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The SAR of brain penetration for a series of heteroaryl urea FAAH inhibitors

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ABSTRACT

The SAR of brain penetration for a series of heteroaryl piperazinyl- and piperadinyl-urea fatty acid amide hydrolase (FAAH) inhibitors is described. Brain/plasma (B/P) ratios ranging from >4:1 to as low as 0.02:1 were obtained through relatively simple structural changes to various regions of the heteroaryl urea scaffold. It was not possible to predict the degree of central nervous system (CNS) penetration from the volumes of distribution (V_d) obtained from pharmacokinetic (PK) experiments as very high V_ds did not correlate with high B/P ratios. Similarly, calculated topological polar surface areas (TPSAs) did not consistently correlate with the degree of brain penetration. The lowest B/P ratios were observed for those compounds that were significantly ionized at physiological pH. However, as this class of compounds inhibits the FAAH enzyme through covalent modification, low B/P ratios did not preclude effective central target engagement.

The brain is the primary moderator of numerous biological processes for which pharmacological intervention may be of clinical benefit. However, it is effectively separated from circulating blood by the blood–brain barrier (BBB) and the blood-cerebrospinal fluid barrier. Of the two, the BBB is the most important as its surface area is several thousand fold greater than that of the blood-cerebrospinal fluid barrier.[1–3] The BBB lines the vasculature of the CNS and consists of endothelial cells with continuous tight intercellular junctions overlaying the basement membrane which in turn overlays astrocytes.[4] The BBB is also armed with an assortment of efflux transporters and metabolic enzymes[5] that collectively tend to keep proteins and other large molecules (mw > 500) as well as small polar or charged species from entering the brain.[6–11] While a certain amount of lipophilic character is required for passive diffusion into the CNS,[12] the relationship between lipophilicity and brain uptake tends to be parabolic in nature, with neither the exceptionally polar nor excessively lipophilic compounds penetrating readily.[13–15] Published reports suggest that a molecule's ability to pass the BBB tends to be poor when it possesses any of the following characteristics: mw > 450 g/mol; Log*P* > 4; hydrogen bond donors > 5; ∑N + O > 10.[16–19] For many classes of CNS drugs, it has been found that the ideal Log*P* lies between 2 and 3.5.[20–26] Correlations between the topological polar surface areas (TPSAs) of molecules and their ability to penetrate the CNS have also been found.[27,28] CNS penetrant compounds typically have TPSAs below 90 Å^2, with many below 60 Å^2.[29]

In the course of an internal FAAH[30,31] program,[32–45] many very similar compounds were profiled in vivo with particular interest paid to their ability to penetrate the BBB. The compounds profiled were heteroaryl piperazinyl and piperadinyl ureas; a class of compounds, reported on previously by us and others (Fig. 1),[46–50] that inhibit the FAAH enzyme through forming a covalent adduct between the active site serine (Ser241) and the urea carbonyl with the concomitant loss of the aryl amine. The compounds are synthetically modular and most are easily prepared in a few steps (see Supporting information).

Table 1 contains a selection of data generated from compounds on which only the heteroaryl amine was varied. Included in the table are the in vitro potency of the molecules reported as apparent IC_{50}s (due to the time dependent nature of the inhibition), PK data from Sprague–Dawley rats when available, the calculated topological polar surface areas (TPSAs) and *A*Log*P* values, and the brain to plasma ratio of compound concentrations (C_b/C_p) obtained after dosing of the compound by oral gavage. Interestingly, the range

