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N-(4-{[4-(1*H*-Benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamide derivatives as small molecule heparanase inhibitors

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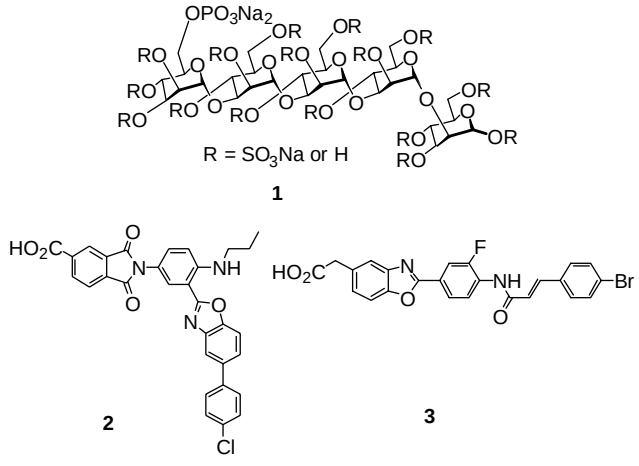
Abstract—A novel class of *N*-(4-{[4-(1*H*-benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamides are described as inhibitors of the endo-β-glucuronidase heparanase. Among them are *N*-(4-{[4-(1*H*-benzoimidazol-2-yl)-phenylamino]-methyl}-phenyl)-3-bromo-4-methoxy-benzamide (**15h**), and *N*-(4-{[5-(1*H*-benzoimidazol-2-yl)-pyridin-2-ylamino]-methyl}- phenyl)-3-bromo-4-methoxy-benzamide (**23**) which displayed good heparanase inhibitory activity (IC_{50} 0.23–0.29 μM), with the latter showing oral exposure in mice.

Heparanase, an endo-β-D-glucuronidase that degrades heparan sulfate glycosaminoglycans in the extracellular matrix (ECM) and the basement membrane, is involved in tumor cell invasion, angiogenesis, and other physiological and pathological processes.[1] Experimental tumor cell metastasis in an animal model is reduced by treatment with heparanase inhibitors.[2] Different from the large number of proteases known to disassemble the ECM,[3] 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 glycosaminoglycans in mammalian tissues,[1b,4] and made heparanase a promising target for the development of antitumor agents, especially antimetastatic agents. Most of the heparanase inhibitors known are sulfated oligosaccharide derivatives that resemble the substrate heparan sulfate glycosaminoglycans.[2,5] The most advanced of them is PI-88, a highly sulfated pentasaccharide (**1**, Scheme 1), which is currently in Phase II clinical trials.[2] As a heparan sulfate analogue, PI-88 has heterogeneous structure and multiple mechanisms of action. Laminarin sulfate and synthetic phosphorothioate oligodeoxynucleotides,[6] suramin and its analogs,[7] benzoic acid analogs,[8] sulfated iminosugars,[9] azapseudodisaccharides,[10] and natural products such as trachyspic acid and its derivatives[11] also inhibit heparanase with good to modest potency.

Recently, small molecules such as 2,3-dihydro-1,3-dioxo-1*H*-isoindole-5-carboxylic acids (**2**)[12] and benzoxazol-5-yl acetic acids (**3**)[13] have been reported to be



Scheme 1.

Keywords: Heparanase inhibitor; Benzimidazole.
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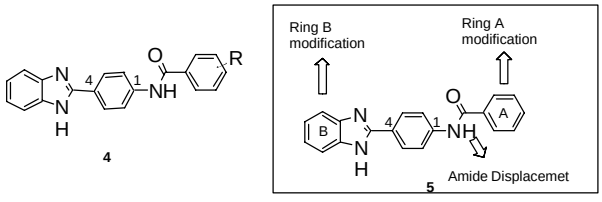
potent heparanase inhibitors. The best compounds from these series have IC_{50}s (compound concentrations that cause 50% inhibition of the enzyme activity) of around 200 nM. These reports prompted us to reveal in this and the following letter our effort aimed at the identification of 'proof-of-concept' small molecule heparanase inhibitors with efficacy in animal models.

