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SHORT COMMUNICATION

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N-aryl 2-aryloxyacetamides as a new class of fatty acid amide hydrolase (FAAH) inhibitors

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ABSTRACT

Fatty acid amide hydrolase (FAAH) is a promising target for the development of drugs to treat neurological diseases. In search of new FAAH inhibitors, we identified 2-(4-cyclohexylphenoxy)-*N*-(3-(oxazolo[4,5-*b*]pyridin-2-yl)phenyl)acetamide, **4g**, with an IC_{50} of 2.6 μM as a chemical starting point for the development of potent FAAH inhibitors. Preliminary hit-to-lead optimisation resulted in 2-(4-phenylphenoxy)-*N*-(3-(oxazolo[4,5-*b*]pyridin-2-yl)phenyl)acetamide, **4i**, with an IC_{50} of 0.35 μM.

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Introduction

The endocannabinoid system is involved in a number of physiological effects including control of pain, appetite and cell proliferation[1]. It contains cannabinoid receptors (CBs) and their stimulating endogenous endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are synthesised on demand from cell membrane arachidonic acid derivatives[2]. AEA and 2-AG have a short lifetime due to rapid hydrolysis by the enzymes fatty acid amide hydrolase (FAAH)[3] and monoacylglycerol lipase (MAGL)[4], respectively. AEA is the primary substrate for FAAH[5]; however, it has a wide substrate specificity and can hydrolyse compounds such as *N*-oleoylethanolamine, a lipid mediator that limits food intake and the anti-inflammatory compound *N*-palmitoylethanolamide[6]. Inhibition of FAAH produces elevated levels of AEA in the brain and periphery, as well as potentially beneficial effects in animal models of anxiety and pain[7–11].

FAAH is a serine hydrolase enzyme with a unique catalytic triad consisting of two serines (Ser^{217} and Ser^{241}) and one lysine (Lys^{142}), which makes it distinct from other serine hydrolases. In contrast to other serine hydrolases, FAAH was revealed to hydrolyse amides faster than esters[12]. A number of FAAH inhibitors has been developed to block the catabolism of AEA[13–15]. A breakthrough came when inhibitors of a class of *N*-alkylcarbamic acid *O*-aryl esters (URB524 and URB597, Figure 1) were found to significantly inhibit the enzyme and to modulate AEA levels in rodents[7,16]. Those inhibitors blocked FAAH activity through irreversible carbamoylation of the catalytic nucleophile Ser^{241}, where the biphenyl group served as a leaving group[17]. Additionally, quantum mechanics and molecular modelling simulation suggested that the process of hydrolysis by FAAH was slowed down because of the stabilised hydrogen bond formation between cyclohexylcarbamic ester and active site of the enzyme[18]. It is also noteworthy that compound URB597 was found to be a selective FAAH inhibitor that did not affect hydrolysis of 2-AG[19]. Benzoxazole-, oxazolopyridine- and benzimidazole-based compounds have previously shown to efficiently inhibit FAAH[13–15]. Nevertheless, the crystal structures of FAAH with covalent and non-covalent inhibitors has revealed multiple pockets near the catalytic core, which offers a wide range of binding modes that can be exploited in the design of novel inhibitors[20,21]. In the present study, we developed synthetic protocols and investigated a series of *N*-(3-(oxazolo[4,5-*b*]pyridin-2-yl) and *N*-(imidazo[4,5-*b*]pyridin-2-yl) moieties connected by a 2-aryloxyacetamide to a aliphatic, phenylic or biphenylic hydrophobic "tail" that mimics the arachidonyl moiety of 2-AG.

Materials and methods

General procedure for the synthesis of compounds 2a–f (Scheme 1)

To the polyphosphoric acid (1.5 g/mmol) was added 2-amino-3-hydroxypyridine or 2,3-diaminopyridine (1.0 equiv) and the relevant benzoic acid (1.0 equiv). The mixture was heated to 200 °C and stirred for 6 h. The reaction was cooled slightly and poured into cold water and the mixture was neutralised to pH 8 with 5 M NaOH. The aqueous layer was extracted with ethyl acetate and

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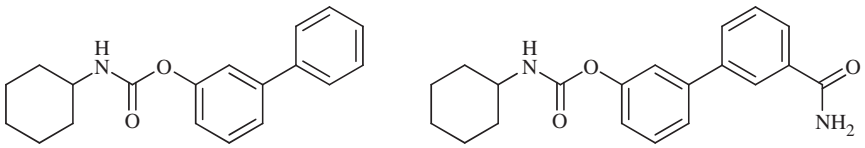
Supplemental data for this article can be accessed here.



URB524 URB527

Figure 1. Known fatty acid amide hydrolase inhibitors.

combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The solid residue obtained was dissolved in EtOAc and triturated with heptanes. The precipitate formed was filtered and dried under vacuum to obtain the desired compounds **2a**–**f**. Further details are given in the Supporting information.

2-(Oxazolo[4,5-b]pyridin-2-yl)aniline (2a)

Yield: 22%; ESI–MS *m/z* calcd for $C_{12}H_9N_3O$ $[M+H]^+$, 212.08; found 212.12; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ8.50 (dd, 1H, $J=4.92$, 1.40 Hz), 8.18 (dd, 1H, $J=8.08$, 1.40 Hz), 7.95 (dd, 1H, $J=8.10$, 1.46 Hz), 7.44–7.41 (*m*, 1H), 7.35–7.31 (*m*, 1H), 7.19 (bs, 2H), 6.94 (dd, 1H, $J=8.38$, 0.66 Hz), 6.73–6.69 (*m*, 1H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 165.63, 156.00, 150.02, 146.46, 141.65, 133.85, 128.51, 120.54, 118.58, 116.93, 116.08, 106.36.

3-(Oxazolo[4,5-b]pyridin-2-yl)aniline (2b)

Yield: 47%; ESI–MS *m/z* calcd for $C_{12}H_9N_3O$ $[M+H]^+$, 212.08; found 212.12; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ8.53 (dd, 1H, $J=4.88$, 1.40 Hz), 8.22 (dd, 1H, $J=8.14$, 1.42 Hz), 7.48–7.43 (*m*, 2H), 7.40–7.37 (*m*, 1H), 7.27 (*t*, 1H, $J=7.90$ Hz), 6.86–6.83 (*m*, 1H), 5.55 (bs, 2H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 166.07, 156.10, 149.96, 146.83, 143.07, 130.33, 126.79, 120.98, 119.31, 118.52, 115.38, 112.73.

4-(Oxazolo[4,5-b]pyridin-2-yl)aniline (2c)

Yield: 57%; ESI–MS *m/z* calcd for $C_{12}H_9N_3O$ $[M+H]^+$, 212.08; found 212.12; ^{1}H NMR (600 MHz, $(CD_3)_2SO$): δ8.42 (d, 1H, $J=4.86$ Hz), 8.07 (d, 1H, $J=8.04$ Hz), 7.91 (d, 2H, $J=8.52$ Hz), 7.33–7.30 (*m*, 1H), 6.72 (d, 2H, $J=8.58$ Hz), 6.16 (bs, 2H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 166.66, 156.90, 153.84, 146.09, 142.74, 130.03, 119.70, 118.26, 114.02, 112.24.

