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Discovery of 5-(2-amino-[1,2,4]triazolo[1,5-a]pyridin-7-yl)-*N*-(*tert*-butyl) pyridine-3-sulfonamide (CZC24758), as a potent, orally bioavailable and selective inhibitor of PI3K for the treatment of inflammatory disease

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ABSTRACT

Herein, we disclose the discovery of a series of 7-substituted triazolopyridines which culminated in the identification of **14** (CZC24758), a potent, orally bioavailable small-molecule inhibitor of PI3Kγ, an attractive drug target for inflammatory and autoimmune disorders. Compound **14** has excellent selectivity across the kinome, demonstrates good potency in cell based assays and furthermore exhibits in vivo efficacy in a collagen induced arthritis model in mouse after oral dosing.

Phosphoinositide 3-kinases (PI3Ks) represent a family of dual specificity enzymes that, by acting as both lipid and protein kinases, regulate numerous biological processes, including cell growth, differentiation, survival, proliferation, migration, and metabolism.[1] These kinases are activated by a wide variety of different stimuli such as growth factors, inflammatory mediators, and antigens. Deregulation of class I PI3Ks, is involved in the pathogenesis of various diseases, representing attractive targets for oncology, inflammatory and cardiovascular diseases, with several molecules being evaluated in early clinical trials in oncology and inflammation.[2]

In our efforts to target the PI3K family for the treatment of inflammatory disease, we were interested in developing selective ligands for the PI3Kγ isoform. This PI3Kγ subtype has been shown to play a role in several immune functions such as granulocyte migration, activation of mast cells, and dendritic cells, as well as the development and differentiation of lymphocytes, thereby suggesting that its inhibition might be beneficial in both inflammatory and autoimmune conditions.[3] Given the high sequence homology within the PI3K family we were aware that achieving substantial selectivity (e.g., >100-fold) for PI3Kγ alone would be challenging. However, as literature evidence suggests that both PI3Kβ and PI3Kδ also play a role in immune function and therefore may have a synergistic anti-inflammatory effect,[4] we were particularly interested in achieving high selectivity over PI3Kα, a target with known antiproliferative effects,[5] and hence more of interest in the cancer area.[6]

Several PI3K inhibitors such as AS-605240,[7] the first small-molecule known to inhibit PI3Kγ, are known in the literature (Fig. 1). The suboptimal pharmacokinetics and the limited selectivity window of this inhibitor, particularly over the PI3Kα isoform as well as other kinases such as DNAPK, severely limited its developability. Therefore we,[8] and others[9] set out to primarily target PI3Kγ inhibitory activity, with selectivity particularly over PI3Kα as well as the rest of the kinome, with novel compounds possessing drug-like properties that would be suitable for oral dosing in vivo.

As part of our efforts to identify potent and selective PI3Kγ inhibitors, we recently described the 6-substituted triazolopyridine **1** (Fig. 1) which showed efficacy in a number of inflammatory models.[10] The X-ray crystal structure of the analogous triazolopyridine **2**[10,11] (Fig. 2) showed that the scaffold occupies the ATP binding pocket with the aminotriazole portion making the usual donor/acceptor interaction with the Val882 of the PI3Kγ protein backbone (hinge). Furthermore, the triazolopyridine scaffold and the pyridine ring efficiently fill the hydrophobic pocket formed by a number of residues that include Ile963 and Tyr867. The methylsulfone and the acetamide groups extend towards the solvent front. We envisioned that the regioisomeric 7-substituted

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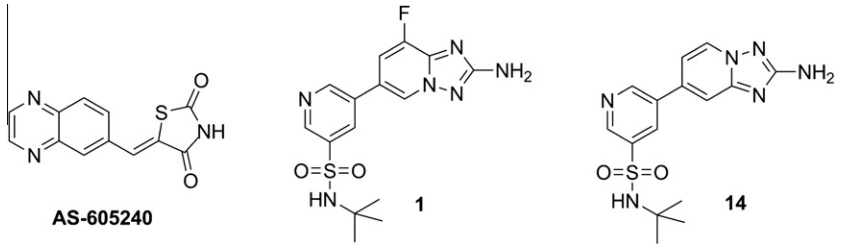


Figure 1. AS-605240, 6-substituted triazolopyridine 1 and 7-substituted triazolopyridine **14**.

