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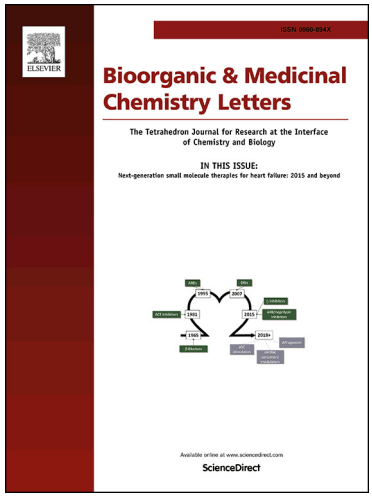

Discovery and evaluation of novel FAAH inhibitors in neuropathic pain model

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Graphical Abstract

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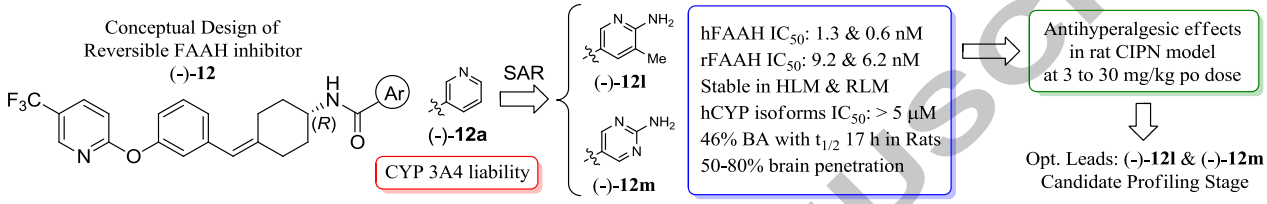

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ABSTRACT

Conceptual design and modification of urea moiety in chemotype PF-3845/ 04457845, the bench marking irreversible inhibitor of fatty acid amide hydrolase (FAAH), led to discovery of a novel nicotinamide-based lead **12a** having reversible mechanism of action. Focused SAR around the pyridine heterocycle (Ar) in **12a** (Table 1 & 2) resulted into four shortlisted compounds, (-)-**12a**, (-)-**12i**, (-)-**12l-m**. The required (-)-enantiomers were obtained via diastereomeric resolution of a novel chiral dissymmetric intermediate **15**. Based on comparative profile of FAAH potency, metabolic stability in liver microsome, liability of inhibiting major hCYP450 isoforms, rat PK, and brain penetration ability, two SAR optimized compounds, (-)-**12l** and (-)-**12m**, were selected for efficacy study in rat model of chemotherapy-induced peripheral neuropathy (CIPN). Both the compounds exhibited dose related antihyperalgesic effects, when treated with 3 to 30 mg/kg po for 7 days. The effects at 30 mg/kg are comparable to that of PF-04457845 (10 mg/kg) and Tramadol (40 mg/kg).

Neuropathic pain (NP) is a chronic pain caused by lesion or disease affecting any part of the nervous system leading to clinical conditions ranging from painful neuropathy to poststroke central pain.[1] Anticonvulsant drugs, Gabapentin and Pregabalin, acting on $\alpha_2\delta$ subunit-containing voltage-dependent calcium channels (VDCCs), and antidepressant agents such as serotonin-norepinephrine reuptake inhibitor (SNRI) Duloxetine are the first-line treatment options for NP.[2-3] However, their efficacies are mediocre, and also associated with several side effects including dizziness, vertigo, nausea, dry mouth and dairrhea.[3c-f] Hence, there is an unmet medical need to discover a new pharmacological class for the treatment of neuropathic pain that can also be safely combined with the current medication. We are particularly interested in condition like pain due to cancer chemotherapy-induced peripheral neuropathy (CIPN) for its growing importance in connection with the cancer chemotherapy.[4]

Fatty acid amide hydrolase (FAAH) is an intracellular membrane-bound enzyme that utilizes unusual catalytic triad Ser-Ser-Lys to degrade and inactivate fatty acid amide family of signaling lipids, including the endogenous cannabinoid ligands *N*-arachidonoylethanolamine (Anandamide, AEA) and 2-arachidonoyl glycerol (2-AG).[5] Since the discovery of FAAH as molecular target in 1996, several chemotypes have been reported and reviewed in the literature as FAAH inhibitors having reversible as well as irreversible mechanism of action.[6-7] Fig. 1 exemplifies some of the well characterized FAAH inhibitors **1-11** including those tested in early phase of clinical trials.[8-19] In general, pharmacological inhibition as well as genetic ablation[20] of FAAH has been shown to be associated with increased levels of endogenous fatty acid amides (FAAs) including AEA, eliciting analgesic effects in various animal models of inflammation and chronic pain primarily via activation of endocannabinoid CB receptors. Therefore, it has been also hypothesized that a FAAH inhibitor may avoid side effects as well as abuse of cannabis and exocannabinoids.[21]

In its first wave, clinical trials with FAAH inhibitors targeted several neuropsychiatric conditions such as schizophrenia, Tourette syndrome, symptoms associated with cannabis withdrawal, and inflammatory pain due to osteoarthritis (OA). In its careful second wave, NP and major depressive disorder (MDD) are being considered as more suitable indications, evidenced by advancement of **7a** (V-158866),[14] **11** (ASP-8477),[19] **6** (SSR-411298)[13] and **10b** (JNJ-42165279)[18b-c] in Phase-2 clinical trials.

