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Discovery of biphenylketones as dual modulators of inflammation and bone loss

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ABSTRACT

Biphenylketones were identified as novel inhibitors of NFκB activation. Structure–activity studies led to the identification of compound **4c**, which had good potency against osteoclasts (IC_{50} = 0.8 μM), showed oral activity, and was able to completely prevent inflammation and bone loss in vivo.

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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 most important is believed to be TNFα and the advent of anti-TNF therapies has revolutionalized the treatment of RA. These therapies include neutralizing antibodies such as infliximab and adalimumab, and decoy receptors such as etanercept. However, all current therapies are expensive biological agents and there is an urgent requirement to identify inexpensive small molecules to act against these targets.

A further complication of RA is the associated bone loss, mediated through the RANKL signalling pathway, which leads to increased osteoclast differentiation and activity. We have previously reported a series of anti-resorptive compounds which prevented bone loss by inhibition of RANKL signalling.[2–4] As both TNFα and RANKL cause up-regulation of NFκB, we chose to further investigate the potential of this class of compounds, to determine if their mode of action shared a common signalling intermediate that would also make them active as anti-inflammatory agents. Through these studies we have identified a novel class of simple small molecules which are highly active in the prevention of inflammation and bone destruction in in vivo models for postmenopausal osteoporosis and RA.

Our previous studies have shown biphenylesters such as **1** (Fig. 1) to be active in the prevention of bone loss and to induce apoptosis in cells of the osteoclast lineage by inhibition of NFκB- and MAP-kinase signalling.[2] However, as a labile ester, this was not a suitable candidate for development. Our initial studies therefore focused on the related biphenylsulfonamides, such as **2** (Fig. 1). These were up to 100-fold more potent in vitro than the esters, but proved of no better potency in our studies on inhibition of RANKL-induced signalling,[5] possibly due to poor cell penetration. Therefore, we decided to investigate other closely related classes of compounds, including the biphenylketones.

Our initial reported synthesis of biphenylketone **4a** involved attack of the lithium salt of a dithiane-protected biphenyl on a silyl-protected tosyl-butylalcohol[6] and was not suitable for the preparation of a larger number of derivatives. Our proposed revised synthesis of biphenylketones involved preparation of 1-(biphenyl)hex-5-en-1-one as an intermediate: using attack of a Grignard, prepared from 5-bromopentene, on a Weinreb amide. Initial attempts to convert this to the alcohol, via hydroboration, led to the reduction of the ketone and formation of **3a**; testing demonstrated that this and **4a** were equipotent in a number of cell-based assays.

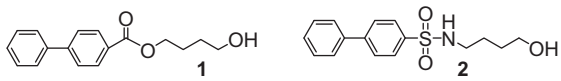

Figure 1. Structures of biphenylester **1** and biphenylsulfonamide **2**.

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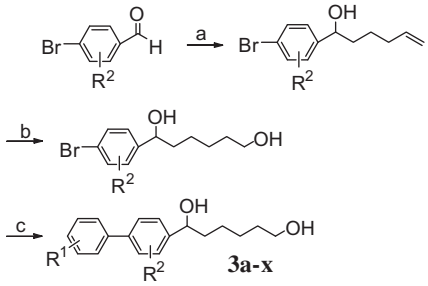
As these diols can be readily prepared by attack of the Grignard on an aldehyde, this suggested a more accessible series of compounds that could be used as a surrogate system for rapid generation of an SAR model.

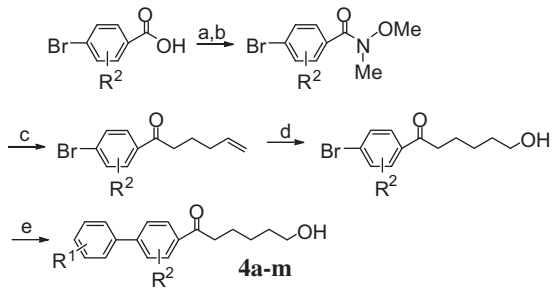
Accordingly, 1-(4-bromophenyl)hexane-1,6-diol was prepared from 4-bromobenzaldehyde and 5-bromopentene followed by hydroboration, and was then coupled to various phenylboronic acids by standard Suzuki coupling methods[7] to give a range of substituted biphenyls for biological testing and SAR generation (Scheme 1).

