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Research paper

Thermodynamics and binding mode of novel structurally related 1,2,4-thiadiazole derivatives with native and modified cyclodextrins

Irina V. Terekhova [a,*], Mikhail V. Chislov [a], Maria A. Brusnikina [a], Ekaterina S. Chibunova [a], Tatyana V. Volkova [a], Irina A. Zvereva [b], Alexey N. Proshin [c]

[a] G.A. Krestov Institute of Solution Chemistry of Russian Academy of Sciences, 1 Akademicheskaya str., 153045 Ivanovo, Russia
[b] Saint Petersburg State University, 7/9 Universitetskaya nab., St. Petersburg 199034, Russia
[c] Institute of Physiologically Active Compounds of Russian Academy of Sciences, 1 Severniy pr., 142432 Chernogolovka, Russia

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ABSTRACT

Study of complex formation of cyclodextrins with 1,2,4-thiadiazole derivatives intended for Alzheimer's disease treatment was carried out using ^{1}H NMR, ITC and phase solubility methods. Structure of cyclodextrins and thiadiazoles affects the binding mode and thermodynamics of complexation. The larger cavity of β- and γ-cyclodextrins is more appropriate for deeper insertion of 1,2,4-thiadiazole derivatives which is accompanied by intensive dehydration and solvent reorganization. Benzene ring of the thiadiazoles is located inside macrocyclic cavity while piperidine ring is placed outside the cavity and can form H-bonds with cyclodextrin exterior. Complexation with cyclodextrins induces the enhancement of aqueous solubility of 1,2,4-thiadiazole derivatives.

1. Introduction

Solubility of novel drug candidates in biological fluids is an important subject in pharmaceutical science. It is well known that majority of newly discovered pharmaceutically active compounds have low aqueous solubility and, consequently, poor bioavailability. This is the main reason causing the delay in preclinical testing and drug development. In this connection, selection of proper technologies and effective solubilizers could resolve this problem and rapidly increase the progress of pharmaceutical industry.

Besides the technological advancements, there are numerous excipients available to overcome poor solubility and bioavailability of drugs. A wide range of high functional excipients for multifunction applications in solid and/or liquid dosage formulation has been used. Water-soluble polymers, oligosaccharides, surfactants, alcohols, etc are the most popular solubilizers at the time [1]. Selection of an effective excipient and its combination with drug is the subject of continued interest to design drugs with improved properties [1,2].

Our attention was focused on novel 1,2,4-thiadiazole derivatives which were recently synthesized and proposed for the treatment of Alzheimer's disease [3,4]. It has been found on the basis of the biological tests that these compounds influence the glutamate-induced calcium ions uptake into synaptosomes of rat brain cortex. In spite of satisfactory biological activity, 1,2,4-thiadiazole derivatives are poorly soluble in the aqueous media. Indeed, solubility of these compounds is not high and lies in the range of 10^{-3}–10^{-4} M. In this work, cyclodextrins (CDs) are proposed as solubilizers for 1,2,4-thiadiazole derivatives. CDs are cyclic oligosaccharides consisting of 6–8 glucopyranose units linked by α-(1,4)-glycosidic bonds. Owing to the sufficient solubilizing capacity of CDs they are widely used in pharmacy for preparation of different dosage forms of poorly soluble compounds [1,2,5,6]. Solubilizing effect of CDs is based on their ability to inclusion complex formation. Hydrophobic cavity of CDs can accommodate drug molecule, and hydrophilic exterior of CDs provides the dissolution of inclusion complexes in water. Therefore, investigation of complex formation of CDs with drugs is of practical importance since it allows to obtain new water-soluble formulations.

The purpose of our work was to reveal the binding affinity of native and modified CDs to inclusion complex formation with novel 1,2,4-thiadiazole derivatives, the structures of which are

* Corresponding author.
E-mail address: ivt@isc-ras.ru (I.V. Terekhova).

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depicted in Fig. 1. These compounds differ by the nature of side group –R located in para-position of the benzene ring. Compounds **I-III** have halogens of different van der Waals volume, electronegativity and other characteristics. Compound **IV** has methyl side group. Moreover, CDs under consideration differ by the size of macrocyclic cavity and the structure of substituents (e.g. methyl, carboxymethyl, hydroxyethyl, etc.). Therefore, the influence of the reagents structure on the binding mode and thermodynamics of complex formation is analyzed in the present study. Furthermore, the solubilizing effect of CDs towards 1,2,4-thiadiazole derivatives caused by the inclusion complex formation is also examined.

