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Discovery of novel cannabinoid receptor ligands by a virtual screening approach: Further development of 2,4,6-trisubstituted 1,3,5-triazines as CB2 agonists

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ABSTRACT

3D ligand-based virtual screening was employed to identify novel scaffolds for cannabinoid receptor ligand development. A total of 112 compounds with diverse structures were purchased from commercial vendors. 12 CB1 receptor antagonists/inverse agonists and 10 CB2 receptor agonists were identified *in vitro*. One of the CB2 agonists, *N*-cyclopentyl-4-ethoxy-6-(4-methylpiperidin-1-yl)-1,3,5-triazin-2-amine (19, $-\log EC_{50} = 7.5$, $E_{max} = 255\%$) was selected for further development. As far as we are aware, the compound's 1,3,5-triazine scaffold represents a new core structure for CB2 agonists. A library of fifty-seven 2,4,6-trisubstituted-1,3,5-triazines was created to clarify the structure–activity relationship study of the analogs.

1. Introduction

Since the two cannabinoid receptors were first identified and cloned, CB1 in 1990 (Matsuda et al., 1990) and CB2 in 1993 (Munro et al., 2003), they have been a subject of intensive study. These G protein-coupled receptors are a part of the endocannabinoid system in combination with the endogenous agonists of cannabinoid receptors (endocannabinoids) and proteins responsible for the synthesis, metabolism and transport of endocannabinoids (Pacher et al., 2006; Basavarajappa 2007; Yates and Barker, 2009).

The endocannabinoid system plays an important role in many crucial physiological functions in both the central and peripheral nervous systems and the peripheral tissues. In addition, a vast variety of diseases and conditions in almost all of the major therapeutic areas has been associated with alterations in this neurotransmitter system. Several reviews have been published on the importance of the endocannabinoid system and its function in different conditions (Di Marzo, 2008; Pacher et al., 2006; Pacher and Mechoulam, 2011).

The endocannabinoid system represents a great opportunity for medicinal chemists, but it is also a major challenge. The CB1 receptors in the brain are responsible for the psychotropic effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive compound in the *Cannabis sativa* plant. This complicates drug development because mind-altering side effects are neither desirable nor acceptable properties if a drug is to reach the market. The CB1 antagonist/inverse agonist SR141716A (rimonabant) highlighted the difficult facets of the endocannabinoid system since the drug had to be withdrawn from the market because it evoked significant psychiatric side-effects (Kirilly et al., 2012). Fortunately, several strategies have been proposed and are being currently intensively studied to avoid psychotropic effects when targeting the endocannabinoid system. These strategies include (1) selective activation or inactivation of CB2 receptors, (2) activation or inactivation of CB1 receptors in the peripheral nerves but not affecting those in the central nervous system, (3) blockade of CB1 by neutral antagonists instead of using inverse agonists and (4) indirect agonism by inhibiting the enzymes metabolizing endocannabinoids or inhibiting the cellular uptake of endocanabinoids (Di Marzo, 2008; Kirilly et al., 2012; Pacher and Mechoulam, 2011). New receptors have also been found which may play a role in the endocannabinoid system and offer novel targets for the drug discovery. For example, G protein-coupled receptor 55 (GPR55) is one recent candidate as a third cannabinoid receptor (Moriconi et al., 2010).

There is a need to create novel scaffolds as starting points for cannabinoid ligand development as well as devising novel pharmacological tools with which to study the endocannabinoid system and thus we used 3D ligand-based virtual screening (VS) in the search for compounds with steric and electrostatic fields similar to SR141716A (rimonabant, 1). After purchasing and testing the most promising compounds of the VS, 12 selective CB1 antagonists/inverse agonists and 10 CB2 agonists were identified with

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IC_{50}/EC_{50} values ranging from low nanomolar to low micromolar concentrations. These selective CB1 antagonists/inverse agonists and selective CB2 agonists are intriguing compounds especially considering the ways in which the endocannabinoid system might be targeted without evoking psychotropic side effects.

One of the found CB2 agonists, *N*-cyclopentyl-4-ethoxy-6-(4-methylpiperidin-1-yl)-1,3,5-triazin-2-amine (19, $-\log EC_{50} = 7.5$, $E_{max} = 255\%$), was chosen for further development. As far as we are aware, the compound's 1,3,5-triazine scaffold is a novel core structure for a CB2 agonist. A small library of 57 analogs was created to study the structure–activity relationships of 2,4,6-trisubstituted-1,3,5-triazines and to enhance the potency of the hit structure.

2. Materials and methods

2.1. Virtual screening

BRUTUS (Tervo et al., 2005; Rönkkö et al., 2006) was used to identify novel scaffolds as starting points for cannabinoid ligand development (scaffold hopping). BRUTUS is a rigid molecular superposition algorithm based on molecular fields. Since the method considers 3D-field properties rather than only simple topological similarities, the resulting compounds can be very diverse in their structures and thus not obvious analogs of the query molecule.

