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Identification of biaryl sulfone derivatives as antagonists of the histamine H_3 receptor: Discovery of (*R*)-1-(2-(4′-(3-methoxypropylsulfonyl)biphenyl-4-yl)ethyl)-2-methylpyrrolidine (APD916)

Graeme Semple *, Vincent J. Santora, Jeffrey M. Smith, Jonathan A. Covel, Rena Hayashi, Charlemagne Gallardo, Jason B. Ibarra, Jeffrey A. Schultz, Douglas M. Park, Scott A. Estrada, Brian J. Hofilena, Brian M. Smith, Albert Ren, Marissa Suarez, John Frazer, Jeffrey E. Edwards, Ryan Hart, Erin K. Hauser, Jodie Lorea, Andrew J. Grottick

Arena Pharmaceuticals, 6166 Nancy Ridge Drive, San Diego, CA 92121, USA

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ABSTRACT

The design of a new clinical candidate histamine-H_3 receptor antagonist for the potential treatment of excessive daytime sleepiness (EDS) is described. Phenethyl-*R*-2-methylpyrrolidine containing biphenyl-sulfonamide compounds were modified by replacement of the sulfonamide linkage with a sulfone. One compound from this series, **2j** (APD916) increased wakefulness in rodents as measured by polysomnography with a duration of effect consistent with its pharmacokinetic properties. The identification of a suitable salt form of **2j** allowed it to be selected for further development.

The biogenic amine histamine acts through four distinct G-protein coupled receptors (GPCR's, H_1–H_4)[1] to modulate a diverse range of biological functions both in the brain and in the periphery. One central role of histamine is in the modulation of sleep and wake states, as demonstrated by the close relationship between the firing rate of histamine neurons and the sleep–wake cycle.[2] Brain permeable H_1 receptor antagonists are sedative, suggesting that the sleep-promoting effects of histamine are largely mediated by the H_1 receptor. H_3 receptors on the other hand are located presynaptically in the CNS and regulate histamine synthesis and release through a process of negative feedback. Hence, inhibiting H_3 receptor function increases histamine release and H_1 receptor activation leading to increased wakefulness.[3] The H_3 receptor also acts as a heteroreceptor to influence the release of other neurotransmitters such as noradrenaline, serotonin, dopamine, glutamine, GABA, and acetylcholine.[4] Recently, the wakefulness effects of an H_3 receptor antagonist have been demonstrated in human subjects with a compound shown to have similar effects in preclinical species.[5]

Multiple structurally diverse series of H_3 antagonists have been described[6] and we have previously reported the design of a series of biphenyl sulfonamide compounds incorporating a phenethyl-*R*-2-methylpyrrolidine moiety (**1**, Fig. 1) as potent and selective antagonists of the H_3 receptor.[7] Compounds of this type however all generated, to a greater or lesser extent, a common metabolite in rodents in vivo. This metabolite (the primary sulfonamide, i.e., **1**, R = H) had significant inverse agonist activity at the H_3 receptor, a long pharmacokinetic half life in rat, and accumulated in brain tissue. As a consequence the profile of the sulfonamide series did not fit with our objective of a short duration of action so as to avoid the potential for extended duration pharmacology that may lead to insomnia in patients. We therefore looked for an alternative series of compounds that could not be metabolized by simple alkyl cleavage from the sulfonamide in the same fashion, and chose to target the closely analogous sulfone derivatives **2**.

The two general methods for the synthesis of our initial series of sulfone derivatives are outlined in Scheme 1. (*R*)-4-(2-(2-Methylpyrrolidin-1-yl)ethyl)phenylboronic acid **5**, was prepared as previously described,[7] and this intermediate underwent Suzuki coupling with bromo-aryl sulfones **8**, that in general were prepared by simple alkylation of 4-bromobenzenethiol (**6**) and oxidation of the resultant thioether **7**, with either 3-chloro perbenzoic acid

* Corresponding author. Tel.: +1 858 453 7200; fax: +1 858 453 7210.
E-mail address: gsemple@arenapharm.com (G. Semple).

