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Bioorganic & Medicinal Chemistry Letters 16 (2006) 409–412
Bioorganic & Medicinal Chemistry Letters

1-[4-(1*H*-Benzoimidazol-2-yl)-phenyl]-3-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-urea derivatives as small molecule heparanase inhibitors

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Received 25 June 2005; revised 11 September 2005; accepted 26 September 2005
Available online 21 October 2005

Abstract—A novel class of 1-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-3-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-ureas are described as potent inhibitors of heparanase. Among them are 1,3-bis-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-urea (**7a**) and 1,3-bis-[4-(5,6-dimethyl-1*H*-benzoimidazol-2-yl)-phenyl]-urea (**7d**), which displayed good heparanase inhibitory activity (IC_{50} 0.075–0.27 μM). Compound **7a** showed good efficacy in a B16 metastasis model.

Heparanase degrades heparan sulfate glycosaminoglycan in the extracellular matrix (ECM) and the basement membrane, and is involved in tumor cell invasion, angiogenesis, and other physiological and pathological processes.[1] Different from the large number of proteases know to disassemble the ECM,[2] there is only one heparanase cDNA sequence coding functional enzyme identified to date.[1a,c] These findings indicated heparanase as a major enzyme that degrades heparan sulfate glycosaminoglycan in mammalian tissues.[1b,3] Among the heparanase inhibitors known[4,5] are sulfated oligosaccharide derivatives, and the most advanced of them is PI-88, which is currently in phase II clinical trials.[4] Only recently, small molecule heparanase inhibitors, exemplified by 1*H*-isoindole-5-carboxylic acid[6a] and benzoxazol-5-yl-acetic acid,[6b] were reported.

Our effort aimed at the development of 'proof-of-concept' small molecule heparanase inhibitors led to the identification of *N*-(4-{[4-(1*H*-benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamides, such as **1** (Scheme 1), as a potent heparanase inhibitor with an IC_{50} (compound concentration that causes 50% inhibition of the enzyme activity) of 0.29 μM.[7] Nevertheless, the highest plasma exposure observed for **1** in mice was 5.5 μM, and this observation deterred us from using it as the 'proof-of-concept' candidate for evaluation in animal models. Our early effort in this area has also identified (benzimidazol-2-yl)-phenyl-phenyl-urea **2**[7] and 4-(1*H*-benzoimidazol-2-yl)-*N*-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-benzamide **3**[8] as heparanase inhibitors with IC_{50}s of 0.93 and 0.95 μM, respectively. We hence envisioned that the symmetrical urea **7** (Scheme 2), which has combined structural features of **2** and **3**, might lead to another series of heparanase inhibitors with desired potencies.

The synthesis of 1,3-bis-[4-(1*H*-arylimidazol-2-yl)-phenyl]-urea **7** is straightforward (Scheme 2). Heating an aryldiamine **4** with 4-amino-benzoic acid **5** in polyphosporic acid at 220 °C for 4 h afforded 4-(1*H*-benzoimidazol-2-yl)-phenylamine **6** in good yield. In the second step, **6** was dimerized at room temperature for overnight with CDI to afford the urea **7**,[9] which was tested in the heparanase enzymatic assay (Table 1).[10]

Results in Table 1 revealed that 1,3-bis-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-urea **7a** has an IC_{50} of 0.27 μM against heparanase. Substitution on the phenyl

