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Furanyl-1,3-thiazol-2-yl and benzoxazol-5-yl acetic acid derivatives: novel classes of heparanase inhibitor

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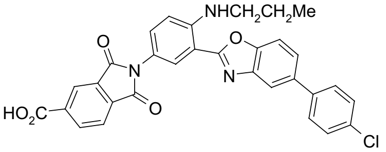
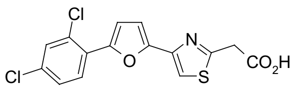
Abstract—Using a furanylthiazole acetic acid as a starting point, a novel series of benzoxazol-5-yl acetic acid derivatives have been identified as heparanase inhibitors. Several compounds possess an IC_{50} of ~200 nM against heparanase, for example, *trans* 2-[4-[3-(3,4-dichlorophenylamino)-3-oxo-1-propenyl]-2-fluorophenyl]benzoxazol-5-yl acetic acid (**16e**). Several of the compounds show anti-angiogenic properties. Improvement to the DMPK profile of compounds has provided compounds of potential use in in vivo models.

Heparanase, an *endo*-β-glucuronidase that cleaves heparan sulfate, is implicated in several physiological and pathological processes.[1,2] It contributes to the remodelling of the extracellular matrix and basement membranes, prerequisite for angiogenesis and egression of metastatic tumour cells; its expression correlating with the metastatic potential of tumour cells and with angiogenesis.[3–7] Although these data promote heparanase as an attractive drug target, progress in this area has been limited by the lack of small molecule inhibitors. The most advanced inhibitor PI-88,[8] which has as its principal component a highly sulfated pentasaccharide, is currently in Phase II clinical trials. The challenge, therefore, is to find small, drug-like molecules with acceptable DMPK properties for evaluation in animal models and as therapeutic leads.

We have recently reported on a series of 2,3-dihydro-1,3-dioxo-1*H*-isoindole-5-carboxylic acids as heparanase inhibitors, exemplified by (**1**), which has an IC_{50} of 200 nM.[9] In a further phase to our research in this area, we discovered the furanylthiazole acetic acid (**2**) to be a heparanase inhibitor (IC_{50}: 25 μM). Although a relatively weak starting point for a medicinal chemistry programme, the paucity of reported heparanase inhibitors, coupled with its scope for structural changes,



(1)



(2)

Keywords: Heparanase; Angiogenesis; Metastasis; β-glucuronidase; Benzoxazole.

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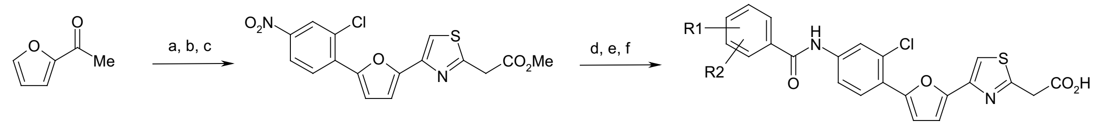


(3)

(4a-h, 5a-e)

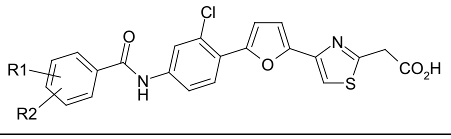
Scheme 1. Reagents and conditions: (a) 2-chloro-4-nitroaniline, $NaNO_2$, $CuCl_2$, aq HCl, 37%; (b) $TBABr_3$, AcOH, CH_2Cl_2, 56%; (c) $H_2NC(=S)CH_2CO_2Me$, *i*-PrOH, 68%; (d) Zn, AcOH, EtOH, 99%; (e) ArCOCl, pyridine; (f) NaOH, H_2O, MeOH.

encouraged us to explore the SAR of this scaffold. Since it was established early on that the 2-chloro substituent appeared critical for retaining inhibitory activity, initial modifications focussed on replacing the 4-chloro substituent. Specifically, introduction of an amino function allowed the introduction of a range of groups via parallel synthesis.