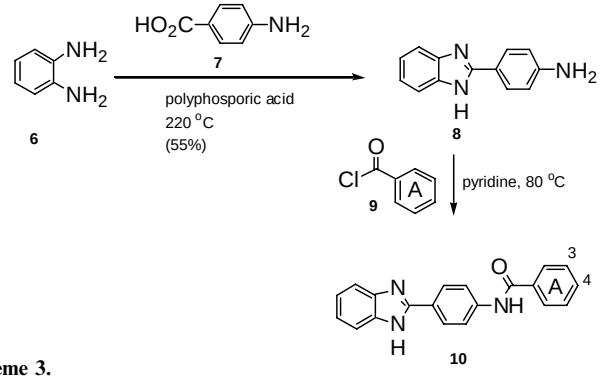
From the hits identified by high throughput screening, **4** (Scheme 2) was selected as the structure–activity relationship (SAR) template mainly based on synthetic feasibility. Early SAR investigation demonstrated that a 1,3-disposition of the benzamide moiety and the pendant benzimidazole ring system in **4** resulted in reduced inhibiting activity. Furthermore, the central phenyl ring in **4** tolerates little change in terms of substitution. Hence, more detailed SAR strategies were devised (**5**, Scheme 2) focusing on: (a) modifications of the aromatic ring A and ring B mainly through substitution changes; and (b) modification of the amide linkage. In most cases, the 1,4-substituted central phenyl ring was kept intact.

In order to examine the effect of modification of ring A on heparanase inhibitory activity, a typical procedure was developed for the preparation of the *N*-[4-(1*H*-benzoimidazol-2-yl)-phenyl]-amides **10** (Scheme 3). Heating the phenyl diamine **6** with 4-amino-benzoic acid **7** in polyphosphoric acid at 220 °C for 4 h afforded 4-(1*H*-benzoimidazol-2-yl)-phenylamine **8** in 55% yield. In the second step, **8** was allowed to react with a substituted benzoyl chloride in pyridine at 80 °C overnight to produce **10**,[14] which was tested for heparanase inhibitory activity in a radio-labeled enzymatic assay[15] (Table 1).

Early SAR investigation on ring A indicated that a 3,4-disubstitution pattern on ring A of **10** seemed to be preferred for heparanase inhibiting activity. Furthermore,

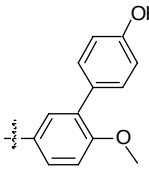


Scheme 2.



Scheme 3.

Table 1. In vitro inhibition of heparanase activity of **10a–h**

Compound	Ring A	Heparanase inhibition IC_{50} (μM)[a]
10a	3-Fluoro-4-methoxy	>15
10b	3-Chloro-4-methoxy	>15
10c	3-Bromo-4-methoxy	4.0
10d	3-Bromo-4-ethoxy	1.2
10e	3-Bromo-4-*n*-propoxy	2.2
10f		11
10g	3-(3′-Pyridyl)-4-methoxy	5.7
10h	3-(1*H*-Indazol-5-yl)-4-methoxy	6.1

[a] Average of three experiments with SD < 15%.

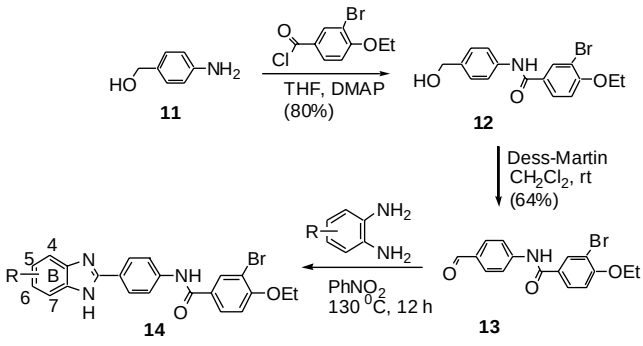
the more preferred substituents for ring A are 3-bromine-4-alkoxy. When the 3-bromine in **10c** was replaced with either fluorine (**10a**) or chlorine (**10b**), the activity against heparanase decreased dramatically. On the other hand, the 4-alkoxy moiety of ring A tolerates more changes, with the 4-methoxy (**10c**), 4-propoxy (**10e**) giving comparable results, and the 4-ethoxy (**10d**) a slightly better IC_{50} of 1.2 μM. This result indicated potential room for extension to the right-hand side of the molecule. We also found that the 3-bromine of compound **10c** could be replaced with certain heteroaryl moieties via Suzuki coupling with boronic acids (**10f–h**), and the activity against heparanase was partially retained.