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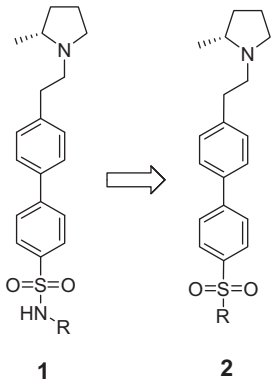


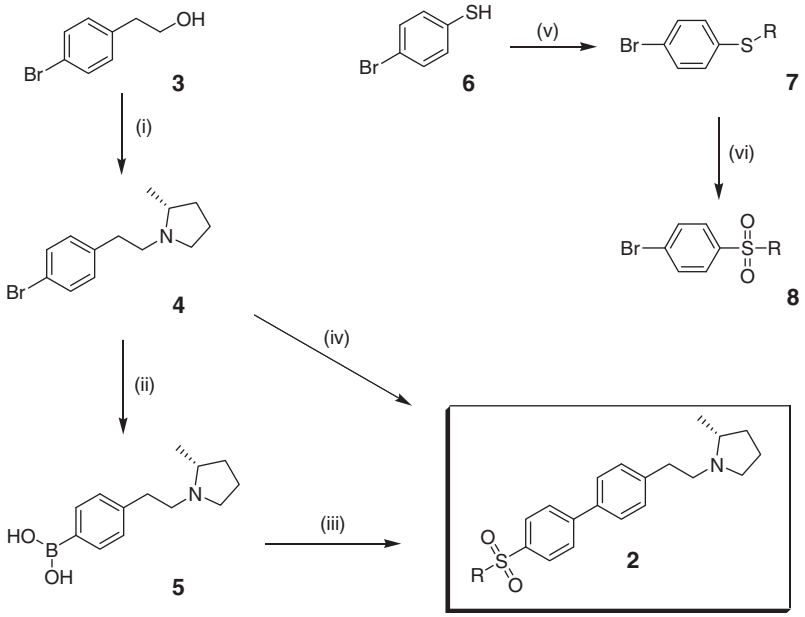
Figure 1. Biphenyl sulfones **2** were designed using knowledge of the metabolism of the previously reported biaryl sulfonamides **1**.

(mCPBA) or oxone®. Alternatively, in the few cases where the boronic acid was commercially available, the Suzuki coupling was performed in the opposite sense, whereby the bromide **4** was coupled with the requisite arylsulfone boronic acid.

We first prepared a number of simple alkyl sulfones (Table 1). These compounds had comparable binding affinity for the rat H_3 receptor (using an isolated rat cortex preparation) with the sulfonamide series.[7] As was also previously observed, a range of different sized groups in this position were all well tolerated and did little to affect binding affinity. The prototypical compound **2a** was also only a weak inhibitor of the hERG channel (Table 1), an off target activity that has plagued some of our previous efforts to identify compounds of interest.[7,8] As with the sulfonamide series, increasing the size of the alkyl group R maintained excellent affinity for the rat H_3 receptor, but caused a significant increase in hERG channel interaction such that all of the other simple alkyl and benzyl examples tested significantly inhibited hERG channel activity in a patch clamp assay at 3 μM. Our previous experience with the sulfonamide series suggested that hERG inhibition could be decreased by the incorporation of ether or alcohol substituents into the alkyl chains. We therefore prepared a series of such analogues in the sulfone series which indeed reduced hERG channel inhibition, but which retained high affinity for rat H_3 receptor (Table 1). Of the linear analogues, **2g**–**k**, in addition to **2a**, appeared to have the most promising early profiles. An additional linear ether analogue, **2o** had somewhat lower affinity for the rat H_3 receptor and was not pursued further. A number of cyclic ether analogues (**2l**–**n**) were also prepared but at least in the case of **2l**, the issues with hERG channel activity resurfaced.

As had been observed previously with several series,[9] the enantiomeric analogues, in which the 2-methylpyrrolidine was of the *S*-configuration, had significantly lower H_3-receptor affinity (e.g., S-antipode of **2g**, K_i = 13.2 nM, n = 4; S.D. = 0.41).

