 mmol) in methanol was added 1 eq $NaBH_4$ by portions while cooling on the ice bath. After compound **2** consumption (controlled by TLC, system petroleum ether/diethyl ether 1:1) KOH (1.2 eq) was added by portions. Subsequently, solution of amine (5 eq) in methanol (1:1 by the volume) was added. Reaction was TLC controlled by conversion of **3** (R_f = 0.65–0.75 system petroleum ether/diethyl ether 1:1) in the reaction mixture. Then the mixture was acidified with an diluted aqueous HCl up to pH = 3, the solvent was removed using a vacuum rotary evaporator, after which the residue was dissolved in 5 mL of water and extracted with methylene chloride (2 times, 10 mL). The organic layer was dried with anhydrous calcium chloride followed by filtering off the drying agent and evaporation. Product was isolated by column chromatography on silica gel using chloroform-methanol eluent.

2-(hexylamino)-1-(4-methoxyphenyl)ethanol (**1g**)

200 mg (54%) of **1g** were obtained. R_f (10% methanol in chloroform) = 0.44. Purity by HPLC ≥ 98%. ^{1}H NMR ($CDCl_3$) δ, ppm: 7.35–7.26 (m, 2H, 2×CH); 6.94–6.78 (m, 2H, 2×CH); 5.01–4.71 (m, 1H, CH); 3.90–3.80 (m, 2H, NH and OH); 3.76 (s, 3H, O-CH_3); 3.07–2.60 (m, 4H, 2×CH_2); 1.70–1.47 (m, 2H, CH_2); 1.40–1.17 (m, 6H, 3×CH_2); 0.95–0.80 (m, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ, ppm: 159.13; 133.70; 127.06; 113.80; 70.24; 56.30; 55.24; 49.11; 31.47; 28.39; 26.67; 22.51;13.99. HRMS: for $C_{15}H_{26}NO_2$ $[M+H]^+$ calculated: 252.1963; found: 252.1967.

1-[4-(benzyloxy)phenyl]-2-piperidin-1-ylethanol (**1k**)

360 mg (77%) of **1k** were obtained. R_f (5% methanol in chloroform) = 0.46. Purity by HPLC ≥ 97% ^{1}H NMR ($CDCl_3$) δ, ppm: 7.48–7.20 (m, 7H, 7×CH); 7.00–6.87 (m, 2H, 2×CH); 5.04 (s, 2H, CH_2-O); 4.95–4.82 (m, 1H, C<u>H</u>-OH); 2.98–2.38 (m, 6H, 3×CH_2); 1.87–1.37 (m, 6H, 3×CH_2). ^{13}C NMR ($CDCl_3$) δ, ppm: 158.31; 136.89; 133.81; 128.54; 127.93; 127.43; 127.13; 114.77; 69.96; 67.97; 66.56; 54.62; 24.93; 23.42. HRMS: for $C_{20}H_{26}NO_2$ $[M+H]^+$ calculated: 312.1963; found: 312.1970.

1-[4-(benzyloxy)phenyl]-2-[(2-hydroxyethyl)amino]ethanol (**1m**)

95 mg (22%) of **1m** were obtained. R_f (20% methanol in chloroform) = 0.33. Purity by HPLC ≥ 98% ^{1}H NMR (DMSO-d_6) δ, ppm: 7.50–7.25 (m, 7H, 7×CH); 7.08–6.95 (m, 2H, 2×CH); 5.09 (s, 2H, CH_2-O); 5.27 (br.s, 1H, C<u>H</u>-OH); 4.89 (br.s, 1H, NH); 4.13–3.95 (m, 1H, CH); 3.74–3.45 (m, 4H, 2×CH_2); 2.85–2.52 (m, 2H, CH_2). ^{13}C NMR (DMSO-d6) δ, ppm: 158.29; 136.96; 129.43; 128.38; 127.78; 127.60; 122.08; 114.74; 69.18; 63.46; 63.00; 57.65; 48.01. HRMS: for $C_{17}H_{22}NO_3$ $[M+H]^+$ calculated: 288.1599**;** found: 288.1604.

