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New method for recognition of sterol signalling molecules: Methinium salts as receptors for sulphated steroids

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ABSTRACT

In this work, we studied indolium and benzothiazolium pentamethine salts **1**–**3** as novel type of receptors for the recognition of sulphated signalling molecules (sulphated steroids: oestrone, pregnenolone and cholesterol sulphate). A recognition study was performed in an aqueous medium (1 mM phosphate buffer (H_2O:MeOH; 99:1 (v/v))) at pH 7.34. The tested salts displayed a high affinity for these sulphated analytes, mainly for cholesterol sulphate. However, no interaction between the salts and control, non-sulphated sterol analytes (cholesterol and bile acid) was observed. The highest affinity for the sulphated steroids was observed for benzothiazole salt **1**. This salt also displayed different spectral behaviour from that observed for carbocyanine salts **2** and **3**. In this presence of cholesterol sulphate, benzothiazole salt **1** displayed significant spectral changes depending on the medium used: a blue shift in the aqueous medium and a red shift in the methanolic one (H_2O:MeOH; 2:1 (v/v)). Subsequently preliminary *in vivo* study showed that, salt **1** significantly inhibits a growth of breast carcinoma on Nu/nu mice model.

1. Introduction

Anions play a fundamental role in a wide range of chemical and biological processes. The development of receptors that are designed for these biologically important compounds such as sulphated steroids (cholesterol sulphate, pregnenolone sulphate and oestrone sulphate) represents an important branch of modern chemistry.

Cholesterol sulphate serve as secondary messengers that affect various bodily functions (e.g., blood clotting, fibrinolysis, epidermal cell adhesion, keratinocyte differentiation, and activation of protein kinase C isoforms) [1]. On the surface of cell membranes, cholesterol sulphate can serve as a binding site for matrix metalloprotease 7 (a strong antigenic factor) [2], high levels of which are associated with cancer, Down's syndrome, diabetes mellitus type l and other intensively studied pathological conditions [3–5].

Pregnenolone sulphate is one of the most biologically important neurosteroids [6–8]. Pregnenolone sulphate has multiple critical effects on brain function, such as cognitive-enhancing, promnesic, antistress and antidepressant effects. At the cellular level, in addition to its effect on postsynaptic receptors, pregnenolone sulphate also has significant regulatory effects on the release of many important neurotransmitters, such as glutamate, γ-aminobutyric acid, acetylcholine, noradrenaline and dopamine. A significant decrease in pregnenolone levels has been associated with certain neurodegenerative diseases such as Parkinson's disease.

Oestrone sulphate forms majority part of oestrogens in blood. The plasma level of oestrone sulphate is 5-fold higher than the plasma levels of other unconjugated oestrogens [9]. This higher concentration can be coupled with a higher risk of serious pathological conditions, e.g., breast cancer [10].

These facts clearly demonstrate the significance of understanding the recognition of sulphated steroids for the development of advanced diagnostic methods. The determination of sulphated steroids can provide valuable information for the diagnosis of serious diseases and the possible prediction of their development [6,10–12]. The attainment of these ambitious goals requires the development and study of suitable tools for the determination and recognition of the synthetic receptors of sulphated steroids.

However, the synthetic receptors of these compounds have not yet been found. The detection of sulphated steroids is accomplished by chromatographic, immunochemical, radio and mass

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spectroscopy methods [5,11–14]. The structural motifs of these receptors could enable, in addition to the development of diagnostic methods, the design of promising therapeutic applications, e.g., for capturing sulphated steroids. The structure of the known protein receptors of sulphated steroids reveals a combination of ionic and hydrophobic interactions [2,15,16]. For example, the binding site of matrix metalloprotease 7 for cholesterol sulphate is based on a combination of arginine, tryptophan and isoleucine [2].

The structure of sulphated steroids indicates a possible structural motif of their synthetic receptors, which could include cationic charges (binding of anionic sulphate group) and should allow for hydrophobic interactions (binding of hydrophobic steroid core and any aliphatic substituent such as an isoprenoidal side chain) [17]. Hydrophobic interactions between aliphatic CH_x groups and aromatic systems some of the weakest known interactions. However, recent studies have shown that polyaromatic receptors can be effectively used for the recognition of aliphatic analytes such as alkyl chains in aqueous environments [18,19]. For the recognition of polycyclic sterol analytes, this phenomenon can significantly affect the selectivity of aromatic receptors for individual steroid derivatives [20].

Another interesting approach was demonstrated by Ideo et al. [21]. The authors observed that some galectins with an affinity for sulphated saccharides also recognise a cholesterol sulphate. This fact inspired us to apply receptors of sulphated polysaccharides for the recognition of sulphated steroids. Indeed, a number of such receptors could be applied for sensing sulphated steroids [22].