Interaction of Ser-241 residue of FAAH catalytic site with its inhibitors have been particularly demonstrated by co-crystal structures,[22] initially with a substrate analog methoxy arachidonoyl fluorophosphonate (MAFP), and subsequently with

non-FA based synthetic inhibitors such as, (i) OL-135[8] (forms reversible oxyanion intermediate), (ii) URB-597[16] and (iii) PF-750/3845[17] (ii and iii form covalently bound irreversible carbamates). We conceptualized that replacing the urea moiety in PF-3845 with an amide functional group, as depicted in newly designed analog **12a** (Fig. 2), might lead to a reversible inhibition, via formation of the tetrahedral oxyanion intermediate.

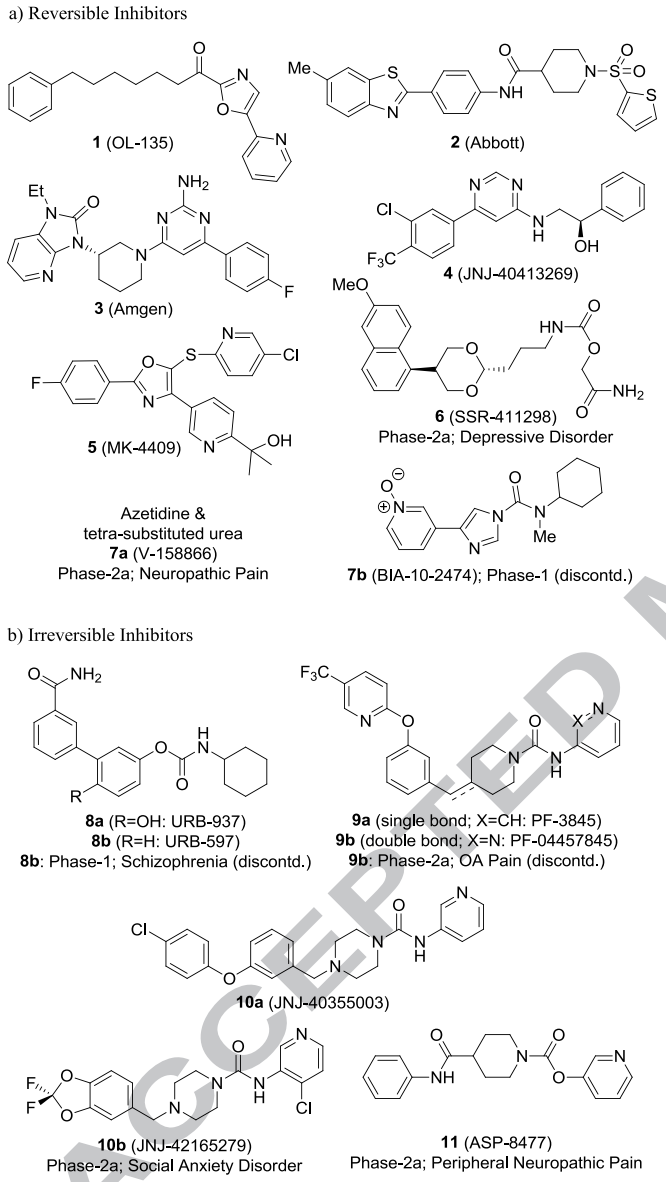


Fig 1. Representative FAAH inhibitors from literature.

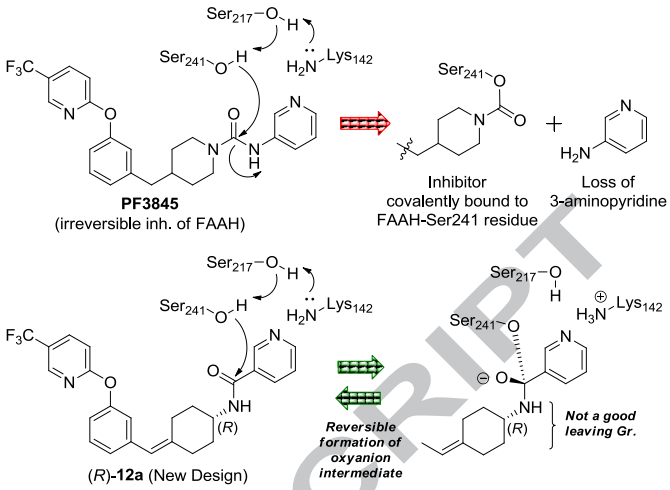


Fig 2. Design of a new reversible inhibitor (*R*)-**12a**.

Such an intermediate would neither have the option of permanent covalent bonding with the Ser-241 nor a hydrolysis, as the aliphatic cyclohexylamine moiety is not a good leaving group. Additionally, the newly designed **12a** scaffold would have the potential of retaining well established selectivity profile of PF-3845/04457845.[17] Suitability of replacing the urea moiety with an amide FG was verified by qualitative docking **12a** to the co-crystal structure of PF-3845 (PDB:3LJ6) (Fig. 2 and supplementary Fig. S1). Based on this in silico exercise, we have concluded that (*R*)-configuration of **12a** could only be docked to the catalytic site with the desired pose as compared to its (*S*)-enantiomer.

Accordingly, a new heteroaryl amide series was invented as FAAH inhibitors broadly claiming the scaffold **12**, and the intellectual property has been protected by PCT application.[23] Objective of this preliminary communication is to disclose a focused set of SAR around **12** which has also yielded two optimized leads suitable for further development.