2-(1H-imidazo[4,5-b]pyridin-2-yl)aniline (2d)

Yield: 29%; ESI–MS *m/z* calcd for $C_{12}H_{10}N_4$ $[M+H]^+$, 211.10; found 211.17; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ13.24 (bs, 1H), 8.31 (d, 1H, $J=3.32$ Hz), 7.96–7.89 (*m*, 2H), 7.30–7.16 (*m*, 4H), 6.84 (dd, 1H, $J=8.28$, 0.88 Hz), 6.65 (*t*, 1H, $J=7.46$ Hz). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 154.72, 149.20, 143.79, 131.43, 128.19, 118.15, 116.87, 115.62, 110.22.

3-(1H-imidazo[4,5-b]pyridin-2-yl)aniline (2e)

Yield: 80%; ESI–MS *m/z* calcd for $C_{12}H_{10}N_4$ $[M+H]^+$, 211.10; found 211.17; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ13.33 (bs, 1H), 8.31 (bs, 1H), 7.97 (bs, 1H), 7.47 (*s*, 1H), 7.33 (d, 1H, $J=7.32$ Hz), 7.23–7.17 (*m*, 2H), 6.72 (d, 1H, $J=7.20$ Hz), 5.33 (bs, 2H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 153.37, 149.18, 143.57, 135.60, 130.21, 129.48, 125.99, 119.04, 117.93, 116.25, 114.32, 112.08.

4-(1H-imidazo[4,5-b]pyridin-2-yl)aniline (2f)

Yield: 90%; ESI–MS *m/z* calcd for $C_{12}H_{10}N_4$ $[M+H]^+$, 211.10; found 211.17; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ12.92 (bs, 1H), 8.21 (bs, 1H), 7.92 (d, 2H, $J=8.00$ Hz), 7.84 (bs, 1H), 7.13 (bs, 1H), 6.72 (d, 2H, $J=7.92$ Hz), 5.58 (bs, 2H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 153.86, 151.33, 149.63, 143.06, 142.26, 135.97, 128.25, 124.82, 118.01, 117.60, 117.04, 116.50, 113.58.

General procedure for the synthesis of compounds 3b–e (Scheme 1)

To a stirred solution of an alcohol derivative (1.0 equiv) in dry DMF (2 mL/mmol) was added NaH (2.0 equiv) and allowed to stir for 1 h at room temperature. To this mixture, sodium iodoacetate (1.5 equiv) was added and continued stirring for 12 h at room temperature. The reaction mixture was diluted with water and washed out with EtOAc. The aqueous layer was acidified with 1 M HCl and extracted with EtOAc, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum to obtain the desired compounds **3b**–**e**.

2-(4-Isopropylphenoxy)acetic acid (3b)

Yield: 65%; ESI–MS *m/z* calcd for $C_{11}H_{14}O_3$ $[M-H]^+$, 193.09; found 193.26; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ7.14 (d, 2H, $J=8.52$ Hz), 6.81 (d, 2H, $J=8.76$ Hz), 4.61 (*s*, 2H), 2.87–2.77 (*m*, 1H), 1.16 (d, 6H, $J=6.92$ Hz). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 170.82, 156.30, 141.35, 127.57, 114.65, 64.96, 33.05, 24.55.

2-([1,1′-Biphenyl]-4-yloxy)acetic acid (3c)

Yield: 60%; ESI–MS *m/z* calcd for $C_{14}H_{12}O_3$ $[M-H]^+$, 227.07; found 227.20; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ13.03 (bs, 1H), 7.62–7.58 (*m*, 4H),7.43 (*t*, 2H, $J=7.68$ Hz), 7.31 (*t*, 1H, $J=7.36$ Hz), 7.00 (d, 2H, $J=8.84$ Hz), 4.72 (*s*, 2H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 170.67, 157.87, 140.22, 133.53, 129.33, 128.21, 127.26, 126.70, 115.36, 64.99.

2-(4-Cyclohexylphenoxy)acetic acid (3d)

Yield: 63%; ESI–MS *m/z* calcd for $C_{14}H_{18}O_3$ $[M-H]^+$, 233.12; found 233.24; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ12.95 (bs, 1H), 7.12 (d, 2H, $J=8.64$ Hz), 6.80 (d, 2H, $J=8.72$ Hz), 4.61 (*s*, 2H), 2.43–2.42 (*m*, 1H), 1.78–1.67 (*m*, 5H), 1.41–1.18 (*m*, 5H). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 170.82, 156.32, 140.63, 127.92, 114.63, 64.95, 43.37, 34.68, 26.87, 26.07.

(E)-2-(hex-2-en-1-yloxy)acetic acid (3e)

Yield: 51%; ESI–MS *m/z* calcd for $C_8H_{14}O_3$ $[M-H]^+$, 157.09; found 157.19; ^{1}H NMR (400 MHz, $CDCl_3$): δ5.78–5.71 (*m*, 1H), 5.58–5.50 (*m*, 1H), 4.09 (*s*, 2H), 4.06 (dd, 2H, $J=6.50$, 0.70 Hz), 2.04 (*q*, 2H, $J=7.22$ Hz), 1.41 (sext, 2H, $J=7.41$ Hz), 0.90 (*t*, 3H, $J=7.38$ Hz). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 172.10, 134.36, 126.70, 71.23, 66.71, 34.18, 22.18, 13.94.

General procedure for the synthesis of compounds 4a–c

The acid derivative (1.0 equiv) was suspended in dry DCM and was added oxalyl chloride (1.5 equiv) and a catalytic amount of

DMF. The mixture was stirred for 2 h at room temperature and evaporated to dryness. The resulting residue was dissolved in dry DMF (5 mL/mmol) and added dropwise to a priorly stirred solution of amine derivative (0.8 equiv) and NaH (1.5 equiv) for 30 min in dry DMF (5 mL/mmol). The reaction mixture was continued stirring for overnight at room temperature, diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The obtained residues were purified with flash column chromatography using 1–5% MeOH in dichloromethane gradient elution to afford the compounds **4a**–**c** in respective yields.

2-([1,1′-Biphenyl]-4-yloxy)-N-(2-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4a)

Yield: 51%; ESI–MS *m/z* calcd for $C_{26}H_{19}N_3O_3$ $[M+H]^+$, 422.15; found 422.22; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 12.50 (bs, 1H), 8.89 (dd, 1H, $J=8.54$, 0.78 Hz), 8.74 (dd, 1H, $J=4.86$, 1.42 Hz), 8.34–8.29 (*m*, 2H), 7.75–7.62 (*m*, 7H), 7.59–7.56 (*m*, 1H), 7.46–7.30 (*m*, 4H), 4.89 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 168.19, 163.84, 157.18, 154.97, 147.53, 142.08, 140.14, 138.67, 134.31, 134.03, 129.35, 129.17, 128.22, 127.34, 126.76, 124.35, 121.87, 120.54, 119.77, 116.12, 113.15, 67.58.