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0960-894X/

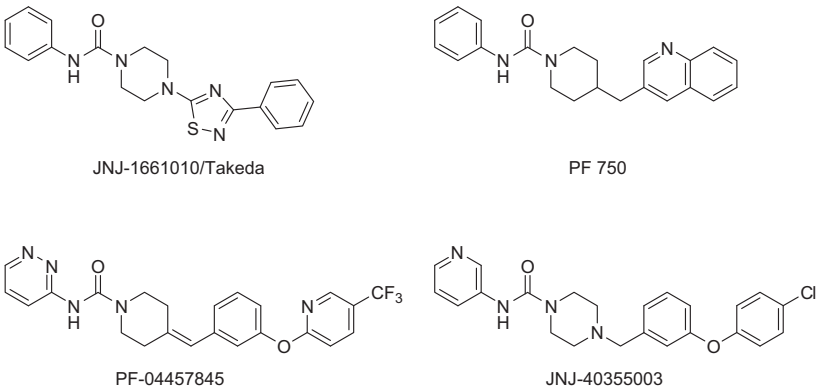


Figure 1. Aryl piperazinyl and piperadinyl ureas.

of C_b/C_p ratios obtained was quite broad and they did not correlate with TPSAs or pK_as. Likewise, the *A*Log*P* values for these compounds (ranging from 2.8 to 4.9) did not offer a predictive trend with regard to brain penetration. The simple phenyl derivative (**1**) serves as a reference point and its C_b/C_p ratio was an impressive 3.64.

It has been reported that the presence of pyridine nitrogens in a molecule tends to lead to decreased brain penetration relative to phenyl.[51–53] In the present series of molecules, replacing the phenyl with a 3-pyridyl group to give **JNJ-40355003** (**2**) modestly decreased CNS penetration to give a C_b/C_p ratio of 2.62. While **JNJ-40355003** retained excellent brain penetration properties, simply adding a chlorine atom to the 4-position of the pyridine ring (**3**) led to a significantly lower C_b/C_p ratio. Addition of the chlorine atom also appears to have introduced greater metabolic instability as (**3**) had a 4-fold higher rate of clearance than **JNJ-40355003**. Counterintuitively, the 4-cyano pyridine derivative (**4**) actually had a higher B/P ratio than **JNJ-40355003**, although it too gets cleared rather quickly. The non-basic pyrazine derivative (**5**) exhibited the highest C_b/C_p ratio (4.2:1) of any of the compounds in this data set despite having two azine nitrogen atoms.

The greatest variation in B/P ratios was obtained with 5-membered heteroarenes. The simple 3-isooxazole derivative (**6**) is highly brain-penetrant (C_b/C_p ratio of 3.3), but replacement of the oxygen with a hydrogen-bond donating N–H results in a compound only slightly favoring partitioning into the brain over the plasma. Perhaps not surprisingly, the tetrazole (**8**), which would be completely deprotonated under physiological conditions (tetrazole p$K_a \approx 4.9$,[54] >99% deprotonated at pH 7.2),[55] is the least brain penetrant compound of this series with a C_b/C_p of 0.02:1 (C_p/C_b of >45:1). However, the very poor CNS penetration ability of (**8**) does not preclude effective central target engagement (vide infra).

Hydrogen-bond donating and acidic groups are not the only chemical features that lead to decreased brain penetration. Fusion of a benzo ring to the isoxazole to give (**9**) also leads to a large decrease in the C_b/C_p ratio, greater in fact, than replacement of the oxygen with an N–H.[56] The two imidazopyridine derivatives (**10** and **11**) are interesting cases as they are significantly protonated[57] at physiological pH (imidazopyridine p$K_a \approx 6.8$,[58] 60% protonated at pH 7.2). These compounds favor the plasma over the brain by about 3:1 and, at least with (**10**), appear to be cleared quickly. This compound too, should serve as a reminder that high volumes of distribution as obtained from simple PK experiments, in this case $V_{ss} = 10.7$, does not necessarily predict good brain penetration.

We have been able to affect large changes in the CNS penetrating ability of these ureas simply through the modification of the pendant heteroarenes of the urea, but we can also modulate C_b/C_p through small changes to the piperazine core and the biaryl ether tail (Table 2). Replacement of the chlorine atom of **JNJ-40355003** with a bromine (**12**), fluorine (**13**) or CF_3 (**14**) group leads to a modest reduction in C_b/C_p. Introduction of a chloropyridine group onto the fluoro derivative (**15**) leads to a substantial further decrease of C_b/C_p, and as was observed with (**3**), gave a compound that was rapidly cleared.

