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## CONCISE ARTICLE

## Design, synthesis, and structure–activity relationships of 1,3,4-oxadiazol-2(3*H*)-ones as novel FAAH inhibitors†

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Novel 5-aryloxy substituted 3-phenyl-1,3,4-oxadiazol-2(3*H*)-ones were prepared and identified as potent inhibitors of FAAH. *In vitro* SAR are discussed. Structural variations of the selected lead compound were explored in order to optimise *in vivo* efficacy and selectivity.

### Introduction

First synthesised in 1899,<sup>1</sup> 3-phenyl-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one (**1a**) attracted little attention until the 1970's, when analogues such as **1b** and **1c** were shown to possess anthelmintic properties.<sup>2</sup> Later, the 5-phenoxy derivatives **2a** and **2b** were demonstrated to have similar biological activity (Fig. 1).<sup>3</sup>

In the early 2000's, 5-alkoxy derivatives (e.g. **3** and **4**) were developed for the treatment of obesity and diabetes, based on their ability to inhibit pancreatic lipase (PL) and hormone-sensitive lipase (HSL).<sup>4</sup> The 3-phenyl-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one fragment also contributes a significant part of the structure of certain benzoylureas (e.g. **5**) reported for the treatment of type 2 diabetes (Fig. 2).<sup>5</sup>

We envisaged that the 3-phenyl-1,3,4-oxadiazol-2(3*H*)-one moiety could also serve as a useful template for the design of improved fatty acid amide hydrolase (FAAH) inhibitors. FAAH, like PL and HSL, is a member of the extensive family of

serine hydrolases<sup>6</sup> and catalyses the degradation of a class of lipid signalling fatty acid amides, including oleamide and anandamide.<sup>7</sup> Anandamide elicits a range of biological responses with potential therapeutic relevance for the treatment of pain and inflammation amongst other disorders, and therefore FAAH has become recognised as a promising therapeutic target.<sup>8</sup> Accordingly, numerous small molecule inhibitors belonging to various

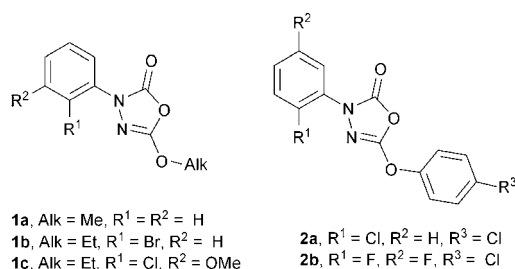


Fig. 1

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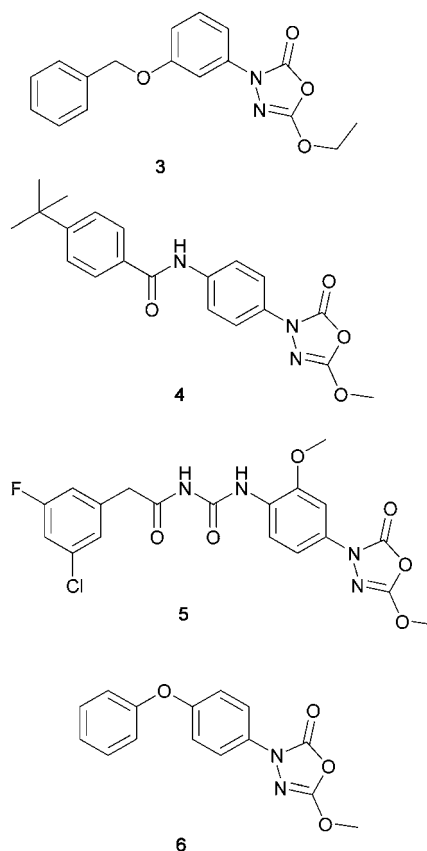


Fig. 2

chemical classes have been reported (for a recent review see<sup>9</sup> and references cited therein).

Our group submitted a patent application (with a priority date of December 2007) claiming a novel series of 3,5-disubstituted 1,3,4-oxadiazol-2(3*H*)-ones as potent FAAH inhibitors,<sup>10</sup> derived from the initial screening hit **7** (3-(3-chlorophenyl)-5-methoxy-1,3,4-oxadiazol-2(3*H*)-one) (Fig. 3). A few weeks before this disclosure reached the public domain, FAAH inhibitory activity was reported for several HSL inhibitors, including seven 3-phenyl-1,3,4-oxadiazol-2(3*H*)-ones,<sup>11</sup> with compound **6** (Fig. 2) clearly the most potent inhibitor *in vitro* with an IC<sub>50</sub> of 6.1 nM.