2-(4-Cyclohexylphenoxy)-N-(2-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4b)

Yield: 42%; ESI–MS *m/z* calcd for $C_{26}H_{25}N_3O_3$ $[M+H]^+$, 428.20; found 428.26; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 12.44 (bs, 1H), 8.88 (d, 1H, $J=7.92$ Hz), 8.69 (dd, 1H, $J=4.88$, 1.40 Hz), 8.34–8.28 (*m*, 2H), 7.72 (*t*, 1H, $J=7.92$ Hz), 7.58–7.55 (*m*, 1H), 7.45–7.37 (*m*, 3H), 7.20 (d, 2H, $J=8.64$ Hz), 4.79 (*s*, 2H), 2.46 (*m*, 1H), 1.78–1.68 (*m*, 5H), 1.40–1.18 (*m*, 5H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 168.39, 163.80, 155.69, 154.97, 147.49, 142.06, 141.20, 138.67, 134.26, 129.16, 127.98, 124.30, 121.84, 120.54, 119.73, 115.39, 113.14, 67.52, 43.41, 34.68, 26.87, 26.07.

(E)-2-(hex-2-en-1-yloxy)-N-(2-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4c)

Yield: 34%; ESI–MS *m/z* calcd for $C_{20}H_{21}N_3O_3$ $[M+H]^+$, 352.17; found 352.30; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): 12.21 (bs, 1H), 8.82 (dd, 1H, $J=8.50$, 0.86 Hz), 8.61 (dd, 1H, $J=4.86$, 1.42 Hz), 8.32–8.26 (*m*, 2H), 7.69 (*t*, 1H, $J=7.90$ Hz), 7.56–7.52 (*m*, 1H), 7.36 (*t*, 1H, $J=7.66$ Hz), 5.96–5.89 (*m*, 1H), 5.81–5.74 (*m*, 1H), 4.22 (dd, 2H, $J=6.22$, 0.86 Hz), 4.14 (*s*, 2H), 2.00 (*q*, 2H, $J=7.06$ Hz), 1.34 (sext, 2H, $J=7.34$ Hz), 0.83 (*t*, 3H, $J=7.38$ Hz). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 170.18, 163.77, 154.93, 147.26, 142.03, 138.74, 135.19, 134.18, 129.12, 126.64, 124.13, 121.79, 120.50, 119.64, 113.08, 72.52, 69.46, 34.12, 22.06, 13.98.

General procedure for the synthesis of compounds 4d–k

The acid derivative (1.5 equiv), N,N′-diisopropyl ethyl amine (6.0 equiv) and TBTU (1.5 equiv) were dissolved in dry DMF (10 mL/mmol) and stirred at room temperature for 30 min. At this point, the amine derivative (1.5 equiv) was added and continued stirring at 70 °C for overnight. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The obtained residues were purified with flash column chromatography using 1–5% MeOH in dichloromethane gradient elution to afford the compounds **4d**–**k** in respective yields.

N-(3-(oxazolo[4,5-b]pyridin-2-yl)phenyl)-2-phenoxyacetamide (4d)

Yield: 43%; ESI–MS *m/z* calcd for $C_{20}H_{15}N_3O_3$ $[M+H]^+$, 346.12; found 346.14; ^{1}H NMR (600 MHz, $(CD_3)_2SO$): δ10.45 (bs, 1H), 8.70 (*s*, 1H), 8.56 (dd, 1H, $J=4.83$, 1.41 Hz), 8.27 (dd, 1H, $J=8.16$, 1.38 Hz), 7.98 (d, 1H, $J=7.86$ Hz), 7.91 (ddd, 1H, $J=8.16$, 2.10, 0.90 Hz), 7.62 (*t*, 1H, $J=7.95$ Hz), 7.49–7.47 (*m*, 1H), 7.35–7.32 (*m*, 2H), 7.04 (d, 2H, $J=7.86$ Hz), 6.99 (*t*, 1H, $J=7.32$ Hz), 4.77 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.65, 165.11, 158.25, 155.91, 147.11, 143.28, 139.80, 130.47, 130.02, 126.85, 124.07, 123.34, 121.72, 121.36, 119.62, 118.93, 115.17, 67.53.

2-(4-Isopropylphenoxy)-N-(3-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4e)

Yield: 51%; ESI–MS *m/z* calcd for $C_{23}H_{21}N_3O_3$ $[M+H]^+$, 388.17; found 388.23; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ10.42 (bs, 1H), 8.71 (*s*, 1H), 8.56 (dd, 1H, $J=4.86$, 1.42 Hz), 8.27 (dd, 1H, $J=8.16$, 1.40 Hz), 7.98 (d, 1H, $J=8.2$ Hz), 7.92–7.89 (*m*, 1H), 7.61 (*t*, 1H, $J=7.98$ Hz), 7.50–7.47 (*m*, 1H), 7.19 (d, 2H, $J=8.56$ Hz), 6.95 (dd, 2H, $J=6.68$, 2.04 Hz), 4.73 (*s*, 2H), 2.88–2.81 (*m*, 1H), 1.18 (d, 6H, $J=6.92$ Hz). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.82, 165.12, 156.37, 155.92, 147.11, 143.28, 141.66, 139.81, 130.46, 127.67, 126.85, 124.06, 123.32, 121.36, 119.61, 118.93, 114.98, 67.69, 33.08, 24.57.

2-([1,1′-Biphenyl]-4-yloxy)-N-(3-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4f)

Yield: 63%; ESI–MS *m/z* calcd for $C_{26}H_{19}N_3O_3$ $[M+H]^+$, 422.15; found 422.09; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ10.50 (bs, 1H), 8.70 (*s*, 1H), 8.56 (dd, 1H, $J=4.88$, 1.40 Hz), 8.26 (dd, 1H, $J=8.18$, 1.41 Hz), 7.99 (d, 1H, $J=7.80$ Hz), 7.91–7.88 (*m*, 1H), 7.66–7.60 (*m*, 5H), 7.50–7.41 (*m*, 3H), 7.31 (*t*, 1H, $J=7.36$ Hz), 7.13 (d, 2H, $J=8.84$ Hz), 4.82 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.61, 165.11, 157.92, 155.92, 147.11, 143.28, 140.18, 139.81, 133.77, 130.49, 129.34, 128.28, 127.30, 126.87, 126.72, 124.05, 123.35, 121.36, 119.62, 118.92, 115.64, 67.63.

