Bioorganic & Medicinal Chemistry Letters 23 (2013) 816–820

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Development of triarylsulfonamides as novel anti-inflammatory agents

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ARTICLE INFO

Article history:
Received 7 November 2012
Revised 16 November 2012
Accepted 18 November 2012
Available online 29 November 2012

Keywords:
TNF-α
NFκB
Inflammation
Rheumatoid arthritis
Bone loss

ABSTRACT

Triaylsulfonamides were identified as novel anti-inflammatory agents, acting by inhibition of RANKL and TNFα signaling. Structure-activity studies led to the identification of compounds with in vitro potencies of <100 nM against J774 macrophages and osteoclasts, but with little activity against osteoblasts or hepatocytes (IC_{50} >50 μM). A representative compound (**4k**, ABD455) was able to completely prevent inflammation in vivo in a prevention model and was highly effective at controlling inflammation in a treatment model.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by painful swelling of joints, stiffness, loss of movement and the destruction of articular cartilage and bone. Whilst the ultimate cause of RA is still not fully understood, various pro-inflammatory cytokines are known to be instrumental in orchestrating the complex signal transduction pathways which initiate and propagate the inflammatory response and tissue destruction.[1] The cytokines regarded as playing a prominent role in the initiation or maintenance of inflammation include TNF-α, IL-1β, IL-6 and IL-17.[1–3] Anti-TNF therapies have revolutionalised the treatment of RA and are the second-line treatment after failure of methotrexate and NSAIDs. These therapies include neutralizing antibodies such as infliximab, certolizumab and adalimumab, and decoy receptors such as etanercept. However, these highly-effective agents are expensive biologicals and a small molecule therapy would enable wider availability of effective treatment. Efforts have been made to develop small molecules drugs against a number of promising targets downstream from TNF-α, including p38 MAPK, JNK and IKK2, but none of these have progressed beyond early stage trials.[4]

About one third of patients do not respond to, or lose response to anti-TNF therapy;[5] biological agents have been successfully targeted against a number of other pro-inflammatory targets including rituximab, which targets CD20,[5] abatacept which targets T-lymphocyte activation[6] and tocilizumab,[3] which targets IL-6. There are also a number of kinases which may make promising targets for orally-active small molecules, the most advanced of these are the Janus kinase inhibitors (JAK)[4,7] including tofacitinib,[8] which is expected to be the first oral therapy for RA, and spleen tyrosine kinase (SYK) inhibitors.[9]

A further complication of RA is the associated bone loss, mediated through the RANKL signaling pathway, which leads to increased osteoclast differentiation and activity; the anti-RANKL antibody denosumab inhibits differentiation and activation, and has shown good clinical therapeutic utility.[10]

We have previously reported a series of compounds that prevented both inflammation and bone loss, in models for rheumatoid arthritis and models for post-menopausal osteoporosis, respectively. These act by combined inhibition of TNF-α and RANKL signaling,[11–14] targets which cause up-regulation of NFκB via common pathways. However, the precise biological target has remained elusive. Our previous studies have shown biphenylesters and biphenylketones such as **1** and **2** (Fig. 1) to be active in the prevention of bone loss and inflammation, and to induce apoptosis in cells of the osteoclast lineage by inhibition of NFκB and MAP-kinase signalling. However, neither of these classes had adequate potency or metabolic stability to be taken forward for pre-clinical development. Biphenylsulfonamides, such as **3** (Fig. 1), presented

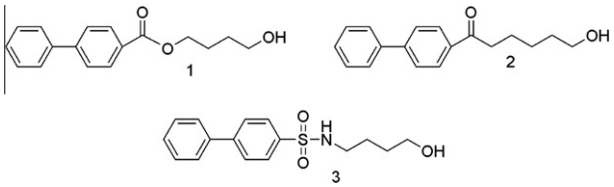

Figure 1. Structures of biphenylesters, ketones and sulfonamides **1**–**3**.