Keywords: Heparanase inhibitor; Benzimidazole; Urea.
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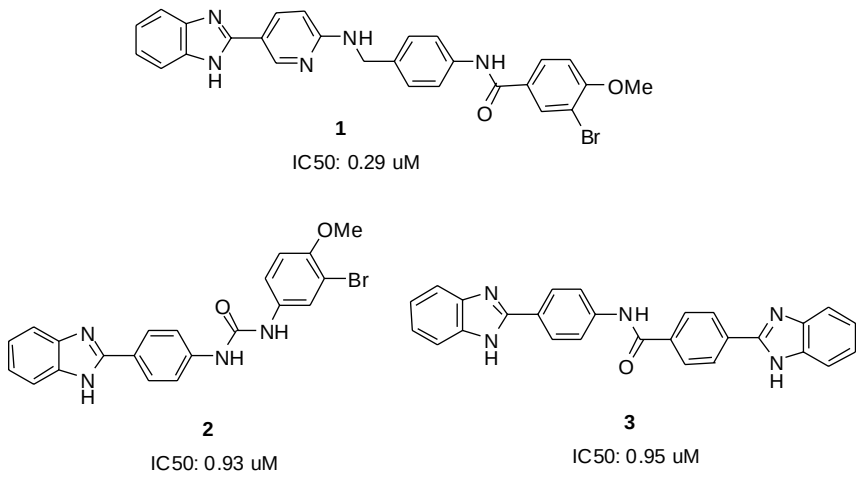
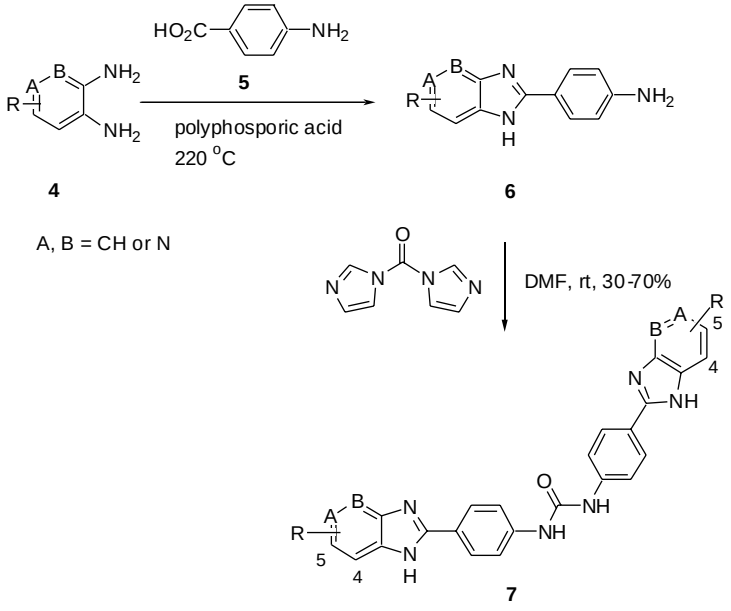
doi:10.1016/j.bmcl.2005.09.069



Scheme 1.



Scheme 2.

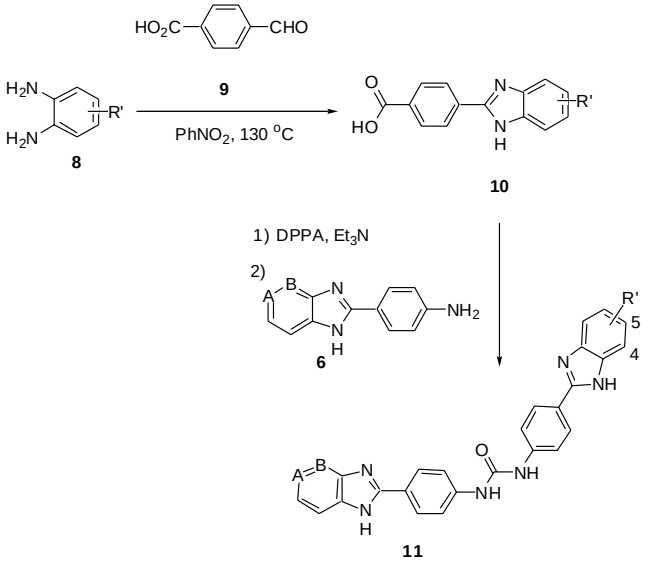
Table 1. In vitro inhibition of heparanase activity of **7a**–**i**