2-(4-Cyclohexylphenoxy)-N-(3-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4 g)

Yield: 57%; ESI–MS *m/z* calcd for $C_{26}H_{25}N_3O_3$ $[M+H]^+$, 428.20; found 428.14; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ10.42 (bs, 1H), 8.71 (*s*, 1H), 8.56 (dd, 1H, $J=4.88$, 1.40 Hz), 8.27 (dd, 1H, $J=8.16$, 1.40 Hz), 7.98 (dd, 1H, $J=6.72$, 1.48 Hz), 7.91 (dd, 1H, $J=8.20$, 1.16 Hz), 7.61 (*t*, 1H, $J=8.00$ Hz), 7.50–7.47 (*m*, 1H), 7.17 (d, 2H, $J=8.68$ Hz), 6.94 (d, 2H, $J=8.72$ Hz), 4.73 (*s*, 2H), 2.44 (*m*, 1H), 1.77–1.67 (*m*, 5H), 1.38–1.19 (*m*, 5H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.84, 165.11, 156.39, 155.92, 147.11, 143.27, 140.94, 139.81, 130.46, 128.02, 126.84, 124.05, 123.31, 121.36, 119.61, 118.92, 114.96, 67.67, 43.39, 34.67, 26.86, 26.06.

(E)-2-(hex-2-en-1-yloxy)-N-(3-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4 h)

Yield: 60%; ESI–MS *m/z* calcd for $C_{20}H_{21}N_3O_3$ $[M+H]^+$, 352.17; found 352.11; ^{1}H NMR (600 MHz, $(CD_3)_2SO$): δ10.09 (bs, 1H), 8.72 (*s*, 1H), 8.56 (d, 1H, $J=4.74$ Hz), 8.27 (d, 1H, $J=8.10$ Hz), 7.96 (d, 1H, $J=7.68$ Hz), 7.91 (dd, 1H, $J=8.19$, 0.93 Hz), 7.59 (*t*, 1H, $J=7.92$ Hz), 7.50–7.47 (*m*, 1H), 5.77–5.73 (*m*, 1H), 5.63–5.58 (*m*, 1H), 4.07 (*s*, 2H), 4.06 (d, 2H, $J=6.30$ Hz), 2.02 (*q*, 2H, $J=7.06$ Hz), 1.38 (sext, 2H, $J=7.34$ Hz), 0.87 (*t*, 3H, $J=7.38$ Hz). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 169.32, 165.18, 155.91, 147.09, 143.26, 139.84, 135.04, 130.34, 126.74, 126.56, 124.16, 123.19, 121.35, 119.60, 119.00, 71.82, 69.37, 34.22, 22.16, 13.99.

2-([1,1′-Biphenyl]-4-yloxy)-N-(4-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4i)

Yield: 42%; ESI–MS *m/z* calcd for $C_{26}H_{19}N_3O_3$ $[M+H]^+$, 422.15; found 421.91; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 10.57 (bs, 1H), 8.53 (d, 1H, $J=4.64$ Hz), 8.23 (*t*, 3H, $J=8.16$ Hz), 7.95 (d, 2H, $J=8.40$ Hz), 7.63 (*t*, 4H, $J=7.10$ Hz), 7.43 (*t*, 3H, $J=6.68$ Hz), 7.32 (*t*, 1H, $J=7.28$ Hz), 7.11 (d, 2H, $J=8.48$ Hz), 4.84 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.75, 165.17, 157.89, 156.17, 146.89, 143.16, 142.90, 140.16, 133.79, 129.35, 129.25, 128.29, 127.32, 126.72, 121.17, 120.98, 120.25, 119.31, 115.62, 67.65.

2-(4-Cyclohexylphenoxy)-N-(4-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4j)

Yield: 44%; ESI–MS *m/z* calcd for $C_{26}H_{25}N_3O_3$ $[M+H]^+$, 428.20; found 427.95; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 10.50 (bs, 1H), 8.53 (dd, 1H, $J=4.86$, 1.18 Hz), 8.24–8.21 (*m*, 3H), 7.94 (d, 2H, $J=8.80$ Hz), 7.46–7.43 (*m*, 1H), 7.16 (d, 2H, $J=8.64$ Hz), 6.92 (d, 2H, $J=8.64$ Hz), 4.73 (*s*, 2H), 2.44 (*m*, 1H), 1.77–1.67 (*m*, 5H), 1.41–1.19 (*m*, 5H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.97, 165.16, 156.37, 156.17, 146.88, 143.15, 142.91, 140.97, 129.22, 128.03, 121.13, 120.97, 120.23, 119.29, 114.93, 67.70, 43.38, 34.67, 26.85, 26.06.

(E)-2-(hex-2-en-1-yloxy)-N-(4-(oxazolo[4,5-b]pyridin-2-yl)phenyl)acetamide (4k)

Yield: 54%; ESI–MS *m/z* calcd for $C_{20}H_{21}N_3O_3$ $[M+H]^+$, 352.17; found 352.05; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): 10.17 (bs, 1H), 8.53 (dd, 1H, $J=4.88$, 1.24 Hz), 8.21 (dd, 3H, $J=9.18$, 1.94 Hz), 7.95 (d, 2H, $J=8.80$ Hz), 7.46–7.43 (*m*, 1H), 5.78–5.71 (*m*, 1H), 5.62–5.55 (*m*, 1H), 4.08 (*s*, 2H), 4.05 (d, 2H, $J=6.24$ Hz), 2.01 (*q*, 2H, $J=6.96$ Hz), 1.38 (sext, 2H, $J=7.35$ Hz), 0.87 (*t*, 3H, $J=7.36$ Hz). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 169.47, 165.22, 156.19, 146.86, 143.14, 143.00, 135.06, 129.13, 126.54, 120.96, 120.94, 120.26, 119.28, 71.80, 69.40, 34.21, 22.15, 14.00.

General procedure for the synthesis of compounds 4 l–n (Scheme 1)

The acid derivative (1.0 equiv) was suspended in dry DCM and was added oxalyl chloride (1.5 equiv) and a catalytic amount of DMF. The mixture was stirred for 2 h at room temperature and evaporated to dryness. The resulting residue was dissolved in dry DMF (5 mL/mmol) and added dropwise to a priorly stirred solution of amine derivative (0.8 equiv) and triethylamine (2.5 equiv) for 30 min in dry DMF (5 mL/mmol). The reaction mixture was continued stirring for overnight at room temperature, diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The obtained residues were purified with flash column chromatography using 1–5% MeOH in dichloromethane gradient elution to afford the compounds **4 l**–**n** in respective yields.

N-(2-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-([1,1'-biphenyl]-4-yloxy)acetamide (4 l)

Yield: 46%; ESI–MS *m/z* calcd for $C_{26}H_{20}N_4O_2$ $[M+H]^+$, 421.17; found 421.09; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.47 (bs, 2H, imidazole-NH, acetamide-NH), 8.85 (d, 1H, $J=8.16$ Hz), 8.45 (bs, 1H), 8.20 (d, 1H, $J=7.32$ Hz), 7.99 (dd, 1H, $J=8.00$, 1.32 Hz), 7.66–7.53 (*m*, 6H), 7.43 (*t*, 3H, $J=7.68$ Hz), 7.31 (*t*, 3H, $J=7.32$ Hz), 4.86 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ168.16, 157.56, 152.97, 144.93, 140.12, 138.13, 133.99, 131.67, 129.34, 128.51, 128.27, 127.33, 126.73, 124.00, 120.81, 119.00, 116.58, 116.06, 68.14.