Data of initial analogs (±)-**12a-h** have been shown in Table 1 wherein SAR was observed for the intended change of the right-hand side pyridin-3-yl ring of **12a** to various other *N*-containing heteroaryl ring (Ar) disposing the ring-*N* at different directions. Based on consistent human and rat FAAH potency as well as intrinsic metabolic stability in HLM and RLM, the analogs **12a**, **12e** and **12g** were qualified for the next round of SAR and optimization. Further focus was made on pyridine-3-yl (**12a**) and pyrimidin-5-yl (**12e**) analogs wherein the ring substitution SAR, particularly with additional -*N* atom, was studied as in **12i-12p**. Whereas, all other analogs maintained the FAAH potencies, **12j-k** lost the rat FAAH potency in a commonly observed phenomena in this series that, any electron withdrawing group which could potentially reduce the basicity of the 3-pyridyl-*N*, resulted in particular loss of rat potency. Subsequently, **12n-p** were also disqualified due to relatively less solubility which were reflected in <10% oral bioavailability in rats (supplementary Table S5a).

Based on above SAR inputs, compounds **12a**, **12i**, **12l-m** were resolved using chiral prep. HPLC and both the enantiomers (with >96% ee) were screened against FAAH inhibition assay. In all the cases, (-)-enantiomers were found to be >100 fold more potent than (+)-enantiomers irrespective of the human or rat FAAH enzyme (supplementary Table S1). Based on the comparative FAAH potency of the enantiomers, and supported

by the docking analysis, an (*R*)- stereochemistry has been tentatively assigned to the more potent (-)-enantiomers.

Profile of the shortlisted (-)-**12a**, (-)-**12i**, and (-)-**12l-m** have been presented in Table 2 which included additional two ADMET evaluation: (a) CYP inhibition; (b) rat PK and brain penetration. Selectivity against inhibition of major human CYP450 isoforms is an important aspect of modern-day drug optimization as the drug otherwise may lead to potential drug-drug-interactions (DDI). This is particularly important in the area of CIPN where multiple drugs are co-administered. CNS or brain penetration was another important requirement due the adequate therapeutic benefits in NP.

Ar								
Compound	**12a**	**12b**	**12c**	**12d**	**12e**	**12f**	**12g**	**12h**
hFAAH IC_{50} (nM)[a]	**2.2±0.04 (n = 8)**	508	14	9.0±1 (n = 6)	**2.6 ±0.2 (n = 3)**	10	**2.6±0.4 (n = 6)**	6
rFAAH IC_{50} (nM)[a]	**27.8±1.9 (n=8)**	ND[b]	15%[c]	194±35.2 (n = 3)	**100±13.1 (n = 3)**	266±64.1 (n = 5)	**64.6±11.3 (n = 7)**	145±53.6 (n = 3)
HLM; RLM MR (nmol/min/mg)[d]	**0.06; 0.06**	--	--	--	**<0.04; 0.07**	--	**0.04; <0.04**	--
Ar								
Compound	**12i**	**12j**	**12k**	**12l**	**12m**	**12n**	**12o**	**12p**
hFAAH IC_{50} (nM)[a]	**5.0±0.1 (n = 5)**	5.1±0.7 (n = 3)	4	**3±0.9 (n = 3)**	**2**	6.7±1.4 (n = 5)	3	2.2±0.2 (n = 5)
rFAAH IC_{50} (nM)[a]	**31.3±5.5 (n = 5)**	80.7±6.9 (n = 3)	52±8.5 (n = 3)	**29±4.2 (n = 3)**	**16**	6.6±0.5 (n = 4)	9	17.2±0.2 (n = 7)
HLM; RLM MR (nmol/min/mg)[d]	**0.07; 0.05**	0.06; 0.04	0.06; <0.04	**0.09; 0.04**	**0.03; 0.02**	0.11; 0.09	0.09; 0.04	0.08; 0.04

As was expected, all the four (-)-enantiomers were near 1 and 10 nM potent in h- and r-FAAH respectively and possessed adequate metabolic stability in HLM and RLM. The observed low intrinsic MR (0.06-0.04 nmol/min/mg) reflected well into the rat PK profile having low plasma clearance (CL), required longer plasma half-life ($t_{1/2}$) and good oral BA (%F). In addition, all the four compounds were found to be available in brain, indicating CNS penetration of the drugs. Compound (-)-**12a**, however, encountered with unacceptable hCYP3A4 inhibition (IC_{50} 0.4 µM) in addition to a border line liability for CYP 2C9 and 2C19.