Extremely limited structure–activity relationship (SAR) data was disclosed in this basic screening study, with the curious exception that replacement of the 5-methoxy group with a 5-phenoxy substituent resulted in a 10-fold decrease in potency *in vitro*. This is in stark contrast to findings within our own series. Furthermore, since only *in vitro* data was reported, no assessment of efficacy of FAAH inhibition *in vivo* could be established. Thus, we herein report the synthesis and explore the SAR of a distinct series of 3,5-disubstituted-1,3,4-oxadiazol-2(3*H*)-ones and reveal an unexpected highly selective preference for peripheral FAAH inhibition *in vivo*.

## Chemistry

The synthetic route to obtain the necessary key intermediates from commercially available reagents is briefly outlined in Scheme 1.

The relatively unstable 3,4-dimethoxy phenylhydrazine **10a** was prepared as previously described<sup>12</sup> by reaction of the Grignard reagent derived from **8** with diethylazodicarboxylate (DEAD) and subsequent hydrolysis of the intermediate **9** thereby obtained.

Commercially unavailable phenyl chloroformates were prepared by reacting appropriately substituted phenols **11a–d** with phosgene in toluene in the presence of *N,N*-dimethylaniline<sup>13</sup> to give **12a–d** in excellent yield (85–95%). Construction of the oxadiazolone ring was accomplished *via* the general method depicted in Scheme 2. Hydrazines **10a–y**, were acylated with selected acyl chlorides **12a–y** in *N*-methylpyrrolidin-2-one (NMP) containing pyridine. Intermediates **13a–y** thus obtained were treated with phosgene in dichloromethane at room temperature to give the target compounds.<sup>14</sup>

## Results and discussion

*In vitro* FAAH inhibition was determined in rat brain homogenates, using tritium (<sup>3</sup>H) labelled anandamide (AEA) by measuring the formation of <sup>3</sup>H-ethanolamine as previously

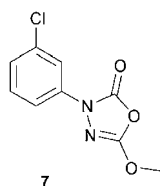
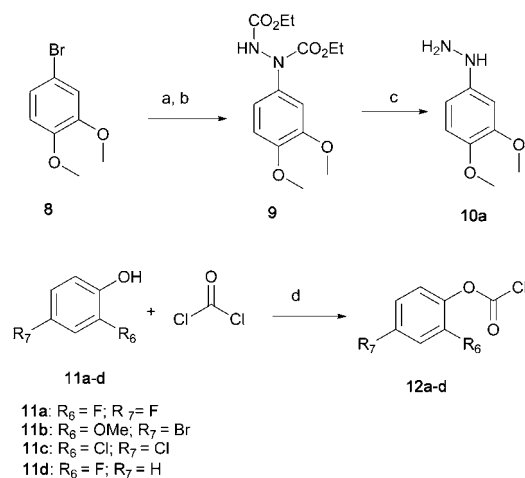


Fig. 3



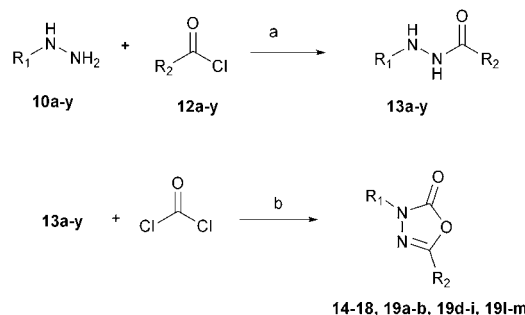
**Scheme 1** Reagents and conditions: (a) Mg, THF, cat. 1,2-dibromoethane, 80 °C. (b) DEAD, THF, –78 °C, then rt. (c) KOH, IPA-water, reflux (d). *N,N*-dimethylaniline, toluene, 0 °C.

described.<sup>15</sup> The results for a representative selection of new compounds are provided in Table 1. The initial screening hit **7** displayed fairly promising activity (95% inhibition at a concentration of 10 μM). The low molecular weight of **7**, in conjunction with a fairly simple synthetic route, led us to consider this molecule as a suitable starting point for a hit-to-lead optimization programme. In the first phase, variation of the side chains (**R<sub>1</sub>** and **R<sub>2</sub>**) by alternative groups was investigated.

Substitution of the methoxy group of **7** with *N*-morpholinyl **14**, phenyl **15**, benzoyl **16**, benzyl **17** or benzyloxy **18** residues completely abolished or drastically reduced FAAH inhibition.

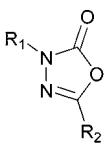
However, it was then discovered that inhibition could be completely restored by incorporation of a 5-phenoxy group as in **19a**. This immediately provided clarification that an *O*-substituent at position 5 of the oxadiazolone ring is absolutely essential for activity against FAAH.