These studies on reduced ketones allowed us to identify structures of interest, for which it was then necessary to synthesize the corresponding ketone. These were prepared from the respective 1-(4-bromophenyl)hex-5-en-1-one, and then selectively hydroborated using 9-BBN and sodium perborate[8] (Scheme 2). Hydroboration usually gave a mixture of starting material and ketone when performed under the given conditions.

As in our previous studies on ester and sulfonamide derivatives,[3,4] we performed initial in vitro screening of candidate compounds by measuring survival of the macrophage cell line J774. The unsubstituted derivatives, **3a** and **4a** were tested in this assay and found to be moderately potent inhibitors of J774 survival, with IC_{50} values of approximately 22 μM, comparable to the original ester and sulfonamide, compounds **1** (18 μM) and **2** (18 μM). With the ester derivatives we had found that a butanediol side chain gave the most potent compounds, and a similar feature was found for the biphenylketones, where a 5 atom separation between the ketone/reduced ketone moiety and the terminal hydroxyl resulted in optimal potency; a one-carbon reduction or extension (**3y** and **3z**, respectively, not shown) resulted in a large decrease in potency (IC_{50} >75 μM and 50 μM, respectively). We had previously conducted studies on the esters to investigate the effects of chain branching and found that addition of a methyl group at either or both ends of the chain gave a slight reduction in potency (IC_{50} = 30 μM), and therefore did further pursue this line of investigation for their biphenylketone analogues.



Scheme 1. Reagents and conditions: (a) 5-bromopentene, Mg, THF (6 h); (b) 0.5 M $NaBH_4$, $BF_3{\cdot}OEt_2$, diglyme (0 °C, 1 h), 3 M NaOH, 30% H_2O_2, H_2O (rt, 0.5 h), K_2CO_3; (c) substituted benzeneboronic acid, $(PPh_3)_4Pd$, ethanol, toluene, 2 M Na_2CO_3 (reflux, 3 h).



Scheme 2. Reagents and conditions: (a) $SOCl_2$, toluene, (reflux, 18 h); (b) HN(Me)OMe, pyridine, $CHCl_3$ (18 h); (c) 5-bromopentene, Mg, THF (3 h); (d) 0.5 M 9-BBN in THF (rt, 4 h), $NaBO_3$ (rt, 1 h), H_2O (50 °C, 4 h); (e) substituted benzeneboronic acid, $(PPh_3)_4Pd$, ethanol, toluene, 2 M Na_2CO_3, (reflux, 3 h).

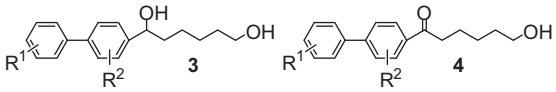
To test whether the substitution pattern for the aromatic rings was important to the activity of the drug, we prepared a range of derivatives with a variety of substituents.

It was apparent that the pattern generally matched that previously found for the sulfonamides and esters,[3,4] but with a much smaller substituent dependency (Table 1) and a shallow SAR. Substitution at the 2′ or 4′ positions generally had little effect on potency, whilst substitution at the 3′ position caused a loss of activity. Only a 2′,4′-dihalo substitution pattern gave an increase in potency: we therefore focused on preparing similar derivatives for the ketone series, compounds **4a**–**m**. These had potencies closely matching those of the reduced ketone analogues, compounds **3a**–**x**.

We next investigated the effects of adding substituents onto the inner ring, and found that again only small increases in potency could be obtained by addition of methyl or halogen groups (Table 1).

Studies carried out to probe the effects of further structural changes were unable to provide any improvements in potency. Partial rigidification of the alkyl chain by incorporation of an aryl

Table 1
In vitro viability assay results for compounds **3a**–**3x** and **4a**–**4m**