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http://dx.doi.org/10.1016/j.bmcl.2012.11.067

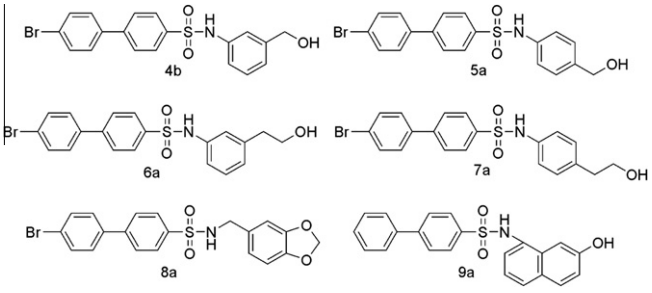


Figure 2. Structures of rigidified biphenylsulfonamides.

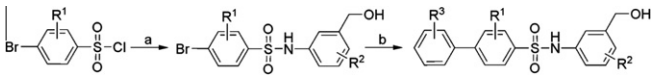
a more promising starting point, having in vitro potency more than ten-fold higher than their ester and ketone analogues and better in vitro stability (30–60 min in human liver microsomes); however, these compounds showed low oral bioavailability (<5%) and had poor activity in the collagen-induced arthritis model,[13] thus they were not pursued as developmental candidates.

To investigate the potential for improving the properties of the biphenylsulfonamides, we took the initial approach of incorporating a phenyl group to rigidify the C4 or C5 alkyl chain, giving triarylsulfonamides of the compound classes **4**–**7** (Fig. 2), by the methods shown in Schemes 1 and 2. When the alkyl chain in the ketone class of compounds **2**, was replaced by a phenyl group, to give a triarylketone, no improvement in potency was obtained;[13] however, with the triarylsulfonamides a modest but promising increase in potency was seen in our primary assay (J774 macrophages viability, 72 h exposure to the test compound[11]): compounds **4b**, **5a**, **6a** and **7a** (Fig. 2) had IC_{50} = 2.5, 3.5, 5.5 and 4.5 μM, respectively. This compares to the 8 and 9 μM of the 4-carbon and 5-carbon alkylsulfonamide analogues (**3**).[12] Compounds **8a** and **9a** (Fig. 2) showed poor activity (IC_{50} >15 μM). As anticipated from previous studies,[11] replacement of the sulfonamide moiety in **4a** and **4j** with a carboxamide also gave compounds with poor activity (**4al** and **4am**, IC_{50} >15 μM, Fig. 3). We conducted a structure activity relationship (Table 1) to investigate the potential for the further development of compounds in classes **4**–**6**. Like osteoclasts, J774 macrophages are dependent on continued NFB activation for survival, thereby providing a valuable screen for compounds with anti-inflammatory activity.[15]

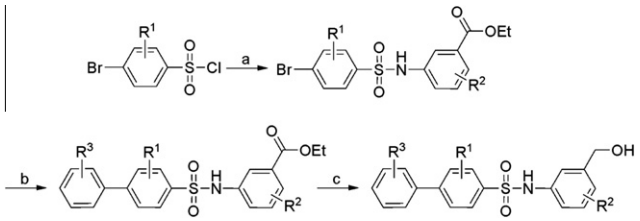
Depending on the commercial availability of starting materials, compounds were synthesized by reaction of an appropriately-substituted 4-bromobenzenesulfonyl chloride with a (3-aminophenyl)methanol (compounds **4a**–**4v** and **4ah**–**4ai**), (4-aminophenyl)methanol (**5a**–**5b**) or with 2-(4-aminophenyl)ethanol (**7a**), and subsequent Suzuki coupling with the required benzeneboronic acid. 4′-Bromobiphenyl derivatives were prepared directly from 4′-bromobiphenylsulfonyl chloride synthesized as described previously.[12,16]

Alternatively, 4-bromobenzenesulfonyl chloride was reacted with the required ethyl 3-aminobenzoate (**4w**–**4ag**) or ethyl 2-(3-aminophenyl)acetate (**6a**–**6c**); Suzuki coupling with a benzeneboronic acid was then performed as before, and the product reduced to the target alcohol in the final step, as shown in Schemes 1 and 2.