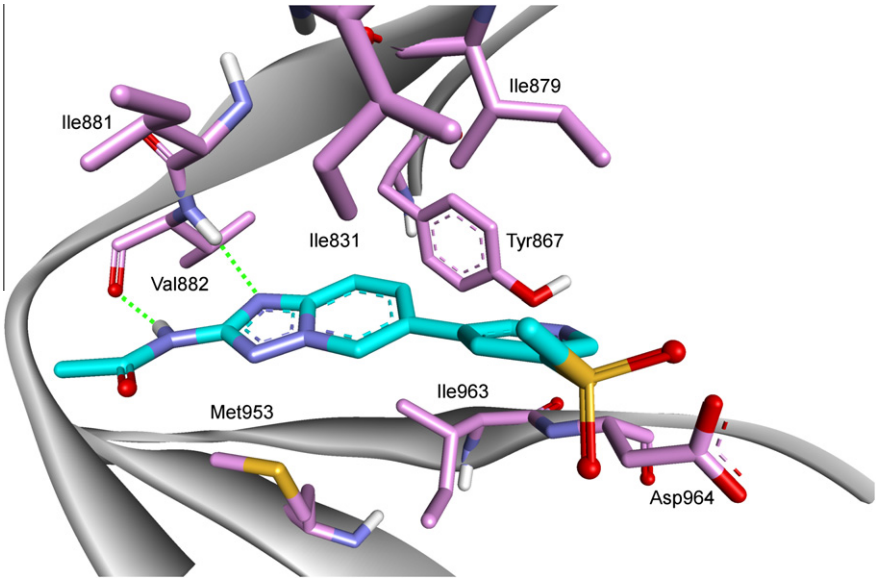


Figure 2. X-ray cocrystal structure (PDB ID: 4AOF) of **2** bound to the ATP pocket of the PI3Kγ kinase domain.

triazolopyridine scaffold should keep all the key interactions shown by the 6-substituted series and hence embarked on exploring the SAR in this scaffold (Fig. 3). This work culminated in the discovery of **14** (CZC24758) an orally bioavailable, potent and selective PI3K inhibitor which showed efficacy in a mouse collagen induced arthritis (CIA) model.

The convergent route for the preparation of 7-substituted triazolopyridines which involved coupling of boronic acids with the key intermediate **6** met with little success[12] (Scheme 1). A more robust synthetic route for the preparation of these analogues involved Suzuki coupling prior to the cyclisation to form the triazolopyridines **9–21** (Scheme 2). The synthesis began by coupling commercially available sulfonyl chlorides with amines to form sulfonamides **23**. We observed inconsistent yields (29% to 60%) and significant impurities in the coupling when the HCl salt of the sulfonyl chloride **22** was used. Using the free base, the sulfonamide intermediates **23** were isolated in almost quantitative yield after simple precipitation from the reaction mixture. Boronic esters were prepared in situ followed by subsequent Suzuki coupling reaction which yielded the intermediates **24**. Thiourea formation and cyclisation with hydroxylamine yielded **9–21** in 79% yield with high purity. This synthetic approach was developed to enable the preparation of multigram quantities required for later-stage experiments in vivo. Compound **9** was synthesized using commercially available 3-bromo-5-(methylsulfonyl)pyridine. The 5-fluorinated triazolopyridine **21** was synthesized in an analogous manner using commercially available 2-amino-4-bromo-6-fluoropyridine.