At this stage we had also begun testing compounds in an acute rat behavioral assay. In this assay,[10] prevention of H_3 agonist (*R*)-α-methyl histamine (RAMH)-induced dipsogenia by pre-administration with an H_3 antagonist is assessed. When the antagonist is dosed per orally, the model can be used to rapidly assess a combination of both oral bioavailability and brain partitioning. Hence, test compounds were administered to rats 0.5 h prior to injection of RAMH (10 mg/kg, SC) and the volume of water consumed over a 20-min period measured. A significant inhibition of agonist-induced drinking was observed for all compounds at either of the two screening doses, 1 or 0.3 mg/kg PO (Table 1, see also Figure 2 for **2g** and **2j**). Thus, a number of compounds remained of interest for further progression, having been shown to be functional antagonists of the H_3 receptor in vivo while maintaining an adequate hERG profile. As we had previously decided that the pharmacokinetic profile of any compound selected would be paramount, and moreover that compound exposure should mirror the in vivo pharmacodynamic antagonist effect in duration, we next examined the PK profiles of a number of compounds using two methods. The first was a routine examination of the PK parameters after both IV and PO administration to rats. The second assessed brain/plasma (b/p) partitioning of test compounds at 0.75, 1 and 2 h post dose using three animals at each time point. The estimate of b/p ratios, as shown in Table 2, was taken from the mean of all nine such b/p determinations for each compound.



Scheme 1. Synthesis of biphenyl sulfone derivatives. Reagents and conditions: (i) (a) MsCl, DIEA, DCM; (b) (*R*)-2-methylpyrrolidine, Na_2CO_3, CH_3CN, Δ; (ii) (a) *n*BuLi, THF, −78 °C; b) $(iPrO)_3B$, −78 °C to rt; (iii) **8**, $Pd(PPh_3)_4$, EtOH, benzene, microwave heating; 20–70% yield; (iv) 4-RSO_2Ph-$B(OH)_2$, $Pd(PPh_3)_4$, EtOH, benzene, microwave heating 20–40% yield; (v) RBr, NaH, DMF; >80% yield; (vi) mCPBA, DCM; >90% yield.

Table 1
Receptor binding affinity, hERG screening data and in vivo efficacy for sulfone analogues

Compound	R	Rat H_3 K_i (nM)	n	Log S.D.	hERG inhibition [% @ 3 μM, n = 2] (IC_{50})	MED in RAMH[a] (mg/kg PO)
2a	–Me	1.3	6	0.20	13	0.3
2b	–Et	0.9	6	0.22	75	0.3
2c	–^{n}Pr	0.6	3	0.10	73	0.3
2d	–^{i}Pr	2.65	3	0.06	65	n.d.
2e	–CH_2Ph	0.6	3	0.11	95	n.d.
2f	–^{c}Pent	1.0	3	0.17	n.d.	n.d.
2g	–$(CH_2)_2$OMe	1.4	20	0.30	29 (7.4 μM)	0.3
2h	–$(CH_2)_2$OH	1.2	10	0.11	25 (7.7 μM)	1
2i	–$(CH_2)_2$OEt	1.0	6	0.19	45	0.3
2j	–$(CH_2)_3$OMe	0.7	24	0.32	14 (11 μM)	0.3
2k	–$(CH_2)_3$OH	0.8	9	0.28	(>30 μM)	1
2l	–CH_2(4-THP)	0.17	6	0.27	64 (3.1 μM)	0.3
2m	–(4-THP)-	1.1	3	0.22	n.d.	1
2n	CH_2(2-THP)	0.2	3	0.20	n.d.	n.d.
2o	–CH_2OMe	2.44	3	0.15	n.d.	n.d.

n.d. = not determined.
[a] Minimum efficacious dose to fully inhibit drinking response induced by SC administration of the H_3 agonist RAMH (R-α-methylhistamine).

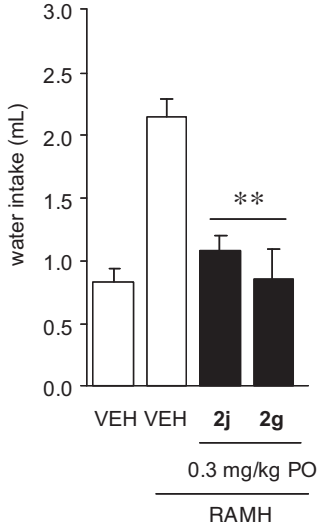


Figure 2. Attenuation of RAMH-induced polydipsia in the rat with an acute 0.3 mg/kg dose of either **2j** or **2g**. Bars represent means ± SEM. $^{**}p$ <0.01.