Compound	A	B	R	Heparanase percent inhibition at 0.83 μM[a] (%)	Heparanase inhibition IC_{50} (μM)[a]
7a	CH	CH	H		0.27
7b	CH	CH	5-Me		0.15
7c	CH	CH	4-Me		0.15
7d	CH	CH	5-Me 6-Me		0.075
7e	CH	CH	5-F	21	NT[b]
7f	CH	CH	5-OMe	16	NT[b]
7g	CH	CH	4-OMe		0.87
7h	N	CH	H		0.90
7i	CH	N	H		3.42

[a] Average of three experiments with SD < 15%.
[b] Not tested.

ring of **7a** proved to have dramatic effects on the heparanase inhibitory activities. Improved activities were observed for methyl substitution, such as **7b**–**d**, with **7d**, a tetra-methyl-substituted urea, having an IC_{50} of 0.075 μM. On the other hand, fluoro (**7e**), or methoxy (**7f**,**g**), substitutions diminished the activities. Pyridine containing ureas, such as **7h**,**i**, had IC_{50}s of 0.90 and 3.42 μM, respectively.

Encouraged by the results obtained from the symmetrical urea series, we then turned our attention to the unsymmetrical ureas **11**. The synthesis of compound **11** is shown in Scheme 3. A phenyl diamine **8** was first reacted with 4-formyl-benzoic acid **9** in nitrobenzene to provide benzimidazole acid **10**. Curtis rearrangement of **10** in the presence of diphenylphosphoryl azide (DPPA) and triethyl amine generated the isocyanate intermediate, which upon reacting with **6** (R = H)



Scheme 3.

provided urea **11**. The activities of **11** against heparanase are summarized in Table 2.

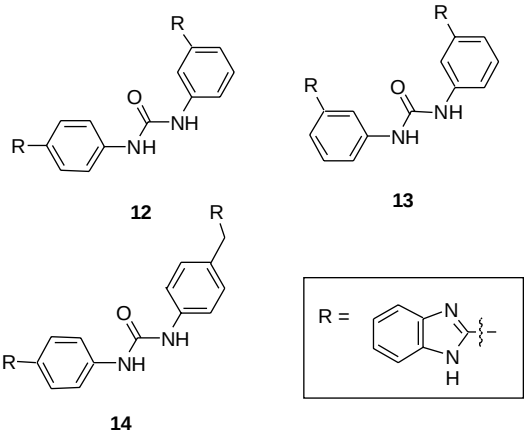
Results from Table 2 indicate that, not surprisingly, the unsymmetrical ureas showed structure–activity relationship (SAR) patterns similar to those of the symmetrical ones, such that methyl substitution (**11a**) improved the heparanase inhibitory activity, and a methoxy substitution (**11b**,**c**) diminished the activity compared to **7a**. In the latter case, again a 4-methoxy (**11c**) is preferred over the 5-methoxy (**11b**). On the other hand, pyridine containing ureas, such as **11e**–**g**, showed decreased heparanase inhibitory activities.

The SAR on the ureas showed a preference for an overall linear, yet 'bent' disposition of the molecule. In order to examine effects of the overall shape and length of the molecule on the heparanase inhibitory activity, several of the more 'bent' molecules, such as **12**–**14** (Scheme 4), were prepared similarly using the synthetic route described in Scheme 3. Consistent with the SAR trends observed in the *N*-(4-{[4-(1*H*-benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamides **1**,[7] the more 'bent' ureas **12**–**14** all have dramatically diminished activities

Table 2. In vitro inhibition of heparanase activity of **11a**–**g**

Compound	A	B	R′	Heparanase percent inhibition at 0.83 μM[a] (%)	Heparanase inhibition IC_{50} (μM)[a]
11a	CH	CH	4-Me		0.15
11b	CH	CH	5-OMe	17	NT[b]
11c	CH	CH	4-OMe		1.15
11d	CH	CH	4-NO_2	20	NT[b]
11e	N	CH	H	17	NT[b]
11f	N	CH	5-OMe	8	NT[b]
11g	CH	N	H		1.0

[a] Average of three experiments with SD < 15%.
[b] Not tested.



Scheme 4.

against heparanase (less than 10% inhibition at 0.83 μM).