Compound **19a** was thus selected as an early lead for further optimization. Various 5-phenoxy-3-phenyl-1,3,4-oxadiazol-2(3*H*)-one derivatives were initially evaluated *in vitro* at two different concentrations (IC<sub>50</sub> values were determined for compounds showing >50% inhibition at 0.1 μM). Whilst lead **19a** exhibited 45% inhibition at a concentration of 0.1 μM, it was found to be completely inactive at a ten-fold lower concentration (Table 2).



**Scheme 2** (a) Pyridine, NMP, 0 °C, then rt. (b) Phosgene, DCM, 0 °C, then rt.

**Table 1** *In vitro* FAAH inhibition by selected 1,3,4-oxadiazol-2(3*H*)-ones in rat brain homogenate

|  |                |                         |                             |
|---|----------------|-------------------------|-----------------------------|
| No.   | R <sub>1</sub> | R <sub>2</sub>          | % of Control <sup>a,b</sup> |
| 7   | –(3-Cl-Ph)     | –OCH <sub>3</sub>       | 5 ± 0                       |
| 14  | –(3-Cl-Ph)     | – <i>N</i> -morpholinyl | 104 ± 14                    |
| 15  | –Ph            | –Ph                     | 110 ± 10                    |
| 16  | –Ph            | –(CO)Ph                 | 84 ± 9                      |
| 17  | –Ph            | –Bn                     | 97 ± 3                      |
| 18  | –Bn            | –OBn                    | 86 ± 3                      |
| 19a   | –Ph            | –OPh                    | 1 ± 0                       |

<sup>a</sup> C<sub>inhib.</sub> = 10 μM. <sup>b</sup> Results are mean ± SEMs of four experiments.

The effects on FAAH inhibitory activity of different substituents on the 3-phenyl- and 5-phenoxy rings of the 1,3,4-oxadiazol-2(3*H*)-one nucleus are shown in Table 2. The 4-methoxy derivative **19b** displayed increased inhibition over the parent **19a**, whereas removal of the methyl group led to the inactive phenol **19c**.

The inclusion of halogen atoms was found to be favourable. The 4-chloro derivative **19d** displayed promising inhibition (69% at 0.1 μM) which was surpassed by the fluoro-analogue **19e** with the 4-bromo-2-methoxy analogue **19f** essentially equipotent. On the other hand, introduction of strongly electron-withdrawing substituents (**19g**, cyano and **19h**, nitro) on the “west-side” of the molecule appeared to be less favourable. A methoxy group at the *ortho* position of the 3-phenyl ring (**19i**) was not tolerated at all. However, the presence of a hydroxyl group at either the *para*-(**19j**) or *meta* position (**19k**) resulted in an approximately two to three fold increase in FAAH inhibition compared to **19a**. The presence of fluorine at either the *ortho*- (**19l**) or *para* (**19m**) positions was also found to be beneficial in terms of potency.

All compounds for which IC<sub>50</sub> values were determined were then subsequently assessed for *in vivo* FAAH inhibition. Compounds **19b**, **19d–f** and **19j–k** were administered orally to mice at a dose of 30 mg/kg. Thereafter, at 1 h post-administration, the animals were sacrificed and FAAH activity was determined in liver and brain. As seen in Table 2, despite the promising *in vitro* activity of most of these compounds (**19b**, **19d–f** and **19l–m**), only the difluoro analogue **19e** showed evidence of *in vivo* efficacy. Somewhat surprisingly, the at least ten-fold less active hydroxy compounds (**19j–k**) *in vitro* showed good *in vivo* inhibition with a clear preference for peripheral FAAH. A hydroxyl group in either the *meta*- or *para* position was found to be essential for high inhibition, indicating that this substituent is likely to be involved in binding to the protein. Furthermore, the presence of this polar, hydrogen bond donating group should decrease the overall lipophilicity of the compounds whilst simultaneously increasing aqueous solubility. These characteristics could contrive to restrict permeation of the molecules across the blood-brain barrier, resulting in selective peripheral

