European Journal of Pharmaceutical Sciences 49 (2013) 423–433

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

1,3,4-Oxadiazol-2-ones as fatty-acid amide hydrolase and monoacylglycerol lipase inhibitors: Synthesis, in vitro evaluation and insight into potency and selectivity determinants by molecular modelling

Heikki Käsnänen [a,b], Anna Minkkilä [a,b], Susanna Taupila [a], Jayendra Z. Patel [a], Teija Parkkari [a], Maija Lahtela-Kakkonen [a], Susanna M. Saario [c], Tapio Nevalainen [a], Antti Poso [a,*]

[a] School of Pharmacy, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland
[b] Medicinal Chemistry, Orion Corporation Orion Pharma, P.O. Box 65, FI-02200 Espoo, Finland
[c] Charles River Discovery Research Services Finland Oy, Microkatu 1, FI-70210 Kuopio, Finland

ARTICLE INFO

Article history:
Received 14 January 2013
Received in revised form 28 February 2013
Accepted 18 March 2013
Available online 2 April 2013

Keywords:
Drug design
FAAH
MAGL
Inhibitors
Molecular docking

ABSTRACT

Inhibition of the key hydrolytic enzymes of the endocannabinoid system, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), has been proposed as potential mode of action for various therapeutic applications. Continuing our previous work, we take the first steps of structure–activity relationship exploration and show that 1,3,4-oxadiazol-2-ones can serve as scaffold for both selective FAAH and MAGL inhibitors, and also function as a dual FAAH/MAGL inhibitor at sub-micromolar IC_{50} values. Moreover, 10-fold selectivity against MAGL over FAAH was achieved with compound **3d** (FAAH and MAGL IC_{50}; 2.0 and 0.22 μM). Lastly, enzyme and ligand features contributing to the potency and selectivity differences are analysed by molecular docking.

1. Introduction

Fatty acid amide hydrolase (FAAH, EC 3.5.1.4) (Cravatt et al., 1996) and monoacylglycerol lipase (MAGL, EC 3.1.1.23, also known as monoglyceride lipase (MGL)) (Dinh et al., 2002) are the principal hydrolytic enzymes responsible for the degradation of the endogenous cannabinoids (endocannabinoids) *N*-arachidonoylethanolamine (anandamide, AEA) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995), respectively. Endocannabinoids are an important class of retrograde messenger lipids that are produced on demand, for instance, in response to elevated intracellular calcium levels. They recognise and activate the cannabinoid CB_1 (Devane et al., 1988) and CB_2 receptors (Munro et al., 1993), the molecular targets for the archetypical phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) (Gaoni and Mechoulam, 1964) evoking several physiological effects, such as antinociception (Di Marzo, 2008). These proteins and lipids constitute, together with specific transporters and additional hydrolases, an intricate signalling machinery termed the endocannabinoid system (ECS) (Blankman et al., 2007; Fu et al., 2011; Kaczocha et al., 2009; Moriconi et al., 2010; Tsuboi et al., 2005; Wei et al., 2006).

The in vivo cannabimimetic effects of AEA and 2-AG remain weak and transient owing to their rapid inactivation by FAAH and MAGL, respectively. On the other hand, blocking the activity of these enzymes by specific inhibitors (Wiskerke et al., 2012) or genetic knock-out (Taschler et al., 2011) increases local levels of endocannabinoids leading to lesser side effect potential compared to global CB receptor activation (Saario and Laitinen, 2007). In terms of pharmacotherapeutic applications of FAAH and MAGL inhibition, the landscape is presumed to be diverse (Di Marzo, 2009). Current in vivo evidence illustrates analgesia, anxiolysis, neuroprotection and tumor size reduction as putative therapeutic outcomes arising from blockade of these enzymes, but there is a plethora of other potential indications as well (Chen et al., 2012; Di Marzo, 2009; Malfitano et al., 2011; Petrosino and Di Marzo, 2010). Interestingly, concurrent dual inhibition of both FAAH and MAGL has been shown to display distinct physiological effects compared to individual inhibition of these enzymes (Long et al., 2009b, 2010; Naidoo et al., 2012).

Although the substrate specificity of FAAH and MAGL is partially intertwined (FAAH is also able to hydrolyse esters) (Patricelli and Cravatt, 1999), and both act as homodimers, these serine hydrolases are quite dissimilar from a structural and sequence

* Corresponding author. Tel.: +358 403552462; fax: +358 17 16 2424.
E-mail address: antti.poso@uef.fi (A. Poso).