Ar				
Compound	**(-)-12a**	**(-)-12i**	**(-)-12l**	**(-)-12m**
hFAAH IC_{50} (nM)[b]	0.7±0.06 (n=3)	1.2±0.03 (n=3)	1.3±0.1 (n=7)	0.6 ±0.03 (n = 3)
rFAAH IC_{50} (nM)[b]	13±1.9 (n=3)	11.6±0.9 (n=3)	9.2±0.8 (n=6)	6.2±1.0 (n = 4)
HLM; RLM MR (nmol/min/mg)[c]	0.04; <0.04	<0.04; 0.06	0.06; 0.04	<0.04; 0.04
hCYP IC_{50} (nM)[d]				
1A2, 2C9, 2C19, 2D6, 3A4	>10, 2.7, 4.6, >10, 0.4 (2.4)	>12.5, 3.4, 9.1, >12.5, >12.5(>12.5)	>12.5, 9.2, >12.5,>12.5, >12.5(>12.5)	>12.5, 6.5, >12.5,>12.5, >12.5(>12.5)
Rat PK:[e]				
CL(mL/min/kg)	2.4±0.2	0.7±0.03	0.8±0.07	0.6±0.1
V_{ss} (L/kg)	0.9±0.1	0.6±0.04	1.2±0.1	0.8±0.2
$t_{1/2}$ (h)	5.1±1.2	9.2±2.6	17 ±2.3	17.2±1.7
%F	35	42	46	46
Brain : plasma	1.5±0.2	0.6±0.01	0.5±0.04	0.8±0.02

Table 2. Profile of the shortlisted (-)-enantiomers.[a]

[a] Chiral prep. HPLC method used for the resolution into the enantiomers (>96% ee). FAAH IC_{50} for the less potent (+)-isomers are given in supplementary Table S1.

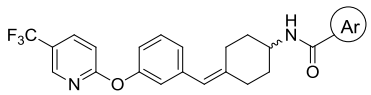
[b] Ref to footnote a) in Table 1.

[c] Ref to footnote d) in Table 1.

[d] Ref to supplementary section for protocol. For hCYP3A4, midazolam as well as testosterone (value in parenthesis) were used as substrates due to different binding sites.

[e] Male SD rats (fasted overnight) were administered compounds at 3 mg/kg iv (solution formulation) and 10 mg/kg po (NaCMC suspension). PK values presented as mean ± SD. Ref to supplementary data for the protocol and formulation details.

Table 1. FAAH inhibition and metabolic stability SAR of analogs around (±)-**12**.



[a] Each IC_{50} value corresponds to an average of at least two experiments (n≥2) each in triplicate data set and obtained as per the experimental procedure described in supplementary section. Values are presented as average±SEM when n>2.

[b] Not Determined.

[c] % inhibition at 100 nM concentration of the inhibitor.

[d] Intrinsic metabolic rate (MR) in Human or Rat Liver Microsome (HLM or RLM) in the presence of NADPH cofactor (in nmol/min/mg of the LM protein). MR value of <0.1 approx correlates to >70% unmetabolized after 30 min. of incubation, and it indicates that the compound is suitable for progression to PK study.

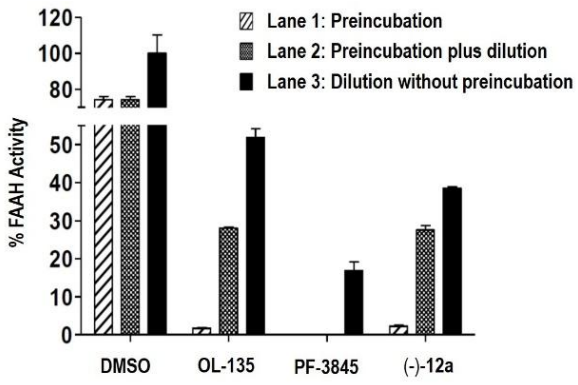


Fig 3. Reversible mechanism of action of (-)-**12a**. Briefly, hFAAH (10x of regular assay conc) and the inhibitor of choice (at 10xIC_{50} conc) were

preincubated for 1 hour followed by addition of arachidonyl-7-amino-4-methyl coumarin amide (AAMCA) as substrate (Lane 1, hatched bar) or a 10-fold dilution of the preincubated enzyme-inhibitor complex into a reaction buffer containing the substrate (Lane 2, crossed grid bar). A reference condition was created without the preincubation period but doing 10-fold dilution process to mimic the IC_{50} situation (Lane 3, solid bar). The resultant % FAAH enzyme activities have been plotted for the three conditions. **PF-3845**: FAAH activity could not be recovered upon dilution after the preincubation (crossed grid bar not detectable), as the inhibitor formed an irreversible complex with the enzyme. However, near 20% FAAH activity was recovered without the preincubation (solid bar). **OL-135** and (-)-**12a**: Near 30% FAAH activity were recovered after the preincubation followed by dilution (crossed grid bars) as the effective conc. of the inhibitors decreased by a factor of 10 (close to its IC_{50} conc), indicating the formation of reversible enzyme-inhibitor complex.

