MedChemComm

CONCISE ARTICLE

Cite this: Med. Chem. Commun., 2014, 5, 932

Received 22nd April 2014 Accepted 15th May 2014 DOI: 10.1039/c4md00181h www.rsc.org/medchemcomm ω -Heteroarylalkylcarbamates as inhibitors of fatty acid amide hydrolase (FAAH)†

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Fatty acid amide hydrolase (FAAH) is a serine hydrolase that terminates the analgetic and anti-inflammatory effects of endocannabinoids such as anandamide. Herein we describe structure–activity relationship studies on a new series of w-heteroarylalkylcarbamate inhibitors of FAAH. The most active compounds exhibit IC_{50} values in the low nanomolar range. Investigations on selectivity and metabolic stability of these inhibitors are also presented.

Anandamide is one principal endocannabinoid in the mammalian organism.^{1,2} It is formed "on demand" during several pathological disorders and mediates analgetic and antiinflammatory effects by activation of the cannabinoid receptors $CB₁$ and $CB₂$. These two receptors are differentially distributed within the various cells.^{3–5} While the CB_1 receptor is preferentially expressed in neuronal tissues, the $CB₂$ receptor occurs mainly in cells of the immune system such as macrophages.

An important enzyme in the endocannabinoid metabolism is fatty acid amide hydrolase (FAAH), which rapidly cleaves the lipid mediator anandamide into arachidonic acid and ethanolamine.⁶–⁹ Inhibition of FAAH is supposed to potentiate the action of anandamide. Therefore, inhibitors of FAAH may represent new agents against pain and inflammation. In the past years many potent inhibitors of FAAH have been found.¹⁰–¹⁴ The majority of these compounds are substances, which form covalent binding interactions with the catalytic serine of the active site of FAAH such as carbamates like 1 (URB 597)¹⁵ and 2, ¹⁶ activated ketone derivatives like the a-ketoheterocycle 3 $(PHOP)^{17}$ and the propan-2-one $4, ^{18}$ (Fig. 1), and urea derivatives like 5 (PF-04457845).¹⁹ The latter compound is being examined in clinical studies for fear response and cannabis withdrawl.²⁰

Several years ago we have described dual inhibitors of cyclooxygenase and 5-lipoxygenase, which contain a pyrrole scaffold with vicinal phenyl substituents $(e.g. 6)^{21}$ (Fig. 2) showing structural similarities with the heterocyclic part of the FAAH inhibitor 2. ¹⁶ This initiated our interest in the question, in which way inhibitory potency of 2 is influenced when the 2-methyl-4,5-diphenylimidazole system of 2 is replaced by a 2,3-diphenyl-5-methylpyrrole substituent. Moreover, this pyrrole ring system offered the opportunity to introduce further

substituents into the ring at the free carbon site, which could lead to additional binding interactions with FAAH. Therefore, we have synthesized the pyrrole analogue of 2 and used it as starting point for structure–activity relationship studies.

The pyrrolyl-substituted N-alkylphenylcarbamates investigated were synthesized using one of the three routes outlined in Scheme 1. In the first instance, the appropriate nitrogen heterocycle was alkylated with a BOC-protected ω -bromoalkylamine followed by cleaving of the BOC group with trifluoroacetic acid and reaction of obtained amine with phenyl chloroformate. Alternatively, the heterocycle was alkylated with a dibromoalkane. The obtained bromoalkyl compound was treated with potassium phthalimide yielding a phthalimide-protected alkylamine. Removal of the protecting group was accomplished using hydrazine hydrate and the formed amine was directly reacted with phenyl chloroformate to yield the target compound. Instead, the phthalimide intermediate was directly prepared by reaction of the nitrogen heterocycle with w-bromoalkyphthalimide as shown for the synthesis of 27 and 30. CONCISE ARTICLE
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Fig. 1 Structure of known inhibitors of FAAH

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[†] Electronic supplementary information (ESI) available: Syntheses and analytical data of the test compounds; procedures for biological evaluation. See DOI: 10.1039/c4md00181h

Fig. 2 Structure of the dual cyclooxygenase–5-lipoxygenase inhibitor 6

Scheme 1 Reagents and conditions: (a) tert-butyl-N-(6-bromohexyl) carbamate–tert-butyl-N-(2-bromoethyl)carbamate, K-tert-butylate, DMSO, 60/80 °C; (b) (1) trifluoroacetic acid, CH_2Cl_2 , 0 °C; (2) phenyl chloroformate, triethylamine, CH₂Cl₂, room temp.; (c) 1,4-dibromobutane, tetrabutylammonium bromide, toluene, 50% aqueous NaOH, 45 °C; (d) potassium phthalimide, DMF, 130 °C; (e) (1) hydrazinehydrate, ethanol, reflux; (2) phenyl chloroformate, triethylamine, CH2Cl2, room temp.; (f) N-(6-bromohexyl)phthalimide, K-tert-butylate, DMSO, 50/70 °C; (g) (1) hydrazine-hydrate, ethanol, reflux; (2) phenyl chloroformate, triethylamine, CH_2Cl_2 , 0 °C to room temp.