0928-0987/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejps.2013.03.015

homology point of view. FAAH is a large (63 kD) integral enzyme belonging to the amidase signature (AS) superfamily (Patricelli et al., 1999). MAGL, on the other hand, is smaller (33 kD), and a member of α/β hydrolase superfamily, and it is found in both cytosolic and membrane-bound fractions (Blankman et al., 2007).

The majority of public FAAH and MAGL inhibitors have been designed from structural modifications of other serine hydrolase inhibitors that contain moderately or highly reactive groups targeted to the nucleophilic catalytic serine (for comprehensive listings and references, see excellent reviews by (Feledziak et al., 2012; Fowler, 2012; Petrosino and Di Marzo, 2010; Seierstad and Breitenbucher, 2008; Vandevoorde, 2008). Examples include classes such as α-keto heterocycles (Boger et al., 2000), carbamates (Tarzia et al., 2003), and carboxamides (Alexander and Cravatt, 2006) for FAAH, and carbamates (Chang et al., 2012; Kapanda et al., 2012; Long et al., 2009a), lactones (Bisogno et al., 2009), and carboxamides (Morera et al., 2012) for MAGL. Recently, however, some non-covalent and non-serine targeting FAAH inhibitors have been disclosed (Gustin et al., 2011; Scott et al., 2011). Additionally, azetidine amides have been introduced as non-covalent inhibitors of MAGL (Schalk-Hihi et al., 2011). An alternative approach for designing MAGL inhibitors has been the use of sulfhydryl-reactive groups targeting the active site cysteines (King et al., 2009; Labar et al., 2007; Saario et al., 2005).

Despite the extensive achievements in ECS field in academia and industry, no marketing authorisation for FAAH or MAGL inhibitors have been granted yet. Consequently, owing to the considerable and diverse therapeutic potential of FAAH and MAGL blockade, novel chemotypes are still desired to be used as tools and to offer alternative starting points for inhibitor research.

Oxadiazolone-based compounds have been reported to show inhibitory activities against various enzymes. Huang and Bushey (1987) demonstrated oxadiazolones as potent insecticides. Sanofi-Aventis has published a series of oxadiazolones as potent pancreatic lipase inhibitors (Schoenafinger et al., 2001, 2003). Moreover, oxadiazolones have been described inhibitors of hormone-sensitive lipase (HSL), FAAH and MAGL (Ben Ali et al., 2006; Muccioli et al., 2008; Minkkilä et al., 2009; Kiss et al., 2011). More recently, Cavalier and colleagues reported that oxadiazolone inhibitors have inhibitory activity against digestive lipases comparable to that of anti-obesity drug orlistat (Point et al., 2012).

In this work, we follow up our previous study (Minkkilä et al., 2009), and explore the structure activity relationships (SAR) of 3-phenyl derivatives of 1,3,4-oxadiazol-2-one core by synthesising 25 novel compounds, and by evaluating their inhibition potencies against FAAH and MAGL. We will show that this scaffold may serve as intriguing founding building block for both selective but also dual inhibitors of these enzymes. In addition, we explore the potency and selectivity determinants of the inhibitors by detailed molecular docking studies.

2. Materials and methods

2.1. Chemistry

Commercially available starting materials were used without further purification. Solvents were distilled or dried with molecular sieves prior to use. All dry reactions were performed under nitrogen in oven-dried glassware. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F_{254} (60 Å, 40–63 μm, 230–400 mesh) precoated aluminium sheets and detected under UV light. Purification of reaction products was carried out by flash chromatography (FC) on J.T. Bakers silica gel for chromatography (pore size 60 Å, particle size 50 nm). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.1 MHz for ^{1}H and 125.1 MHz for ^{13}C. Chemical shifts are reported in ppm on the δ scale from an internal standard of solvent ($CDCl_3$ 7.26 and 77.0 ppm; DMSO-d_6 2.50 and 39.52 ppm, the shifts for the deuterated standards are given compared to TMS). The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet, dd = doublets of doublets, qn = quintet. Elemental analyses (CHN) were recorded using a Thermo Quest CE Instrument EA 1110 CHNSO elemental analyser.