N-(2-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-(4-cyclohexylphenoxy)acetamide (4m)

Yield: 21%; ESI–MS *m/z* calcd for $C_{26}H_{26}N_4O_2$ $[M+H]^+$, 427.21; found 427.07; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.43 (bs, 2H, imidazole-NH, acetamide-NH), 8.82 (d, 1H, $J=7.68$ Hz), 8.42 (bs, 1H), 8.19 (d, 1H, $J=6.92$ Hz), 7.91 (d, 1H, $J=7.16$ Hz), 7.53 (*t*, 1H, $J=7.55$ Hz), 7.32–7.27 (*m*, 4H), 7.17 (d, 2H, $J=8.56$ Hz), 4.75 (*s*, 2H), 2.43 (*m*, 1H), 1.78–1.67 (*m*, 5H), 1.37–1.21 (*m*, 5H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.97, 155.69, 152.92, 144.31, 140.76, 137.67, 131.10, 128.10, 127.60, 123.52, 120.35, 118.36, 116.39, 114.93, 67.77, 42.95, 34.22, 26.42, 25.62.

(E)-N-(2–(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-(hex-2-en-1-yloxy)acetamide (4n)

Yield: 45%; ESI–MS *m/z* calcd for $C_{20}H_{22}N_4O_2$ $[M+H]^+$, 351.18; found 351.11; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): 13.61 (bs, 1H, imidazole-NH), 13.25 (bs, 1H, acetamide-NH), 8.80 (dd, 1H, $J=8.28$, 0.96 Hz), 8.40 (dd, 1H, $J=4.66$, 1.18 Hz), 8.18(d, 1H, $J=7.34$ Hz), 8.01 (dd, 1H, $J=7.92$, 1.12 Hz),7.52 (*t*, 1H, $J=7.89$ Hz), 7.31–7.26 (*m*, 2H), 5.74 (*m*, 2H), 4.19 (d, 2H, $J=3.36$ Hz), 4.10 (*s*, 2H), 2.00–1.95 (*m*, 2H), 1.31 (sext, 2H, $J=7.33$ Hz), 0.82 (*t*, 3H, $J=7.38$ Hz). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 169.93, 152.83, 144.86, 138.29, 135.07, 131.59, 128.51, 126.67, 123.71, 120.65, 118.85, 116.38, 72.27, 69.73, 34.16, 22.07, 13.96.

General procedure for the synthesis of compounds 4o–t

The acid derivative (1.5 equiv), *N,N*-diisopropylethylamine (6.0 equiv) and TBTU (1.5 equiv) were dissolved in dry DMF (10 mL/mmol) and stirred at room temperature for 30 min. At this point, amine derivative (1.5 equiv) was added and continued stirring at 70 °C for overnight. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The obtained residues were purified with flash column chromatography using 1–5% MeOH in dichloromethane gradient elution to afford the compounds **4o**–**t** in respective yields.

N-(3-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-([1,1'-biphenyl]-4-yloxy)acetamide (4o)

Yield: 60%; ESI–MS *m/z* calcd for $C_{26}H_{20}N_4O_2$ $[M+H]^+$, 421.17; found 420.96; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ13.58–13.20 (bs, 1H, imidazole-NH), 10.35 (bs, 1H), 8.62 (*s*, 1H), 8.33 (bs, 1H), 8.05 (bs, 1H), 7.92(d, 1H, $J=7.40$ Hz), 7.79(d, 1H, $J=7.92$ Hz), 7.66–7.62 (*m*, 4H), 7.54 (*t*, 1H, $J=7.74$ Hz), 7.43 (*t*, 2H, $J=7.70$ Hz), 7.32 (*t*, 1H, $J=7.36$ Hz), 7.27–7.24 (*m*, 1H), 7.13 (d, 2H, $J=8.72$ Hz), 4.82 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.32, 157.95, 156.77, 153.55, 152.72, 149.83, 144.56, 144.29, 140.19, 139.43, 136.02, 133.74, 130.71, 129.91, 129.35, 128.28, 127.72, 126.72, 122.42, 122.36, 122.28, 119.77, 118.79, 118.64, 115.64, 67.64.

N-(3-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-(4-cyclohexylphenoxy)acetamide (4p)

Yield: 44%; ESI–MS *m/z* calcd for $C_{26}H_{26}N_4O_2$ $[M+H]^+$, 427.21; found 427.00; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.48 (bs, 1H,

imidazole-NH), 10.28 (bs, 1H), 8.60 (*s*, 1H), 8.34 (bs, 1H), 8.02 (bs, 1H), 7.91 (d, 1H, $J = 7.68$ Hz), 7.77 (d, 1H, $J = 8.20$ Hz), 7.52 (*t*, 1H, $J = 7.96$ Hz), 7.26–7.23 (*m*, 1H), 7.17 (d, 2H, $J = 8.44$ Hz), 6.94 (d, 2H, $J = 8.24$ Hz), 4.71 (*s*, 2H), 2.38 (*m*, 1H), 1.77–1.67 (*m*, 5H), 1.38–1.19 (*m*, 5H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.55, 156.42, 153.54, 152.70, 149.81, 144.60, 144.27, 140.92, 139.42, 136.02, 130.68, 129.88, 128.01, 126.77, 122.38, 119.80, 118.78, 118.63, 114.95, 67.67, 43.39, 34.67, 26.86, 26.06.

(E)-N-(3-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-(hex-2-en-1-yloxy)acetamide (4q)

Yield: 66%; ESI–MS *m/z* calcd for $C_{20}H_{22}N_4O_2$ $[M + H]^+$, 351.18; found 351.11; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.54–13.17 (bs, 1H, imidazole-NH), 9.93 (bs, 1H), 8.60 (*s*, 1H), 8.33 (bs, 1H), 8.05 (bs, 1H), 7.89 (d, 1H, $J = 7.64$ Hz), 7.77 (d, 1H, $J = 7.56$ Hz), 7.51 (*t*, 1H, $J = 7.64$ Hz), 7.27–7.24 (*m*, 1H, $J = 7.70$ Hz), 5.79–5.72 (*m*, 1H), 5.64–5.57 (*m*, 1H), 4.06–4.05 (*m*, 4H), 2.02 (*q*, 2H, $J = 6.97$ Hz), 1.38 (sext, 2H, $J = 7.35$ Hz), 0.88 (*t*, 3H, $J = 7.36$ Hz). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 168.99, 156.78, 153.62, 152.80, 149.82, 144.53, 144.26, 139.45, 136.02, 134.97, 130.61, 129.83, 129.77, 127.70, 126.60, 122.37, 122.27, 119.75, 118.85, 118.62, 71.79, 69.39, 34.22, 22.17, 14.01.