FAAH inhibition. Thus, subsequent efforts focussed on this hydroxyl series (**19j–k**), leading to the elaboration of derivatives **19n–y** shown in the lower half of Table 2. From the *in vitro* SAR, introduction of electron-withdrawing substituents on the 5-phenoxy ring was found to be clearly beneficial, such that all of these compounds presented IC<sub>50</sub> values in the low nanomolar range. In parallel with the *in vitro* assay, the ability of compounds **19n–y** to inhibit FAAH in mice was investigated. The *para*-nitro substituted derivative **19n** was found to be equipotent to the parent hydroxy compound **19j**. Replacement of the nitro group with chlorine as in **19o** gave a compound that demonstrated an approximately two-fold improvement in potency for liver FAAH but which also performed reasonably well in the brain. Incorporation of a second chlorine atom (**19p**) provided a further increase in liver FAAH inhibition with more enhanced selectivity. Conversely, the *ortho*-fluoro analogue **19q** failed to provide enhanced inhibition over the parent compound **19j**, although the *para*-fluoro isomer **19r** exhibited slightly higher activity. Combining the substitution patterns of compounds **19q** and **19r** resulted in the 2,4-difluoro derivative **19s**, which was equipotent to the dichloro analogue **19p** in the periphery with no traceable effect in the brain. As mentioned earlier, the introduction of a fluorine atom in the *ortho* position of the 3-phenyl ring (**19l**) provided a dramatic increase in *in vitro* potency. Based on this finding, we were prompted to synthesise the corresponding 3-(2-fluorophenyl) analogue of **19o**. Gratifyingly, the resulting compound **19t** exhibited five-fold greater efficacy over the parent **19o** with considerably improved peripheral selectivity.

For completeness, a few examples of *meta*-hydroxy substituted derivatives (**19u–w**) were prepared for *in vivo* comparison to their *para*-hydroxy counterparts (**19o** and **19r–s**). Compounds **19u–w** were generally endowed with more enhanced liver FAAH inhibition over **19o** and **19r–s**, and furthermore they were found to be exquisitely peripherally selective inhibitors of FAAH. Thereafter, we were encouraged to consider introducing a further hydroxyl group to these molecules. Although the more lipophilic difluoro catechol **19x** demonstrated some degree of central inhibition, the mono-chloro catechol **19y** exhibited potent, highly selective peripheral FAAH inhibition.

Finally, we examined the time-dependent FAAH inhibition by those compounds that provided over 90% *in vivo* FAAH inhibition. Table 3 details the differences in the FAAH inhibitory profiles of compounds **19p**, **19t** and **19x–y** in mouse liver and brain homogenates.

Compounds **19p** and **19t** were found to achieve maximum inhibitory effect in the liver at 1 h after oral administration at a dose of 30 mg kg<sup>–1</sup>. Thereafter they sustained constant inhibition of FAAH over the next 7 h, followed by a gradual return to baseline levels at 24 h post-administration. The extent of central FAAH inhibition by oxadiazolones **19p** and **19t** was markedly lower compared to peripheral inhibition and hovered around the 50% level up to 8 h post-dose. Catechols **19x–y** were orally administered to mice at a lower dose of 10 mg/kg. Both compounds achieved 90–95% FAAH inhibition in the liver within 1 h post-dose and had no measurable effect (Table 2) in the brain at any time-point. This finding may be of therapeutic relevance, as the purely peripheral selective nature of FAAH inhibition by compounds **19x–y** could serve to reduce potential side effects caused by inhibition of FAAH in the central nervous system.

**Table 2** *In vitro* (in rat brain homogenate) and *in vivo* (in homogenates of mouse liver and brain) FAAH inhibition by selected 5-Phenoxy-3-phenyl-1,3,4-oxadiazol-2(3*H*)-ones

