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Investigation of the lipophilic behaviour of some thiazolidinediones Relationships with PPAR-γ activity

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Abstract

Various lipophilicity aspects of five well-known PPAR-γ ligands, belonging to the thiazolidinedione (TZD) class, ciglitazone (CSZ), troglitazone (TGZ), netoglitazone (NGZ) and the ampholytic pioglitazone (PGZ) and rosiglitazone (RGZ), have been explored. The compounds were found to be highly lipophilic as assessed by direct octanol–water partitioning experiments and further confirmed by reversed phase HPLC measurements under different conditions. Immobilised artificial membrane (IAM) chromatographic indices were also determined as an alternative expression of lipophilicity. They were found to show less diversity forming two clusters. Experimental log *D*/log *P* values were compared to those predicted by three widely used calculation systems. For the two ampholytic TZDs, the lipophilicity and retention/pH profiles were established over a broad pH range and compared to the corresponding calculated profiles. Lipophilicity indices derived under the different conditions were further compared to biological activity, concerning in vitro transactivation (pEC_{50}) and binding affinity (pK_i) data, taken from literature. The most active TZD (RGZ) in both transactivation and binding assay proved to be the less lipophilic analogue. An equation relating pEC_{50} data to experimental log $D_{7.4}$ or reversed-phase log k_w values could be established, while pK_i data did not lead to satisfactory correlation.

Keywords: Thiazolidinediones; PPAR-γ ligands; Lipophilicity; *n*-Octanol/water partitioning; RP-HPLC; IAM chromatography

1. Introduction

Thiazolidinediones (TZDs) represent a new class of oral anti-diabetic agents currently used for the treatment of type II diabetes mellitus [1]. Beyond their anti-diabetic therapeutic benefits, TZDs have recently exhibited a wide spectrum of actions, including anti-inflammatory [2] and anti-neoplastic properties [3]. TZDs mostly exert their effects by selectively binding to and activating the nuclear peroxisome-proliferator activated receptor-γ (PPAR-γ) [4], while receptor independent actions have not been excluded [5]. In general, the members of TZD class posses a few essential pharmacophore elements which comprise an acidic group linked to a central flat ring and a large lipophilic substructure [6]. To date, a large number of TZD derivatives, synthesized through various structural modifications of these essential features, have been tested for PPAR-γ functional activity. However, experimental studies regarding their lipophilicity profile, which could contribute to the optimization and the understanding of their action, are still missing in literature. The fact that PPAR-γ is dramatically highly expressed in adipose tissue supports an essential role of lipophilicity for its ligands. The minimum hydrophobicity concept, formulated by Hansch et al. 20 years ago is considered as a general guideline in Drug Design [7], while for drugs intended for oral administration Lipinski's rule of 5 suggests an upper limit for lipophilicity [8]. In this aspect, the question arises how much lipophilicity should be incorporated in the ligands so that they satisfy the receptor and receptor environment requirements and comply with principles nowadays generally accepted.

The most widely used index of lipophilicity is the *n*-octanol/water partition or distribution coefficient (log *P* or log *D*) [9,10]. This choice offers a quite representative simulation of drug partitioning into bio-membranes [11]. Due, however, to difficulties involved in direct *n*-octanol/water partitioning experiments, reversed-phase liquid chromatography (RP-HPLC) has alternatively been applied for drug lipophilicity assessment. This

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technique offers several practical advantages compared to the traditional shake-flask method, including speed, reproducibility, broader dynamic range and insensitivity to impurities or degradation products [12,13]. Extrapolated RP-HPLC indices ($\log k_w$) are usually calibrated towards the *n*-octanol/water system and appropriate conditions are chosen, so that the best parallelism with $\log P/\log D$ is achieved [13–16]. In addition, a rich arsenal of calculation procedures has been developed for rapid estimation of $\log P$ [17–19]. For complex structures, however, their performance is often disputable. Predictions may be even less accurate in the case of $\log D$ for molecules containing ionizable groups. On the other hand, *n*-octanol/water system and RP-HPLC reflect non specific interactions with cell membranes, but they fail to predict specific ones [20]. For this purpose, the development of immobilised artificial membrane (IAM) chromatography has unfolded new perspectives in the application of HPLC for rapid evaluation of drug partitioning into bio-membranes, since it is thought to mimic the phospholipid bilayers more closely. In fact, IAM columns contain phospatidylcholine incorporated on a silica-propylamine backbone, simulating better the ion-pairing and hydrogen bonding interactions [21–23].