2. Experimental section

2.1. Materials

α-CD (⩾98%), β-CD (⩾99%), hydroxypropyl-β-cyclodextrin (HP-β-CD, ⩾98%), hydroxyethyl-β-cyclodextrin (HEt-β-CD, ⩾98%), methyl-β-cyclodextrin (M-β-CD, ⩾98%) and γ-CD (⩾90%) were obtained from Sigma Aldrich and were used as received. Carboxymethyl-β-cyclodextrin sodium salt (CM-β-CD, ⩾95%) was purchased from Cyclolab (Hungry). Average substitution degree per glucose unit was 0.6, 0.7, 1.6 and 0.5 for HP-β-CD, HEt-β-CD, M-β-CD and CM-β-CD, respectively. CDs were stable crystallohydrates, the water content in which determined by thermogravimetry (thermogravimetric analyzer TG Netzsch 209 F1 Libra) was equal to 1.2, 12.0, 2.0, 0.1, 0.2, 2.8 and 2.3% for α-CD, β-CD, HP-β-CD, HEt-β-CD, M-β-CD, CM-β-CD and γ-CD, respectively.

1,2,4-Thiadiazole derivatives were synthesized by standard and accessible methods in the Institute of Physiologically Active Compounds of the Russian Academy of Sciences (Chernogolovka) [7]. The purity of these compounds was ⩾ 98%.

2-[5-(4-Fluorophenylamino-[1,2,4]thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine (**I**). Light crystals. Yield 69%. Calc.,%: C 61.35, H 7.72, N 17.89 $C_{20}H_{30}FN_5S$. Found, %: C 61.23, H 7.87, N 17.92. ^{1}H NMR [200 MHz] δ: 0.71 (2H, m, C(3)HH, C(5)HH), 1.02, 1.04 (12H, s, 4xCH_3), 1.07 (3H, d, J = 6.6 Hz, CH_2CHCH_3), 1.72 (2H, dd, J = 3.0, 12.6 Hz, C(3)HH, C(5)HH), 2.72 (2H, dd, J = 2.3, 6.6 Hz, CH_2CHMe), 2.91 (1H, m, C(4)H), 3.34 (1H, m, CH_2CHMe), 7.04 (2H, t, J = 8.6 Hz, H_{arom}), 7.58 (2H, dd, J = 4.7, 9.1 Hz, H_{arom}).

2-[5-(4-Bromophenylamino-[1,2,4]thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine (**II**). Yellow powder. Yield 72%. Calc., %: C 53.09, H 6.68, N 15.48 $C_{20}H_{30}BrN_5S$. Found, %: C 53.28, H 6.56, N 15.64. ^{1}H NMR [200 MHz] δ: 0.73 (2H, m, C(3)HH, C(5)HH), 1.02 and 1.05 (12H, c and c, 4xCH_3), 1.08 (3H, d, J = 6.5 Hz, CH_2CHCH_3), 1.72 (2H, dd, J = 3.1, 12.5 Hz, C(3)HH, C(5)HH), 2.75 (2H, dd, J = 2.3, 6.5 Hz, CH_2CHMe), 2.91 (1H, m, C(4)H), 3.33 (1H, m, CH_2CHMe), 7.40 and 7.52 (4H, d and d, J = 8.8 Hz, H_{arom}).

2-[5-(4-Chlorophenylamino-[1,2,4]thiadiazol-3-yl]-1-methylethyl}-(2,2,6,6-tetramethylpiperidine-4-yl)-amine (**III**). White powder. Yield 70%. Calc., %: C 58.88, H 7.41, N 17.16 $C_{20}H_{30}ClN_5S$. Found, %: C 58.80, H 7.40, N 17.34. ^{1}H NMR [200 MHz] δ: 0.86 (2H, m, C(3)HH, C(5)HH), 1.14 and 1.20 (12H, s and s, 4xCH_3), 1.27 (3H, d, J = 6.65 Hz, CH_2CHCH_3), 1.87 (2H, d, J = 12.5 Hz, C(3)HH, C(5)HH), 2.84 (2H, d, J = 6.5 Hz, CH_2CHMe), 3.03 (1H, m, C(4)H), 3.45 (1H, m, CH_2CHMe), 7.40 and 7.50 (4H, d and d, J = 8.4 Hz, H_{arom}).