Introduction of a chloropyridine group onto the fluoro derivative (**13** → **15**) leads to a similarly potent compound that exhibits a lower C_b/C_p, and as was observed with (**3**), was rapidly cleared. However, the high clearance of (**15**) did not prevent the increase in the concentrations of FAAs even as its plasma levels were rapidly decreasing.

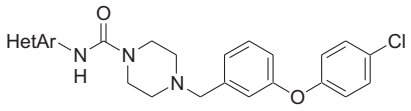
Introduction of a chlorine atom to the benzyl ring of the biaryl ether had a significant deleterious impact on the PK properties of (**16**) relative to (**2**). The PK profile of (**16**) suggested very slow absorption from the gut was taking place, such that after 4 h, compound levels in the plasma and brain were still rising and the C_b/C_p gradually increasing (Fig. 2).

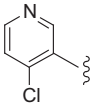
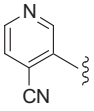
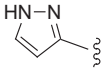
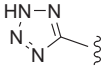
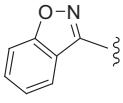
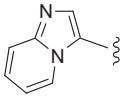
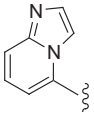
Surprisingly, a much smaller effect was observed when the piperazine nitrogen was replaced with a C–H. Compound (**17**) gave a C_b/C_p ratio very close to that of **JNJ-40355003**. Interestingly, reintroduction of a nitrogen atom into the biaryl ether (**18**) had a robust deleterious effect on CNS penetration (C_b/C_p ratio of 0.45).

While lacking a direct comparator for (**19**), it would appear that moving the side-chain of the piperidine ring from the 4- to the 3-position did not benefit CNS penetration.

As mentioned above, a strong preference for partitioning into the brain over the plasma is not always necessary for effective target engagement. Using the most CNS penetrant compound (**5**) as a reference (Fig. 3), it was determined that residual FAAH activity was reduced to below 10% within a half-hour and remained suppressed throughout the experiment. The profile for the highly potent, but plasma preferring compound (**10**), is somewhat different. Thirty minutes after dosing, (**10**) has only inhibited approximately 60% of FAAH present in the brain, but by 1 h there is less than 10% active enzyme and by 4 h residual activity is below 5%. Even the tetrazole (**8**; C_b/C_p ratio of 0.02:1) is able to inhibit FAAH centrally to below 10% residual enzyme activity but it took approximately 4 h to do so. PEA and OEA concentrations began increasing at lesser degrees of FAAH inhibition because they are hydrolyzed by the enzyme more slowly than AEA, which is the preferred substrate of FAAH. It is possible that AEA levels increased 7–10 h post dosing, but data for those time points were not collected. Interestingly, a complete return of enzymatic activity took place by 24 hours post dose, whereas complete reversal of inhibition with the more CNS penetrant **JNJ-40355003** took approximately 48 h. Mechanism-based enzyme inhibitors don't necessarily need to exhibit a high C_b/C_p to engage the target, but low concentrations in the brain will affect the rate at which inhibitors *encounter the*