N-*(4-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-([1,1'-biphenyl]-4-yloxy)acetamide(4r)*

Yield: 58%; ESIMS *m/z* calcd for $C_{26}H_{20}N_4O_2$ $[M + H]^+$, 421.17; found 421.02; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.31 (bs, 1H, imidazole-NH), 10.40 (bs, 1H, acetamide-NH), 8.31 (d, 1H, $J = 4.48$ Hz), 8.20 (d, 2H, $J = 8.72$ Hz), 7.96 (bs, 1H), 7.85 (d, 2H, $J = 8.72$ Hz), 7.64 (*t*, 4H, $J = 7.38$ Hz), 7.44 (*t*, 2H, $J = 7.68$ Hz), 7.32 (*t*, 1H, $J = 7.36$ Hz),7.23–7.20 (*m*, 1H), 7.12 (d, 2H, $J = 8.76$ Hz), 4.82 (*s*, 2H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.44, 157.92, 144.24, 140.90, 140.17, 133.77, 129.35, 128.29, 127.94, 127.31, 126.72, 125.28, 120.13, 118.51, 115.63, 67.67.

N-*(4-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-(4-cyclohexylphenoxy)acetamide(4s)*

Yield: 49%; ESI–MS *m/z* calcd for $C_{26}H_{26}N_4O_2$ $[M + H]^+$, 427.21; found 427.07; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 13.33 (bs, 1H, imidazole-NH), 10.33 (bs, 1H, acetamide-NH), 8.31 (d, 1H, $J = 4.12$ Hz), 8.19 (d, 2H, $J = 8.64$ Hz), 7.97 (d, 1H, $J = 7.44$ Hz), 7.84 (d, 2H, $J = 8.68$ Hz), 7.24–7.20 (*m*, 1H), 7.16 (d, 2H, $J = 8.56$ Hz), 6.92 (d, 2H, $J = 8.60$ Hz), 4.71 (*s*, 2H), 2.44 (*m*, 1H), 1.77–1.67 (*m*, 5H), 1.41–1.19 (*m*, 5H). ^{13}C (150 MHz, $(CD_3)_2SO$): δ 167.67, 156.39, 152.72, 149.91, 144.29, 143.90, 140.95, 136.09, 128.02, 126.41, 125.22, 120.12, 119.51, 118.49, 114.94, 67.72, 43.38, 34.67, 26.86, 26.06.

(E)-N-(4-(1H-imidazo[4,5-b]pyridin-2-yl)phenyl)-2-(hex-2-en-1-yloxy)acetamide(4t)

Yield: 44%; ESI–MS *m/z* calcd for $C_{20}H_{22}N_4O_2$ $[M + H]^+$, 351.18; found 351.11; ^{1}H NMR (400 MHz, $(CD_3)_2SO$): 13.34 (bs, 1H, imidazole-NH), 9.98 (bs, 1H, acetamide-NH), 8.31 (d, 1H, $J = 3.92$ Hz), 8.17 (d, 2H, $J = 8.72$ Hz), 7.98 (bs, 1H), 7.85(d, 2H, $J = 8.72$ Hz), 7.23–7.20 (*m*, 1H), 5.78–5.71 (*m*, 1H), 5.63–5.56 (*m*, 1H), 4.06–4.04 (*m*, 4H), 2.02 (*q*, 2H, $J = 7.01$ Hz), 1.38 (sext, 2H, $J = 7.32$ Hz), 0.88 (*t*, 3H, $J = 7.36$ Hz). ^{13}C (100 MHz, $(CD_3)_2SO$): δ 169.14, 156.98, 153.57, 152.74, 149.88, 144.30, 143.87, 140.96, 136.15, 135.03, 127.88, 127.78, 126.56, 126.39, 125.07, 120.14, 119.39, 118.49, 118.22, 71.79, 69.42, 34.22, 22.16, 14.00.

Results and discussion

Chemistry

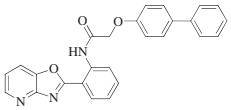
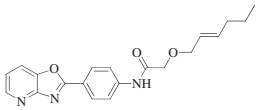
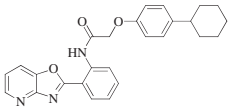
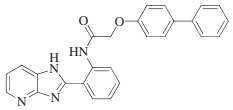
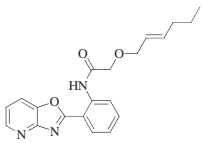
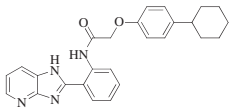
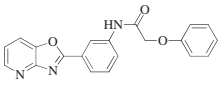
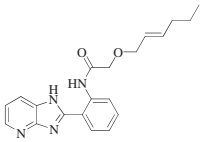
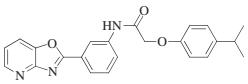
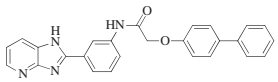
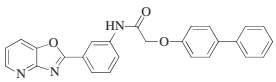
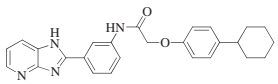
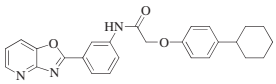
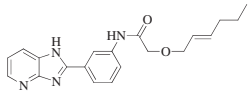
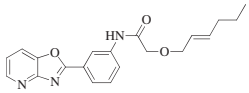
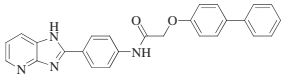
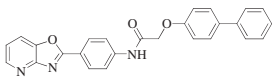
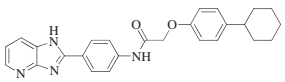
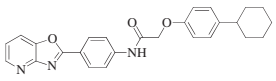
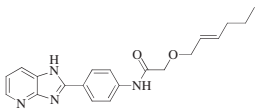
Oxazolo[4,5-*b*]pyridine (**4a**–**k**) and 1*H*-imidazo[4,5-*b*]pyridine derivatives (**4 l**–**t**) with 2-alkoxy and 2-aryloxyacetamidesubstituents in *ortho*, *meta* and *para* position (Table 1) were synthesised using the short and efficient route shown in Scheme 1. Previous methods for the preparation of oxazolopyridine derivatives were limited to one positional isomer and only demonstrated to work for phenols[22]. Moreover, synthetic pathways for compounds based on the imidazopyridine scaffold required protection of the imidazole NH group to avoid diacylation during the anilide bond formation[23]. Our synthetic pathway efficiently gives access to aryloxy- and alkyloxy acetamides in all positional isomers without the need for the protection of the imidazole NH group.

The intermediates **2a**–**c** and **2d**–**f** were obtained by condensation of respective aminopyridines **1a** and **1b** with corresponding amino benzoic acid derivatives in the presence of polyphosphoric acid (PPA)[22,24]. The acid building blocks **3b**–**e** were synthesised from the modified procedure by reacting aryl- or aliphatic alcohols with sodium iodoacetate in the presence of NaH in moderate yields[25]. The final *meta* and *para* compounds **4d**–**k** and **4o**–**t** were obtained in moderate yields from corresponding amines **2b**, **2c**, **2e** and **2f** and acid derivatives **3a**–**e** by using *N,N,N′,N′*-tetramethyl-*O*-(benzotriazol-1-yl)uroniumtetrafluoroborate (TBTU) as coupling agent and *N,N*-diisopropylethylamine (DIPEA) as base. The *ortho*-oxazolopyridineanilides **4a**–**c** were synthesised by reacting the respective acid chloride with compound **2a** in presence of NaH as a base and the *ortho*-imidazopyridineanilides **4 l**–**n** by reacting the respective acid chloride with **2d** using triethylamine as a base.