| No. | R <sub>3</sub> | R <sub>4</sub>   | R <sub>5</sub>    | R <sub>6</sub>    | R <sub>7</sub>    | 0.1 μM <sup>a,c</sup> | 0.01 μM <sup>a,c</sup> | Liver <sup>b,c</sup> | Brain <sup>b,c</sup> | IC <sub>50</sub> nM <sup>d</sup> |
|-----|----------------|------------------|-------------------|-------------------|-------------------|-----------------------|------------------------|----------------------|----------------------|----------------------------------|
| 19a | H              | H                | H                 | H                 | H                 | 56 ± 7                | 97 ± 9                 | ND                   | ND                   | ND                               |
| 19b | H              | H                | H                 | H                 | –OCH <sub>3</sub> | 7 ± 1                 | 61 ± 2                 | 94 ± 4               | 108 ± 6              | 16 (11; 21)                      |
| 19c | H              | H                | H                 | H                 | –OH               | 80 ± 14               | ND                     | ND                   | ND                   | ND                               |
| 19d | H              | H                | H                 | H                 | Cl                | 6 ± 2                 | 31 ± 4                 | 81 ± 9               | 110 ± 1              | 5 (4; 5)                         |
| 19e | H              | H                | H                 | F                 | F                 | 1 ± 0                 | 14 ± 1                 | 39 ± 10              | 94 ± 13              | 3 (2; 4)                         |
| 19f | H              | H                | H                 | –OCH <sub>3</sub> | Br                | 1 ± 0                 | 16 ± 1                 | 83 ± 6               | 101 ± 9              | 4 (3; 6)                         |
| 19g | –CN            | H                | H                 | H                 | H                 | 96 ± 3                | ND                     | ND                   | ND                   | ND                               |
| 19h | H              | –NO <sub>2</sub> | H                 | H                 | H                 | 78 ± 5                | 103 ± 10               | ND                   | ND                   | ND                               |
| 19i | H              | H                | –OCH <sub>3</sub> | H                 | H                 | 112 ± 19              | ND                     | ND                   | ND                   | ND                               |
| 19j | –OH            | H                | H                 | H                 | H                 | 28 ± 3                | 91 ± 8                 | 46 ± 15              | 97 ± 6               | 52 (43; 63)                      |
| 19k | H              | –OH              | H                 | H                 | H                 | 14 ± 3                | 90 ± 15                | 33 ± 7               | 95 ± 2               | 32 (21; 46)                      |
| 19l | H              | H                | F                 | H                 | H                 | 3 ± 0                 | 43 ± 8                 | 72 ± 8               | 97 ± 2               | 7 (5; 10)                        |
| 19m | F              | H                | F                 | H                 | H                 | 3 ± 0                 | 46 ± 2                 | 65 ± 10              | 86 ± 2               | 9 (6; 12)                        |
| 19n | –OH            | H                | H                 | H                 | –NO <sub>2</sub>  | 0 ± 0                 | 5 ± 1                  | 45 ± 16              | 96 ± 4               | 2 (1; 2)                         |
| 19o | –OH            | H                | H                 | H                 | Cl                | 0 ± 0                 | 6 ± 1                  | 20 ± 5               | 38 ± 13              | 3 (2; 3)                         |
| 19p | –OH            | H                | H                 | Cl                | Cl                | 0 ± 0                 | 5 ± 1                  | 9 ± 8                | 59 ± 31              | 1 (1; 2)                         |
| 19q | –OH            | H                | H                 | F                 | H                 | 1 ± 0                 | 53 ± 12                | 44 ± 18              | 94 ± 6               | 10 (8; 13)                       |
| 19r | –OH            | H                | H                 | H                 | F                 | 2 ± 0                 | 51 ± 5                 | 30 ± 2               | 82 ± 8               | 14 (10; 20)                      |
| 19s | –OH            | H                | H                 | F                 | F                 | 0 ± 0                 | 4 ± 0                  | 12 ± 3               | 86 ± 11              | 2 (2; 2)                         |
| 19t | –OH            | H                | F                 | H                 | Cl                | 0 ± 0                 | 5 ± 1                  | 4 ± 1                | 64 ± 23              | 1 (1; 2)                         |
| 19u | H              | –OH              | H                 | H                 | Cl                | 1 ± 0                 | 25 ± 3                 | 10 ± 4               | 100 ± 7              | 4 (2; 6)                         |
| 19v | H              | –OH              | H                 | H                 | F                 | 3 ± 0                 | 57 ± 12                | 24 ± 2               | 99 ± 6               | 11 (6; 18)                       |
| 19w | H              | –OH              | H                 | F                 | F                 | 0 ± 0                 | 10 ± 2                 | 12 ± 2               | 94 ± 5               | 2 (2; 4)                         |
| 19x | –OH            | –OH              | H                 | F                 | F                 | 0 ± 0                 | 25 ± 3                 | 4 ± 1                | 66 ± 6               | 4 (2; 7)                         |
| 19y | –OH            | –OH              | H                 | H                 | Cl                | 0 ± 0                 | 34 ± 5                 | 5 ± 1                | 83 ± 39              | 6 (3; 10)                        |

<sup>a</sup> % of Control. <sup>b</sup> 30 mg kg<sup>–1</sup>, po, FAAH activity was determined 1 h after administration. <sup>c</sup> Results are mean ± SEMs of four experiments. <sup>d</sup> IC<sub>50</sub> values are presented with 95% of confidence intervals.