The synthesis of these analogues centred on a diazotisation reaction of 2-chloro-4-nitroaniline on 2-acetyl furan, with subsequent α-bromination and thiazole ring formation providing the key intermediate (**3**). It was then a straightforward matter to reduce the nitro group and introduce selected amides to provide (**4a**–**h**) and (**5a**–**e**) (Scheme 1). Compounds were evaluated for their heparanase inhibitory activity and their ability to inhibit angiogenesis as previously described.[9] A modified benzamide moiety resulted in approximately an order of magnitude increase in potency, for example (**4b**), which had an IC_{50} of 2.5 μM against heparanase and appreciable activity (IC_{50}: 2 μM) in inhibiting angiogenesis (Table 1). However, a brief survey of substituent changes on the benzamide moiety revealed only a modest impact on potency.

Extending the amide moiety to a cinnamoyl group resulted in a 10–20-fold increase in in vitro potency against heparanase, compared to the benzamide series (Table 2). However, for the relatively small group of analogues evaluated within the cinnamoyl series there was little impact of the nature of phenyl ring substitution on the level of heparanase inhibition. Noteworthy is the fact that the 4-bromo derivative (**5b**) showed much better activity in the angiogenesis assay than any of the other analogues.

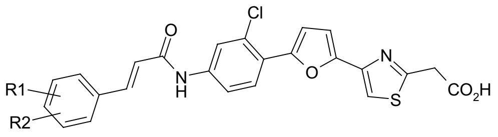
Table 1. In vitro inhibition of heparanase and anti-angiogenic activity of (**4a**–**h**)



Compound	R1	R2	Heparanase inhibition[a] IC_{50}, μM	Angiogenesis inhibition[a] IC_{50}, μM
4a	H	H	>30	NT
4b	H	4-Cl	2.5	2.0
4c	H	4-CF_3	3.5	7.5
4d	2-Cl	4-Cl	3.5	0.5
4e	H	4-OCF_3	1.5	0.5
4f	3-Cl	4-Cl	1.0	NT
4g	H	4-MeO	8.0	NT
4h	3-CN	H	4.0	>20

[a] Mean of at least two determinations; NT: not tested.

Table 2. In vitro inhibition of heparanase and anti-angiogenic activity of (**5a**–**e**)

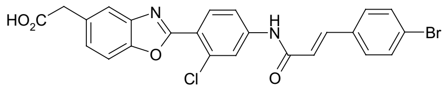


Compound	R1	R2	Heparanase inhibition[a] IC_{50}, μM	Angiogenesis inhibition[a] IC_{50}, μM
5a	H	H	0.45	15
5b	H	4-Br	0.4	1
5c	2-Cl	4-Cl	0.3	>20
5d	H	4-OCF_3	0.6	20
5e	H	4-MeO	0.4	5

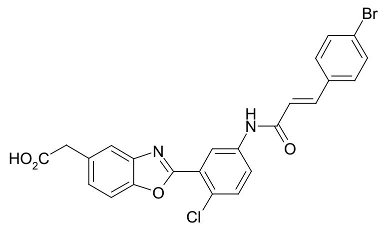
[a] Mean of at least two determinations; NT: not tested.

Although these data were encouraging, the synthetic access to the furanylthiazole acetic acids was often capricious. Furthermore, the best compound to date, (**5b**), was poorly bioavailable and had a short half-life (0.5 h) in mice. Accordingly, replacements for this unit were sought. Molecular overlays of bicyclic heteroaromatic acetic acids with furanylthiazole acetic acids (e.g., (**5b**)) suggested that such structural 'morphing' might provide an acceptable surrogate for the latter system. To validate this approach, the synthesis of two 'pilot' structures was undertaken: (**11i**) and its positional isomer (**7**).