The SAR of compounds in class **4** showed broad similarities to those described for compound classes **1**–**3**; thus only a small number of derivatives were made and the focus was on hydrophobic, electron-withdrawing substituents. As for class **3**, a 2′,4′-difluoro (**4j**; 300 nM) or 2′,4′-dichloro (**4k**; 500 nM) substitution on the left hand ring and 3-methyl group on the middle ring led to the most potent compounds (**4s**; 80 nM), giving a combined 25-fold increase in potency over the unsubstituted parent compound. The addition of a third ring gave further opportunities for optimisation; a 2-



Scheme 1. Synthesis of triarylsulfonamides **4a**–**4v**, **4ah**–**4ai**: Reagents and conditions (a) (3-aminophenyl)methanol, DCM, pyridine (room temp, 18 h); (b) substituted benzeneboronic acid, $(PPh_3)_4Pd$, ethanol, toluene, 2 M Na_2CO_3, (reflux, 3 h).



Scheme 2. Synthesis of triarylsulfonamides **4w**–**4ag**: Reagents and conditions (a) ethyl 3-aminobenzoate, DCM, pyridine (room temp, 18 h); (b) substituted benzeneboronic acid, $(PPh_3)_4Pd$, ethanol, toluene, 2 M Na_2CO_3, (reflux, 3 h); (c) 1 M $LiAlH_4$, THF, (50 °C, 5 h).

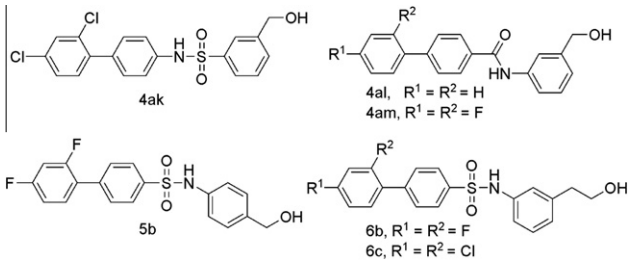


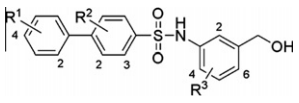
Figure 3. Structures of compounds **4al**–**4ak**, **5b**, **6b**–**6c**.

methyl group proved ideal, giving a further 3 to 4-fold increase in potency and compound **4t** showed 25 nM potency against J774 macrophages. Representative derivatives based on other substituents including 4-methyl (**4v**), 6-chloro (**4w**), 2-chloro (**4x**), 2-hydroxy (**4ad**), and 5-hydroxymethyl (**4ae**) gave a decrease in potency; 4-chloro (**4y**) and 4-methoxy (**4z**) had little effect on potency. 4-Hydroxy (**4ac**) or 6-hydroxymethyl (**4ag**) substitution gave small increases in potency; when combined with the 2′,4′-difluorobiphenyl motif these also looked promising candidates for further development.

Activity was validated in our secondary assay, mouse osteoclast formation, in a mouse osteoblast–bone marrow co-culture.[11] Compounds showed similar potency and rank order to that seen in the J774 assay, from **4b** IC_{50} = 3000 nM to **4t** = 35 nM (osteoclasts identified by TRAcP staining[11] and subsequent cell counting). **4k** and **4s** also showed little effect on mouse osteoblast viability after 72 h, as measured by AlamarBlue.[11] As osteoclasts and osteoblasts are responsible for bone resorption and formation, respectively, this represents a good therapeutic window and suggests low general toxicity. Further studies in human hepatocytes demonstrated that there was no reduction in cell viability after 72 h incubation with 50 μM **4j** or **4k** in an MTT assay. Results are shown in Table 2. For further assay details, see Supplementary data.