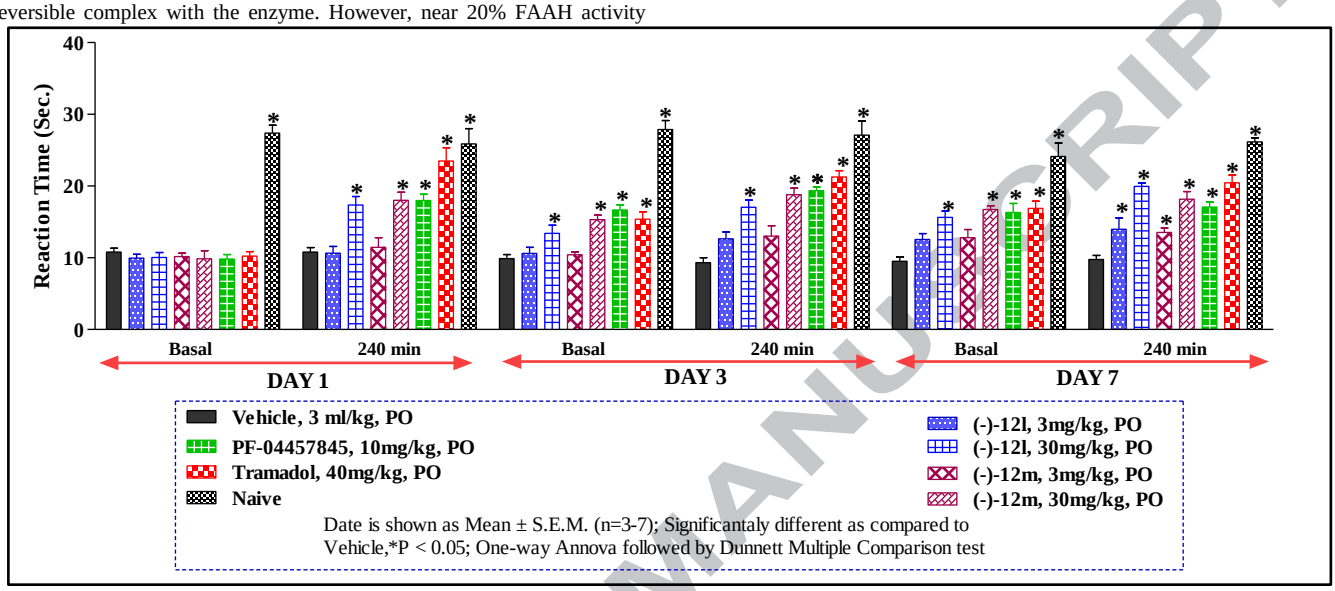
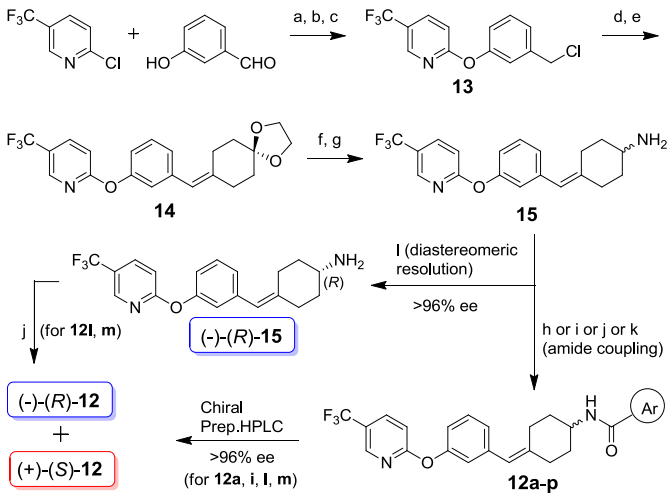


Fig 4. Effect of (-)-**12l** and (-)-**12m** (synthesized by diastereomeric resolution method; >96% ee) on thermal hyperalgesia in a rat model of CIPN. The model was developed in SD rats by administration of cisplatin (Cytoplatin Injection, Cipla) intraperitoneally once a week at a dose of 3 mg/kg for 4 weeks (cumulative dose, 12 mg/kg). After that, thermal hyperalgesia was assessed using plantar test apparatus at 25 IR intensity and cut off time of 45 seconds. Testing comprised alternate presentations of radiant heat from below to hindpaw, focused on the mid-plantar surface. The heat source cut off automatically with paw withdrawal and the reaction time (Paw Withdrawal Latency) was recorded. Paw withdrawal latency was defined as the mean of three trials applied to paw. All the treated and the vehicle groups were assessed for the thermal hyperalgesia on day 1, 3, 7 at the time of 0 h (before dosing, basal) and 4 h (240 min) after once daily (qd) oral (po) treatment. Vehicle control: Tween 80® - 1% (v/v); 0.5% (w/v) NaCMC in water q.s.; dose vol. 3 mL/kg. Reversible FAAH inhibitors: (-)-**12l**, (-)-**12m** at 3 and 30 mg/kg po qd dose. Irreversible FAAH inhibitor: **PF-04457845** at 10 mg/kg po qd dose. A standard positive control: Tramadol at 40 mg/kg po qd dose. Dosing formulation for the test compounds: oral suspension in Tween 80® - 1% (v/v); 0.5% (w/v) NaCMC in water q.s; dose vol. 3 mL/kg. Ref to supplementary data for the detailed protocol.

The desired optimized lead like properties were achieved in compounds (-)-**12l** and (-)-**12m** with a balanced profile of FAAH potency (h-IC_{50}: 1.3 & 0.6 nM; r-IC_{50}: 9.2 & 6.2 nM respectively), selectivity against five major hCYP450 isoforms (>5000 fold), and rat PK properties (46% BA, 50-80% brain penetration). Finally, for the development of FAAH inhibitors, selectivity against a related enzyme, monoacylglycerol lipase (MAGL),[6b,6f] is very important as the latter is primarily responsible for hydrolysis of another endocannabinoid 2-AG, essential for the brain and CNS functions. We did not see any inhibition of MAGL tested in vitro up to 10 μM of (-)-**12l** and (-)-**12m** (>10000 fold selectivity).