Urea **7a** showed less than 50% proliferation inhibition on B16-BL6 melanoma cells in vitro at 100 μM, and hence was selected for further biological evaluation. When dosed intraperitoneally (ip) to mice ($n = 3$) as a suspension in cremophore/5% DMSO/CMC, compound **7a** showed a plasma concentration of 31 μM at 1 h, and 23 μM by 4 h, after dosing. These levels were considerably higher than its IC_{50} of 0.27 μM against heparanase. In the B16 metastasis model[5e] (Fig. 1), compound **7a** was dosed ip to C57 mice at 30 mg/kg as a suspension in cremophore/5% DMSO/CMC. About 30–60 min after the injection of the compound, B16-BL6 melanoma cells (2×10^5/mouse) were injected into the tail vein of the mice. Twenty-one days later, the mice were sacrificed and their lungs were examined for nodules. Results in Figure 1 demonstrate that the group of mice treated with compound **7a** ($n = 10$) showed around 50% inhibition of lung metastasis compared to the vehicle-treated group ($n = 8$) or the untreated group ($n = 10$). This result is comparable to that obtained from PSS (single ip dose of 16 mg/kg, $n = 10$), which is a sulfated poly-

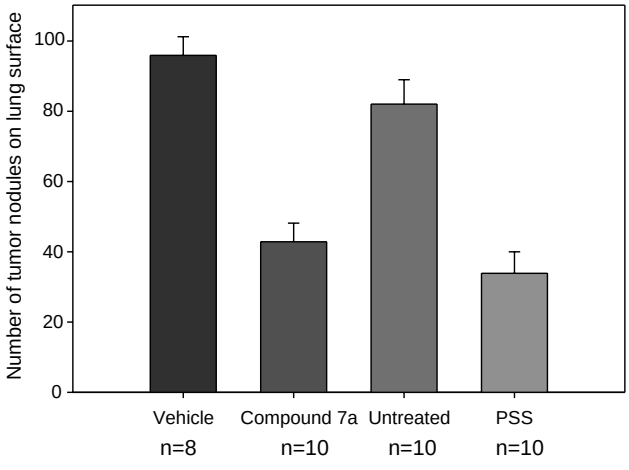


Figure 1.

saccharide heparanase inhibitor with a heparanase inhibitory IC_{50} of 15 nM in the enzymatic assay.

In summary, a novel class of 1-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-3-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-ureas are described as potent inhibitors of heparanase. Among them are 1,3-bis-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-urea (**7a**), and 1,3-bis-[4-(5,6-dimethyl-1*H*-benzoimidazol-2-yl)-phenyl]-urea (**7d**), which displayed good heparanase inhibitory activity (IC_{50} 0.075–0.27 μM), with **7a** showing good efficacy in the B16 metastasis model. Active effort is in progress to further prove the link between the inhibition of heparanase activity and the efficacy observed in the B-16 study, and the results will be reported in due course.

Acknowledgments

We are grateful to Dr. Peter Bohlen for his support and Dr. Marc Labelle for his helpful advice and discussion.

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8. Unpublished results.
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10. Heparanase activity assays: human heparanase protein was purified from human platelets using a modified protocol (Freeman, et al. *Biochem. J. 330*, **1998**, 1341). Heparan sulfate (HS, Seikagaku) was labeled with sodium boro[^{3}H]hydride (Amersham-Pharmacia Biotech). The specific activity was determined as 98.4 cpm/ng HS. The purified 3H-HS was then immobilized on CNBr-activated sepharose beads (Pharmacia) according to manufacturer's instructions. Heparanase activity was determined using 96-well plates. Human platelet heparanase (2.67 nM) was pre-mixed with a compound of the present study (various concentrations for IC_{50} studies) in a total volume of 125 μl. ^{3}H-HS-sepharose slurry (25 μl, 4 nM) was then added into the mixture and incubated overnight at 37 °C. The reaction buffer was transferred to 96-well Luma plates (Perkin-Elmer). The plates were air-dried and the radioactivity was directly detected in a TopCounter (Perkin-Elmer).