European Journal of Pharmaceutical Sciences 67 (2015) 85–96

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Synthesis, *in vitro* and *in vivo* evaluation of 1,3,5-triazines as cannabinoid CB2 receptor agonists

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ARTICLE INFO

Article history:
Received 8 September 2014
Received in revised form 1 November 2014
Accepted 6 November 2014
Available online 14 November 2014

Keywords:
Cannabinoid receptor
CB2R agonists
Triazines
Radioligand

ABSTRACT

The cannabinoid receptors type 2 (CBR2) are attractive therapeutic targets of the endocannabinoid signaling system (ECS) as they are not displaying the undesired psychotropic and cardiovascular side-effects seen with cannabinoid receptor type 1 (CB1R) agonists. In continuation of our previous work on 2,4,6-trisubstituted 1,3,5-triazines as potent CB2 agonists, we synthesized an additional series of more polar analogues (**1**–**10**), which were found to possess high CB2R agonist activity with enhanced water solubility. The most potent compound in the series was *N*-(adamantan-1-yl)-4-ethoxy-6-(4-(2-fluoroethyl)piperazin-1-yl)-1,3,5-triazin-2-amine (**9**) with EC_{50} value of 0.60 nM. To further evaluate the biological effects of the compounds, the selected compounds were tested *in vitro* against four different cell lines. A human retinal pigment epithelial cell line (ARPE-19) was used to evaluate the cytotoxicity of the compounds whereas an androgen-sensitive human prostate adenocarcinoma cell line (LNCaP), a Jurkat leukemia cell line and a C8161 melanoma cell line were used to assess the antiproliferative activity of the compounds. The most interesting results were obtained for *N*-(adamantan-1-yl)-4-ethoxy-6-(4-methylpiperazin-1-yl)-1,3,5-triazin-2-amine (**6**), which induced cell viability decrease in prostate and leukemia cell lines, and diminished proliferation of C8161 melanoma cells. The results could be reversed in leukemia cells with the selective CB2R antagonist AM630, whereas in prostate cells the AM630 induced a significant cell viability decrease with a mechanism probably unlinked to CB2 cannabinoid receptor. The antiproliferative effect of **6** on the melanoma cells seemed not to be mediated via the CB1R or CB2R. No cytotoxicity was detected against ARPE-19 cell line at concentrations of 1 and 10 μM for compound **6**. However, at 30 μM concentration the compound **6** decreased the cell viability. Finally, in order to estimate *in vivo* behavior of these compounds, ^{18}F labeled PET ligand, *N*-cyclopentyl-4-ethoxy-6-(4-(2-fluoro-18-ethyl)piperazin-1-yl)-1,3,5-triazin-2-amine ([^{18}F]**5**), was synthesized and its biodistribution was determined in healthy male Sprague–Dawley rats. As a result, the tracer showed a rapid (<15 min) elimination in urine accompanied by a slower excretion via the hepatobiliary route. In conclusion, we further demonstrated that 1,3,5-triazine scaffold serves as a suitable template for the design of highly potent CB2R agonists with reasonable water solubility properties. The compounds may be useful when studying the role of the endocannabinoid system in different diseases. The triazine scaffold is also a promising candidate for the development of new CB2R PET ligands.

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http://dx.doi.org/10.1016/j.ejps.2014.11.003
0928-0987/

1. Introduction

The endocannabinoid signaling system (ECS) participates in numerous diseases affecting humans and thus it offers many potential targets for drug development. The ECS is known to consist, at least, of two cannabinoid receptors (CB1R and CB2R), their endogenous ligands (endocannabinoids), the enzymes synthesizing and degrading the endocannabinoids, and the putative endocannabinoid transporters (Lambert and Fowler, 2005; Pacher et al., 2006). Even though, the major players of the ECS have already been known for quite some time, there is still a significant amount of research that needs to be done to fully understand their roles in the system.

CB1R-mediated psychotropic side effects is the main drawback in the ECS-based drug development. However, one possible strategy is to selectively modulate only the peripheral CB2R which is anticipated to be devoid of the central nervous system (CNS) side effects (Han et al., 2013). The CNS and peripheral CB2R expression levels are increased in various diseases in a tissue- and time-dependent manner (Pacher and Kunos, 2013; Pacher and Mechoulam, 2011; and the references cited therein). This may enable the design of well-targeted therapeutics, and the CB2R may also serve as a potential biomarker for disorders (Evens et al., 2012).

In our previous study, we reported a series of 2,4,6-trisubstituted 1,3,5-triazines which act as potent CB2R agonists (Yrjölä et al., 2013). However, high lipophilicity of these ligands limited their applicability in *in vitro* and *in vivo* experiments. We decided to solve the problem by incorporating polar functional groups to the triazine skeleton, which lowered lipophilicity and beneficially retained potency of the compounds for the CB2R. Overall, in this study we have synthesized a new more polar series of triazine analogues (**1**–**10**) and determined the distribution coefficients (log*D*) and water solubilities for **3**, **6**, **7** and **10**. The compounds were also tested *in vitro* using four different cell lines that have been shown to express CB1 and/or CB2 receptors. A human retinal pigment epithelial cell line (ARPE-19) was used to evaluate the cytotoxicity of the compounds, whereas an androgen-sensitive human prostate adenocarcinoma cell line (LNCaP), a Jurkat leukemia cell line and a C8161 melanoma cell line were used to assess the compounds' antiproliferative activity (Wei et al., 2009; Sarfaraz et al., 2005; Rieder et al., 2010; Blázquez et al., 2006). Finally, in order to estimate *in vivo* behavior of these compounds, we synthesized fluorine-18 labeled triazine derivative ([^{18}F]**5**) and determined its biodistribution in healthy rats.