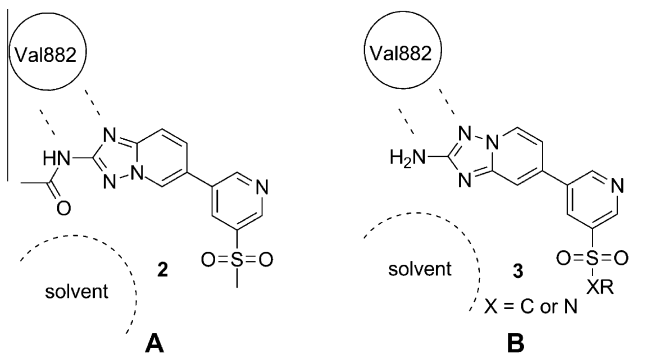
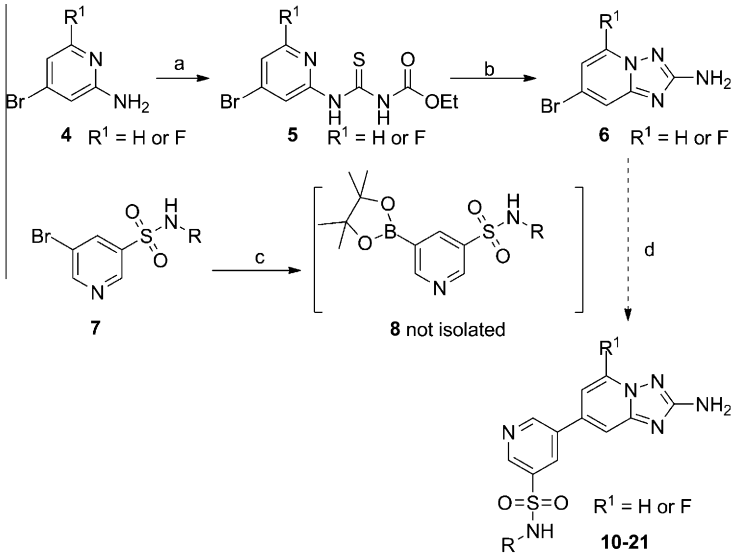
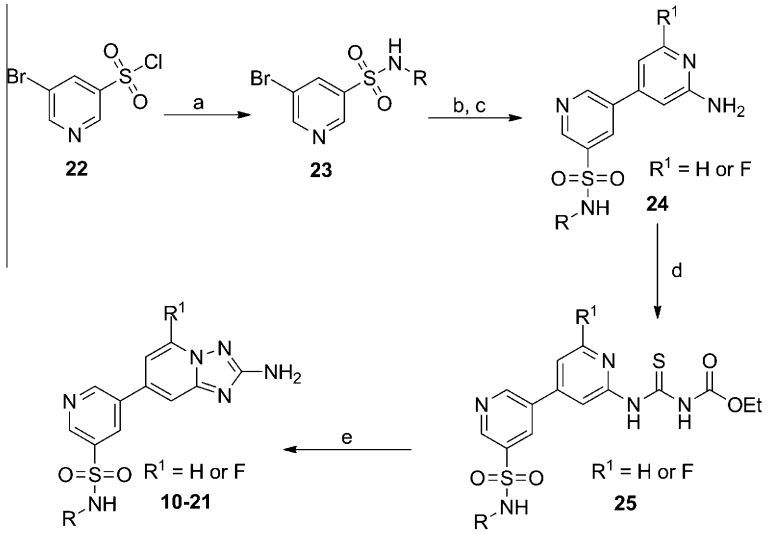


Figure 3. Cartoon representation showing hydrogen bonding interactions of 2 in PI3Kγ (**A**) and proposed binding of 7-substituted triazolopyridine scaffold (**B**).

The compounds **9–21** were initially tested using the *Kinobeads* assay developed for lipid kinases[10] to determine potency and selectivity followed by their cellular potency in a PI3Kγ/PI3Kδ dependent neutrophil migration assay (Table 1).[10] SAR analysis was carried out with a focussed set of compounds using the prior knowledge from the 6-substituted series.[10] As in the previous series the 7-substituted series showed good potency for PI3Kγ. The methyl sulfonamide showed a small increase in potency compared to the methyl sulfone (pIC_{50} **9**: 7.1 vs pIC_{50} **11**: 7.6), but larger substituents on the sulfonamide, although tolerated, failed to give a significant increase in potency (pIC_{50}: 7.5–7.9). The diethylsulfonamide **19** was the most potent compound identified (pIC_{50}: 8.1) although disappointingly it showed a lower potency in the cellular assay (pIC_{50}: 5.9). The majority of analogues gave a similar selectivity profile across the PI3K family. As was observed in the



Scheme 1. Reagents and conditions: (a) ethoxycarbonyl isothiocyanate, DCM, 35 °C; (b) $NH_2OH{\cdot}HCl$, DIPEA, MeOH/EtOH, 90 °C; (c) bis(pinacolato)diboron, Pd(dppf)Cl_2·DCM, KOAc, dioxane, 120 °C, microwave; (d) Pd(dppf)Cl_2·DCM, EtOH, 2 M Na_2CO_3, 140 °C, microwave.