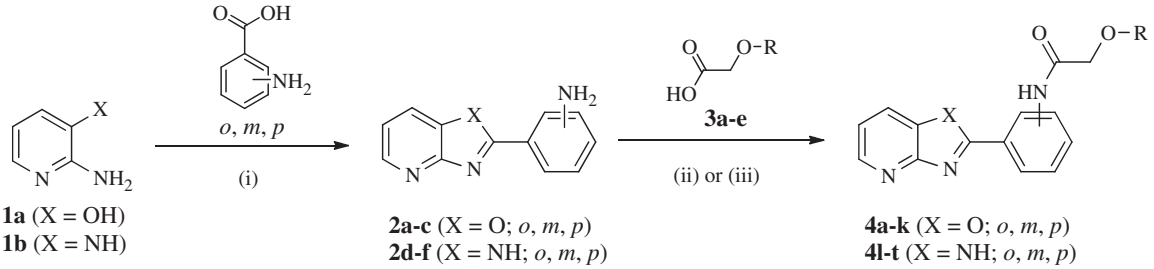
Inhibition of FAAH

All synthesised compounds **4a**–**t** were evaluated for their *in vitro* FAAH inhibitory profile using rat brain homogenates as enzyme source and 0.5 μM [3H] AEA as substrate[26,27]. The data are summarised in Table 1, and examples of the inhibition curves obtained for compounds of different potency are shown in Figure 2. A structure–activity relationship (SAR) analysis revealed that the oxazolo[4,5-*b*]pyridine-*ortho*-anilides **a**–**c** were void of activity while in the set of oxazolo[4,5-*b*]pyridine-*meta*-anilides **4d**–**h** all compounds inhibited FAAH. Compound **4f** with a biphenyl ether was the most potent and completely blocked AEA hydrolysis with an IC_{50} of 0.63 μM. Among the oxazolo[4,5-*b*]pyridine-*para*-anilides **4i**–**k**, the results were less clear. In this set, we identified the oxazolo[4,5-*b*]pyridine-*para*-anilide with a biphenyl ether group **4i** as the most potent compound with an IC_{50} of 0.35 μM. However, this compound did not reach 100% inhibition even at high concentrations. The other compounds all proved to be less effective as FAAH inhibitors. To further investigate the importance of the oxazolo[4,5-*b*]pyridine core, the *ortho, meta* and *para* isomers **4 l**–**t** with hex-2-en-1-yl, biphenyl and 4-cyclohexylphenyl groups on the 1*H*-imidazo[4,5-*b*]pyridine anilide were synthesised an evaluated. In this set, the most potent compound proved to be the biphenyl *para*-substituted compound **4r** with an IC_{50} value of 0.62 μM and maximum inhibition of 80%, thus equipotent with the oxazolo derivative **4i**. The *ortho*-oxazolo derivatives **4a**–**c** were all inactive in contrast to the imidazo isosteres **4 l**–**n** with IC_{50} values ranging from 0.65 to 5.5 μM. The *meta*-substituted imidazo compounds **4o**–**q** were all less potent than their corresponding oxazoloisosteres **4f**–**h**. For the *para*-substituted compounds, no clear trends were observed and potent

Table 1. FAAH inhibitory profile of synthesised compounds.

ID	Structure	IC_{50} (μM)[a]	Maximum inhibition (%)[b]	ID	Structure	IC_{50} (μM)[a]	Maximum inhibition (%)[b]
4a		NA[c]	3 ± 5	**4k**		NA	46 ± 9
4b		NA	−1 ± 3	**4l**		1.1	70 ± 2
4c		NA	7 ± 3	**4m**		0.65	82 ± 3
4d		18	100	**4n**		5.5	100
4e		2.1	100	**4o**		13	71 ± 9
4f		0.63	100	**4p**		47	100
4g		2.6	100	**4q**		73	100
4h		17	100	**4r**		0.62	80 ± 2
4i		0.35	75 ± 4	**4s**		1.5	62 ± 4
4j		NA	19 ± 10	**4t**		64	100

[a]Based on data pooled from three independent experiments using 5–7 compound concentrations.
[b]Maximal attainable inhibition according to the preferred curve fit given as mean ± standard error.
[c]NA: not active.



Scheme 1. Reagents and conditions: (i) PPA, 200 °C, 6 h; (ii) $(COCl)_2$, NaH/Et_3N, DMF, rt, overnight (for **4a–c** and **4l–n**); (iii) TBTU, DIPEA, DMF, 70 °C, overnight (for **4d–k** and **4o–t**).

as well as poor inhibitors were found among both oxazolo and imidazo compounds, for example, **4i** and **4r** compared to, for example, **4k** and **4t**. The data show that the oxazolo and imidazo compounds display slightly different SARs based on the positioning of these groups and the amide on the central aromatic ring. Based on the low IC_{50} and maximum inhibition, the oxazolo scaffold, for example, **4f** is superior when carrying large cyclic substituents in *meta* position.

The data summarised in Table 1 was according to an assay where the compounds were not pre-incubated with the enzyme prior to the addition of substrate, in order to give an estimate of the initial affinity between inhibitor and FAAH. However, for most of the compounds, concentration–response curves were also obtained using a 60-min pre-incubation period, in order to determine whether or not the observed inhibition was time-dependent. A small degree of time-dependent inhibition was seen with **4t** (IC_{50} values of 43 vs. 64 μM for 60 vs. 0 min of pre-incubation). For compound **4i**, the IC_{50} valued were essentially the same (0.28 vs. 0.35 μM for 60 vs. 0 min of pre-incubation), but the maximum observed inhibition was lower for the pre-incubated samples (39 ± 2% vs. 75 ± 4%, respectively). A reduced maximal inhibition was also seen with **4 l**, **4m** and **4r,** whereas essentially identical inhibition curves were seen with **4n**–**q** and **4s**.

Compounds **4a**–**c** were not active, even after pre-incubation. These data support the conclusion that the compounds are reversible FAAH inhibitors. Under our assay conditions with a 60-min pre-incubation, the irreversible inhibitor URB597 gave an IC_{50} value of around 2 nM[28–30], which is in line with previously reported data of 4.6 ± 1.6 nM[7].