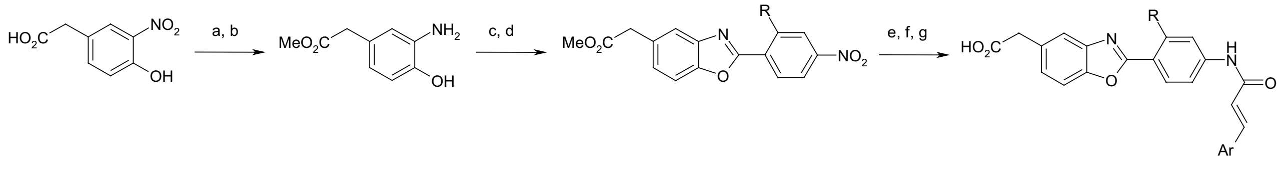
It was pleasing to find that (**11i**) displayed heparanase inhibitory activity (IC_{50}: 0.4 μM) comparable with that



(11i)



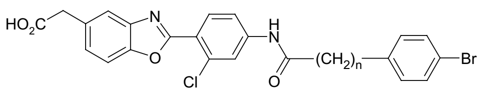
(7)



(9) **(10)** **(11a-k,12a-p)**

Scheme 2. Reagents and conditions: (a) MeOH, HCl, Δ, 90%; (b) H_2, Pd–C, EtOAc, 92%; (c) $O_2NC_6H_3(R)COCl$, THF; (d) TsOH, toluene, Δ; (e) Zn, AcOH, EtOH; (f) ArCH=CHCOCl, NEt_3, THF; (g) LiOH, H_2O, THF.

for (**5b**), although its activity in the angiogenesis assay (IC_{50}: 5 μM) was somewhat less than that for its furanylthiazole counterpart. In contrast, compound (**7**) was significantly less active against heparanase (IC_{50}: 8.5 μM). These results prompted further exploration of the SAR around (**11i**) with the principal aims of improving the anti-angiogenic activity and the pharmacokinetic profile. The synthesis of this structural class employed a two-stage coupling/dehydrative methodology for generating key benzoxazole intermediates (**10**) from the aminophenol (**9**), thus allowing ready introduction of different substituents in the central aromatic ring. It was then a relatively straightforward matter to reduce the nitro group, couple the resulting aniline to cinnamic acid chlorides and finally liberate the free benzoxazole-acetic acids (**11a–j**, **12a–p**) (Scheme 2).



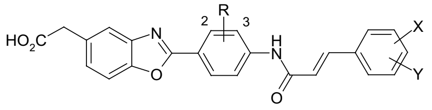
(**8a**) n = 0
(**8b**) n = 1
(**8c**) n = 2

We initially probed the nature of the link between the amide group and the pendant aromatic ring. In fact, this appears to be critical for the observed activity. Thus, the *p*-bromobenzamide (**8a**) loses much activity (IC_{50}: >10 μM) and the benzyl (**8b**) and dihydrocinnamoyl (**8c**) analogues had IC_{50}s of 3 μM and 4 μM, respectively. It was thus confirmed that further optimisation should focus on the cinnamoyl series.

It was important to ascertain whether the 2-chloro substituent in the central aromatic ring was actually contributing to the activity profile to address these two sets of compounds (**11a–e**) and (**11g–j**). The data for these compounds (Table 3) show that the heparanase inhibitory activity is essentially unaffected by the introduction of the 2-chloro substituent. From this exercise, no improvement on the profile of (**11i**) was achieved in terms of heparanase inhibition and anti-angiogenic effects. However, it was the introduction of a 3-fluoro substituent that had a more significant effect on the latter aspect.

Thus, although the heparanase inhibitory potency of the 3-fluoro series mirrored their unsubstituted counterparts, the 3- and 4- bromo analogues, (**12k**) and (**12d**), respectively, showed greatly enhanced anti-angiogenic properties (IC_{50}: ~1 μM) (Table 4). Noteworthy is the 3,4-dichloro analogue (**12n**), which showed the greatest potency to date in the angiogenesis assay, in comparison to the corresponding 2,4-dichloro derivative, which was inactive. The reason for this is not readily apparent.