Scheme 2. Reagents and conditions: (a) RNH_2, pyridine, rt; (b) bis(pinacolato)diboron, Pd(dppf)Cl_2·DCM, KOAc, dioxane, 120 °C, microwave; (c) 2-amino-4-bromopyridine or 2-amino-4-bromo-6-fluoropyridine, Pd(dppf)Cl_2·DCM, EtOH, 2 M Na_2CO_3, 140 °C, microwave; (d) ethoxycarbonyl isothiocyanate, DCM, 35 °C; (e) $NH_2OH{\cdot}HCl$, DIPEA, MeOH/EtOH, 90 °C.

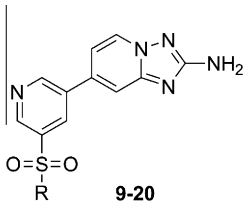
6-substituted series, we found that simple alkyl sulfonamides gave the best balance of PI3Kγ potency and cellular activity.

Substitution of the parent 6-substituted triazolopyridine core with a fluorine at the C-8 position gave an increase in selectivity for PI3Kγ.[10] As expected the fluoro analogue of **14**, the 5-fluorinated triazolopyridine **21**, showed improved selectivity, comparable potency and selectivity profile to 6-substituted analogue **1**.[10] However, the 5-fluorinated compound **21** was not pursued further due to instability in human/rat/mouse hepatocytes (2.5/7.5/36.1 ml/min/g liver).

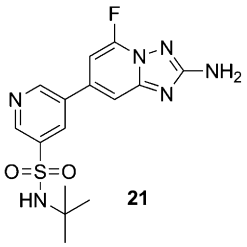
Having identified leads showing good in vitro PI3Kγ inhibitory activity and cellular potency, we carried out an evaluation of the pharmacokinetic (PK) properties of selected compounds in rat. Compounds **11** and **14** were chosen as they were two of the most potent in the cellular assay. The in vitro metabolic stability of these leads in rat liver microsomes (Table 2) predicted that the compounds would exhibit reasonable metabolic stability in vivo. Indeed, they showed low clearance (12–22 mL/min/kg) when dosed intravenously (i.v.) in rats and furthermore were orally bioavailable (56–81%) with good exposure. In a pharmacokinetic study in a Cynomolgus monkey, **14** had an in vivo clearance of 1.6 mL/min/kg, a half life of 6.1 h and was 88% orally bioavailable.

Encouraged by the good cell potency and superior oral exposure, we fully characterized **14** in other off-target activities and broad selectivity across the kinome. In a panel of 158 kinases, **14** displayed greater than 100-fold selectivity against 155 kinases. Within the PI3K family **14** showed some in vitro selectivity over the other potential inflammatory targets PI3Kβ and PI3Kδ and excellent selectivity over the unwanted kinase PI3Kα (Fig. 4).[13] Further profiling showed that **14** was negative in Ames[14], was clean in a patch clamp hERG assay (>250 μM) and devoid of CYP enzymatic inhibitory activities (>20 μM inhibition against CYP1A,

Table 1
PI3K family *Kinobeads* assay and cellular data for **9** to **21**



9-20



21

Compound	R	PI3Kγ[a] (pIC_{50})	PI3Kα[a,b] (ΔpIC_{50})	PI3Kβ[a,b] (ΔpIC_{50})	PI3Kδ[a,b] (ΔpIC_{50})	NeuMig[a] (pIC_{50})
9	Me	7.1	1.7	1.4	1.4	
10	NH_2	7.7	1.8	1.4	1.5	6.1
11	NHMe	7.6	1.9	1.4	1.6	6.3
12	NHPr	7.5	1.9	1.5	>1.7	6.2
13	NH^iPr	7.6	1.9	1.2	1.5	6.3
14	NH^tBu	7.9	1.7	1.3	1.3	6.3
15	$NHCH_2CF_3$	7.7	2.1	1.9	2.1	5.9
16	$NHCMe_2CF_3$	7.7	2.0	1.5	1.5	5.6
17	NHPh	7.4	1.4	1.5	1.6	5.0
18	NHBn	7.0	2.0	1.6	1.7	
19	NEt_2	8.1	2.1	1.6	1.9	5.9
20		7.6	1.9	1.3	1.5	5.7
21		7.8	2.4	1.5	2.1	6.1

[a] The values are averages of at least two independent experiments.
[b] Selectivity versus PI3Kγ.