[1-Methyl-2-(5-p-tolylamino-[1,2,4]thiadiazol-3-yl)-ethyl]-(2,2,6,6-tetramethylpiperidine-4-yl)-amine (**IV**). Light crystals. Yield 69%. Calc., %: C 65.08, H 8.58, N 18.07. $C_{21}H_{33}N_5S$. Found, %: C 65.01, H 8.52, N 18.09. ^{1}H NMR [200 MHz] δ: 0.71 (2H, m, C(3)HH, C(5)HH), 1.02, 1.05 (6H, s + s, 2xCH_3), 1.07 (3H, d, J = 6.5 Hz, CH_2CHCH_3), 1.12, 1.14 (6H, s + s, 2xCH_3), 1.75 (2H, dd, J = 3.1, 12.5 Hz, C(3)HH, C(5)HH), 2.73 (2H, dd, J = 2.3, 6.5 Hz, CH_2CHMe), 2.91 (1H, m, C(4)H), 3.32 (1H, m, CH_2CHMe), 7.09 and 7.38 (4H, d + d, J = 8.6 Hz, H_{arom}), 10.56 (1H, br.s, ArNH).

Phosphate buffer (pH 7.4) was prepared on the basis of KH_2PO_4 and $Na_2HPO_4{\cdot}12H_2O$ of analytical grade (⩾99%). The pH of solutions was controlled by means of Mettler Toledo S220 SevenCompact pH-meter. Bidistilled water was used throughout the work.

2.2. ^{1}H NMR spectroscopy

The ^{1}H NMR spectra were recorded with a Bruker-AV-500 spectrometer operating at 500 MHz and temperature of 298.15 K. Samples were prepared in D_2O (99.9% isotopic purity). Cyclohexane was applied as an external reference.

For evaluation of stability constants of the complexes, the ^{1}H NMR spectra of 1,2,4-thiadiazole derivatives were recorded in the presence of variable concentrations of CDs. Chemical shift changes $\Delta\delta$ were calculated as follows:

$$\Delta\delta = \delta_{complexed} - \delta_{free} \quad (1)$$

Stability constants were derived from the concentration dependences of $\Delta\delta$ using nonlinear least squares curve-fitting procedure.

2.3. Spectroscopic determination of stoichiometry of the complexes

Stoichiometry of the complexes was determined using Job's method [8]. Stock solutions of CD ($6{\cdot}10^{-5}$ mol/kg) and 1,2,4-thiadiazole derivative ($6{\cdot}10^{-5}$ mol/kg) were prepared and mixed in such a way that the sum of total concentration of host and guest was constant. The mole fraction of 1,2,4-thiadiazole derivative $\left(R = \frac{X_{thiadiazole}}{X_{thiadiazole}+X_{CD}}\right)$ was varied from 0.1 to 1.0. The UV–vis spectra of solutions were recorded on UV–vis spectrometer (Shimadzu 1800, Japan). The corrected absorbance $(\Delta A \cdot X_{thiadiazole})$ at fixed wavelength was plotted against R.

2.4. Isothermal titration calorimetry

Thermodynamic parameters of complex formation were determined using an isothermal titration calorimeter TA Nano (TA Instruments, New Castle, USA). Calorimetric experiments were carried out in phosphate buffer (pH 7.4) at 298.15 K. The syringe was filled by 20 mM of CD solution or 15 mM in case of β-CD and the measurement cell contained 1 ml of the solution of 1,2,4-thiadiazole derivative (0.2 mM). All solutions were degassed for 10 min before the titration experiments. The 9.99 μL of CD solution were injected stepwise. The stirring speed was set to 300 rpm, the equilibration period between the injections was 1400 s. The results

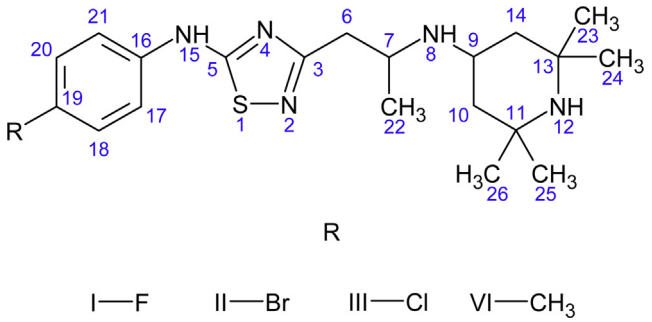

Fig. 1. 1,2,4-Thiadiazole derivatives under study.