Identification of novel steroidal inhibitors of AKR1C4

Sofija S. Bekić[1], Andrea R. Nikolić[1], Jovana J. Ajduković[1], Marija N. Sakač[1], Edward T. Petri[2] and Andjelka S. Ćelić[2]

[1]University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

[2]University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

INTRODUCTION

Steroids are polycyclic compounds that play a vital role in the area of drug discovery. Steroid derivatives have a wide spectrum of biological activities, such as anticancer, antibacterial, antioxidant, antiviral, antiparasitic, and anti-inflammatory. They can be effective inhibitors of aldo-keto reductases (AKRs): NADPH-dependent oxidoreductases involved in the biosynthesis and metabolism of steroid hormones, as well as in the detoxification of xenobiotics. Some AKR isoforms are overexpressed in human tumors, such as breast, prostate and colon cancer, glioma, neuroblastoma and acute myeloid leukemia. Substrates for AKRs may include carbonyl-containing cytostatic agents, leading to the inactivation of these drugs and chemoresistance. Specific inhibition of these metabolic enzymes has been established as an attractive strategy for anti-cancer drug development. Under physiological conditions AKR1C4 isoform is essential for bile acid biosynthesis and steroid metabolism and inhibition should be avoided, but due to its altered activity in some types of cancers, it is considered to be therapeutic target. Furthermore, AKR1C4 is also reported to be involved in the reduction of some drugs, such as opioid receptor antagonists and corticosteroids, indicating the importance of its inhibition.

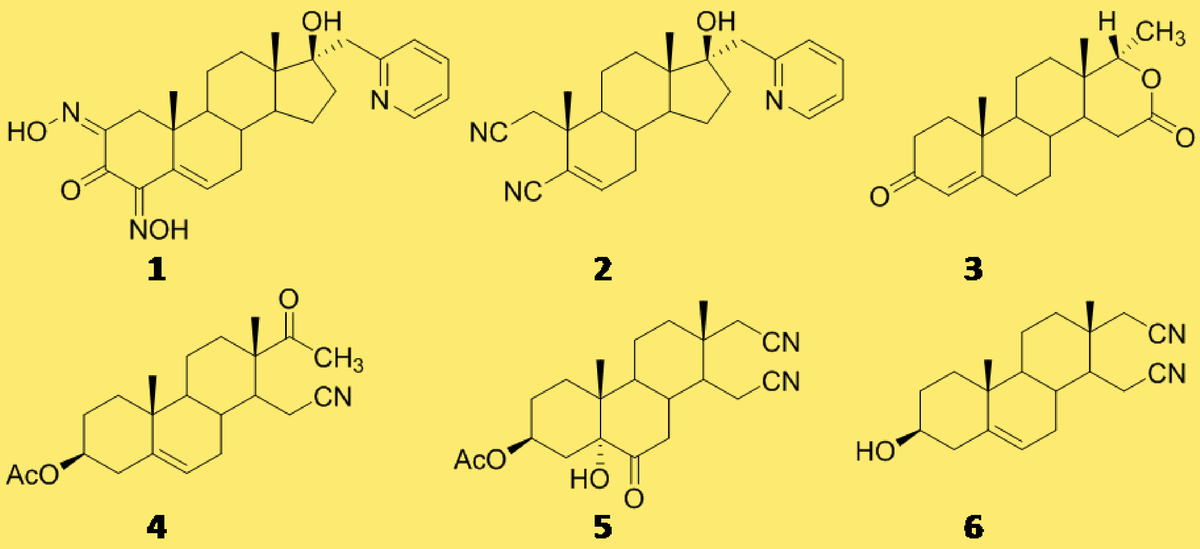

***Figure 1**. Chemical structures of A- and D-ring modified steroids **1-6**.*

AIM

The aim of this study was to evaluate inhibition potential of A- and D-ring modified steroid derivatives **1-6** (**Figure 1**) against the recombinant AKR1C4.

MATERIAL AND METHODS

The pET28b(+)-AKR1C4 construct was a kind gift from dr. Chris Bunce from The University of Birmingham. Inhibition potential of A- and D-ring modified steroids against recombinant AKR1C4 was evaluated by fluorimetry, monitoring the decrease in NADPH fluorescence. His-tagged human recombinant AKR1C4 was expressed in *Escherichia coli* BL21 cells and purified using immobilized metal affinity chromatography, followed by size-exclusion chromatography (**Figure 2**). Enzyme activity was measured as the decrease in fluorescence emission of NADPH at 460 nm following excitation at 340 nm in the presence of 9,10-phenanthroquinone (PQ).