Table 3. In vitro inhibition of heparanase and anti-angiogenic activity of (**11a–k**)



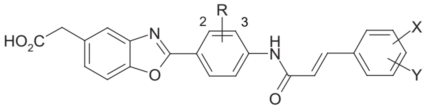
Compound	R	X, Y	Heparanase inhibition[a] IC_{50}, μM	Angiogenesis inhibition[a] IC_{50}, μM
11a	H	H	3	NT
11b	H	4-F	2.5	NT
11c	H	3-Br	0.75	>20
11d	H	4-Br	0.6	20
11e	H	3-MeO	2.5	NT
11f	H	4-MeO	3.0	NT
11g	2-Cl	H	>30	NT
11h	2-Cl	3-Br	0.75	20
11i	2-Cl	4-Br	0.4	7.5
11j	2-Cl	3-MeO	2.5	NT
11k	2-Cl	2,4-Di-Cl	0.35	5

[a] Mean of at least two determinations; NT: not tested.

We next examined reversal of the cinnamic acid amide moiety to provide structures (**15a–l**) (Table 5). Such compounds were readily prepared via the route outlined in Scheme 3. This route centred on a successful Heck reaction of the 2-(4-bromophenyl)benzoxazoles (**13**) to provide the cinnamic acid esters (**14**). Selective cleavage of the *t*-butyl ester followed by amide formation and saponification afforded the benzoxazole acetic acids (**15a–l**) and (**16a–g**) (Table 6).

When the central phenyl ring was unsubstituted, the observed SAR against heparanase was similar to the 'forward' cinnamic acid amide orientation (cf. (**11a**, **11c–f**) and (**15a–d**)). Notably, introducing a fluoro substituent *ortho* to the 4-methoxy group of (**15d**) resulted in a 10-fold increase in anti-heparanase activity (cf. (**15j**)). The 4-bromo derivative (**15b**) showed potent activity in the angiogenesis assay (IC_{50}: 0.25 μM). However, the most potent heparanase inhibitors were obtained when a

Table 4. In vitro inhibition of heparanase and anti-angiogenic activity of (**12a–n**)



Compound	R	X, Y	Heparanase inhibition[a] IC_{50}, μM	Angiogenesis inhibition[a] IC_{50}, μM
12a	3-F	H	5.5	NT
12b	3-F	4-F	3.0	NT
12c	3-F	4-Cl	2.5	NT
12d	3-F	4-Br	0.4	1
12e	3-F	4-CF_3	2.0	NT
12f	3-F	4-MeO	3.0	NT
12g	3-F	4-CN	0.8	1
12h	3-F	3-Cl	0.7	7.5
12i	3-F	2-F, 3-Cl	0.3	5
12j	3-F	3-Cl, 4-F	0.75	2
12k	3-F	3-Br	0.8	1
12l	3-F	2-Cl	2.5	NT
12m	3-F	2,4-Di-Cl	0.35	>20
12n	3-F	3,4-Di-Cl	0.8	0.2

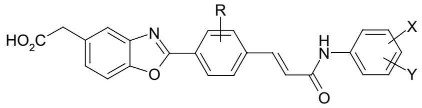
[a] Mean of at least two determinations; NT: not tested.

2-F substituent was introduced into the central phenyl ring. Thus, (**16a**), (**16d**) and (**16e**) all displayed IC_{50}s of 200 nM with (**16e**) showing moderate activity in the angiogenesis assay (IC_{50}: 2 μM).

When administered to mice, both (**12d**) and (**15b**) showed a plasma concentration of ~10*x* the heparanase IC_{50} following oral dosing at 20 mg/kg. Compound (**12d**) showed no significant inhibition of the major human cytochrome P450 isozymes (Cyp3A4 and Cyp2C9, IC_{50}: >30 μM), had improved aqueous solubility over the furanylthiazole acetic acids (**5b**) and displayed modest cell penetration in a Caco2 cell model of intestinal drug transport[10] ($A \rightarrow B$: 0.47×10^6 cm s^{-1}, $B \rightarrow A$: 0.24×10^6 cm s^{-1}).

