European Journal of Medicinal Chemistry 136 (2017) 523–542

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Novel propanamides as fatty acid amide hydrolase inhibitors

Alessandro Deplano [a, 1], Carmine Marco Morgillo [c, 1], Monica Demurtas [a], Emmelie Björklund [b], Mariateresa Cipriano [b], Mona Svensson [b], Sanaz Hashemian [b], Giovanni Smaldone [d], Emilia Pedone [e], F. Javier Luque [f], Maria G. Cabiddu [g], Ettore Novellino [c], Christopher J. Fowler [b], Bruno Catalanotti [c, *], Valentina Onnis [a]

[a] Department of Life and Environmental Sciences, Unit of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, via Ospedale 72, Cagliari I-09124, Italy
[b] Department of Pharmacology and Clinical Neuroscience, Umeå University, SE-901 87 Umeå, Sweden
[c] Department of Pharmacy, Università Degli Studi di Napoli Federico II, Napoli, Italy
[d] IRCCS SDN, Via Emanuele Gianturco 113, 80143 Napoli, Italy
[e] Institute of Biostructures and Bioimaging, CNR, Naples, Italy
[f] Departament de Nutrició, Ciències de l'Alimentació i Gastronomia and Institut de Biomedicina (IBUB), Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Santa Coloma de Gramenet, Spain
[g] Department of Chemical and Geological Sciences, University of Cagliari, Italy

ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form
2 May 2017
Accepted 11 May 2017
Available online 12 May 2017

Keywords:
FAAH inhibitors
Heteroaryl propanamides
Fatty acid amide hydrolase
Endocannabinoids
Anandamide

ABSTRACT

Fatty acid amide hydrolase (FAAH) has a key role in the control of the cannabinoid signaling, through the hydrolysis of the endocannabinoids anandamide and in some tissues 2-arachidonoylglycerol. FAAH inhibition represents a promising strategy to activate the cannabinoid system, since it does not result in the psychotropic and peripheral side effects characterizing the agonists of the cannabinoid receptors. Here we present the discovery of a novel class of profen derivatives, the *N*-(heteroaryl)-2-(4-((2-(trifluoromethyl)pyridin-4-yl)amino)phenyl)propanamides, as FAAH inhibitors. Enzymatic assays showed potencies toward FAAH ranging from nanomolar to micromolar range, and the most compounds lack activity toward the two isoforms of cyclooxygenase. Extensive structure-activity studies and the definition of the binding mode for the lead compound of the series are also presented. Kinetic assays in rat and mouse FAAH on selected compounds of the series demonstrated that slight modifications of the chemical structure could influence the binding mode and give rise to competitive (**TPA1**) or non-competitive (**TPA14**) inhibition modes.

1. Introduction

Anandamide (AEA), 2-acylethanolamines and 2-arachidonoyl glycerol are signaling lipids belonging to the class of endocannabinoids, endogenous agonists of the cannabinoid receptor type-1 (CB_1) and type-2 (CB_2). The cannabinoid system regulates many physiological functions, both in the central and peripheral nervous systems and in peripheral organs. In order to maintain the normal functionality of nerve systems, many fatty acid amides, including AEA, are degraded by fatty acid amide hydrolase (FAAH), a member of the serine hydrolase enzyme family, characterized by the unusual catalytic triad Ser^{217}-Lys^{142}-Ser^{241}.