The databases from Chembridge and Asinex were searched with BRUTUS using a known CB1 receptor antagonist/inverse agonist SR141716A (rimonabant, 1) as the query. A small selection of 112 compounds was chosen and purchased for *in vitro* testing after visual inspection focusing on the synthetic feasibility and drug-like properties. Details of the screening procedure are described in Section 5.1.

2.1.1. Active compounds found based on virtual screening - Choosing the hit structure for further study

The purchased 112 compounds were tested for their CB1 and CB2 receptor agonist and antagonist activities (see Section 2.3. *Biological testing* for details). Subsequently, 12 selective CB1 antagonists/inverse agonists and 10 selective CB2 agonists were evaluated (Fig. 1). Even though the CB1 antagonist/inverse agonist SR141716A (1) was used as the query in the search, it was not surprising to identify also CB2 activating compounds. CB1 and CB2 receptors share only 44% overall amino acid sequence identity and 68% identity in the transmembrane domains but nonetheless many ligands are unable to distinguish between them (Lutz, 2002). Overall, the identified compounds represent novel structures with good selectivity and potency as starting points for cannabinoid ligand development.

We chose the compound *N*-cyclopentyl-4-ethoxy-6-(4-methylpiperidin-1-yl)-1,3,5-triazin-2-amine (19) for further study because of its potency, selectivity, small molecular weight (MW 305.42) and synthetic feasibility. This compound was the only one to show also some CB1 agonist activity among the identified CB2 agonists, although it was clearly more selective for CB2 than CB1 ($-\log EC_{50}$ values for CB2 and CB1: 7.5 and 5.9, respectively). The 1,3,5-triazine scaffold of the compound represents a new core structure for CB2 agonists and the small molecular weight of the hit compound permits diverse modifications to the structure.

2.2. Chemistry

2,4,6-Trisubstituted 1,3,5-triazine analogs were synthesized using the procedures described in the literature (Coburn et al., 2009). In general, the compounds were synthesized by displacement of chlorine atoms in cyanuric chloride with various substituents. This synthesis route is very feasible because cyanuric chloride is an inexpensive reagent and its chlorine atoms can be substituted one at a time as the reactivity of the triazine nucleus decreases with the number of electron-donating substituents (Thurston et al., 1951). Scheme 1 shows an example of synthesis of a 2,4,6-trisubstituted 1,3,5-triazine analog (26b). Overall, a library of 38 synthesized and 19 commercially available analogs was created.

2.3. Biological testing

All synthesized and commercial compounds were tested for their CB1 and CB2 receptor activities by using the [^{35}S]GTPγS binding assay according to procedures described in literature (Savinainen et al., 2003; Savinainen et al., 2005). Dose–response curves were determined for compounds that demonstrated activity over 140% basal at 10 μM concentration. Nonlinear regression analysis with the equation for a sigmoidal concentration–response curve was used to calculate $-\log EC_{50}$ and E_{max} values (GraphPad Prism 5). Values are mean ± SEM of at least two independent experiments performed in duplicate.

3. Results and discussion

As a result, 28 analogs of the hit structure (19) showed CB2 agonist activity with EC_{50} values ranging from low nanomolar to low micromolar concentrations (Tables 1–7). The CB2 receptor mediated response was tested using nontransfected CHO cells (Savinainen et al., 2005) and the CB2 receptor selectivity over CB1 was tested in rat cerebellar membranes at 10 μM concentrations (data not shown).

3.1. The three-fold symmetry of the analogs

The hit compound (19) or all of its synthesized or commercial analogs do not contain chiral centers. However, the molecules possess three-fold symmetry, which makes structure–activity relationship studies quite challenging. The structures cannot be superimposed unambiguously if compounds differ in more than one substituent. The analog library was designed in a such way that most of the synthesized compounds differ only with one or two substituents from the lead structure, whereas the commercial compounds offer more variability in the substituents.

In order to help the comparison of the structures, the substituents of the hit compound (19) are referred as follows: ethoxy (R^1), *N*-cyclopentyl (R^2) and 4-methylpiperidin-1-yl (R^3) (Fig. 2). Analogs are also classified according to the substituents which are changed compared to the starting structure; Compounds 24a–g (varying group R^1, Table 1), 25a–i (R^2, Table 2), 26a–g (R^3, Table 3), 27a–h (R^2 and R^3, Table 4), 28a–e (R^1 and R^3, Table 5), 29a–c (R^1 and R^2, Table 6) and 30a–r (varying all groups R^1–R^3, Table 7).

3.2. Changing the substituent in R^1 position (compounds 24a–g, Table 1)

All changes made in position R^1 decreased activity. However, the compound 24 g was the only completely inactive structure with its *N*-phenyl substituent, whereas other compounds were active in at least the high nanomolar range. The least disturbing modification was changing the ethoxy substituent to a propyloxy group (24c, $-\log EC_{50} = 7.1$). The important role of oxygen atom was revealed when ethoxy was changed to a plain *n*-propyl substituent (24b) or *n*-propyloxy was changed further to *n*-propylamine (24d). When changing *n*-propyloxy to its branched isomer (24e), the potency decreased. However, when the methyl groups of the