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Synephrine Analogues as Glucocorticoid Receptor Agonists †

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Abstract: This work carried out the synthesis of several new synephrine analogues by universal method. Some of the synthesized compounds showed cytotoxicity on myeloid leukaemia cells K562 and lymphoma cell line Granta-519. Molecular docking using the glucocorticoid receptor (GR) model (PDB identifier 1P93) was performed to understand the possible underlying mechanism of compound action. The simulation showed the 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 leukaemia; mantle cell lymphoma

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1. Introduction

Glucocorticoids (GC) are steroid hormones regulating many cellular and physiological functions and they are 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 the application of main antitumor therapy methods, including chemotherapy and radiation therapy [3,4]. In addition, GC are widely used in the therapy of leukaemia and lymphoma as cytostatic drugs [5]. GC implement their biological effect via activation of glucocorticoid receptors which leads to the suppression of tumor cells' growth and proliferation, inducing their apoptosis [6–8].

Synthetic GC dexamethasone (Dex) is a glucocorticoid receptor agonist commonly used as a direct chemotherapy agent in the case of certain malignant neoplasm types'; however, it has several shortcomings. The effect of Dex outside the zone of therapeutic interests may cause a 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 the Dex sterane backbone with a less rigid hydrocarbon skeleton, predominantly preserving the spatial arrangement of original functional groups. We have assumed that such analogues could be obtained based on the synephrine molecule, which mimics the Dex backbone (Figure 1).

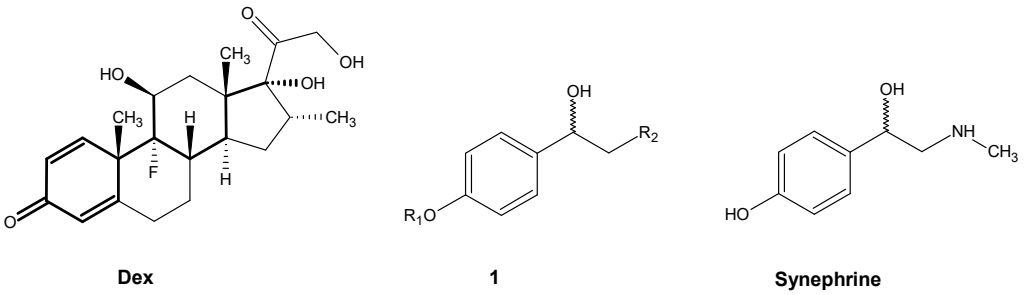

Figure 1. Structures of Dex, synephrine, and its synthetic analogues **1**.

These suggestions were examined by the molecular docking approach using the glucocorticoid receptor model. Simulation has shown selective binding of synephrine analogues in the hydrophobic pocket of the 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 of the synthesis of intermediate epoxide and its following interaction with aliphatic primary and secondary amines (Figure 2).

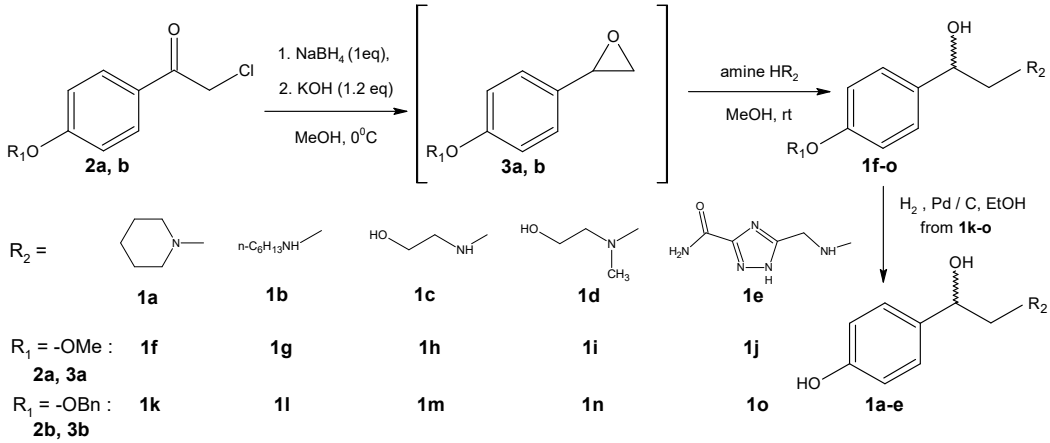

Figure 2. Synthesis of synephrine derivatives **1a–o.**

The starting compound **2** was obtained according to known methods [15,16]. The 4-Alcoxychloroacetophenones **2** was reduced with the excess of $NaBH_4$ in methanol followed by the treatment with KOH and the addition of a fivefold excess of amine without isolation of intermediate products. The target compounds **1f–o** were isolated by column chromatography on silica gel after the 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 a synthesis of individual enantiomers only in the case of active compound detection. Individual enantiomers can be obtained by stereoselective reduction of corresponding ketones **2** [17].