Blockade of FAAH activity is a suitable strategy to modulate the endocannabinoid system compared to exogenous cannabinoid receptor agonists, whose therapeutic profile is heavily limited by CB_1-mediated psychotropic and peripheral side effects such as impairment in cognition, motor coordination, and psychoses. It is now well established that the endocannabinoid system is involved in the regulation of mood, and in animal models of anxiety, and that FAAH inhibitors show a potentially useful profile [1]. Additionally, FAAH inhibitors may be useful in disorders ranging from cannabinoid dependence [2] to preventing or reducing the inflammatory process associated with Aβ deposition in Alzheimer's Disease [3] and in the treatment of comorbidity between psychological disorders and cardiac disease [4]. The principal classes of covalent and non-covalent inhibitors as well as the FAAH inhibitors entered into

* Corresponding author.
E-mail address: brucatal@unina.it (B. Catalanotti).
[1] These authors equally contribute to the present paper.

http://dx.doi.org/10.1016/j.ejmech.2017.05.033
0223-5234/

clinical studies have been recently reviewed [5]. Clinical studies so far undertaken indicate that FAAH inhibitors are generally well tolerated [6,7]. A serious brain injury was reported in a phase I study with the FAAH inhibitor BIA 10-2474, but this appears to be an off-target effect of the drug, not correlated to FAAH inhibition [8]. Indeed, a computer-based proteome-docking approach suggested that BIA 10-2474 could interact with several targets in a manner likely to cause brain haemorrhaging [9].

We previously reported that some NSAIDs as ibuprofen display modest FAAH inhibitory activity. The conversion of the carboxylic group of ibuprofen into heterocyclic amide namely 2-(4-isobutylphenyl)-*N*-(3-methylpyridin-2-yl)propanamide (**Ibu-AM5**) that showed high FAAH inhibitory activity, while retaining COX inhibitory properties of the parent ibuprofen [10–12]. Encouraged by these finding, we have been prompted to investigate further new **Ibu-AM5** analogs as potential FAAH inhibitors. Since superimposition of **Ibu-AM5** with FAAH co-crystallized inhibitors indicated that the isobutyl chain of **Ibu-AM5** overlap aromatic hydrophilic moieties, as a rational development we designed and synthesized a new series of compounds where the isobutyl group is replaced by a 2-(trifluoromethyl)pyridin-4-ylamino group (Fig. 1). In the present study we report the discovery, synthesis, pharmacological and biochemical characterization of these compounds, as well as their putative binding mode of the lead and finally the efforts to identify key structural features of this novel series of *N*-(heteroaryl)-2-(4-((2-(trifluoromethyl)pyridin-4-yl)amino)phenyl) propanamides as FAAH inhibitors. With the aim to define the critical requirements for FAAH inhibitory activity we have explored the effects of structural modification in the heteroaryl group, in the linker between the heteroaryl group and the phenylcarbonyl moiety, the effect of replacement of the methyl on the C-2 with smaller and larger substituents, and finally the effect of introducing different polar rings in the (trifluoromethyl)pyridine region.

2. Results and discussion

2.1. Chemistry

The synthesis of the target amides was undertaken as outlined in Schemes 1-6. The first step of the synthetic approach to the new series of TPA amides was based on a previously described procedure that allows the construction of the trifluoromethylpyridine ring linked by an amino nitrogen to an aromatic ring [13]. This synthetic approach was modified and successfully applied for the preparation of 2-(4-((2-(trifluoromethyl)pyridin-4-yl)amino)phenyl)propanoic acid (**7**) starting from the easily available 2-(4-nitrophenyl)propionic acid (**1**), which was first converted into its methyl ester and then submitted to reduction of the nitro group. The resulting methyl 2-(4-aminophenyl)propanoate **2** reacted with 1,1,1-trifluoro-4-methoxypent-3-en-2-one (**3**) in 1:1.5 molar ratio in refluxing acetonitrile (MeCN) solution to yield the enaminone **4** in good yields. Upon reacting with an excess of *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) in refluxing toluene, the enaminone **4** was converted into 1,1,1,trifluorohexadienone **5** in 88% yield. The treatment of intermediate **5** with ammonium acetate in DMF at 100 °C produced pyridine ring closure to ester **6** in 75% yield. Hydrolysis of compound **6** in hydroalcoholic sodium hydroxide solution afforded acid **7**.