The prototypical compound **2a** was shown to have good brain partitioning and excellent oral bioavailability in these studies. However, the compound had a high volume of distribution and somewhat long half life (Table 2). In addition, there was a clear delay in the time to achieve C_{max} after oral administration. Thus we did not believe that this compound would have the appropriate PK profile for our selected indication. Both the volume of distribution and the plasma half-life could be reduced by increasing the size of the sulfone substituent. However, as has already been noted above, these modifications generally increased hERG inhibition. Interestingly, **2c** was rapidly converted to the hydroxylated analogue **2k** in vivo, such that the parent compound was barely observed in plasma. Although **2k** was seen to be cleared relatively rapidly, the delay in T_{max} and the modest brain partitioning meant that we did not consider dosing of **2c** as a prodrug of **2k** to be a viable approach.

Dosing of the two ether analogues **2g** and **2j** that had each satisfied both the in vitro and in vivo activity and hERG selectivity requirements, showed them both to be rapidly adsorbed with moderate elimination half-lives and significantly lower volumes of distribution than the prototype antagonist in this series, **2a**. In addition both compounds showed excellent partitioning into the brain in our screening assay (Table 2). At this stage we favored **2j** based on its modestly higher dose-adjusted C_{max} and somewhat greater b/p ratio and as this compound had moderate plasma protein binding (74% in both rat and human plasma) we expected that a significant portion of the whole brain concentration would be unbound.

Further experiments confirmed that both **2j** and **2g** completely prevented the RAMH-induced drinking response after oral doses of 0.3 mg/kg but not 0.1 mg/kg, where both compounds showed only a partial effect. At the 0.3 mg/kg dose in rat, the brain concentration of **2j** at the 1 h time point (the time approximately coinciding with the measurement of the drinking effect) was measured to be 17 ng/mg tissue. Assuming that the free fraction in brain was approximately equal to that in plasma, this would provide a free concentration of 9–10 nM in brain. Based on the binding K_i for the rat receptor of 0.7 nM, this concentration in brain should provide >90% receptor occupancy.

Compounds 2j and **2g** were then progressed into the first duration of action study which again used the RAMH-induced drinking assay. In this paradigm the compounds were dosed orally (0.6 mg/kg; i.e., twice the minimum fully efficacious dose) at selected time points prior to injection of RAMH. The inhibition of RAMH-induced drinking diminished with increasing time before injection of the agonist, from maximal inhibition at the 0.5 and 1 h time points to less than 20% inhibition when RAMH was injected 6 h post administration of either **2g** or **2j** (Fig. 3), leading us to conclude that the pharmacodynamic profile of both of these compounds showed a good correlation with the pharmacokinetic profile of short T_{max} and moderate $T_{½}$.

We were finally able to differentiate between the two compounds by their cross-species metabolism and PK profiles. In these studies, **2j** had good to excellent oral bioavailability and a pharmacokinetic half-life in dog, monkey, and mouse (Table 3) which would predict suitability for once daily oral administration in human. For both compounds however, two major metabolites were identified that were common to all species following oral administration. The first, resulting from oxidative cleavage of the methyl pyrrolidine moiety to produce the phenyl acetic acid derivatives was of little concern as neither had any affinity for the H_3 receptor (see Supplementary data). The second metabolite, resulting from simple demethylation of the ether group (i.e., **2k** was generated from **2j** and **2h** was generated from **2g**), were most predominant in mouse and rat and exposure was much higher for **2h** (**2k** measured as 52% of the AUC of **2j** in mouse and 27% in rat; **2h** measured as 145% of the AUC of **2g** in mouse and 100% in rat). As these com-

Table 2
PK parameters for selected compounds in rat

Compound	Dose (mg/Kg)	T_{max} (h)	Plasma C_{max} (μg/mL)	iv $T_{1/2}$ (h)	Vss (L)	%F[c]	b/p[a] (dose, mg/kg)
2a	10	4	0.218	3.6	19.8	~100	3.03 (10)
2b	3	0.3	0.057	1.4	14.3	46	1.75 (3)
2c	3	0.1	0.006	0.3	5.9	<0.1	- (3)
2g	10	0.3	0.184	0.7	7.6	68.5	1.73 (10)
2h	1	0.5	0.122	3.2	5.0	53.6	0.72 (1)
2j	3	0.5	0.104	1.9	7.5	27.3	3.3 (3)
2k	–	3[b]	0.449[b]	–	–	–	0.7 (N.A.[b])

N.A. = Not Applicable.
[a] Mean of ratio of brain (μg/mg) and plasma (μg/mL) concentration at 3 time points (0.75, 1.5 and 3 h) following a 3 mg/kg PO dose.
[b] Parameters for exposure of **2k** following administration of **2c** (3 mg/kg PO).
[c] Bioavailability based on the ratio of AUCpo/AUCiv.