	R_1	R_2	IC_{50}, μM[a]		
			J774	OC	OB
3a	H	H	22	8	>100
3b	4-Br	H	20	–	–
3c	2-F	H	22	–	–
3d	4-F	H	18	–	–
3e	2-F, 4-F	H	8	–	–
3f	2-Me	H	24	–	–
3g	3-Me	H	>100	–	–
3h	4-Me	H	22	–	–
3i	2-OH	H	40	–	–
3j	4-OH	H	15	–	–
3k	4-SMe	H	24	–	–
3l	3-Cl	H	>100	–	–
3m	4-Cl	H	17	–	–
3n	2-Cl, 4-Cl	H	8	–	–
3o	4-CF_3	H	13	–	–
3p	4-OCF_3	H	14	–	–
3q	4-OEt	H	14	–	–
3r	4-NMe_2	H	28	–	–
3s	2-OMe, 4-F	H	70	–	–
3t	2-Me, 4-F	H	20	–	–
3u	2-F, 4-F	2-Me	8	1.3	>100
3v	4-F	3-Cl	8	–	–
3w	4-F	3-F	14	–	–
3x	H	2-F	17	–	–
4a	H	H	22	9	>100
4b	2-F, 4-F	H	6	2	>100
4c	2-Cl, 4-Cl	H	8	0.8	>100
4d	2-F, 4-F	2-Me	8	1.5	–
4e	2-F, 4-F	3-Me	4	–	–
4f	2-F, 4-F	3-Cl	7	3	>100
4g	3-F,4-F	H	20	–	–
4h	2-Me, 4-F	H	20	–	–
4i	2-OMe, 4-OMe	H	50	13	–
4j	3-OMe, 4-OMe	H	60	14	–
4k	2-CF_3,4-CF_3	H	23	–	–
4l	2-Cl, 4-F	H	15	–	–
4m	3-Me	H	100	–	–

[a] Values are means of three experiments as determined by alamar blue viability assay. J774 = J774 macrophages, OC = osteoclasts, OB = osteoblasts.

ring was expected to provide more information about the active conformation and ideally give an increase in potency, by reduction of the number of inactive conformations available to the molecule. Compounds were prepared by the same methodology as described for the alkyl derivatives, using 3-bromostyrene to form the Grignard: unusually, either of the rigid derivatives **5a** (6 μM) or **5b** (6 μM) (Fig. 2) showed any significant difference in potency from the parent compound **4c**. Likewise rigidification of the biphenyl system, by replacement with a phenanthrene (IC_{50} = 25 μM) or fluorene (33 μM)) had little effect on potency.

We also investigated the effects of replacement of each of the phenyl rings with pyridyls. As these are electron-poor, the results shown in Table 1 led us to expect that these might be well tolerated in either ring. However, introduction of a heteroatom in the outer ring by way of a 2-pyridyl (40 μM), 3-pyridyl (40 μM), 4-pyridyl (15 μM), did not give any meaningful increases in potency.

Derivatives with inner ring pyridyls were prepared, using 6-bromonicotinic acid and 5-bromopicolinic acid as starting materials and the same methodology as shown in Scheme 2, to give compounds **6a** and **6b** (Fig. 3). In both cases the addition of the ring nitrogen caused a reduction in potency (**6a** = 55 μM, **6b** = 60 μM). Attempts to prepare the ketone analogue of **6b** were unsuccessful, with the carbonyl undergoing reduction under the given hydroboration conditions.

The ketone was reacted with hydroxylamine or *O*-methylhydroxylamine to give the oximes **7a** and **7b** (Fig. 4): neither of which differed in potency from the parent compound (10 μM). A small increase in potency (4 μM) was seen when the ketone was alkylated with methylmagnesium bromide to give compound **8a** and a small decrease (18 μM) upon similar addition of an ethyl group **8b** (not shown, prepared by a method analogous to Scheme 1, using 4′-bromopropiophenone as the starting material). The central hydroxyl group was methylated prior to hydroboration to give compound **9**, which gave a reduction in potency (30 μM). This suggests a relatively unselective binding interaction and that many groups capable of acting as hydrogen bond acceptors can interact. One exception to this is the amide, which we have previously shown to have poor potency (>100 μM) against macrophages and osteoclasts.[4] Finally, the hydrogen bond acceptor was completely removed by hydrogenation of compound **3a**, to give a derivative

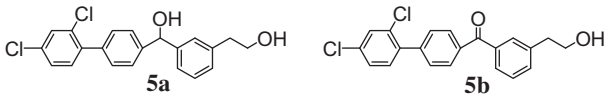


Figure 2. Structures of rigidified derivatives **5a** and **5b**.

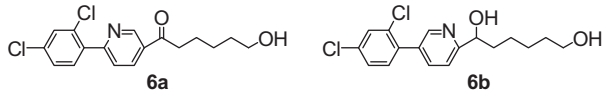


Figure 3. Structures of derivatives **6a** and **6b** bearing a pyridyl group.