The results in Table 1 led to the selection of **10d** as a template for further optimization of ring B (Table 2). A convergent synthesis of compounds **14** was developed to allow modification of ring B in the last step of the synthesis (Scheme 4). First, 4-aminobenzyl alcohol **11** was reacted with 3-bromo-4-ethoxy-benzoyl chloride to form alcohol **12**. A Dess Martin oxidation of **12** yielded aldehyde **13**, which was allowed to condense with a substitut-

Table 2. In vitro inhibition of heparanase activity of **14a–i**

Compound	R	Heparanase percent inhibition at 25 μM[a]	Heparanase inhibition IC_{50} (μM)[a]
10d	H		1.2
14a	5-OMe	73	NT[b]
14b	5-OMe 6-OMe	35	NT[b]
14c	5-Cl 6-Cl	30	NT[b]
14d	5-Cl 6-Me	30	NT[b]
14e	4-Me		2.3
14f	5-Me		1.6
14g	4-Me 5-Me		1.3
14h	5-Me 6-Me		1.1
14i	4-Me 7-Me		2.7

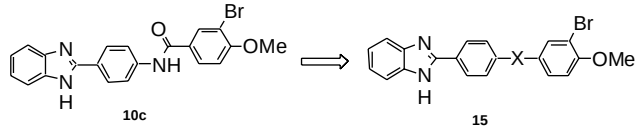
[a] Average of three experiments with SD < 15%.
[b] Not tested.



Scheme 4.

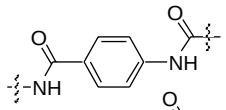
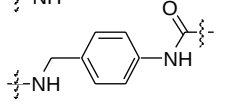
ed phenyl diamine, followed by spontaneous oxidation in nitrobenzene to yield **14** in good yields. Results in Table 2 revealed that methoxy (**14a**, **14b)**, or chlorine substituents on ring B (**14c**) resulted in a dramatically decreased activity against heparanase when compared to **10d**. However, substitution of ring B with a methyl (**14e**, **14f)**, or a dimethyl (**14g**–**i)**, largely retained the activity.

When dosed ip to mice ($n = 3$) at 50 mg/kg, **10d** showed a plasma concentration of 0.53 μM at 1 h and dropped down to 0.38 μM by 4 h. Better exposure was observed for the methoxy derivative **10c** (plasma concentration 2.24 μM at 1 h and 0.67 μM by 4 h after ip dosing). The observed sub-optimal plasma exposures, combined with their modest activity against heparanase (IC_{50} of 1.2 and 4.0 μM, respectively), excluded **10d**,**c** as 'proof-of-concept' candidates for evaluation in animal models. Nevertheless, these results prompted us to study the effect of replacing the amide linkage of **10c** with the aim of improving anti-heparanase activity and pharmacokinetic properties (Scheme 5 and Table 3).



Scheme 5.

Table 3. In vitro inhibition of heparanase activity of **15a**–**h**

Compound	X	Heparanase inhibition IC_{50} (μM)[a]
10c	–NHCO–	4.0
15a	–N(Me)CO–	>15
15b	–CONH–	>15
15c	$–NHSO_2–$	>15
15d	$–NHCH_2–$	>15
15e	$–NHCOCH_2–$	>15
15f	–NHCONH–	0.93
15g		0.91
15h		0.23

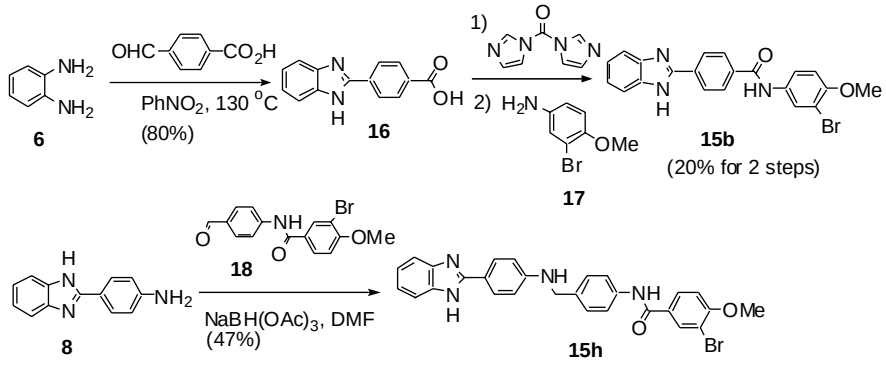
[a] Average of three experiments with SD < 15%.