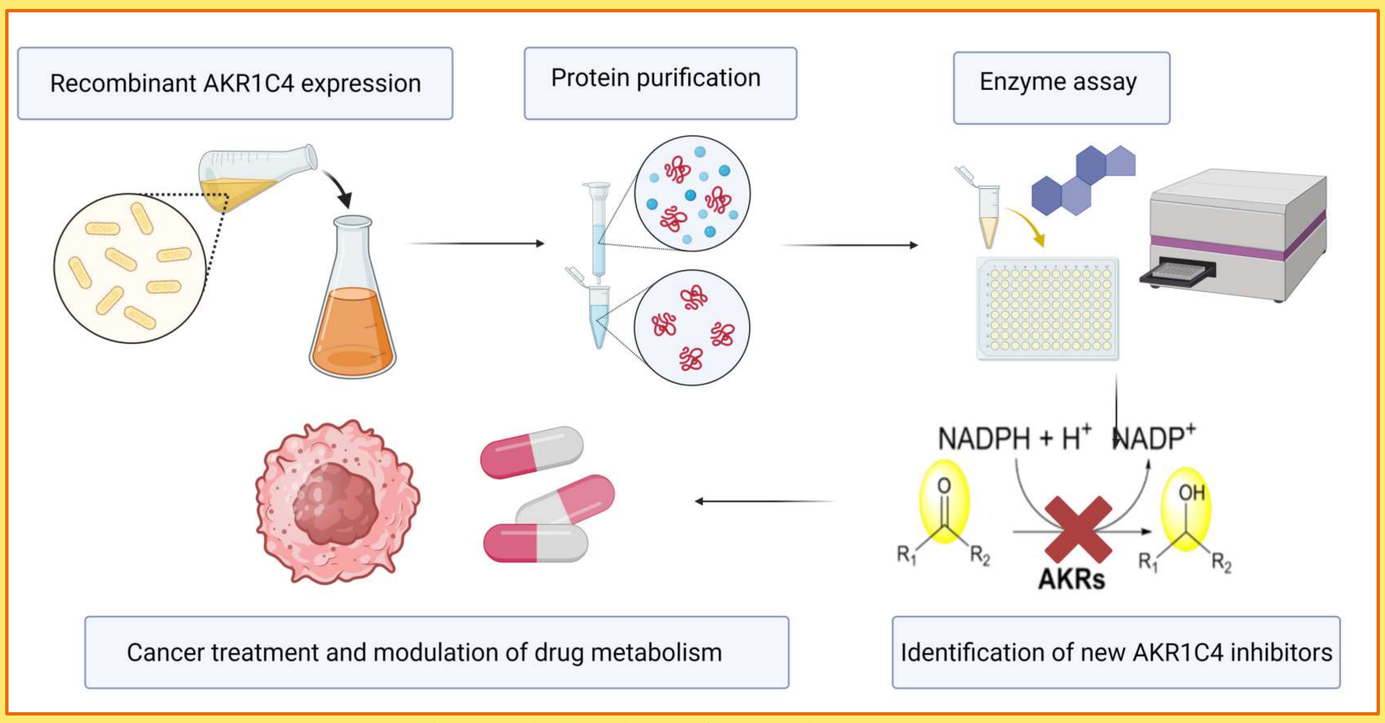

***Figure 2**. Design of experiments.*

RESULTS AND DISCUSSION

In the presence of steroid derivatives **1**, **3**, **4** and **5** activity of recombinant AKR1C4 was significantly inhibited (**Figure 3.** and **Table 1**), more than in the presence of known AKR1C inhibitor, ibuprofen, suggesting that these steroid derivatives are promising AKR1C4 inhibitors. Compound **2**, which differs from compound **1** only in the structure of the A ring, showed even a 10-fold lower inhibitory potential, indicating the potential importance of this part of the steroid molecule in binding to the active center of AKR1C4.

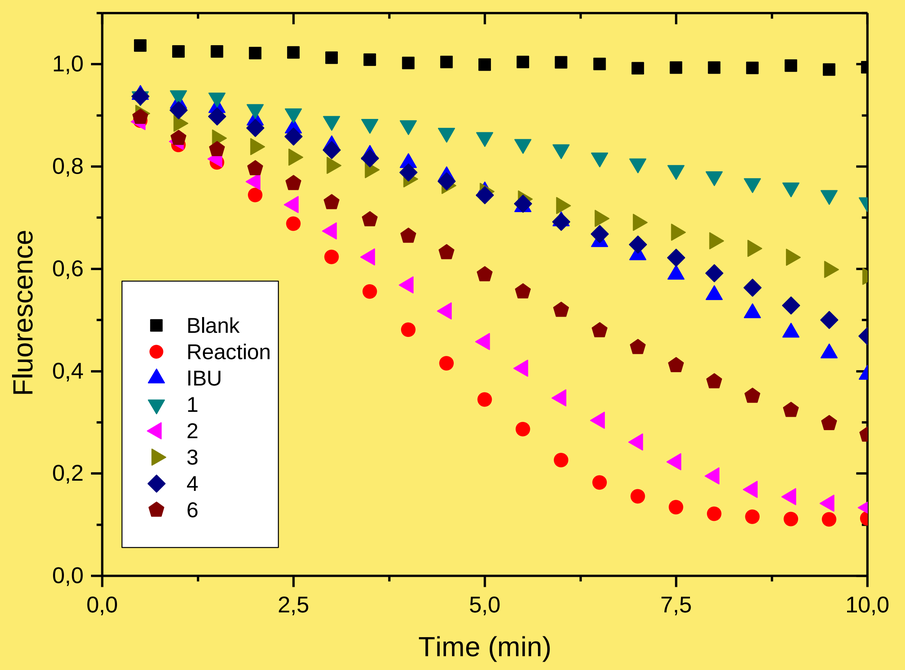

***Figure 3.** Inhibition of recombinant human AKR1C4 by A- and D-ring modified steroids **1-6** and ibuprofen (IBU). Blank consisted of all reaction components except the enzyme.*

***Table 1**. AKR1C4 inhibition potential of compounds **1-6** and ibuprofen.*

AKR1C4 INHIBITION (%)						
IBU	1	2	3	4	5	6
45.09	78.40	7.76	67.18	52.79	46.35	30.83

CONCLUSION

Considering that imbalanced expression of human AKRs is associated with the development and progression of various diseases, *in vitro* screening and identification of AKR1C4 inhibitors is of great importance in the design of potential therapeutics. Our preliminary results suggest that steroid derivatives have high potential to inhibit AKR1C4 and could serve as a starting point for the design of novel anticancer drug candidates targeting AKRs with high specificity.

ACKNOWLEDGMENT

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