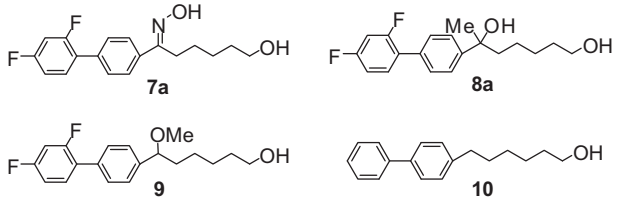


Figure 4. Structures of compounds **7a**, **8a**, **9** and **10** in which the carbonyl has been further modified.

10. Studies of this compound in the J774 macrophage assay were complicated by low solubility, and did not permit calculation of an IC_{50}. However, as compound **10** did not inhibit TNFα signalling, any activity is presumed not to be against the same target as compounds **3** and **4**.

These findings broadly agree with earlier studies,[3,4] but the very flat SAR gives little scope for optimization and the potency is somewhat below that normally desirable for a lead compound, albeit in a cell-based assay, rather than as assessed by direct inhibition of a molecular target. A greater improvement in potency was seen in viability studies on genuine osteoclasts,[4] which are dependent on RANKL-induced signalling for their survival. Compounds **4a**–**4d** and **4f** have respective potencies of 9, 2, 0.8, 1.5 and 3 μM (Table 1). These potencies are still below that which would be ideal in a lead series, but do have a large therapeutic window, as shown by their lack of activity against osteoblasts (Table 1), suggesting that they may have low general cytotoxicity and may have good selectivity for the putative biological target.

Further use of these compounds as probes in mechanism of action studies, demonstrated their unusual activity as inhibitors of NFκB signalling. Compounds **4c** and **4d** inhibited both RANKL- and TNF-induced signalling in mouse macrophages at 10 μM (Fig 5), whilst compounds **4m** and **10** showed no effect even at 50 μM. Our proposed mechanism of action is that these compounds inhibit recruitment of components of the signalling complex to the RANK and TNFα receptors, and thus suppress NFκB- and MAP-kinase signalling in osteoclasts and macrophages. Supporting evidence and further details will be presented in a separate publication.

In the absence of a fully validated molecular target, we chose to study these compounds directly in our models for osteoporosis and rheumatoid arthritis. For these studies, the ketone derivatives were preferred as they lack the complication of a chiral centre.

A range of biphenylketones was selected and tested for their oral anti-resorptive activity and potential as a treatment for osteoporosis, using the ovariectomy-induced bone loss model as described previously.[4] The results are shown in Figure 6 and show that **4c** was the most effective of the compounds chosen at prevention of bone loss. In the untreated mice, a drop in trabecular BMD of 34% was seen in comparison with sham controls, in contrast to a 21% decrease in mice treated with **4a**, a 10% decrease with **4b**, a 3% decrease with **4f** and a 6% increase on BMD in mice treated with **4c**.

From this study, we selected compound **4c** (**ABD328**)[9] to be further investigated for anti-inflammatory activity and for potential as an anti-arthritic agent, using the collagen-induced arthritis model as described previously.[10] Treatment with compound **4c** (10 mg/kg/day, orally or 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 experi-

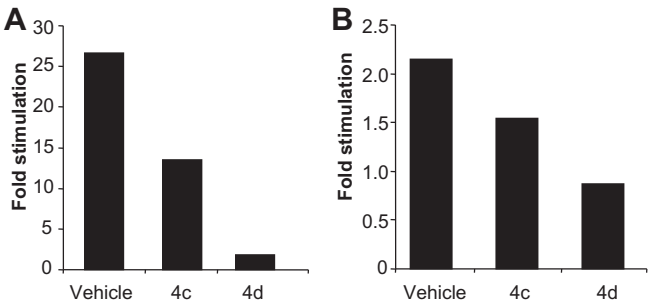


Figure 5. Compounds **4c** and **4d** inhibit both TNFα- (A) and RANKL- (B) induced phosphorylation of IκB. Mouse macrophages were pretreated for 1 h with compounds (10 μM) or vehicle, stimulated with cytokines for 5 min and IκB phosphorylation assessed using Western blotting.