Synephrine analogues 1 a-e (general synthesis procedure)

To 0.5 mmol of **1k–o** dissolved in 5 mL of EtOH, an 10% mass excess of Pd on C was added. The suspension was mixed at room temperature in a hydrogen atmosphere. After compound **1k–o** consumption (controlled by TLC, system 5% methanol in chloroform) the reaction mixture was filtered through a layer of celite and evaporated.

4-(1-hydroxy-2-piperidin-1-ylethyl)phenol (**1a**)

91 mg (82%) of **1a** were obtained. R_f (25% methanol in chloroform) = 0.30. Purity by HPLC ≥ 98% ^{1}H NMR (DMSO-d_6) δ, ppm: 7.19–7.05 (m, 2H, 2×CH); 6.73–6.63 (m, 2H, 2×CH); 4.60–4.52 (m, 1H, C<u>H</u>-O*H*); 2.46–2.22 (m, 6H, 3×CH_2); 1.57–1.30 (m, 6H, 3×CH_2). ^{13}C NMR

(DMSO-d_6) δ, ppm: 158.19; 134.74; 127.07; 114.64; 69.08; 67.11; 54.32; 25.52; 23.94. HRMS: for $C_{17}H_{28}NO_2$ $[M+H]^+$ calculated: 222.1494; found: 222.1501.

4.3. In Silico Studies

3D-geometry of compounds was optimized using MM2 force field in Chem3D software (Perkin Elmer Informatics, Inc., Waltham, MA, USA).

The crystal structure of GR was obtained from the Protein Data Bank (PDB ID: 1P93). The selected structure of the complex has a resolution of 2.7 Å and does not contain gaps in the main protein chain near the ligand-binding domain. Removal of solvent molecules, adding hydrogen atoms, assigning atom types and combining non-polar hydrogen atoms, calculating partial Gasteiger charges, Kollmann charges were done using AutoDockTools 1.5.7 software (The Scripps Research Institute, La Jolla, CA, USA).

During the docking process, all the torsion bonds of the ligands were free to rotate, while the protein remained rigid. A 40 × 40 × 40 grid was created with 1 Å spacing centered on the GR active site. Docking calculations were performed in Autodock Vina software (The Scripps Research Institute, La Jolla, CA, USA) using the Lamarckian genetic algorithm (LGA). Visualization and graphical representation of the results of ligand interaction were done in Discovery Studio Visualizer software (version 21.1.0.20298, Dassault Systems Biovia Corp., San-Diego, CA, USA).

4.4. Biology

Cell viability (cell proliferation). Cells were cultured in 24-well plates (50,000 cells/well) and treated with solvent (DMSO), dexamethasone (25 uM), 1a (50 μM), 1g (50 μM), 1k (100 μM), 1m (100 μM) for 24, 48 or 72 h for observe cytostatic action. After the incubation cells were mixed 1:1 with 0.4% trypan blue in PBS solution. Viable cells were immediately counted using TC20 automatic cell counter ("Bio-Rad", USA).

Author Contributions: Conceptualization, A.M.; E.L. and M.Y.; methodology and data curation, A.M.; E.Z. and L.G.; investigation, E.O.; E.M.; D.G.; V.M.; writing—original draft preparation, A.M.; E.O.; writing—review and editing, E.Z.; L.G.; E.L. and M.Y.; visualization, E.O.; funding acquisition, E.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian science foundation grant, grant number 23-15-00321.

Acknowledgments: This work was performed using the equipment of the Shared Science and Training Center for Collective Use RTU MIREA and supported by the Ministry of Science and Higher Education of the Russian Federation.

Conflicts of Interest:

References

1. Khadka, S.; Druffner, S.R.; Duncan, B.C.; Busada, J.T. Glucocorticoid regulation of cancer development and progression. *Front. Endocrinol.* **2023**, *14*, 1161768.
2. Lin, K.T.; Wang, L.H. New dimension of glucocorticoids in cancer treatment. *Steroids* **2016**, *111*, 84–88.
3. Chari, A.; Martinez-Lopez, J.; Mateos, M.V.; Bladé, J.; Benboubker, L.; Oriol, A.; Arnulf, B.; Rodriguez-Otero, P.; Pineiro, L.; Jakubowiak, A.; et al. Daratumumab plus carfilzomib and dexamethasone in patients with relapsed or refractory multiple myeloma. *Blood* **2019**, *134*, 421–431.
4. Huang, X.; Zheng, Z.; Zeng, B.; Xiao, H.; Zheng, H.; Lin, Z.; Song, J.; Li, A.; Chi, P.; Yang, Y.; et al. Impact of glucocorticoids on the efficacy of neoadjuvant chemoradiotherapy and survival of patients with locally advanced rectal cancer: A retrospective study. *BMC Cancer* **2023**, *23*, 238.
5. Herr, I. Glucocorticoiduse in prostate cancer and other solid tumours: Implications for effectiveness of cytotoxic treatment and metastases. *Lancet Oncol*. **2006**, *7*, 425–430.
6. Budunova, I.V.; Kowalczyk, D.; Pérez, P.; Yao, Y.J.; Jorcano, J.L.; Slaga, T.J. Glucocorticoid receptor functions as a potent suppressor of mouse skin carcinogenesis. *Oncogene* **2003**, *22*, 3279–87.
7. Mayayo-Peralta, I.; Zwart, W.; Prekovic, S. Duality of glucocorticoid action in cancer: Tumor-suppressor or oncogene? *Endocr. Relat. Cancer* **2021**, *28*, 157–171.
8. Schoepe, S.; Schäcke, H.; May, E.; Asadullah, K. Glucocorticoid therapy-induced skin atrophy. *Exp. Dermatol*. **2006**, *15*, 406–20.

9. Schlossmacher, G.; Stevens, A.; White, A. Glucocorticoid receptor-mediated apoptosis: Mechanisms of resistance in cancer cells. *J. Endocrinol*. **2011**, *211*, 17–25.
10. Bal, M.P.; de Vries, W.B.; van der Leij, F.R.; van Oosterhout, M.F.; Berger, R.M.; Baan, J.; van der Wall, E.E.; van Bel, F.; Steendijk, P. Neonatal glucocorticosteroid treatment causes systolic dysfunction and compensatory dilation in early life: Studies in 4-week-old prepubertal rats. *Pediatr. Res*. **2005**, *58*, 46–52.
11. Zheng, Y.; Ishiguro, H.; Ide, H.; Inoue, S.; Kashiwagi, E.; Kawahara, T.; Jalalizadeh, M.; Reis, L.O.; Miyamoto, H. Compound A Inhibits Bladder Cancer Growth Predominantly via Glucocorticoid Receptor Transrepression. *Mol Endocrinol*. **2015**, *29*, 1486–1497.
12. Yemelyanov, A.; Czwornog, J.; Gera, L.; Joshi, S.; Chatterton, R.T.; Jr, Budunova, I. Novel steroid receptor phyto-modulator compound a inhibits growth and survival of prostate cancer cells. *Cancer Res*. **2008**, *68*, 4763–4773.
13. Tilova, L.R.; Savinkova, A.V.; Bocharov, A.K.; Kuzin, K.A.; Borisova, O.I.; Zhidkova, E.M.; Kirsanov, K.I.; Belitsky, G.A.; Yakubovskaya, M.G.; Yaminova, L.V.; et al. Synthesis of novel selective glucocorticoid receptor agonist and evaluation of its anti-cancer activity in the model of hematologic malignancies in vitro. *Mod. Probl. Sci. Educ.* **2016**, *6*, 110.
14. Chen, J. Synthesis and Uses of Synephrine Derivatives. United States Patent 0250944, 2005.
15. Gu, X.L.; Liu, H.B.; Jia, Q.H.; Li, J.F.; Liu, Y.L. Design and synthesis of novel miconazole-based ciprofloxacin hybrids as potential antimicrobial agents. *Monatshefte Chem.-Chem. Mon.* **2014**, *146*, 713–720.
16. Zeng, H. Preparation Method of 2-(4-benzyloxy phenyl)ethanol. China Patent113200825, 2021.
17. Bailey, W.F.; Bischoff, J.J. Formation of cyclic ethers in the double Baeyer-Villiger oxidation of ketals derived from cyclic ketones. *J. Org. Chem.* **1985**, *50*, 3009–3010.