In summary, the data presented here describe a series of benzoxazoles as potent inhibitors of heparanase. The DMPK profile of some of these suggests that progress has been made towards identifying compounds, which should serve as useful biological tools and may also provide a basis for the design of novel therapeutic agents.[11]

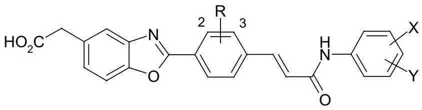
Table 5. In vitro inhibition of heparanase activity of (**15a–l**)



Compound	R	X, Y	Heparanase inhibition[a] IC_{50}, μM
15a	H	3-Br	0.5
15b	H	4-Br	0.7
15b	H	2-OMe	>10
15c	H	3-OMe	3.0
15d	H	4-OMe	3.0
15e	H	2-$OCHF_2$	8.5
15f	H	4-$OCHF_2$	1.5
15g	H	2,4-Di-Cl	0.4
15h	H	4-F	2.5
15i	H	2-Cl	3.0
15j	H	3-F, 4-OMe	0.3
15k	H	3-Cl, 4-OMe	3.0
15l	H	3,4-Di-OMe	>10

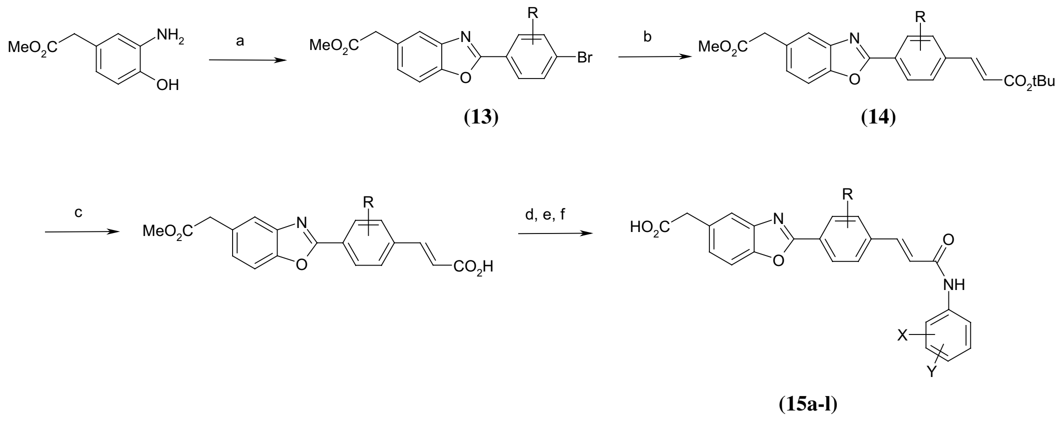
[a] Mean of at least two determinations; NT: Not tested.

Table 6. In vitro inhibition of heparanase activity of (**16a–g**)



Compound	R	X, Y	Heparanase inhibition[a] IC_{50}, μM
16a	2-F	3-Br	0.2
16b	2-F	4-Br	0.4
16c	2-F	2,4-Di-F	0.75
16d	2-F	2,4-Di-Cl	0.2
16e	2-F	3,4-Di-Cl	0.2
16f	2-F	3-F, 4-OMe	0.45
16g	2-F	4-CN	0.3

[a] Mean of at least two determinations; NT: Not tested.



Scheme 3. Reagents and conditions: (a) $BrC_6H_3(R)COCl$, dioxane, μW 210 °C; (b) $H_2C{=}CHCO_2t\text{-}Bu$, $Pd(OAc)_2$, *o*-Tol_3P, NEt_3, DMF, μW 150 °C; (c) TFA, CH_2Cl_2; (d) $(COCl)_2$, DMF, THF; (e) $BrArNH_2$, NEt_3, THF; (f) LiOH, H_2O, THF.

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