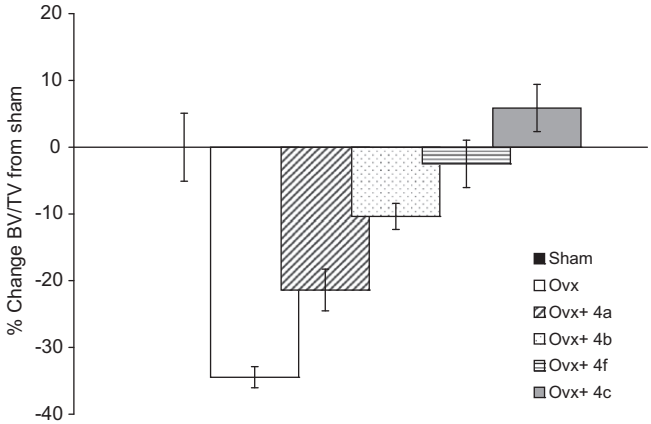


Figure 6. Effects of biphenylketones on ovariectomy-induced bone loss. Each of **4a**, **4b**, **4c** and **4f** were administered orally to ovariectomized mice (Ovx) at 20 mg/kg/day by gavage. Data are expressed as percentage change from sham operated control animals ± SD, n = 6–8 per group. BV/TV: bone volume as percentage of total tissue volume.

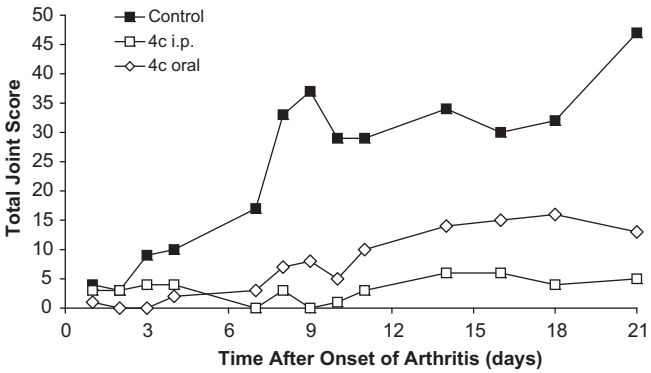


Figure 7. Graph showing the sum of the joint inflammation scores for each study group over a 21-day period following the first signs of inflammation in an arthritis model: (■) control group, corn oil ip; (□) **4c** (10 mg/kg/day, ip); (◇) **4c** (10 mg/kg/day, orally); n = 10 animals per group.

ment 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 7 shows that the untreated study group has a final total joint score of 47, whereas the group treated with **4c** orally has a score of 13 and the group treated with **4c** ip has a score of 5.

In a separate study, we also compared compound **4d** and its sulfonamide analogue **2a** (**ABD295**,[3] not shown). In this study the control group had a final total score of 29, **4d** fully prevented the onset of inflammation with a score of 3, whilst **2a** had very little effect (total score of 17, but a smaller difference from control on previous days). The results (Fig. 8, shown in Supplementary data) demonstrate that such sulfonamides are significantly less active in this model, as matched by their lack of activity in the NFκB- and MAP-kinase activation assays.[5] The results also highlight that the anti-inflammatory properties may be mediated by a different mechanism to that responsible for the anti-resorptive properties in closely related compounds, and that the differing activities are not separated by the use of macrophages and osteoclasts alone. However, they do still provide a useful primary screen, especially when combined with a suitable assay which measures activation of the TNF-NFκB signalling pathway.

These results demonstrate that biphenylketones such as **4c** [11] show great potential as dual modulators in the treatment of rheumatoid arthritis and associated bone loss. Whilst these compounds are perhaps not ideal in terms of a modest in vitro potency and structure not obviously drug-like, these compounds do represent an invaluable starting point in the search for effective small molecule anti-inflammatory agents.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.055.

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11. *Data for 4c (ABD328):* White solid; mp 63–65 °C (Et_2O/petrol); ^1H NMR ($CDCl_3$): δ 1.45 (2H, m), 1.60 (2H, m), 1.80 (2H, m), 3.01 (2H, t, J = 6.5 Hz), 3.68 (2H, t, J = 6.5 Hz), 7.28 (2H, d, J = 8.2 Hz), 7.30 (1H, s), 7.50 (2H, m) and 8.01 (2H, d, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$): δ 23.9, 25.5, 32.5, 38.6, 62.7, 127.4, 128.0, 129.7, 129.9, 131.9, 133.1, 134.5, 136.3, 138.0, 142.8 and 199.9. Anal. ($C_{18}H_{18}Cl_2O_2$): C, H; MS, m/z: calcd, 337.24; found, 337.28, 339.30 (M, M+2).