A combination of the two abovementioned approaches can lead to the application of cationic heteroaromatic compounds with confirmed sulphate selectivity for the recognition of sulphated steroids, such as polymethinium salts. In fact, polymethinium salts represent one type of receptor that could satisfy the abovementioned conditions. These compounds display excellent spectral properties (high extinction coefficients), and they have been intensively studied for their anion recognition capability. A number of recent studies have shown that these salts display high affinity and selectivity for various biologically important anionic analytes [23–27], such as sulphate and sulphated polysaccharides [27], and exhibit significant spectral changes. These findings indicate the high potential of polymethinium salts for the recognition of other important anionic analytes such as sulphated steroids. Therefore, we decided to study the utility of polymethinium salts for this application.

2. Material and methods

2.1. Receptors and steroids

The receptors **1**–**3** were prepared according synthetic protocol described in our previous studies [23,24]. Steroids were acquired from Sigma–Aldrich (Czech Republic, Prague).

2.2. Analytical study of receptors *1*–*3*

The association constants of receptors **1**–**3** with steroids were studied by UV–Vis spectroscopy in aqueous (1 mM phosphate buffer (H_2O:MeOH; 99:1 (v/v))), or a methanolic medium (1 mM phosphate buffer (H_2O:MeOH; 2:1 (v/v))) at pH 7.34. Conditional constants (*Ks*) were calculated from absorbance changes in the salts using their maximum absorbance (ΔA) by nonlinear regression via the program Letagrop Spefo, 2005. The concentration of the salts used was 1.8 μmol/L, and the concentration of the analytes was varied from 0 to 0.4 mmol/L.

3. Results

Suitable pentamethinium salts **1**–**3** were studied as receptors for oestrone, pregnenolone and cholesterol sulphate under aqueous conditions (water:methanol 99:1) by UV–Vis spectroscopy (see Fig. 1). The structure of every tested steroid is shown in Fig. 2. In addition to the aforementioned analytes, we used cholesterol, cholic acid and taurocholic acid as comparative structures due to their structural similarity. In this study, we did not observe any interactions for the nonsulphated analytes (cholesterol and cholic acid) (Table 1). On the other hand, the sulphated steroids displayed a strong interaction with receptors **1**–**3**, which was accompanied by significant spectral changes. Significant differences in the interaction of cholesterol sulphate with receptor **1**, relative to the interactions observed for the three structurally similar analytes (taurocholic acid, oestrone and pregnenolone sulphate), were observed. With respect to taurocholic acid, oestrone and pregnenolone sulphate, a decrease in the intensity of the spectral band and a corresponding slight shift toward higher wavelengths were observed (Fig. 3). For carbocyanine salts **2** and **3**, no interaction with taurocholic acid or the nonsulphated steroids (cholesterol and cholic acid) was observed. In the presence of oestrone and pregnenolone sulphate, the spectral behaviour of these salts was similar to that observed for salt **1**.

Dramatic spectral changes were observed for receptor **1** after the addition of cholesterol sulphate. We observed a significant decrease in the intensity of the main absorption band and an increase in the intensity of a new maximum at lower wavelength. In addition, a new band of negligible intensity located at a higher wavelength (750 nm) appeared (Fig. 4).

To obtain a better understanding of the effect of hydrophobic interactions on the affinity of receptor **1** toward cholesterol sulphate, study of the receptor in methanolic medium (1 mM phosphate buffer (H_2O:MeOH; 2:1) as performed. It is well known that molecular recognition between aliphatic guests (e.g., steroids and alkyl chains) and aromatic receptors is often controlled by hydrophobic interactions [18,28]. We assumed that supplementing the test medium with an organic solvent would suppress these hydrophobic interactions. In keeping with our expectation, a

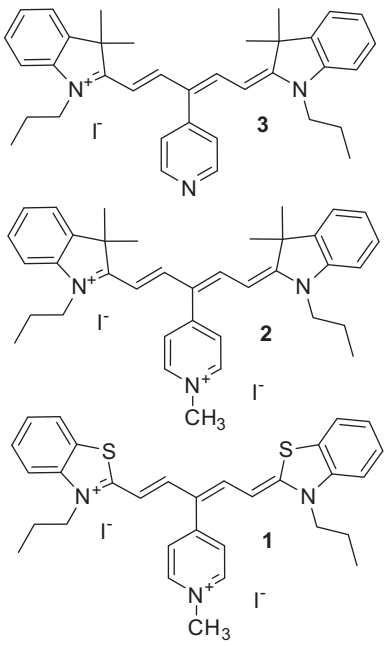

Fig. 1. Structures of receptors used in this study.