Compound **15a** was obtained by treating **10c** with sodium hydride, followed by methylation with methyl iodide at room temperature. Compounds **15c**, **15d**, and **15f** were synthesized from **8** via standard sulfonamide formation with the corresponding sulfonyl chloride, reductive amination with the corresponding aldehyde using $NaBH(OAc)_3$ in dichloroethane, and urea formation with an isocyanate. Compounds **15e**,**g** were prepared from **8** via standard amide formation with the corresponding acid chlorides. Compounds **15b** and **15h** were prepared as shown in Scheme 6. The phenyl diamine was first reacted with 4-formylbenzoic acid in nitrobenzene to provide acid **16**. Reacting **16** with CDI in DMF, followed by treatment with aniline **17**, provided **15b**. Reacting **8** with aldehyde **18**, which was prepared similarly as **13** (Scheme 4), under standard reductive amination condition provided **15h**.

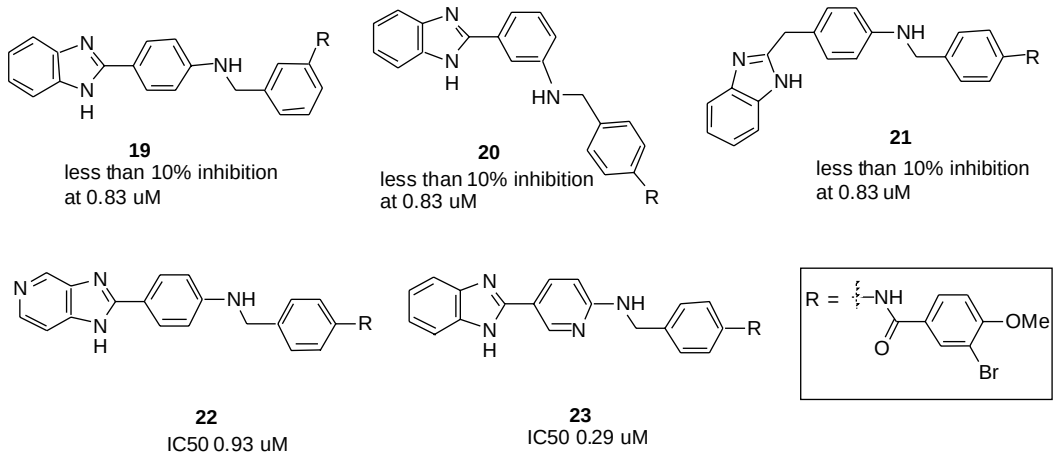
Results from Table 3 revealed that compound **15a**, the N methylated product of **10c**, has a dramatically decreased activity against heparanase compared to **10c**. Replacement of the amide in **10c** with a 'reversed amide' (**15b**), or a sulfonamide (**15c**), or an amino methyl linkage (**15d**) all diminished the heparanase inhibitory activity. Extending the amide linkage in **10c** with an extra methylene unit was also met with disappointment (**15e**). However, a urea replacement of the amide (**15f**) improved the heparanase inhibitory activity by about 3-fold, and a similar improvement of activity was observed for **15g**. Remarkably, the more flexible compound **15h** has an IC_{50} of 0.23 μM against heparanase.

Compound **15h** was then selected as a template for further modification, and the representative results from this study are shown in Scheme 7. Compounds **19**–**23** were prepared similarly as **15h** (Scheme 6) by using the corresponding amines or aldehydes. Results in Scheme 7 indicated that a overall linear, yet slightly 'bent,' disposition of the molecule is important for activity against heparanase, as the more 'bent' molecules, such as **19** and **20**, have diminished activities. Similar reasons may have also caused the loss of activity for compound **21**, which has an additional methylene unit inserted between the benzimidazole and the central phenyl ring. Replacement of the phenyl group to the left hand side of the molecule led to **22** that had an IC_{50} of 0.93 μM against heparanase. Remarkably, the central phenyl group could be replaced with a pyridine moiety and the resultant **23** had an IC_{50} of 0.29 μM. When dosed p.o. to mice ($n = 3$) as a suspension in ethanol/Tween 80/PEG 400 (E/T/P) at 30 mg/kg, compound **23** showed a plasma concentration of 3.8 μM at 1 h, going up to 5.5 μM by 4 h. Interestingly, changing formulation or dosing **23** intraperitoneally did not lead to improved plasma exposure.

In summary, a novel class of *N*-(4-{[4-(1*H*-benzoimidazol-2-yl)-arylamino]-methyl}-phenyl)-benzamides are described as heparanase inhibitors. Among them are **15h** and **23** which displayed good heparanase inhibitory activity (IC_{50} 0.23–0.29 μM), with the latter showing oral



Scheme 6.



Scheme 7.

exposure in mice. Furthermore, the SAR information gathered during this study provided useful insights into the design of potent small molecule heparanase inhibitors suitable for animal model evaluation (see following article).