Table 1
The effect of heteroaryl substituents on CNS penetration



Compd	HetAr	hFAAH apparent IC_{50}[a] (nM)	rFAAH apparent IC_{50}[a] (nM)	PK properties			TPSA (Å^2)	ALog*P*	pK_a[h]	C_b/C_p ratio (dose)
				Cl (L/h/kg)	V_{ss} (L/kg)	F (%)				
(**1**)		6 ± 4	298 ± 86	–	–	–	44.8	4.7	–	3.64 ± 0.33 (10 mg/kg)[c]
(**2**) **JNJ-40355003**		1.4 ± 0.41	33 ± 8.7	0.69[b]	3.2	53–69	57.7	3.5	5.25	2.62 ± 0.24 (0.6, 6 & 20 mg/kg)[g]
(**3**)		1.3 ± 0.55	20 ± 9	2.79[b]	3.33	18	57.7	4.2	3.85	1.18 ± 0.30 (20 mg/kg)
(**4**)		17 ± 5.5	17 ± 4.3	–	–	–	81.5	3.4	1.9	3.28 ± 0.26 (20 mg/kg)[d]
(**5**)		8.7 ± 6.3	84 ± 34	0.93[b]	4.11	35	70.6	2.9	0.6	4.24 ± 0.21 (20 mg/kg)
(**6**)		30 ± 8.5	615 ± 140	0.6[b]	2.6	50	70.8	3.1	−2.97	3.34 ± 0.32 (20 mg/kg)
(**7**)		32 ± 5.4	154 ± 81	0.5[b]	2	75	73.5	3.3	2.5	1.28 ± 0.11 (3, 10 & 30 mg/kg)[f]
(**8**)		81 ± 12	46 ± 11	0.2[c]	1.1	100	99.3	2.8	4.9	0.023 ± 0.004 (6 mg/kg)
(**9**)		10 ± 2.3	8 ± 2.5	0.56[b]	2.48	44	70.8	4.9	−4.7	0.86 ± 0.05 (3 mg/kg)
(**10**)		46 ± 16	118 ± 53	3.8[d]	10.7	60	62.1	3.6	6.8	0.33 ± 0.06 (20 mg/kg)
(**11**)		2.7 ± 0.4	5.7 ± 1.1	–	–	–	62.1	3.6	6.8	0.33 ± 0.11 (20 mg/kg)[e]

[a] 1 h incubation with the enzyme, errors are the standard error of the mean (SEM).
[b] Vehicle was 75% pharmasolve, 20% cremaphore and 5% aqueous dextrose.
[c] 30% SBCD with 1 M HCl.
[d] 50% PEG 400/water.
[e] Purified water.
[f] At 2 h time point.
[g] 5% aqueous dextrose.
[h] pK_a values are for the heterocycle and substituent (if any) absent the urea.

target and thus the time to onset of pharmacological effect. In those instances where target inhibition is slowly or non-reversible, then it's possible for the pharmacology induced by the molecule to persist beyond the time it takes for it to be cleared from the organism. For those indications where onset of centrally mediated pharmacology needs to be rapid (such as analgesia), a quickly absorbed compound with a high C_b/C_p ratio may be preferred. For chronic conditions, a compound with a lower C_b/C_p ratio may be preferable in order to minimize the potential for off-target side effects resulting from accumulation of the compound in the brain.

Due to recent events in the clinic with BIA 10-2474, it is important to extensively characterize the selectivity and secondary pharmacology of any FAAH inhibitor (particularly a mechanism-based inhibitor) that has the potential to enter the clinic. In addition, while there is the potential for the secondary pharmacology to influence the PK and C_b/C_p ratio of a compound, it is hypothesized that this effect would be minimal. The secondary pharmacology of every compound described in this manuscript has not been studied, but those that have and other compounds within this class of compound were shown to be highly selective after having been