The SAR of compounds from class **5** was less clear-cut: and the 2′,4′-difluorobiphenyl derivative (**5b**, Fig. 3) did not show the same degree of improvement in potency (3 μM) as was found with compound **4j** and these were not further investigated. Compounds from classes **6** and **7** showed poor metabolic stability, for example, the 2′,4′-difluorobiphenyl and 2′,4′-dichlorobiphenyl derivatives **6b** and **6c** (Fig. 3) had half lives of just 17 and 13 min, respectively, in human liver microsomes.

Table 1
In vitro viability, stability and solubility assay results for compounds **4a**–**4am**



	R_1	R_2	R_2	IC_{50}, [a] μM	$T_{1/2}$ [b] (min)	Solubility [c] (μg/ml)
4a	—	—	—	2.2	8	11
4b	4-Br	—	—	2.5	42	22
4c	4-Br, 2-F	—	—	1	—	—
4d	4-CF_3	—	—	2.5	—	—
4e	4-Cl	—	—	2.5	—	—
4f	4-F	—	—	3.5	—	—
4g	4-NMe_2	—	—	>5	—	—
4h	4-Me	—	—	1.2	10	21
4i	2-F	—	—	1.3	9	57
4j	2-F, 4-F	—	—	0.3	30	50
4k	2-Cl, 4-Cl	—	—	0.5	28	40
4l	4-Cl, 2-F	—	—	0.7	42	73
4m	4-SO_2Me		—	>5	—	—
4n	4-NH_2Ac		—	>5	—	—
4o	4-F	3-OCF_3	—	1.0	—	—
4p	4-F	3-Cl	—	1.2	26	25
4q	2-F, 4-F	2-Me	—	0.1	30	—
4r	4-F	—	2-Me	0.7	14	24
4s	2-F, 4-F	—	2-Me	0.08	20	43
4t	2-F, 4-F	2-Me	2-Me	0.025	15	—
4u	4-Br	—	2-Me	0.75	34	12
4v	4-Br	—	4-Me	>5	9	47
4w	4-Br	—	6-Cl	>5	—	—
4x	4-Br	—	2-Cl	5	56	14
4y	4-Br	—	4-Cl	2	45	11
4z	4-Br	—	4-OMe	2.5	—	—
4aa	4-Br	—	6-OH	2.5	10	4
4ab	4-Br	—	4-OH	1.0	5	12
4ac	2-F, 4-F	—	4-OH	0.2	38	12
4ad	4-Br	—	2-OH	>5	7	19
4ae	2-Cl, 4-Cl	—	5-CH_2OH	2.5	—	—
4af	4-F		6-CH_2OH	1.0	—	—
4ag	2-F, 4-F		6-CH_2OH	0.15	14	32
4ah	5-Indolyl	—	—	>5	—	—
4ai	6-Indolyl	—	—	2.5	—	—
4aj	*N*-Methylated **4k**			2.2	—	—
4ak	Reversed sulfonamide of **4k**			>10	>200	40
4al	Carboxamide of **4a**			>15		
4am	Carboxamide of **4j**			>15		

[a] Mean of three experiments as determined by AlamarBlue viability assay in J774 macrophages.[11]
[b] $T_{1/2}$ measured in human liver microsomal preparations, concentration measured by LC–MS/MS at five timepoints from 0–45 min.
[c] Solubility measured in FaSSIF, filtered and conc. measured by HPLC/UV.

Table 2
In vitro viability assay results for selected class **4** compounds

	IC_{50} (μM)[a]			
	J774[a]	OC[b]	OB[c]	Hepatocytes[d]
4a	2.2	1.1	—	—
4b	2.5	3.0	—	—
4j	0.3	0.2	—	>50
4k	0.5	0.5	70	>50
4s	0.8	0.12	>100	—
4t	0.025	0.035	—	—

[a] Values are means of three experiments as determined by [a]AlamarBlue viability assay in J774 macrophages.
[b] TRAcP staining in osteoclasts (OC).
[c] AlamarBlue viability assay in osteoblasts (OB).
[d] MTT viability assay in human hepatocytes.