We determined the thermodynamic aqueous solubility in phosphate-buffered saline (10 mM, pH 7.48) at 37 °C of a subset of compounds essentially as described previously[31]. It is clear that all the tested compounds (**4f**, **4i**, **4m**, **4r** and **4s**) are poorly soluble under these conditions with solubility ranging from 3 to 20 μM. Comparison of **4i** and **4r** indicates that there is no difference in solubility, 18 and 20 μM, respectively, between oxazolo and imidazo compounds. Compounds with the cyclohexylphenoxy substitutent were generally less soluble than compounds with the biphenyl substituent, as seen for **4s** (3 μM) and **4r** (20 μM).

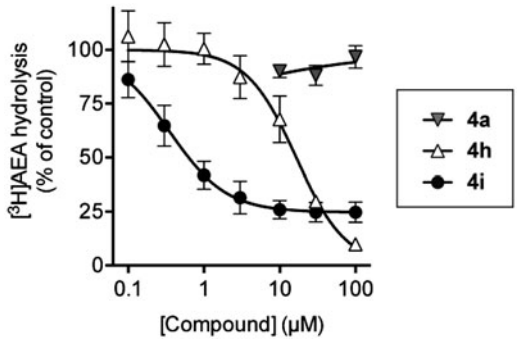


Figure 2. Inhibition of 0.5 μM [3H]AEA hydrolysis in rat brain hydrolysis by **4a**, **4 h** and **4i**. Shown are means ± sem. (when not enclosed by the symbols, $n = 3$). The curves are those of best fit using the log[inhibitor] versus response, variable slope algorithm available in the GraphPad Prism software (v6.0 h for the Macintosh). The curves were constrained to a maximum value of 100% and the minimum value was either set at 0% or allowed to float. The preferred model was then selected by the software using Akaike's informative criteria.

Molecular modelling

To evaluate potential binding poses of the compounds to FAAH, **4a**–**t** were docked to a crystal structure of FAAH (PDB code: 3QJ9)[32] using a docking protocol validated on the crystal ligand's docking performance (Figure 3(a)). Further details on method and results are given in the Supporting information. The crystal ligand 1-{(3S)-1-[4–(1-benzofuran-2-yl)pyrimidin-2-yl]piperidin-3-yl}-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one bound to FAAH with the benzofurane in the ligand binding site entrance interacting with Trp531 through a T-shaped aromatic interaction, and the benzimidazole-2-one deep inside the protein closer to the catalytic triad (Figure 3(a)). The pyrimidine bound to the protein via a water-mediated hydrogen bond, but no other classical hydrogen bonds were present, indicating a protein–ligand interaction mainly dominated by hydrophobic forces. Analysis of the docking binding poses revealed that molecules with the amide in *para*-position to the oxazolo[4,5-*b*]pyridine moiety (**4i**–**k)** or to the 1*H*-imidazo[4,5-*b*]pyridine (**4r**–**t**) bound with the aromatic parts at the same binding site as those of the ligand present in the crystal structure (Figure 3(b)). Molecules with the amide in *ortho*-position (**4a**–**c** and **4 l**–**m**), the oxazolo[4,5-*b*]pyridine or 1*H*-imidazo[4,5-*b*]pyridine rings bound in the same place as the crystal ligand benzofurane, while the other aromatic parts did not overlay (Figure 3(c)). Thus, the *ortho* molecules did not display the same binding mode as the crystal ligand and the hex-2-en-1-yl, biphenyl and 4-cyclohexylphenyl groups (Figure 3(c)) extending towards the catalytic triad (Figure 3(a)). The *meta* molecules displayed a mix of binding modes varying between those similar to the crystal ligand and those similar to the *ortho* molecules. Notably, the potent *meta*-molecule **4i** bound with all aromatic parts overlaying with the crystal ligand's ditto, very similar to compound **4r** (Figure 3(b)) indicating a plausible binding conformation. The docking scores (Glidescore)[33] and the binding free energies (MM-GBSA)[34] of the docked compounds were compared to the measured IC_{50} values. The crystal ligand was the strongest binder according to MM-GBSA ($\Delta G_{bind} = -95$ kcal/mol) and the reported IC_{50} for that compound was 0.1 μM[32] compared to our strongest inhibitors (ΔG_{bind} of −47 to −71 kcal/mol) with IC_{50} of 0.35–0.65 μM. Nevertheless, the binding free energies could not be used as a reliable indicator of binding strength between the molecules and FAAH. For instance, the difference seen between *ortho*-molecules carrying the oxazolo[4,5-*b*]pyridine ring, which were inactive, and the ones carrying the 1*H*-imidazo[4,5-*b*]pyridine, which included strong to weak inhibitors, could not be differentiated based on the binding free energy. Moreover, the potent inhibitor **4f** was

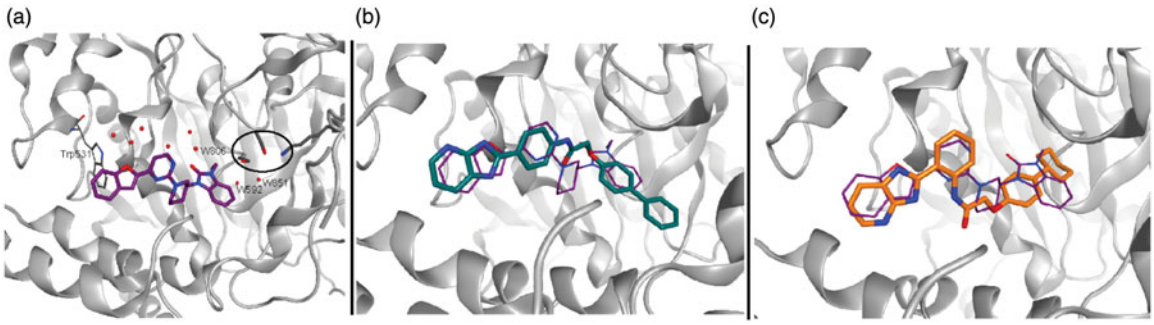


Figure 3. The FAAH protein with (a) the inhibitor from crystal structure with PDB code 3QJ9 in stick, the catalytic triad marked with a circle, and water molecules removed prior to docking indicated with name; (b) the highest ranked docking pose of **4r** in stick and crystal pose in wire; (c) the highest ranked docking pose of **4m** in stick and crystal pose in wire.

deemed weak according to ΔG_{bind} (−47 kcal/mol), while the equipotent inhibitors **4i** and **4r** had a ΔG_{bind} of −71 and −67 kcal/mole, respectively. Thus, the binding event of these inhibitors to FAAH is intricate, possibly including water-mediated interactions that has not been accounted in the energy calculations. The binding poses of the molecules, and especially for some of the *meta* and *para* molecules, are similar to the one seen in the crystal structure, although the affinity for FAAH cannot be completely and accurately described with MM-GBSA calculations.

Conclusions

In conclusion, for the first time, *N*-aryl 2-aryloxyacetamides were identified as FAAH inhibitors. These chemical scaffolds may serve to be useful as templates for the design of novel inhibitors of this physiologically important enzyme.

Supporting information

Information on the docking method, results and references, general chemistry and ^{1}H and ^{13}C spectra of final compounds **4a**–**t**.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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