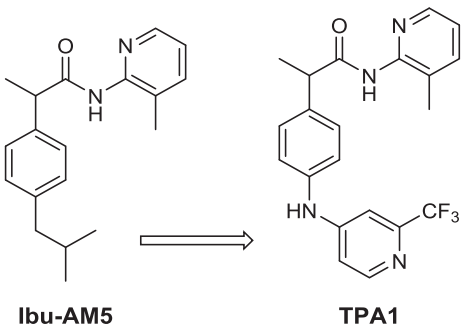

Fig. 1. Design of the representative member TPA1 from Ibu-AM5.

Finally, treatment of acid **7** with the appropriate amine in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) in MeCN solution gave amides **TPA1-16, 18-21** in moderate to good yields.

In order to study the structure-activity relationship of TPA compounds and to discover the better modification to improve the activity as FAAH inhibitors, we modified all moieties of the molecule.

First we focused our attention upon the 3-methylpyridin-2-yl moiety. To evaluate the effect of the presence of the methyl group and of its position on the pyridine ring upon the FAAH inhibitory properties. Specifically, the 2-aminopyridine, 3-aminopyridine and 4-aminopyridine analogs **TPA4-6** and the 5-methyl **TPA2** and 4-methyl **TPA3** analogs were prepared as reported in Scheme 1. To evaluate the effect of pyridine replacement by other rings, the phenyl analog **TPA7**, the pyrimidine (**TPA8**), pyrazine (**TPA9**), quinoline (**TPA10**) and arylpiperazino analogues (**TPA18–21**) were also prepared (Scheme 1). Finally, the 3-methylpyrido group was replaced with halogens and trifluoromethyl group (**TPA11–14**).

To investigate the importance for FAAH inhibition of the distance between the carbonyl group and the pyridine ring, the acid **7** was condensed with 2-picolylamine and 3-picolylamine to obtain amides **TPA15** and **TPA16** respectively (Scheme 1). Further linker extension was accomplished through introduction of a second amide moiety to give **TPA17**. As indicated in Scheme 2, **TPA17** was prepared by condensation between **7** and ethyl glycinate hydrochloride using the EDC method. The obtained ester **8** was hydrolyzed under basic conditions to obtain compound **9**. This acid was finally condensed with the 2-amino-3-methylpiridine using the EDC method to obtain the amide **TPA17.**

To examine the importance of the substituent on C-α to the carbonyl group firstly, a slight modification of the procedure used to afford acid **7** was employed. Thus, the unsubstituted derivative **TPA22** was prepared starting from the methyl ester of the 2-(4-aminophenyl)acetic acid (**10**). The obtained acid **14** was subsequently condensed with 2-amino-3-methylpyridine using EDC method (Scheme 3).

To introduce a second methyl or a cycloalkyl group on the C-α 2-(4-nitrophenyl)acetic acid was converted into its methyl ester derivative **15**, which was treated with iodomethane in DMF solution in the presence of sodium hydride to obtain intermediate **16**.The nitro group of **16** was reduced into its corresponding amine (**17**). Compound **17** was used as starting material for the construction of the trifluoromethylpyridine ring through the reaction sequence above described and then the acid **21** was converted into **TPA23** by EDC method (Scheme 4).

TPA24–27 were prepared in an analogous manner starting from the nitro derivative **15** and the appropriate di-halogen derivative, namely 1,2-dibromoethane, 1,3-diiodopropane, 1,4-diiodobutane, 1,5-diiodopentane respectively that were reacted modifying a described method [14] (Scheme 5).

Finally, we investigated the importance for FAAH inhibition of the trifluoromethylpyridine ring. To this purpose, the ester **2** was hydrolyzed to the acid **46.** That is subsequently treated with 4-chloro-8-trifluoromethylquinoline and 4-chloro-7-trifluoromethylquinoline to obtain the corresponding acids **47** and **48**, which are condensed with 2-amino-3-methylpyridine by