The related derivatives with indole, indazole, imidazole and benzotriazole heterocycle were prepared similarly to Method C of Scheme 1 using K-tert-butylate–DMSO, NaH–DMSO, NaH– DMF, K_2CO_3 -DMSO and K_2CO_3 -acetonitrile, respectively, for the N-alkylation of the appropriate heterocycle with $N-(\omega$ -bromoalkyl)phthalimide.²²

The indole derivatives with 3-pyridyl carbamate residues (52 and 54) were synthesized with the latter method utilizing 3-pyridyl chloroformate, synthesized from diphosgene and pyridin-3-ol, instead of phenyl chloroformate in the last step (Scheme 2).

Inhibition of FAAH hydrolase by the test compounds was determined with an assay using microsomes from rat brain as enzyme source and N-(2-hydroxyethyl)-4-pyren-1-ylbutanamide as fluorogenic substrate.²³ Inhibitory potencies of the tested compounds were assessed by comparing the amount of 4-pyren-1-ylbutanoic acid released from the substrate in their absence and presence after an incubation time of 60 min by reversedphase HPLC with fluorescence detection (see ESI[†]). With this assay for the diphenylimidazole 2 an IC_{50} of 0.35 μ M was measured (Table 1). Replacement of the imidazole scaffold of 2 by a pyrrole (9) led to an about sevenfold loss of activity. Shortening of the alkyl chain connecting the carbamate group and the pyrrole heterocycle from six to four and two carbon atoms further decreased inhibitory potency. With an IC_{50} of 6.2 μ M the butyl derivative 13 was only about half as active as the hexyl compound 9. The derivative with an ethyl linker (15) even was inactive at the highest test concentration of 10 μ M. Because removing of the methyl group in position 2 of the imidazole ring of 2 did not change the activity of the compound,²⁴ the impact of the corresponding methyl group of the pyrrole 9 on inhibitory potency was not examined.

Introduction of polar ethoxycarbonyl, acetyl and cyano substituents at the free position of the pyrrole ring of 9 was also detrimental. The inhibition values of obtained compounds 18, 21 and 24 were in the magnitude of 10 μ M (Table 2). Interestingly, moving the phenyl group from position 3 to position 4 of the pyrrole ring, to give compound 27, as well as completely removing the phenyl ring, to give compound 30, significantly increased FAAH inhibition. The activity of these two inhibitors was comparable to that of the diphenylimidazole 2.

Similar results were obtained with analogous indole derivatives (Table 3). With IC_{50} values of about 5 μ M the 2,3-diphenylindole 32 and the 3-phenylindole 34 possessed the lowest potency of the series. The 2-phenylindole 36 was about tenfold more active than the 3-phenylderivative 34. Thus, a phenyl substituent is much better tolerated in position 2 than in position 3 of the indole scaffold. The indole derivative 38, which has no phenyl-substituents in 2- and 3-position, possessed the highest activity $(IC_{50}$ 0.25μ M) showing that phenyl substituents are even unfavourable.

Scheme 2 Reagents and conditions: (a) $N-(\omega-\text{bromoalkyl})$ phthalimide, NaH, DMSO, 100 °C; (b) (1) hydrazine-hydrate, ethanol, reflux, 2 h; (2) trichloromethyl chloroformate, pyridin-3-ol, ethyl(diisopropyl) amine, CH_2Cl_2 , THF, - 30 °C to room temp.

Table 1 Inhibition of FAAH

 a Values are the means of at least two independent determinations, errors are within $\pm 20\%$. $\overset{b}{}$ n.a.: not active at 10 uM.

Table 2 Inhibition of FAAH

 a Values are the means of at least two independent determinations, errors are within \pm 20%. $\frac{b}{21\%}$ inhibition at 10 µM. $\frac{c}{33\%}$ inhibition at $10 \mu M$.

Next the effect of an introduction of nitrogen atoms in position 2 and 3 of the indole ring of 38 was studied. The indazole and benzotriazole derivatives 40 and 42 bearing a nitrogen in position 2 of the heterocycle were about three- to fourfold less active than the indole 38 (Table 4). In contrast, the imidazole derivative 44 possessing an additional nitrogen only in position 3 was equipotent to the indole 38.