**Table 3** FAAH inhibition by **19p**, **19t** and **19x–y** in homogenates of mouse at defined intervals

| Liver time course, % of inhibition <sup>a</sup> |        |         |         |          |
|---|--------|---------|---------|----------|
| No.   | 1 h    | 4 h     | 8 h     | 24 h     |
| 19p <sup>b</sup>                                | 9 ± 8  | 18 ± 14 | 11 ± 10 | 103 ± 16 |
| 19t <sup>b</sup>                                | 4 ± 1  | 5 ± 0   | 7 ± 2   | 79 ± 27  |
| 19x <sup>c</sup>                                | 6 ± 1  | 18 ± 1  | 27 ± 10 | 79 ± 5   |
| 19y <sup>c</sup>                                | 10 ± 2 | 17 ± 6  | 38 ± 7  | 91 ± 9   |

| Brain time course, % of inhibition <sup>a</sup> |         |         |         |         |
|---|---------|---------|---------|---------|
| No.   | 1 h     | 4 h     | 8 h     | 24 h    |
| 19p <sup>b</sup>                                | 59 ± 31 | 72 ± 22 | 52 ± 33 | 99 ± 10 |
| 19t <sup>b</sup>                                | 64 ± 23 | 34 ± 6  | 57 ± 25 | 80 ± 20 |
| 19x <sup>c</sup>                                | 102 ± 3 | 100 ± 5 | 103 ± 3 | 104 ± 2 |
| 19y <sup>c</sup>                                | 99 ± 3  | 90 ± 6  | 96 ± 1  | 102 ± 4 |

<sup>a</sup> Results are mean ± SEMs of four experiments. <sup>b</sup> 30 mg kg<sup>–1</sup>, po. <sup>c</sup> 10 mg kg<sup>–1</sup>, po.

## Conclusion

Novel substituted 5-phenoxy-3-phenyl-1,3,4-oxadiazol-2(3*H*)-ones **19a–m** derived from the initial *in vitro* screening hit **7** were

found to be potent *in vitro* inhibitors of FAAH. Compounds **19j–k** displayed promising *in vivo* inhibition over compounds **19b**, **19d**, **19f** and **19l–m**. Consequently, an optimised series of 3-(hydroxyphenyl)-5-phenoxy-1,3,4-oxadiazol-2(3*H*)-ones **19n–y** were prepared and found to possess enhanced *in vivo* FAAH inhibition over the parent phenols **19j–k**. Catechols **19x–y** showed potent and peripherally selective *in vivo* FAAH inhibition at a dose of 10 mg kg<sup>–1</sup> and may be beneficial in the treatment of certain cardiovascular disorders such as hypertension and heart failure. Compounds **19n–y** are currently being further evaluated for their therapeutic potential.

## Experimental section

### Chemistry

The purity of test compounds in all cases was higher than 95%. Analytical TLC was performed on precoated silica gel plates (Merck 60 Kieselgel F 254) and visualized with UV light. Solvents and reagents were purchased from Aldrich, Merck, and Fluka and were used without further purification.

### 3,4-Dimethoxy phenylhydrazine 10a

To a stirred mixture of 4-bromo-1,2-dimethoxybenzene **8** (10 g, 46.1 mmol) and magnesium turnings (1.232 g, 50.7 mmol) in THF (185 mL) was added 1,2-dibromoethane (0.20 g, 1.06 mmol). The reaction was heated at reflux for 2 h, and then cooled to  $-78^{\circ}\text{C}$ , whereupon diethyl diazene-1,2-dicarboxylate (8.02 g, 7.25 mL, 46.1 mmol) was added dropwise. After stirring for 15 min in the cold, followed by 2 h at room temperature, acetic acid (0.6 g) was added followed by water. The mixture was extracted with ethyl acetate and the organic phase was dried over  $\text{MgSO}_4$ , then filtered and evaporated. Chromatography (petroleum ether-ethyl acetate, 2 : 1) followed by trituration in a mixture of diethyl ether-petroleum ether gave the title compound **9** as a beige powder (10.4 g, 72%).

**Cleavage of ethyl carbamate protecting groups.** To a solution of the carbamate derivative **9** (9.7 g, 31.1 mmol) in isopropanol (250 mL) was added 2.5 M aqueous potassium hydroxide solution (66.5 mL, 166 mmol). The reaction was refluxed for 4 h, whereupon isopropanol was removed by evaporation under reduced pressure. The residue was acidified by adding 1 N HCl solution and then extracted twice with diethyl ether. The aqueous phase was then basified with excess 10% sodium hydroxide solution and extracted with dichloromethane. The organic phase was dried over  $\text{MgSO}_4$ , then filtered and evaporated under reduced pressure to furnish compound **10a** as a beige powder (1.66 g, 32%).