Table 2
Rat microsomal stability and PK properties of lead compounds

	CL_{int}[a]	F (%)	CL_{obs} (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	C_{max} (μM)	AUC (μM h)
11[b]	0.32	56	21.7	2.2	1.2	5.0	6.7
14[c]	0.31	81	11.7	1.3	1.2	5.9	15.3

[a] Rat microsomal clearance (ml/min/g liver).
[b] Wistar rats male; 5 mg/kg p.o. (5% DMSO/5% Cremophor/0.5% carboxymethylcellulose); 1 mg/kg i.v. (5% DMSO/5% Cremophor).
[c] Wistar rats male; 5 mg/kg p.o. (5% DMSO); 1 mg/kg i.v. (5% DMSO).

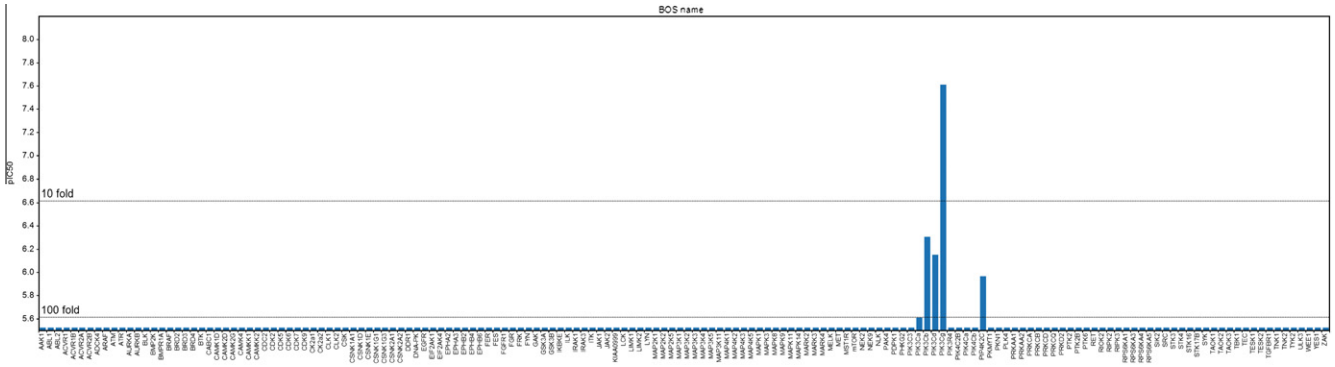


Figure 4. Kinase selectivity profiling of compound **14** showing 10-fold and 100-fold selectivity.

2D6, 2C9, 2C19 and 3A4 (midazolam and testosterone as substrates), thereby indicating that it has very low potential for drug-drug interactions.

On the basis of its good on-target potency, off-target selectivity, and PK profiles, **14** was chosen for further in vivo evaluation. The efficacy of **14** was assessed in a mouse IL-8 air-pouch model to evaluate its ability to reduce the recruitment of inflammatory cells in vivo.[3b] The compound was administered orally 0.5 h before IL-8 was injected into the air pouch. After a further 4 h the pouch was lavaged and the granulocytes counted. The IL-8-induced migration of granulocytes into the air pouch was inhibited >70% after dosing at 3 mg/kg and 10 mg/kg, while no significant inhibition was

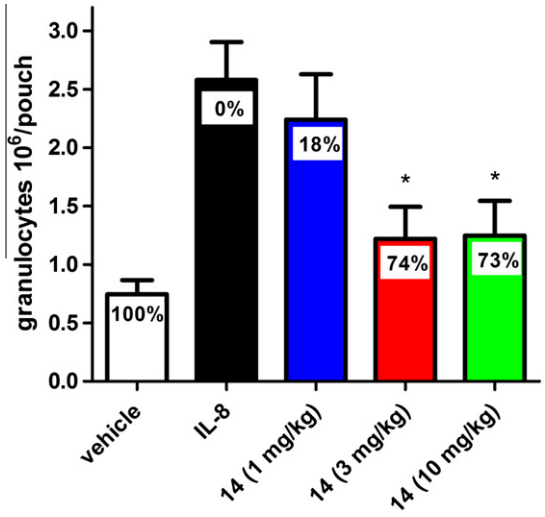


Figure 5. Compound **14** in the IL-8 air-pouch mouse model: $N = 4$ for vehicle control group, $N = 10$ for IL-8 control group, $N = 5$ for 1 mg/kg group, $N = 8$ for 3 mg/kg group, $N = 7$ for 10 mg/kg group. **14** administered orally. Vehicle: 5% DMSO, 0.5% carboxymethylcellulose. $^{*}p \leqslant 0.05$, one-way ANOVA analysis.