A reversible mechanism of action by this chemotype was established based on a principle of qualitative recovery of FAAH enzyme activity upon dilution of a preincubated enzyme-inhibitor complex. As briefed in Fig. 3, recovery of hFAAH activity, for hydrolysis of its substrate AAMCA, was possible in case of preincubation with the known reversible inhibitors **OL-135**, and (-)-**12a**. On the contrary, the enzyme activity could not be recovered when preincubated with the covalent and irreversible inhibitor **PF-3845**. Reversible as well a non-substrate like MoA for the FAAH inhibition by (-)-**12a** was further confirmed in a separately designed experiment by near 100% recovery of (-)-**12a** (quantified by mass spectroscopic method) over a period of 1 h incubation with hFAAH enzyme. In contrast, there was a gradual loss of **PF-3845** observed under the similar condition (supplementary Table S2).

Finally, the optimized leads **(-)-12l** and (-)-**12m** were tested in rat model of CIPN (3 and 30 mg/kg, po, qd, administered for 7 days) and the results have been shown in Fig. 4. Under acute set up, after single oral administration at 30 mg/kg, both the compounds exerted significant improvement of the NP condition and thermal hyperalgesia by exhibiting increased Paw Withdrawal Latency and reaction time compared to vehicle (measured at 4 h post dosing on day 1). The pain relieving effect was maintained in



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 100 °C, 18 h, 95%; (b) $NaBH_4$, MeOH, 0 °C, 1 h, 99%; (c) $SOCl_2$, CH_2Cl_2, 0 °C to RT, 1 h, 99%; (d) $P(OEt)_3$, 140 °C, 18 h, 85%; (e) 1,4-dioxaspiro[4.5]decan-8-one, NaH, THF, 0 °C to RT, 18 h, 63%; (f) 1(M) HCl, acetone, 60 °C, 2-4 h, 100%; (g) (i) 2(M) NH_3 in EtOH, Ti(*O*-*i*-Pr)$_4$, RT, 6 h; (ii) $NaBH_4$, RT, 18 h; (iii) NH_4OH, 60% combined; (h) $ArCO_2H$, EDCI.HCl, HOBt, NMM, DMF, RT, 14-18 h, 40-50% (for **12a-h**, **12n-p**); (i) ArCOCl, Et_3N, DCM, 0 °C to 26 °C, 2 h, 79% (for **12a**); (j) $ArCO_2H$, PyBOP, DIPEA, CH_2Cl_2, RT, 18 h, 60-72% (for **12i, 12k-m**); (k) RCOOH, oxalyl chloride, Et_3N, CH_2Cl_2, 0 to 26 °C, 2 h, 82% (for **12j**); (l) (i) (+)-*O*,*O*'-di-*p*-toluoyl-*D*-tartaric acid, EtOH, 70 °C, 2 h, then cool to RT, ppt collected; (ii) Crystallization using MeOH:EtOH (3:1) followed by recrystallization using MeOH:EtOH (1:1); (iii) 5% NaOH, AcOEt (pH 10), 10-15 °C, 30 min. >98% chiral purity by HPLC.

a subacute condition upon repeat dosing for 6 days and measured after 24h of post dosing (same as basal reading on day 7). Though the antihyperalgesic effect of the compounds at 3 mg/kg po could not be seen upon single administration, it indeed established the effect on day 7 in a dose dependent manner (measured at 4 h post dosing on day 7). Overall, the reversible FAAH inhibitors (-)-**12l** and (-)-**12m** at 30 mg/kg po manifested the therapeutic effects comparable to the tested irreversible FAAH inhibitor **PF-04457845** (at 10 mg/kg po dose) and a standard positive control Tramadol (at 40 mg/kg po dose).

Synthetic outline of the FAAH inhibitors **12a-p** has been shown in Scheme-1 (ref. to supplementary section for experimental). A novel dissymmetric amine **15** acted as a common intermediate which was synthesized from commercially available synthons, 2-chloro-5-(trifluoromethyl) pyridine and 3-hydroxybenzaldehyde in seven steps via the formation of pyridyloxybenzylchloride intermediate **13** (steps a, b, c) followed by the spiro-olefinic intermediate **14** (steps d, e). Deprotection of ketal in **14** and subsequent reductive amination using ammonia and Ti(*O*-*i*-Pr)$_4$ (steps f, g) furnished the racemic amine **15**,[24] which upon amide coupling with appropriate carboxylic acids or acid chlorides (steps h/i/j/k) yielded the racemic compounds **12a-p**. Enantiomers of **12a**,[25] **12i**,[26] **12l-m**[27-28] were initially separated using chiral preparative HPLC and the required enantiomers were used for in vitro and PK studies as mentioned in Table 2. Subsequently, for multigram requirement of (-)-**12l-m** (CIPN efficacy study and toxicology experiments), the racemic intermediate **15** was resolved into (-)-(*R*)-**15** via diastereomeric salt formation using (+)-*O*,*O*'-di-*p*-toluoyl-*D*-tartaric acid (step l) and was used for the intended amide coupling reactions (step j).

In conclusion, a novel scaffold **12** has been discovered as FAAH inhibitor with reversible mechanism of action. Design strategy was to maintain the overall structural features of PF-3845/04457845 to take advantage of the well-established selectivity profile of PF-chemotype. The reversible and non-substrate like mechanism of action has been ascertained based on a qualitative recovery of the active enzyme and near quantitative recovery of (-)-**12** upon preincubation with hFAAH. After a systematic screening, two compounds (-)-**12l** and (-)-**12m** have been identified as optimized leads and therefore nominated for preclinical candidate profiling towards an indication of neuropathic pain (NP). Irreversible inhibitors particularly with longer plasma half-life can potentially generate circulating antibody upon chronic administration. The current FAAH inhibitors are expected not to encounter with such problems.