2.2. In Silico Studies

Molecular docking has shown several significant non-covalent interactions demonstrated by studied compounds in the GR ligand-binding domain. Thus, π-alkyl interactions with Met604 and 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 and Thr739 (Figure 3). Dex location determined in the experiment coincides with literature data reported by other authors [12].

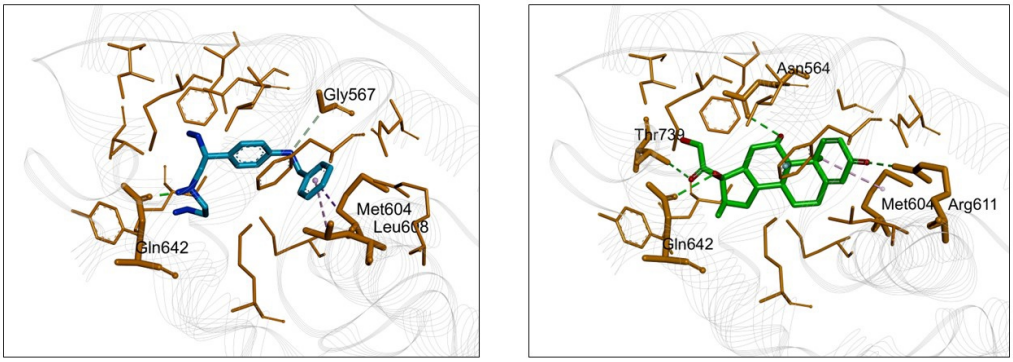

Figure 3. Structure of the GR active site with the analogue of synephrine **1m** in comparison to Dex (shown in blue and green, respectively).

2.3. Biology Section

In vitro, experiments were held to estimate the cytotoxicity of new compounds on the model of the hematopoietic system malignant neoplasms. Cell proliferation was evaluated using myeloid chronic leukaemia cells K562 and B cell lymphoma cell line Granta-519 (Table 1).

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

Cell Line	Time, h	Compound No.				
		1a (C = 50 μM)	**1g (C = 50 μM)**	**1k (C = 100 μM)**	**1m (C = 100 μM)**	**Dex (C = 25 μM)**
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 the cell line. Synthesized compounds demonstrated an effect similar to that of Dex, which suggests that the mechanism of their action is similar to the mechanism of Dex. In the case of the cell line K562, the studied compounds demonstrated an inhibitory effect on cell proliferation in 48 h (**1k**–**m**) and 72 h (**1g**), which is completely similar to the action of the reference drug. For the Granta-519 cell line, such a pattern could also be traced for **1a**,**g**,**k**, and m, but these 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 Dex effect on the myeloid leukaemia cell line K562 and lymphoma cell line Granta-519. The in silico modelling data suggests 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.

The ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz on Bruker DPX-300 spectrometer. High-resolution mass spectra (HRMS) were recorded using 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, 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 in portions. Subsequently, a solution of amine (5 eq) in methanol (1:1 by volume) was added. The 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 a 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. The product was isolated by column chromatography on silica gel using chloroform–methanol eluent.

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

In total, 200 mg (54%) of **1g** was obtained. R_f (10% methanol in chloroform) = 0.44. Purity by HPLC $\geq$ 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**)

In total, 360 mg (77%) of **1k** was obtained. R_f (5% methanol in chloroform) = 0.46. Purity by HPLC $\geq$ 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**)

In total, 95 mg (22%) of **1m** was obtained. R_f (20% methanol in chloroform) = 0.33. Purity by HPLC $\geq$ 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, a 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**)

In total, 91 mg (82%) of **1a** was obtained. R_f (25% methanol in chloroform) = 0.30. Purity by HPLC $\geq$ 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>-OH); 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

The 3D geometry of compounds was optimized using an 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. Removing solvent molecules, adding hydrogen atoms, assigning atom types, combining non-polar hydrogen atoms, and calculating partial Gasteiger charges and Kollmann charges were done using the 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 centred on the GR active site. The docking calculations were performed using the Autodock Vina software (The Scripps Research Institute, La Jolla, CA, USA) utilizing the Lamarckian genetic algorithm (LGA). The visualization and graphical representation of the results of ligand interaction were conducted using the Discovery Studio Visualizer software (version 21.1.0.20298, Dassault Systems Biovia Corp., San Diego, CA, USA).

4.4. Biology

Cell viability (cell proliferation): The cells were cultured in 24-well plates (50,000 cells/well) and treated with solvent (DMSO), dexamethasone (25 μM), **1a** (50 μM), **1g** (50 μM), **1k** (100 μM), and **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. The viable cells were immediately counted using the TC20 automatic cell counter ("Bio-Rad", Hercules, California, USA).

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