Compounds of class **4** showed modest in vitro metabolic stability in human liver microsomes: none of the more potent derivatives had a half life of >45 min; metabolite analysis, following 45 min incubation with human hepatocytes with detection by LC/ESI/TOF-MS (see Supplementary data), demonstrated that the benzylic methylene was the main site for metabolism, being oxidised to the corresponding carboxylic acid. The benzoic acid analogue of **4k** (**10b**, Fig. 4), prepared by hydrolysis of the ester in methanolic sodium hydroxide, was more stable ($T_{1/2}$ = 130 min) but had low potency in the J774 macrophage assay (IC_{50} >50 μM) and thus wasn't considered likely to be an active metabolite responsible for in vivo activity. The lack of in vitro activity could have been associated with poor cell permeability and the ethyl ester **10a** (Fig. 4) was also tested, but found to have low potency (20 μM). The reversed sulfonamide (**4ak**) was prepared for **4k** and, suprisingly, this transformation was sufficient to completely stabilise the molecule in HLM, presumably by reducing the electron density of the benzyl group. Unfortunately, this was also accompanied by a substantial loss of potency (>10 μM).

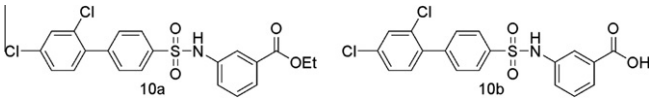


Figure 4. Structures of compounds **10a** and **10b**.

Table 3
In vitro and in vivo DMPK for compound **4k**

Parameter	**4k** Results			
	CLint (μL/min/mg protein)			$T_{1/2}$ (min)
Human mixed microsomal stability		50.5		27.5
Human mixed hepatocytes stability		60.6		22.9
		A–B		B–A
Caco-2 permeability (P_{app} nm/s)		15		55
	C_{max} (ng/mL)	T_{max} (min)	AUC (μg.h/mL)	F%
Rat PK (2.5 mg/kg po)	30	50	0.11	8

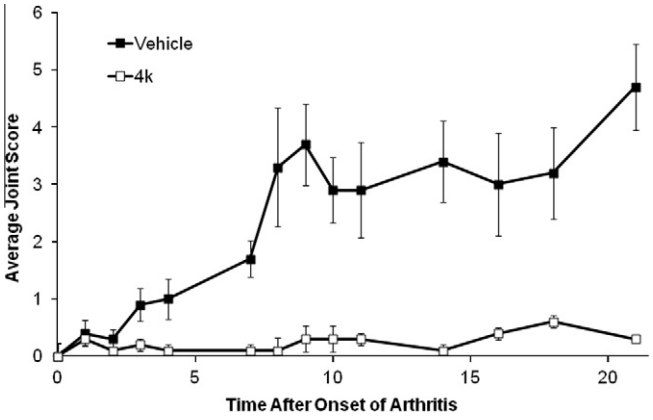


Figure 5. Graph showing the sum of the joint inflammation scores for each study group over a 21-day period following the first signs of inflammation in an arthritis model: (■) control group, no drug; (□) **4k** (10 mg/kg/day, ip); $n = 10$ animals per group.

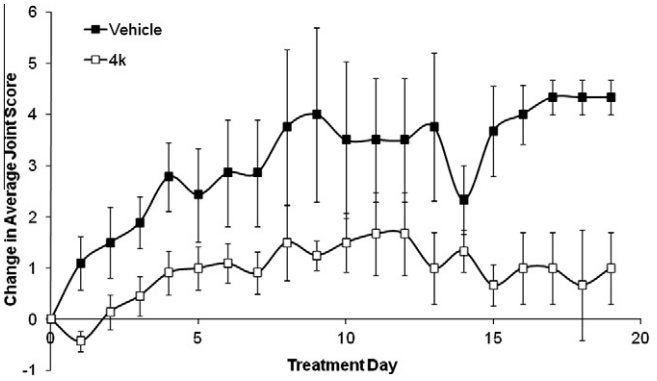


Figure 6. Graph showing the average change in joint inflammation scores for each study group over a 19-day period following the development of more severe inflammation (joint score >3): (■) control group, no drug; (□) **4k** (10 mg/kg/day, ip); $n = 10$ animals per group.