2. Materials and methods

2.1. Design of the 2,4,6-trisubstituted 1,3,5-triazines

The structures of a small library of 10 new triazine derivatives (**1**–**10**) are outlined in Fig. 1. The structures were designed based on the results of our previous structure–activity relationship study of 2,4,6-trisubstituted 1,3,5-triazines as CB2R agonists (Yrjölä et al., 2013). The original hit structure, *N*-cyclopentyl-4-ethoxy-6-(4-methylpiperidin-1-yl)-1,3,5-triazin-2-amine (**11**), is also presented in Fig. 1 (Yrjölä et al., 2011). The ethoxy group was maintained in all new compounds (**1–10**) and the *N*-cyclopentyl substituent was kept intact in five structures (**1–5**). The *N*-cyclopentyl was replaced with *N*-adamantyl group in four compounds (**6–9**) as it is known to be beneficial for the CB2R activity (Lu et al., 2005). Physicochemical properties of the compounds were modified by introducing more polar substituents, morpholine (**1**), thiomorpholine-1,1-dioxide (**2, 7, 10**), piperazine (**4, 8**) and 1-methylpiperazine (**3, 6**) on the triazine skeleton. All ten compounds were screened for their ability to activate the CB1R/CB2R (Table 1).

2.2. General synthetic procedures

The compounds **1**–**10** were synthesized as reported in our previous work (Yrjölä et al., 2011). Commercially available chemicals were used without further purification. Reactions were monitored by thin layer chromatography (TLC) which was performed on Merck silica gel F254 precoated aluminum sheets. Petroleum ether-ethyl acetate (PE-EA; 9:1, 5:1 or 1:1) or dichloromethane-methanol (DCM-MeOH; 6:1) was used as an eluent and spots were detected by UV light and ninhydrin stain. The products were purified by column chromatography using silica gel. Elution was performed using PE-EA or DCM-MeOH with gradient elution increasing the proportion of a polar solvent. Yields were not optimized.

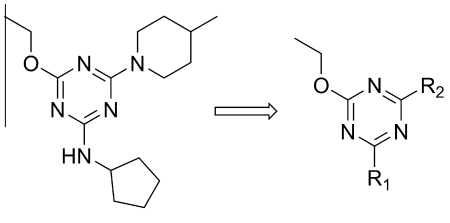
NMR spectra were recorded on a Bruker Avance instrument (500.1 MHz for ^{1}H, 125.8 MHz for ^{13}C). Chemical shifts are reported in δ (ppm) values with tetramethylsilane or the solvent resonance as an internal standard. Coupling constants *J* are given in Hz. Multiplicities are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br). ESI mass spectra were performed on Finnigan MAT LCQ quadrupole ion trap mass spectrometer. Elemental analyses were carried out using a ThermoQuest CE Instruments EA 1110 CHNS-O elemental analyzer. The purity (>95%) of compounds **2**, **5** and **8**–**10** were determined by using Agilent 1100 series HPLC with DAD detector (254 nm). Column: Zorbax Eclipse XBD-C18, 4.6 × 50 mm, 1.8 μm, Agilent Technologies.

2.3. Intermediates A, B and C

The synthesis of intermediates 2,4-dichloro-6-ethoxy-1,3,5-triazine (intermediate A), 4-chloro-*N*-cyclopentyl-6-ethoxy-1,3,5-triazin-2-amine (intermediate B) and *N*-(adamantan-1-yl)-4-chloro-6-ethoxy-1,3,5-triazin-2-amine (intermediate C) have been described earlier (Yrjölä et al., 2013).

2.4. General procedure for synthesizing intermediate D and final products *1–4*, *6*, *7* and *10*

A mixture of an appropriate amine and *N,N*-diisopropylethylamine (DIPEA) or triethylamine (Et_3N) in THF was added to a solution of cyanuric chloride or compound derived from cyanuric



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1, R_1 = cyclopentyl*NH*, R_2 = morpholine
2, R_1 = cyclopentyl*NH*, R_2 = thiomorpholine-1,1-dioxide
3, R_1 = cyclopentyl*NH*, R_2 = *N*-Me-piperazinyl
4, R_1 = cyclopentyl*NH*, R_2 = piperazinyl
5, R_1 = adamantyl*NH*, R_2 = *N*-FCH_2CH_2-piperazinyl
6, R_1 = adamantyl*NH*, R_2 = *N*-Me-piperazinyl
7, R_1 = adamantyl*NH*, R_2 = thiomorpholine-1,1-dioxide
8, R_1 = adamantyl*NH*, R_2 = piperazinyl
9, R_1 = adamantyl*NH*, R_2 = *N*-FCH_2CH_2-piperazinyl
10, R_1 = thiomorpholine-1,1-dioxide, R_2 = 4-Me-piperidinyl

Fig. 1. The hit structure (**11**) used as a starting point to create a series of 2,4,6-trisubstituted 1,3,5-triazines (Yrjölä et al., 2011).