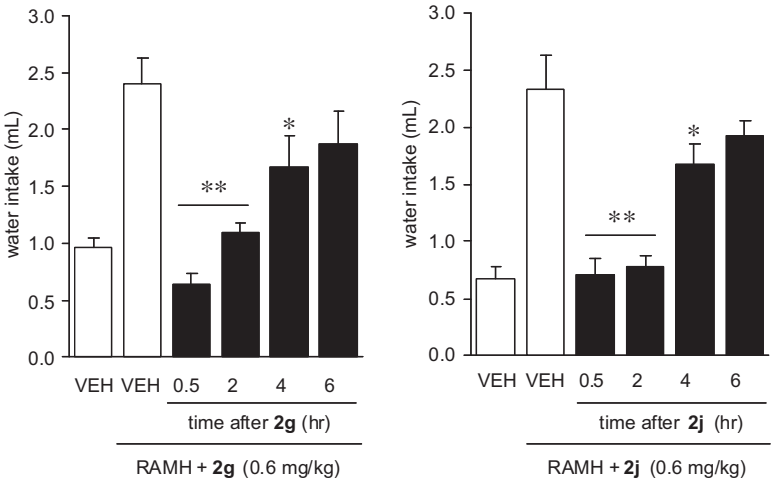


Figure 3. Blockade of RAMH-induced polydipsia over time after an acute 0.3 mg/kg dose of **2g** (left panel) and **2j** (right panel). Bars represent means ± SEM. $^{*}p$ <0.05, $^{**}p$ <0.01.

pounds were active at the receptor, it is conceivable, particularly in the case of **2h**, that they would have made some contribution to the pharmacological effect in these species. Both metabolites **2h** and **2k** were cleared at approximately the same rate as the parent so the risk of accumulation was deemed to be low. Fortunately, whereas the active metabolites were rather abundant in rodents, only very low levels (<5% AUC of parent) of **2k** were observed in dog and monkey, whereas the exposure of the metabolite **2h** following dosing of **2g** remained significantly higher in both these species (20% and 12% of **2g** in monkey and dog, respectively). Unfortunately because of the very low turnover of **2j** in liver microsomes from any species ($T_{½}$ >1 h in all species), any correlation to provide an estimate of how much of the active metabolite **2k** may be generated in human was not possible, but the risk of observing any contribution of the metabolite to a pharmacological effect in human was regarded as very low for **2j** but of moderately higher risk for **2g** based on the data from the cross species PK experiments. We therefore elected to move **2j** forward for further profiling.

Compound 2j was tested for its ability to increase wakefulness in the rat using subjects implanted with chronically indwelling cortical electrodes. Specifically, **2j** was administered orally at the same dose as in the antagonist duration of action study (0.6 mg/kg) during the animals subjective night (approximately four hours after lights on) and wake time measured over an 8 h period. As can be seen in Fig. 4, **2j** increased total wake time significantly during the first 2 h after administration, but consistent with the data obtained from the in vivo antagonist duration of action experiments, the effect was no longer significant at the 4 h time point and beyond. A dose response study (data not shown) also confirmed that 0.3 mg/kg was the minimum efficacious dose to increase wakefulness over the first 1 h, however the effect was not significant at this dose if the first 2 h were taken into consideration. Thus, we believed that we had demonstrated that **2j** was a potent but relatively short acting antagonist of the histamine H_3 receptor that was able to increase wakefulness in the rat after oral administration.

As all the screening thus far had used rat tissue binding, we next tested the affinity of **2j** for the cloned human H_3 receptor and con-

Table 3
Selected PK parameters for **2j** in 4 species

Species	PO Dose (mg/kg)	T_{max} (hr)	C_{max} (μg/mL)	$T_{1/2}$ (hr)	%F[b]	Cl (L/hr/kg) (% of HBF)[a]	Vss (L/kg)
Mouse	3	1.0	0.08	1.5	35.4	4.36 (78%)	2.54
Rat	3	0.5	0.10	1.9	27.3	4.46 (106%)	7.54
Monkey	3	2.3	0.55	6.9	67.5	0.37 (14%)	2.16
Dog	3	0.8	0.60	5.0	~100	0.82 (43%)	2.89

[a] % of hepatic blood flow in each species.
[b] Bioavailability based on the ratio of AUCpo/AUCiv. The iv dose in each species was 2 mg/kg.