### General procedure for the synthesis of oxadiazolones 14–18, 19a–b and 19d–i, 19l–m

**Preparation of phenyl chloroformates 12a–d.** To an ice-cooled solution of the appropriately substituted phenol **11a–d** (20 mmol) and a 20% w/w solution of phosgene in toluene (11.58 mL, 22 mmol), was added dropwise a solution of *N,N*-dimethylaniline (2.424 g, 20 mmol) in toluene (9 mL). After stirring at room temperature for 3 h, nitrogen was bubbled through the reaction mixture for 30 min. The mixture was then quenched with ice-water. The organic phase was separated and washed with 1 N HCl solution and water respectively. After drying over  $\text{MgSO}_4$  the organic phase was filtered and the toluene was removed under reduced pressure to give the title product.

**Preparation of phenyl 2-phenyl phenylhydrazinecarboxylates 13a–y.** To an ice-cooled solution of the appropriately substituted phenyl hydrazine **10a–y** (10 mmol) and pyridine (3.95 g, 50 mmol) in *N*-methylpyrrolidinone (15 mL) was added portionwise the corresponding acylating agent **12a–y** (12 mmol). The reaction mixture was stirred at room temperature for 1 h, and then poured onto a mixture of ice and 1 N HCl. The resulting precipitate was filtered off, washed with water and dried under vacuum.

**Preparation of oxadiazolones 14–18, 19a–b, 19d–i, 19l–m.** To an ice-cooled mixture of the appropriately substituted 2-phenyl phenylhydrazinecarboxylate **13a–y** (19.61 mmol) and pyridine (8.25 mL, 8.06 g, 102 mmol) in dichloromethane (150 mL) was added dropwise a 20% w/w solution of phosgene in toluene (24.76 mL, 47.1 mmol). The reaction mixture was stirred for 15 min in the cold and then for 45 min. at room temperature. Nitrogen was then bubbled through the reaction mixture for 30

min, whereupon it was diluted with water at  $0^{\circ}\text{C}$ . The two phases were separated and the organic phase was washed with 1 N HCl solution and water respectively. After drying over  $\text{MgSO}_4$  and filtration, the solvent was removed under reduced pressure. The crude oxadiazolone was either purified by column chromatography or recrystallised from isopropanol.

**Preparation of oxadiazolones 19c, 19j–k and 19n–y.** A stirred solution of the appropriately substituted *O*-protected intermediate (9.85 mmol) in dichloromethane (30 mL) under nitrogen was cooled to  $-78^{\circ}\text{C}$  and boron tribromide (1.86 mL, 19.70 mmol) was added dropwise. The resulting mixture was stirred in the cold for 5 min and then allowed to warm up to room temperature and stirred for 2 h. The reaction mixture was then quenched with ice-water at  $0^{\circ}\text{C}$ . The resulting precipitate was filtered off, washed with water and dried under vacuum. The crude oxadiazolone was either purified by column chromatography or recrystallised from isopropanol.

### Pharmacology

***In vitro* assay.** *In vitro* FAAH activity was determined accordingly to the following method:

Frozen brains (without cerebellum) from Wistar rats were used, and each brain was homogenized in 15 mL 1 mM  $\text{MgCl}_2$ , 20 mM HEPES pH 7.0 with Potter Elvehjem (8 strokes at 500 rpm). Homogenates were centrifuged for 20 min at 36000g at  $4^{\circ}\text{C}$  (Beckman, 70Ti rotor). Pellets were resuspended in 15 mL of the same buffer and centrifuged under the same conditions. Pellets were resuspended in 15 mL of the same buffer and incubated for 15 min at  $37^{\circ}\text{C}$  after which they were centrifuged for 20 min at 36000g at  $4^{\circ}\text{C}$ . Each pellet was then resuspended in 15 mL 3 mM  $\text{MgCl}_2$ , 1 mM EDTA, 50 mM Tris pH 7.4 and protein determined with BioRad Protein Assay (BioRad) using a standard curve of BSA ( $50\text{--}250\text{ }\mu\text{g mL}^{-1}$ ). The membrane suspensions were aliquoted and stored at  $-80^{\circ}\text{C}$ .