An additional consideration was compound solubility: many of the most potent compounds in class **4** had limited solubility (25–75 μg/ml in FaSSIF). We had hoped to be able to improve this by addition of polar groups, but neither the addition of the hydroxyl of **4ac**, nor the hydroxymethyl of **4ag** gave any increase in solubility. Attempts to synthesise the related 2 or 4-hydroxymethyl derivatives were unsuccessful: the final reduction of a hindered 2-CO_2Et group to the alcohol was not achieved under the given conditions; meanwhile the presence of a 4-CO_2Me prevented reaction with the sulfonyl chloride.

In the absence of a fully-validated molecular target or a biochemical assay allowing for direct study, we felt it necessary to ensure in vivo anti-inflammatory activity and potential as an anti-arthritic agent prior to any further developmental work, using the collagen-induced arthritis model as described in the Supplementary data,[17] and selected compound **4k** for this. Preliminary in vitro testing showed that **4k** had moderate permeability in the Caco-2 assay with some signs of efflux; $T_{1/2}$ in heptocytes of 23 min and modest bioavailability of 8% (see Table 3).

In a prevention model using 8-week old male DBA/1 mice (Harlan, UK), treatment with compound **4k** (10 mg/kg/day ip, delivered in corn oil) was started when joint inflammation became apparent in the first animals (in this experiment 15 days after injection of collagen), and the experiment was terminated 3 weeks later. The animals were scored for joint inflammation at least three times per week using the scoring system whereby 0 = normal, 1 = mild inflammation of individual digits, 2 = moderate redness and swelling and 3 = severe redness and swelling of entire paw. For each animal, the scores for all four joints were added (maximal score = 12). Figure 5 shows that the untreated study group has a total joint score of 47, whereas the group treated with **4k** ip has a score of three.

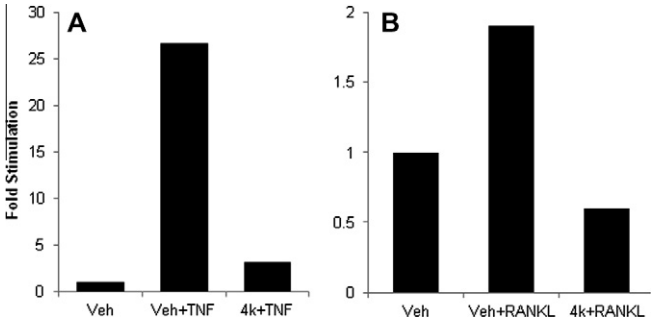


Figure 7. Compound **4k** inhibits both TNFα-(**A**) and RANKL-(**B**) induced phosphorylation of IκB. Mouse macrophages were pre-treated for 1 h with compound **4k** (10 μM) or vehicle, stimulated with cytokines for 5 min and IκB phosphorylation assessed using Western blotting.

Compound **4k** was also investigated in a treatment model also using 8-week old male DBA/1 mice (Harlan, UK), in which the drug was not given until each animal has developed more severe inflammation (joint score of ⩾3 per animal. Figure 6 shows that treatment with **4k** was highly effective in limiting any further increase in the level of inflammation in a model for established arthritis, (delta = 1), whilst the untreated animals showed a continued worsening of inflammation (delta = 4.3).

As expected from previous mechanistic studies on the biphenylketones, compound **4k** inhibited both TNFα and RANKL-induced phosphorylation of IκB in M-CSF-dependent bone marrow macrophage cultures. Macrophage cultures were pre-treated for 1 h with **4k** at 10 μM, stimulated with RANKL (100 ng/ml) or TNF (10 ng/ml) for 5 min and IκB phosphorylation was analysed by Western blotting (Fig. 7).