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24. 4-[[3-[[5-(Trifluoromethyl)-2-pyridyl]oxy]phenyl]methylene]cyclohexanamine **(15)**: Thick liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.13-1.23 (m, 1H, -CH_2-), 1.25-1.34 (m, 1H, -CH_2-), 1.87-1.94 (m, 1H, -CH_2-), 1.95-2.04 (m, 2H, -CH_2-), 2.25 (dt, *J* = 12.9, 3.4 Hz, 1H, >C<u>H</u>-NH_2), 2.38 (d, *J* = 13.7 Hz, 1H, -CH_2-), 2.84-2.95 (m, 2H, -CH_2-), 3.48 (s, 2H, -NH_2), 6.26 (s, 1H, olefinic), 6.97-7.01 (m, 3H, Py-3H & Phenoxy-*ortho*-H), 7.08 (d, *J* = 7.6 Hz, 1H, Phenoxy-*para*-H), 7.37 (t, *J* = 7.8 Hz, 1H, Phenoxy-*meta*-H), 7.89 (dd, *J* = 8.5, 2.2 Hz, 1H, Py-4H), 8.45 (s, 1H, Py-6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.1, 35.3, 36.9, 37.6, 50.1, 111.3, 118.9, 121.5 (q, *J* = 33.3 Hz, 1C), 121.8, 122.1, 123.7 (q, *J* = 270 Hz, 1C), 126.1, 129.4, 136.7 (q, *J* = 3.1 Hz, 1C), 140.2, 142.4, 145.6 (q, *J* = 4.1 Hz, 1C), 153.0, 165.9. LC-MS (ES) m/z 349.2 (M+1). HRMS (ESI) *m/z* calcd for $C_{19}H_{20}F_3N_2O$ $[M+H]^+$ 349.1522, found 349.1523. **(-)-15**: Chiral purity: >98 % (by HPLC). $[\alpha]^{20}_D$ -28.8° (*c* 0.5, MeOH).
25. *N*-[4-[[3-[[5-(Trifluoromethyl)-2-pyridyl]oxy]phenyl]methylene]cyclohexyl]pyridine-3-carboxamide (**12a**): Mp 140-142 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.37 (dq, *J* = 11.0, 5.6 Hz, 1H, -CH_2-), 1.48 (dq, *J* = 11.3, 5.1 Hz, 1H, -CH_2-), 2.15-2.59 (m, 3H, -CH_2-), 2.37-2.49 (m, 2H, -CH_2-), 2.88-2.94 (m, 1H, -CH_2-), 4.18-4.28 (m, 1H, >C<u>H</u>-NH_2), 6.00 (d, *J* = 7.4 Hz, 1H, amide-NH), 6.32 (s, 1H, olefinic), 6.99-7.03 (m, 3H, Py-3H & Phenoxy- *ortho*-H), 7.00 (d, *J* = 7.9 Hz, 1H, Phenoxy-*para*-H), 7.37-7.41 (m, 2H, Phenoxy-*meta*-H & RHS-Py-5H), 7.90 (dd, *J* = 8.8, 2.4 Hz, 1H, Py-4H), 8.10 (dt, *J* = 7.8, 1.7 Hz, 1H, RHS-Py-4H), 8.45 (s, 1H, Py-6H), 8.72 (dd, *J* = 4.9, 1.7 Hz, 1H, RHS-Py-6H), 8.94 (d, *J* = 1.7 Hz 1H, RHS-Py-2H). LC-MS (ES) m/z 454.1 (M+1). HRMS (ESI) *m/z* calcd for $C_{25}H_{22}F_3N_3O_2$ $[M+H]^+$ 454.1737, found 454.1742. (-)-**12a**: Mp 135-136 °C. $[\alpha]^{20}_D$ -22.8° (*c* 0.2, $CHCl_3$).
26. 6-Amino-*N*-[4-[[3-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenyl] methylene]cyclohexyl]pyridine-3-carboxamide (**12i**). Mp 156-157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28-1.39 (m, 1H, -CH_2-), 1.41-1.50 (m, 1H, -CH_2-), 2.09-2.20 (m, 3H, -CH_2-), 2.35-2.45 (m, 2H, -CH_2-), 2.90 (d, *J* = 14.2 Hz, 1H, -CH_2-), 4.15-4.23 (m, 1H, 1H, >C<u>H</u>-NH_2), 4.79 (brs, 2H, RHS-Py-6-NH_2), 5.80 (d, *J* = 7.6 Hz, 1H, amide-NH), 6.31 (s, 1H, olefinic), 6.49 (d, *J* = 8.5 Hz, 1H, RHS-Py-5H), 6.99-7.02 (m, 3H, Py-3H & Phenoxy- *ortho*-H), 7.10 (d, *J* = 7.6 Hz, 1H, Phenoxy-*para*-H), 7.38 (t, *J* = 7.8 Hz, 1H, Phenoxy-*meta*-H), 7.85-7.91 (m, 2H, Py-4H & RHS-Py-4H), 8.44

(brs, 2H, Py-6H & RHS-Py-2H). LC-MS (ESI) m/z 469.3 (M+1). HRMS (ESI) *m/z* calcd for $C_{25}H_{24}F_3N_4O_2$ $[M+H]^+$ 469.1846, found 469.1849. (-)-**12i**: Mp 145-147 °C. $[\alpha]^{20}_D$ -56.3° (*c* 0.2, $CHCl_3$).