2.2. General procedures for preparation of 1,3,4-oxadiazol-2(3H)-ones

1,3,4-Oxadiazol-2(3*H*)-ones were prepared applying a similar method as described previously (Minkkilä et al., 2009): Methyl chloroformate (1.1 equiv.) was cautiously added dropwise to a solution of hydrazine carboxylate (for the preparation, see Supplementary data) (1.0 equiv.) and NMP (0.9 equiv.) in pyridine at 0 °C under nitrogen. The reaction mixture was stirred for 2 h at 0 °C and 1 h at rt, and then diluted with water. The product was extracted with EtOAc, and organic phase washed with brine and dried over Na_2SO_4. The solvent was evaporated and residue dried under vacuum. Purification by FC (eluent EtOAc/petroleum ether (PE), fraction 40–60 °C) and crystallization from EtOAc/hexane gave the title compounds.

2.2.1. 5-Methoxy-3-(3-methoxyphenyl)-1,3,4-oxadiazol-2(3H)-one (**1a**)

White solid (100 mg, 19%); ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 2H), 7.31 (t, J = 8.4 Hz, 1H), 6.78–6.76 (m, 1H), 4.11 (s, 3H), 3.84 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 160.2, 155.8, 148.2, 137.3, 130.0, 111.4, 110.1, 103.8, 57.7, 55.4; Elemental analysis calcd for $C_{10}H_{10}N_2O_4C$, 54.05; H, 4.54; N, 12.61, found C, 54.25; H, 4.59; N, 12.51.

2.2.2. 5-Methoxy-3-[3-(benzyloxy)phenyl]-1,3,4-oxadiazol-2(3H)-one (**1b**)

Light solid (288 mg, 55%); ^{1}H NMR ($CDCl_3$) δ 7.50–7.30 (m, 8H), 6.84 (dd, J = 8.2, 1.8 Hz, 1H), 5.10 (s, 2H), 4.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.3, 155.7, 148.2, 137.2, 136.6, 130.0, 128.6, 128.1, 127.6, 112.0, 110.3, 104.8, 70.1, 57.7; Elemental analysis calcd for $C_{16}H_{14}N_2O_4C$, 64.42; H, 4.73; N, 9.39, found C, 64.77; H, 4.80; N, 9.38.

2.2.3. 5-Methoxy-3-(3-octylphenyl)-1,3,4-oxadiazol-2(3H)-one (**1d**)

Light solid (70 mg, 46%); ^{1}H NMR ($CDCl_3$) δ 7.61–7.60 (m, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 4.11 (s, 3H), 2.64 (t, J = 7.7 Hz, 2H), 1.64–1.60 (m, 2H), 1.31–1.26 (m, 10H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 155.8, 148.3, 144.3, 136.1, 128.9, 125.8, 117.9, 115.4, 57.7, 36.0, 31.9, 31.4, 29.4, 29.3, 29.2, 22.7, 14.1; Elemental analysis calcd for $C_{17}H_{24}N_2O_3$ 2.3% hexanes C, 67.08; H, 7.95; N, 9.20, found C, 67.09; H, 8.22; N, 9.17.

2.2.4. 5-Methoxy-3-[3-(trifluoromethyl)phenyl]-1,3,4-oxadiazol-2(3H)-one (**1e**)

Yellow solid (270 mg, 38%); ^{1}H NMR ($CDCl_3$) δ 8.08–8.05 (m, 2H), 7.55 (t, J = 8.0 Hz, 1H), 7.48 (d, J_{CF} = 7.7 Hz, 2H), 4.14 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 156.0, 148.0, 136.6, 131.7 (q, $^2J_{CF}$ = 32.7 Hz), 129.8, 123.7 (q, $^1J_{CF}$ = 272.5 Hz),122.0 (q, $^3J_{CF}$ = 3.7 Hz), 120.7, 114.7 (q, $^3J_{CF}$ = 4.0 Hz), 57.9; Elemental analysis calcd for $C_{10}H_7F_3N_2O_3C$, 46.16; H, 2.71; N, 10.77, found C, 46.25; H, 2.62; N, 10.76.

2.2.5. 5-Methoxy-3-(3-chlorophenyl)-1,3,4-oxadiazol-2(3H)-one (**1f**)

White solid (220 mg, 42%); ^{1}H NMR ($CDCl_3$) δ 7.82 (t, J = 1.9 Hz, 1H), 7.76–7.34 (m, 1H), 7.34 (t, J = 8.1 Hz, 1H), 7.20–7.19 (m, 1H), 4.12 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 155.9, 148.0, 137.1, 135.0, 130.2,