Taken together, replacement of the diphenylpyrrole heterocycle from the starting compound 9 by an unsubstituted indole (38) led to an about tenfold increase of FAAH inhibitory potency. At this point we wanted to evaluate the role of the alkyl linker of 38 on activity in more detail. Both shortening and elongation of the hexyl chain by one carbon increased activity about twofold: the IC_{50} values of the pentyl and heptyl derivatives 47 and 49

Table 3 Inhibition of FAAH

 a Values are the means of at least two independent determinations, errors are within \pm 20%.

were about 0.09 μ M (Table 5). A more pronounced variation of the chain length to four (45) or eight (51) carbons resulted in a reduction of FAAH inhibition.

In literature a series of highly potent 3-pyridyl carbamate inhibitors of FAAH is described.²⁵ Therefore, we finally wanted to investigate the effect of a replacement of the phenyl group of the carbamates 47 and 38 by a 3-pyridyl residue. This structural variation led to a drastic increase of activity (Table 5). With IC_{50} values of 0.0036 μ M and 0.0052 μ M obtained compounds 52 and 54 were about as active as the α -ketoheterocycle 3 (PHOP), which counts to the most active inhibitors of FAAH known today. MedChemComm Your May 2014. Downloaded on 16 May 2014. Downloaded on 16 May 2014. Downloaded the main of 16 May 2014. Downloaded the main of 16 May 2022 11 May 2022 12:32 PM 2022 12:32 PM 2022 12:32 PM 2022 12:32 PM 2022 1

Besides FAAH, monoacylglycerol lipase (MAGL) is another important enzyme during the process of endocannabinoid inactivation. Just like FAAH, MAGL contains a catalytic serine in the active site. The main substrate of MAGL is 2-arachidonoyl glycerol. To get some information about the specificity of the developed substance class, some of the new FAAH inhibitors were also tested for MAGL inhibition.²⁶ As shown in Table 5, the evaluated phenyl ω-(indol-1-yl)alkylcarbamates did not inhibit MAGL at the highest test concentration of 10 μ M. Contrary, with

 a Values are the means of at least two independent determinations, errors are within \pm 20%.

Table 5 Inhibitory potency against FAAH and MAGL and stability in biological environments of several ω -indolylalkylcarbamates

^a Values are the means of at least two independent determinations, errors are within $\pm 20\%$. ^b Percent of parent remaining after incubation with rat liver S9 fractions for 30 min, in presence of the co-factor NADPH ϵ Percent of parent remaining after incubation with porcine blood plasma for 30 min; values are means \pm standard deviations of independent determinations ($n = 3$). ^d n.a.: not active at 10 µM. ^e n.t.: not tested.

 IC_{50} values in the submicromolar range, the corresponding pyridyl carbamates 52 and 54 inhibited MAGL significantly. However, the inhibitory potency of these substances against FAAH is still 60- to 90-fold higher than against MAGL.

Another enzyme of the serine hydrolase family is cytosolic phospholipase $A_2\alpha$ (cPLA₂ α), which catalyzes the first step of the so called arachidonic acid cascade by cleaving membrane phospholipids in arachidonic acid and lysophospholipids. At the highest test concentration of 10 μ M cPLA₂ α was not inhibited²⁷ by the indole compounds listed in Table 5. These results indicate that the inhibitors investigated display a certain specificity with regard to their ability to inhibit serine hydrolase enzymes.

Since carbamates are known to be susceptible to hydrolysis in biological environments²⁸⁻³⁰ leading to the inactivation of the compounds, we also measured the stability of some of the new substances in rat liver S9 fractions in presence of the co-factor NADPH³¹ and in porcine blood plasma (Table 5). After incubation in the liver S9 fractions for 30 min, still about 50% of the selected indole compounds were present in the incubation mixture. For comparison, the metabolism rate of the known FAAH inhibitor URB 597, which also exhibits a carbamate structure, was significantly higher. Here, only about 13% of the parent compound could be detected after the metabolic reactions. Incubation in blood plasma for 30 min revealed striking different results. The pyridin-3-yloxy carbamates 52 and 54 had totally disappeared at the end of the trial most likely due to an extensive hydrolysis or transcarbamoylation. The stability of the phenoxycarbamates depended on the chain length of the alkyl spacer. The pentylsubstituted compound 47 exhibited high stability, with 91% parent remaining after incubation. In contrast, only 39% of the heptyl derivative 49 could be detected under the same conditions.

Conclusions

In conclusion, starting from the lead compounds 2 and 9, respectively, a pronounced increase of FAAH inhibitory potency could be attained by only a few structural variations. The most active compounds synthesized (52 and 54) exhibited IC_{50} values in the low nanomolar range.

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