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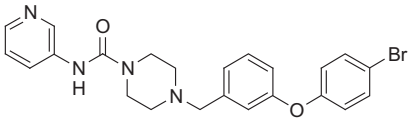
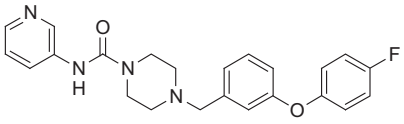
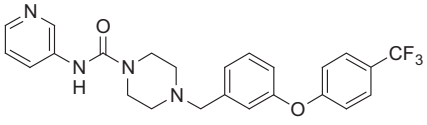
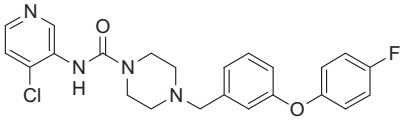
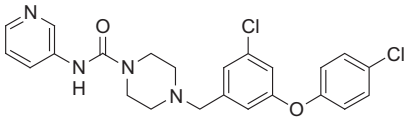
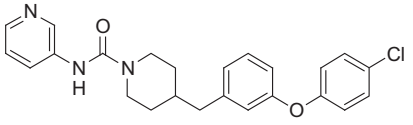
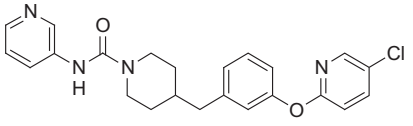
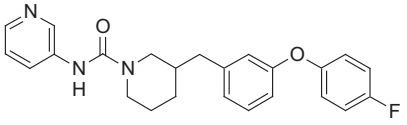
Table 2
The effect of small changes to the core and biaryl ether tail on CNS penetration

Compd	hFAAH apparent IC_{50}[a] (nM)	rFAAH apparent IC_{50}[a] (nM)	TPSA (Å^2)	*A*Log*P*	C_b/C_p ratio (dose)
(**12**) 	2.4 ± 1.3	20 ± 6.0	57.7	3.6	1.58 ± 0.33[f] (20 mg/kg)[b]
(**13**) 	16 ± 5	290 ± 12	57.7	3.1	1.57 ± 0.14 (20 mg/kg)[c]
(**14**) 	1.0 ± 0.5	18 ± 3.5	57.7	3.8	1.99 ± 0.13[g] (20 mg/kg)[b]
(**15**) 	3.3 ± 0.8	48 ± 4	57.7	3.8	0.67 ± 0.16 (20 mg/kg)[d]
(**16**) 	8.0 ± 2.1	76 ± 16	57.7	4.2	1.26 ± 0.09 (20 mg/kg)[b]
(**17**) 	4.3 ± 2.2	215 ± 15	54.5	4.5	2.26 ± 0.26 (20 mg/kg)[c]
(**18**) 	1.0 ± 0	22 ± 2.9	67.4	3.8	0.45 ± 0.07 (20 mg/kg)[d]
(**19**) 	28 ± 8	41 ± 14	54.5	4.1	0.33 ± 0.03 (20 mg/kg)[e]

[a] 1 h incubation with the enzyme, errors are the s.e.m.
[b] Vehicle = water.
[c] Vehicle = 20% hydroxypropyl cyclodextrin.
[d] Vehicle = 50% PEG 400/water.
[e] Dosed as a suspension in 75% pharmasolve, 20% cremaphore and 5% aqueous dextrose;
[f] PK parameters Cl = 0.8 L/h/kg, V_{ss} = 2.8 L/kg, $F \approx 100\%$.
[g] PK parameters Cl = 0.6 L/h/kg, V_{ss} = 4.0 L/kg, $F \approx 100\%$.

extensively profiled (radioligand binding, functional assays, esterase/serine hydrolase enzymatic inhibition assays, and activity-based protein profiling (proteomics)). These data will be included in a subsequent manuscript describing the further characterization of biology and pharmacology of these compounds.

In conclusion, we have shown that the brain penetrating ability of heteroaryl piperazine and piperadine ureas can be modulated through small structural changes to the heteroarenes, the piperazine or piperidine core, and the biaryl ether tail. Compounds exhibiting C_b/C_p ratios ranging as high as 4.2:1 to as low as 0.02:1 have been preparable. The degree of brain penetration was not predictable from PK experiments as large volumes of distribution did not correlate with a high C_b/C_p. As the described compounds are mechanism-based inhibitors of the FAAH enzyme, low C_b/C_p ratios did not preclude central target engagement, but did slow the onset of inhibition.