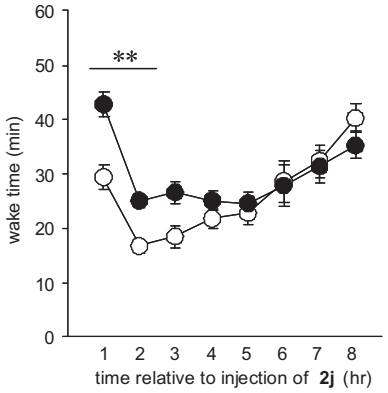


Figure 4. Effect of **2j** (0.6 mg/kg PO) on wakefulness in the rat over an eight hour period. Bars represent means ± SEM. $^{**}p$ <0.01, vehicle (○), **2j** (●).

firmed that it was in a similar range to that observed for the rat (human H_3 K_i = 4.2 ± 1.6 nM; n = 3). In addition, in a functional assay (GTPγS binding) also using the cloned human H_3 receptor, **2j** behaved as a potent full inverse agonist (IC_{50} = 0.7 nM; n = 2). Broader testing against a standard panel of 80 receptors (that included the closely related histamine H_1, H_2 and H_4 receptors) revealed no binding in excess of 50% of control, at a concentration of 10 μM, for any other target tested.

Compound 2j did not inhibit any of the five major CYP isoforms (IC_{50} >40 μM), showed no mutagenic potential in a micro-Ames test, and was only a moderate inhibitor of the hERG channel in a patch clamp assay (IC_{50} = 11 μM; n = 2). In dose-escalating PK studies in rat, the exposure (both C_{max} and AUC) was dose-proportional up to 250 mg/kg, which was close to the maximum tolerated dose.

The high exposure observed in the dog PK studies described above led us to use this species for cardiovascular safety studies. No treatment-related effects of **2j** on mean arterial blood pressure were observed in conscious animals, and only a mild increase in heart rate at 10 and 30 mg/kg PO was noted, with reversion toward negative control levels within 60 min after dosing. Plasma levels for the observed mild effects were around 1000-times greater than the plasma levels required to increase wakefulness in rat.

At this stage, although we had established a suitable PK and preliminary safety profile for further development, the hydrochloride salt of **2j** that we had hitherto used for all of the preclinical studies was deemed to be unsuitable for preclinical development based on its hygroscopicity at elevated relative humidity. In addition, the free base was also not considered to be a viable option based on its very low melting point. Interestingly, **2g**, which differs from **2j** by only one carbon atom formed a highly soluble (>30 mg/mL in water) crystalline, non-hygroscopic hydrochloride salt (mp 182 °C). Hence we conducted a salt screen for **2j** in which we tested a matrix of 12 solvents and 15 potential counter ions. From this process we identified only two solid products that were characterized as a citrate salt (poorly crystalline, mp ~100 °C) and a dicitrate salt that in early studies was a highly soluble (>150 mg/mL in water), non-hygroscopic and highly crystalline salt of sufficiently high melting point (148–150 °C) for further development. It was also demonstrated that the in vivo antagonist effect of the dicitrate and hydrochloride salts were identical (see Supplementary data).

We were initially concerned about the physical integrity of this unusual salt form under stress conditions, but we observed no change in the PXRD pattern after grinding with a mortar and pestle (see Supplementary data), including in the presence of a range of excipients. In addition, the stability of the salt form was further confirmed by compressing the ground salt at high pressure to mimic a tableting process and on particle size reduction (Fitz mill, 8000 rpm, 20 mesh screen). Finally, slurrying of the crystalline material with a range of solvents (40 °C, 4 days) did not induce a change in crystalline form as assessed by PXRD, DSC or IR.

Thus, having identified a viable salt form and with positive pharmacology data in hand that matched the pharmacokinetic profile appropriately, **2j** (APD916) was selected for preclinical development and was eventually progressed into clinical studies. The results of these studies and a further, more extensive pharmacological profile will be reported elsewhere.[11]

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.075.

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