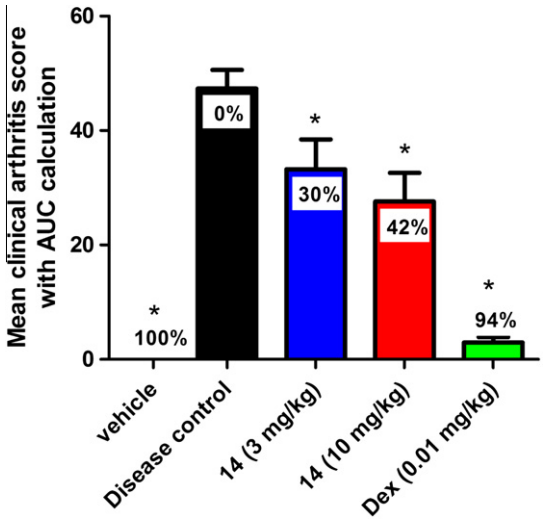


Figure 6. CIA mouse model with **14**: $N = 10$ mice for treatment and disease control groups, $N = 5$ mice for Dexamethasone group, $N = 4$ mice for vehicle control group. **14** administered orally 10 mg/kg and 3 mg/kg BID. Vehicle: 5% DMSO, 0.5% carboxymethylcellulose. $^{*}p \leqslant 0.05$, Student's *t*-test to vehicle.

observed at 1 mg/kg dose (Fig. 5). In the same model the 6-substituted-8-fluoro triazolopyridine **1** showed 29% inhibition at 3 mg/kg.

The efficacy of **14** was also evaluated in a mouse collagen-induced arthritis (CIA) model, an autoimmune disease model which shares pathogenic similarities to human rheumatoid arthritis. Compound **14** was examined in a therapeutic model where treatment started when each group of mice had reached a mean clinical score of 1.5.[15] Mice were scored in a blinded manner and dosed orally twice daily for 14 days. Treatment with compound **14** significantly inhibited the progression of disease compared to the vehicle control at 3 and 10 mg/kg (Fig. 6).[16] The C_{max} following the final dose was 2.5 μM and 10.2 μM for the 3 mg/kg and 10 mg/kg doses, respectively.

In summary, we have described the synthesis and SAR of a series of 7-substituted triazolopyridines, which are potent PI3Kγ inhibitors. Some members of this series have shown good pharmacokinetic profiles in rat and compound **14** (CZC24758) demonstrated efficacy in a therapeutic CIA model after oral dosing, thereby confirming the potential use of PI3K inhibitors in inflammatory disease.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.090.

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11. PDB ID code: 4AOF.
12. Synthesis of 6-substituted triazolopyridine series was highly successful using the convergent route as described previously.[5] However, in the 7-substituted triazolopyridine series, the last Suzuki step was capricious with variable yields despite screening numerous conditions. There was no improvement when boronic ester of the triazolopyridine **6** was made in situ to carry out the Suzuki reaction.
13. Compound **14** was tested against Cellzome off-target panel, which includes >150 kinases, as well as other proteins. List of kinases/proteins screened are shown in Supplementary data.
14. Compound **14** was tested against TA1535, TA100, TA98 and TA102 strains ±S9 metabolic activation.
15. Arthritis was induced by immunizing male DBA/1OlaHsd mice with Bovine Type II collagen in Freund's complete adjuvant. Disease severity was evaluated by scoring all four paws for each animal, with a maximum possible score being 5.0: No arthritis, 1:1 hind or fore paw joint affected or minimal diffuse erythema and swelling, 2:2 hind or fore paw joints affected or mild diffuse erythma and swelling, 3:3 hind or fore paw joints affected or moderate diffuse erythma and swelling, 4: Marked diffuse erythma and swelling, or 4 digit joints affected, 5: severe diffuse erythma and severe swelling entire paw, unable to flex digits. 21–35 days later, onset of arthritis occurred and mice were randomized by clinical scores into groups once the mean clinical arthritis score had reached 1.5. Mice were orally dosed with compound **14** at 3 or 10 mg/kg BID for 14 days. Disease progression was significantly inhibited by **14** compared to vehicle control (P <0.05, by Student's t-test) at 3 and 10 mg/kg.
16. The histopathological analysis of the joints resulted in a significant inhibition of cartilage damage, bone resorption, pannus formation and joint inflammation at both 3 and 10 mg/kg, 41% and 46% respectively.