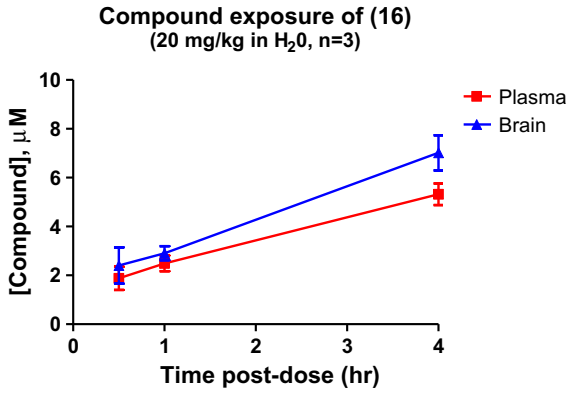


Figure 2. Compound exposures for the slowly absorbed (**16**).

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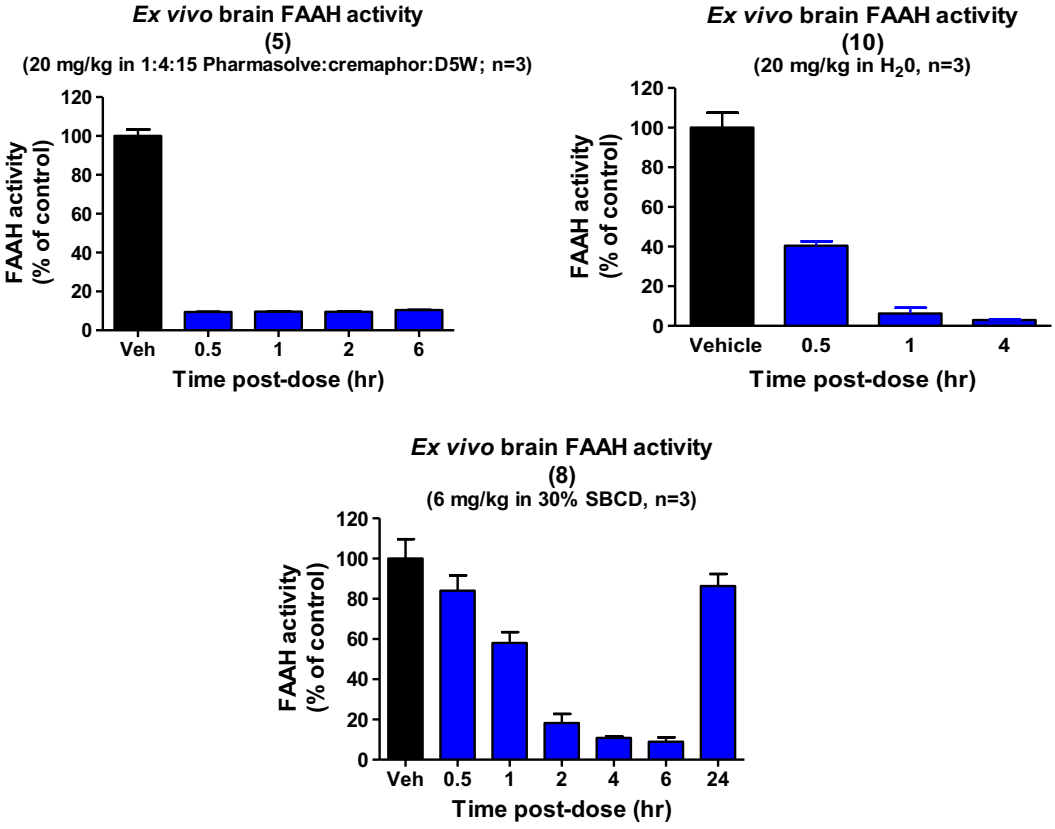

Figure 3. Time-course of brain FAAH inhibition by (**5**), (**10**) and (**8**).

Supplementary data

Discussion on the synthesis of new compounds and intermediates, as well as their respective characterization data (annotated ^{1}H NMR and MS data), is included in the Supporting information. Also included are brain and plasma compound concentration curves, and fatty acid amide elevation and residual enzyme activity graphs.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.001.

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