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References and notes

1. (a) Vlodavsky, I.; Freidmann, Y.; Elkin, M.; Aingorn, H.; Atzmon, R.; Ishai-Michaeli, R.; Bitan, M.; Pappo, O.; Peretz, T.; Michal, I.; Spector, L.; Pecker, I. *Nat. Med.* **1999**, *5*, 793; (b) Hulett, M. D.; Freeman, C.; Hamdorf, B. J.; Baker, R. T.; Harris, M. J.; Parish, C. R. *Nat. Med.* **1999**, *5*, 803; (c) Fairbanks, M. B.; Mildner, A. M.; Leone, J. W.; Cavey, G. S.; Mathews, W. R.; Drong, R. F.; Slightom, J. L.; Bienkowski, M. J.; Smith, C. W.; Bannow, C. A.; Heinrikson, R. L. *J. Biol. Chem.* **1999**, *274*, 29587; (d) Parish, C. R.; Freeman, C.; Hulett, M. D. *Biochim. Biophys. Acta* **2001**, *1471*, M99.
2. Parish, C. R.; Freeman, C.; Brown, K. J.; Francis, D. J.; Cowden, W. B. *Cancer Res.* **1999**, *59*, 3433.
3. Duffy, M. J. *Clin. Cancer Res.* **1996**, *2*, 613.
4. Kussie, P. H.; Hulmes, J. D.; Ludwig, D. L.; Patel, S.; Navarro, E. C.; Seddon, A. P.; Giorgio, N. A.; Bohlen, P. *Biochem. Biophys. Res. Commun.* **1999**, *61*, 183.
5. (a) Irimura, T.; Nakajima, M.; Nicolson, G. L. *Biochem.* **1986**, *25*, 5322; (b) Saiki, I.; Murata, J.; Nakajima, M.; Tokura, S.; Azuma, I. *Cancer Res.* **1990**, *50*, 3631.
6. Miao, H. Q.; Elkin, M.; Aingorn, E.; Ishai-Michaeli, R.; Stein, C. A.; Vlodavsky, I. *Int. J. Cancer* **1999**, *83*(3), 424.
7. (a) Marchetti, D.; Reiland, J.; Erwin, B.; Roy, M. *Int. J. Cancer* **2003**, *104*(2), 167; (b) Nakajima, M.; DeChavigny, A.; Johnson, C. E.; Hamada, J.; Stein, C. A.; Nicolson, G. L. *J. Biol. Chem.* **1991**, *266*(15), 9661.
8. Ishida, K.; Wierzba, M. K.; Teruya, T.; Simizu, S.; Osada, H. *Chem. Biol.* **2004**, *11*, 367.
9. (a) Kawase, Y.; Takahashi, M.; Takatsu, T.; Arai, M.; Nakajima, M.; Tanzawa, K. *J. Antibiot.* **1996**, *9*, 1; (b) Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem.* **2000**, *65*, 2.
10. Takahashi, S.; Kuzuhara, H.; Nakajima, M. *Tetrahedron* **2001**, *57*, 6915.
11. (a) Shiozawa, H.; Takahashi, M.; Takatsu, T.; Kinoshita, T.; Tanzawa, K.; Hosoya, T.; Furuya, K.; Takahashi, S.; Furihata, K.; Seto, H. *J. Antibiot.* **1995**, *48*, 357; (b) Ishida, K.; Hirai, G.; Murakami, K.; Teruya, T.; Simizu, S.; Sodeoka, M.; Osada, H. *Mol. Cancer Ther.* **2004**, *3*(9), 1069.
12. Courtney, S. M.; Hay, P. A.; Buck, R. T.; Colville, C. S.; Porter, D. W.; Scopes, D. C.; Pollard, F. C.; Page, M. J.; Bennett, J. M.; Hircock, M. L.; McKenzie, E. A.;

Stubberfield, C. R.; Turner, P. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3269.
13. Courtney, S. M.; Hay, P. A.; Buck, R. T.; Colville, C. S.; Phillips, D. J.; Scopes, D. C.; Pollard, F. C.; Page, M. J.; Bennett, J. M.; Hircock, M. L.; McKenzie, E. A.; Bhamanb, M.; Felix, R.; Stubberfield, C. R.; Turner, P. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2295.
14. All synthetic intermediates were characterized by ^{1}H NMR and MS; all final products were characterized by ^{1}H NMR and LCMS.
15. Heparanase activity assays: human heparanase protein was purified from human platelets using a modified protocol (Freeman et al., *Biochem. J.* **1998**, *330*, 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 ^{3}H-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).