In the present study, we exploited the above mentioned alternatives in order to investigate in detail the lipophilicity of five well-known TZDs, ciglitazone (CSZ), troglitazone (TGZ), netoglitazone (NGZ), pioglitazone (PGZ), rosiglitazone (RGZ). For this purpose the distribution coefficients ($\log D$) in *n*-octanol/water system were measured and compared with $\log D$ values estimated by three widely used calculation systems. RP-HPLC lipophilicity indices determined under different conditions were evaluated for their ability to reproduce the *n*-octanol/water $\log D$ values. In addition, the behavior of TZDs in IAM chromatography was assessed. Lipophilicity measures obtained from the different systems were further used in relation to literature biological data.

2. Materials and methods

2.1. Materials

Ciglitazone-CGZ, troglitazone-TGZ, netoglitazone-NGZ, pioglitazone-PGZ and rosiglitazone-RGZ were purchased from Cayman Chemical Company, Michigan, USA. Their structures are presented in Fig. 1.

Octanol was extra pure purchased by Panreac Quimica, Spain. Methanol and acetonitrile were HPLC grade and were purchased from Lab-Scan Science Ltd., Ireland. Sodium hydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, sodium chloride and 3-morpholinepropanesulfonic acid (MOPS) were purchased from Merck, Darmstadt, Germany. Water was deionised and further purified by means of a Milli-Q Plus Water purification system, Millipore Ltd.

2.2. Octanol/water partitioning experiments

Octanol/water distribution coefficient values were measured by the shake-flask method, using a standard procedure as described in [24]. Briefly, the protocol is as follows:

TZDs were first diluted in DMSO at a concentration of 5 mg/ml (stock solution). Aqueous TZD solutions were then prepared by the addition of appropriate volume of stock solutions in order for a concentration approximately 10^{-5} M to be obtained. The final concentration of DMSO in the aqueous solution did not exceed 0.2%. The pH of the aqueous solutions was pre-

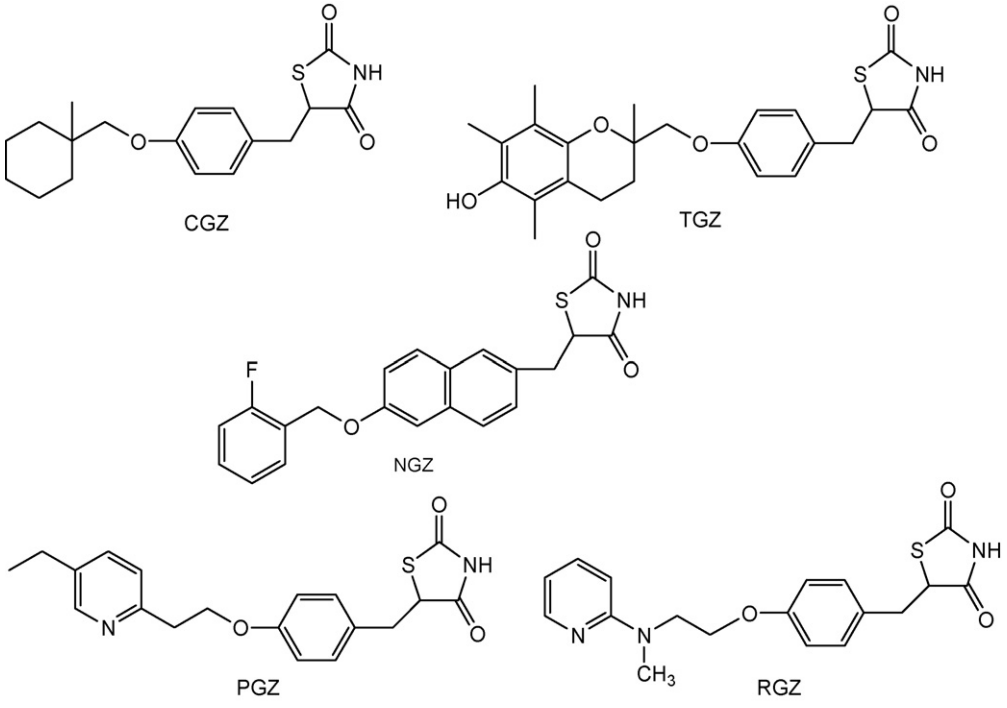

Fig. 1. Structure of TZDs.