27. 6-Amino-5-methyl-*N*-[4-[[3-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenyl] methylene]cyclohexyl]pyridine-3-carboxamide (**12l**). Mp 165-168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25-1.38 (m, 1H, -CH_2-), 1.41-1.51 (m, 1H, -CH_2-), 2.10-2.21 (m, 6H, -CH_2-, RHS-Py-5-CH_3), 2.35-2.45 (m, 2H), 2.87-2.92 (m, 1H), 4.15-4.25 (m, 1H, >C<u>H</u>-NH_2), 4.74 (brs, 2H, RHS-Py-6-NH_2), 5.79 (d, *J* = 7.3 Hz, 1H, amide-NH), 6.31 (s, 1H, olefinic), 6.99-7.02 (m, 3H, Py-3H & Phenoxy- *ortho*-H), 7.10 (d, *J* = 7.5 Hz, 1H, Phenoxy-*para*-H), 7.38 (t, *J* = 7.6 Hz, 1H, Phenoxy-*meta*-H), 7.72 (s, 1H, RHS-Py-4H), 7.90 (dd, *J* = 8.6, 2.4 Hz, 1H, Py-4H), 8.30 (s, 1H, RHS-Py-2H), 8.45 (s, 1H, Py-6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.9, 27.1, 33.5, 34.2, 35.3, 48.3, 111.4, 115.9, 119.1, 121.1, 121.5 (q, *J* = 33.3 Hz, 1C), 121.8, 123.7 (q, *J* = 269.4 Hz, 1C), 122.7, 126.1, 129.5, 136.7 (q, *J* = 3.1 Hz, 1C), 140.0, 141.5, 145.0, 145.5 (q, *J* = 3.8 Hz, 1C), 153.0, 159.3, 165.5, 165.8, 165.9. LC-MS (ESI) m/z 483.1 (M+1). HRMS (ESI) *m/z* calcd for $C_{26}H_{26}F_3N_4O_2$ $[M+H]^+$ 483.2002, found 483.2004. (-)-**12l**: Mp 142-144 °C. $[\alpha]^{20}_D$ -60.4° (*c* 0.2, $CHCl_3$).

28. 2-Amino-*N*-[4-[[3-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenyl] methylene]cyclohexyl]pyrimidine-5-carboxamide (**12m**). Mp 187-190 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27-1.37 (m, 1H, -CH_2-), 1.40-1.49 (m, 1H, -CH_2-), 1.80-1.96 (m, 2H, -CH_2-), 2.06 (t, *J* = 12.8 Hz, 1H, -CH_2-), 2.27 (t, *J* = 13.2 Hz, 1H, -CH_2-), 2.38 (d, *J* = 13.6 Hz, 1H, -CH_2-), 2.80 (d, *J* = 13.6 Hz, 1H, -CH_2-), 3.93-4.02 (m, 1H, >C<u>H</u>-NH_2), 6.29 (s, 1H. olefinic), 6.99 (s, 1H, amide-NH), 7.03 (d, *J* = 8.1 Hz, 1H, Phenoxy-*ortho*-H), 7.10-7.17 (m, 3H, Phenoxy-*ortho*-H, pyrimidine-3 & 5-H), 7.22 (d, *J* = 8.6 Hz, 1H Py-3H), 7.40 (d, *J* = 7.8 Hz, 1H, Phenoxy-*para*-H), 8.02 (t, *J* = 7.9 Hz, 1H, Phenoxy-*meta*-H), 8.22 (dd, *J* = 8.6, 2.0 Hz, 1H, Py-4H), 8.56 (s, 1H, Py-6H), 8.63 (s, 2H, pyrimidine-NH_2). ^{13}C NMR (100MHz, $CDCl_3$) δ 27.1, 33.5, 34.2, 35.2, 48.5, 111.5, 118.6, 119.2, 121.6 (q, *J* = 33.3 Hz, 1C), 121.9, 123.0, 123.8 (q, *J* = 269.4 Hz, 1C), 126.2, 129.6, 136.8 (q, *J* = 3.1 Hz, 1C), 139.9, 141.1, 145.6 (q, *J* = 3.9 Hz, 1C), 153.1, 157.9 (2C), 163.5, 164.0, 165.9. LC-MS (ESI) m/z 470.2 $[M+H]^+$. (-)-**12m**: Mp 173-175 °C. $[\alpha]^{20}_D$ -72° (*c* 0.5, MeOH).

Supplementary Data

Molecular docking study; synthetic details of compounds **12**; brief protocols of pharmacological and DMPK experiments; additional FAAH MoA data associated with the article.

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Highlights:

- Novel scaffold as FAAH inhibitor with reversible mechanism of action
- Proof of MoA by recovery of active enzyme upon preincubation with inhibitor
- Synthesis via diastereomeric resolution of novel chiral dissymmetric intermediate
- Drug-like DMPK properties of Opt. Leads (-)-**12l-m** suitable for preclinical development
- Antihyperalgesic effects, in rat CIPN model at 3 to 30 mg/kg po dose.