The FAAH activity was determined using AEA (labelled with  $^3\text{H}$  in the ethanolamine part of the molecule) as substrate and measuring the  $^3\text{H}$ -ethanolamine formed. Reaction mix (total volume of 200  $\mu\text{L}$ ) contained: 2  $\mu\text{M}$  AEA (2  $\mu\text{M}$  AEA + 5 nM  $^3\text{H}$ -AEA), 0.1% fatty acid free BSA, 5  $\mu\text{g}$  protein, in 1 mM EDTA, 10 mM Tris pH 7.6 and 10  $\mu\text{M}$  or 100 mM compounds. Stock solutions of the compounds to test (10 mM) were prepared in 100% DMSO and the DMSO concentration in the assay was 0.1%. After a 15 min preincubation period at  $37^{\circ}\text{C}$ , reaction was started by the addition of the substrate solution (cold EAE + radiolabelled EAE + BSA). Reaction was carried out for 10 min before termination by the addition of 400  $\mu\text{L}$  activated charcoal suspension (8 g charcoal in 32 mL 0.5 M HCl in continuous agitation). After a 30 min incubation period at room temperature with agitation, charcoal was sedimented by centrifugation in microfuge (10 min at 13000 rpm). 200  $\mu\text{L}$  of the supernatant were added to 800  $\mu\text{L}$  Optiphase Supermix scintillation cocktail previously distributed in 24-well plates. Counts per minute (cpm) were determined in Microbeta TriLux scintillation counter (10 min counting or until  $\sigma = 2$ ). In each assay blanks (no protein, usually below 200 cpm) and controls (no compound) were present. The results are reported as % of control after blank subtraction.

**In vivo assay.** *In vivo* FAAH inhibitory activity was determined accordingly to the following method:

**Animal treatment.** The animals used for experiments were male NMRI mice (weighing 27–44 g) obtained from Interfauna Ibérica (Spain). Mice were kept 5 per cage, under controlled environmental conditions (12 h light/dark cycle and room temperature  $22 \pm 1$  °C). Food and tap water were allowed *ad libitum* and the experiments were all carried out during daylight hours.

Animals were administered 30 mg kg<sup>-1</sup> test compounds *via* oral route (8 ml kg<sup>-1</sup>; compound suspended in 0.5% carboxymethylcellulose (CMC) or solubilized in water) or 8 ml kg<sup>-1</sup> 0.5% CMC (controls) using animal feeding stainless steel curve needles (Perfectum, USA). Fifteen minutes before sacrifice animals were anaesthetised with pentobarbital 60 mg kg<sup>-1</sup> administered intraperitoneally. A fragment of liver and brain without cerebellum were removed and put in plastic vials containing membrane buffer (3 mM MgCl<sub>2</sub>, 1 mM EDTA, 50 mM Tris HCl pH 7.4). Tissues were stored at –30 °C until analysis. Animals were fasted overnight before administration of compounds except for time periods of >18h, where food was removed on the morning of the day of administration and the compound was administered in the afternoon of the same day. Animals were then given water but nothing else.

All animal procedures were conducted in the strict adherence to the European Directive for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609CEE) and Portuguese legislation (Decreto-Lei 129/92, Portaria 1005/92 e 1131/97). The number of animals used was the minimum possible in compliance with current regulations and scientific integrity.

**Reagents and solutions.** Anandamide [ethanolamine -1-<sup>3</sup>H-] (40–60 Ci/mmol) was obtained from American Radiochemicals. All other reagents were obtained from Sigma-Aldrich. Optiphase Supermix was obtained from Perkin Elmer and activated charcoal was obtained from Sigma-Aldrich.

**Tissue preparation.** Tissues were thawed on ice and were homogenised in 10 volumes of membrane buffer (3 mM MgCl<sub>2</sub>, 1 mM EDTA, 50 mM Tris HCl pH 7.4) with either Potter-Elvehjem (brains - 8 strokes at 500 rpm) or Heidolph Diax (livers - 2 strokes at position 5 for 20 s with 30 s pauses). Total protein in tissues was determined with the BioRad Protein Assay (BioRad) using a standard curve of BSA (50–250 µg ml<sup>-1</sup>).

**Enzymatic assay.** Reaction mix (total volume of 200 µl) contained: 2 µM AEA (2 µM AEA + 5 nM <sup>3</sup>H-AEA), 0.1% fatty acid

free BSA, 15 µg (brain), 5 µg (liver) or 50 µg (lung) protein, in 1 mM EDTA, 10 mM Tris pH 7.6. After a 15 min pre-incubation period at 37 °C, reaction was started by the addition of the substrate solution (cold AEA + radiolabelled AEA + BSA). Reaction was carried out for 10 min (brain and lung) or 7 min (liver) before termination by the addition of 400 µl activated charcoal suspension (8 g charcoal in 32 ml 0.5 M HCl in continuous agitation). After a 30 min incubation period at room temperature with agitation, charcoal was sedimented by centrifugation in microfuge (10 min at 13000 rpm). 200 µl of the supernatant were added to 800 µl Optiphase Supermix scintillation cocktail previously distributed in 24-well plates. Counts per minute (cpm) were determined in a MicrobetaTriLux scintillation counter.

In each assay blanks (without protein) were prepared. The percentage of remaining enzymatic activity was calculated with respect to controls (no compound) and after blank subtraction.

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