These results demonstrated that triarylsulfonamides such as ABD455[18,19] (**4k**) show potential in the treatment of rheumatoid arthritis and may be promising starting points for the development of much-needed small molecule TNFα modulators for the treatment of inflammatory diseases.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.067.

References and notes

1. Smolen, J. S.; Steiner, G. *Nat. Rev. Drug Disc.* **2003**, *2*, 473.
2. Mills, K. H. G.; Dunne, A. *Nat. Med.* **2009**, *15*, 1363.
3. Scheinecker, C.; Smole, J.; Yasothan, U.; Stoll, J.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2009**, *8*, 273.
4. O' Neill, L. A. J. *Nat. Rev. Drug Disc.* **2006**, *5*, 549.
5. Mack, G. S. *Nat. Biotech.* **2008**, *1053*, 26.
6. Moreland, L.; Bate, G.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2006**, *5*, 185.
7. Opar, A. *Nat. Rev. Drug Disc.* **2010**, *9*, 257.
8. Dolgin, E. *Nat. Rev. Drug Disc.* **2011**, *10*, 717.
9. Sheridan, C. *Nat. Biotech.* **2008**, *26*, 143.
10. Redlich, K.; Smolen, J. S. *Nat. Rev. Drug Disc.* **2012**, *11*, 234.
11. Van 't Hof, R. J.; Idris, A. I.; Ridge, S. A.; Dunford, J.; Greig, I. R.; Ralston, S. H. *J. Bone Miner. Res.* **2004**, *19*, 1651.
12. Greig, I. R.; Idris, A. I.; Ralston, S. H.; Van 't Hof, R. J. *J. Med. Chem.* **2006**, *49*, 7487.
13. Greig, I. R.; Coste, E.; Ralston, S. H.; Van 't Hof, R. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5548.
14. Idris, A. I.; Mrak, E.; Greig, I. R.; Guidobono, F.; Ralston, S. H.; Van 't Hof, R. J. *Biochem. Biophys. Res. Commun.* **2008**, *371*, 94.
15. Luckman, S. P.; Coxon, F. P.; Ebetino, F. H.; Russell, R. G.; Rogers, M. J. *J. Bone Miner. Res.* **1998**, *13*, 1668.
16. O'Brien, P. M.; Ortwine, D. F.; Pavlovsky, A. G.; Picard, J. A.; Sliskovic, D. R.; Roth, B. D.; Dyer, R. D.; Johnson, L. L.; Man, C. F.; Hallak, H. *J. Med. Chem.* **2000**, *43*, 156.
17. Leung, B. P.; Sattar, N.; Crilly, A.; Prach, M.; McCarey, D. W.; Payne, H.; Madhok, R.; Campbell, C.; Gracie, J. A.; Liew, F. Y.; McInnes, I. B. *J. Immunol.* **2003**, *170*, 1524.
18. Greig, I. R.; Ralston, S. H.; van 't Hof. R. J. Patent Application, WO2008/114022.
19. *Data for* ***4k*** *(ABD455)*: white solid; mp 132–133 °C (Et_2O/petrol); ^1H NMR (DMSO-d_6): δ 4.39 (2H, s), 5.20 (1H, s), 6.96 (1H, d, J = 7.6 Hz), 7.03 (1H, d, J = 8.2 Hz), 7.14 (1H, s), 7.20 (1H, d, J = 8.2 Hz), 7.44 (1H, d, J = 8.8 Hz), 7.50 (1H, d, J = 8.8 Hz), 7.60 (2H, d, J = 7.9 Hz), 7.75 (1H, s), 7.86 (2H, d, J = 7.9 Hz) and 10.40 (1H, s). ^{13}C NMR (DMSO-d_6): δ 64.7, 120.0, 120.6, 123.9, 127.1, 127.5, 129.6, 130.0, 130.1, 131.9, 133.0, 134.9, 136.6, 137.1, 138.5, 142.5 and 143.0. Anal. ($C_{19}H_{15}Cl_2NO_3S$): C, H; MS, m/z: Calcd, 407.01. Found: 407.01 (M).