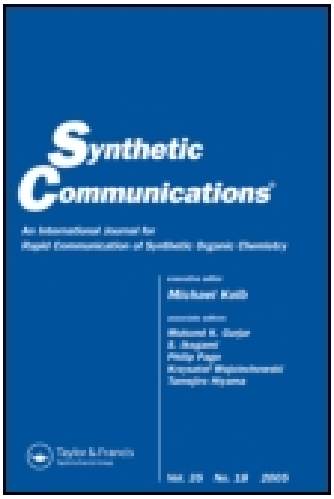

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Synthetic Route to a Benzooxazole Derivative with Heparanase Inhibitory Activity

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SYNTHETIC ROUTE TO A BENZOOXAZOLE DERIVATIVE WITH HEPARANASE INHIBITORY ACTIVITY

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The synthesis of a benzooxazol-5-yl acetic acid derivative (9) with strong heparanase and angiogenesis inhibitory activity, and thus possible commercial interest, is described in detail.

Keywords: Angiogenesis; benzooxazole; heparanase; inhibitor; metastasis

INTRODUCTION

Heparanase is an enzyme implicated in several physiological and pathological processes including tumor angiogenesis and metastasis.[1–3] Few compounds with heparanase inhibitory activity have been reported in the literature,[4–6] and most of these are sugar derivatives, which have modest activity. Compound **9**, however, is a small molecule (Fig. 1) that shows potent and selective heparanase, as well as angiogenic, inhibitory activities.[7]

Despite its biological activity, no detailed synthesis or data for **9** are available. Herewith, we report an efficient protocol for the synthesis of **9**.

The seven steps of our synthesis are outlined in Scheme 1. 4-Hydroxy-3-nitrophenylacetic acid was first protected as its methyl ester in 90% yield in two steps according to a published procedure, and the nitro group of **1** was then reduced to obtain the amino derivative **2**.[8] Compound **2** was then coupled to 3-fluoro-4-nitro benzoyl chloride (**3**). Refluxing the amide **4** with *p*-toluenesulfonic acid (*p*-TsOH) in toluene[9] afforded the ring-closed product **5** in 60% yield. Reduction of the nitro group in **5** was accomplished using standard catalytic hydrogenation conditions. When a scale larger than 300 mg of the starting material **5** was used, the yield of the product **6** was in fact improved from $<50\%$ to quantitative using H_2Pd/C instead the Zn/acetic acid procedure suggested in literature.[7] Compound **6** was coupled to 4-bromocinnamoyl chloride (**7**) in CH_2Cl_2. The product **8** was carefully purified, after washing with tetrahydrofuran (THF) water, by chromatography on silica gel starting with CH_2Cl_2 as eluent and then gradually increasing the polarity to 50:1 CH_2Cl_2–methanol. Because of the limited solubility of **8** in most organic solvents, hydrolysis

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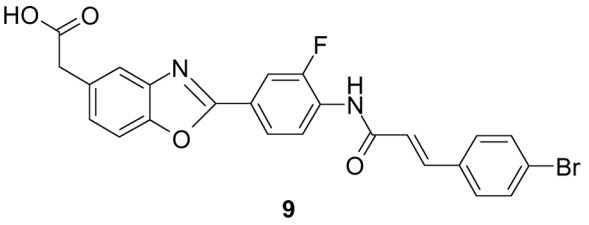
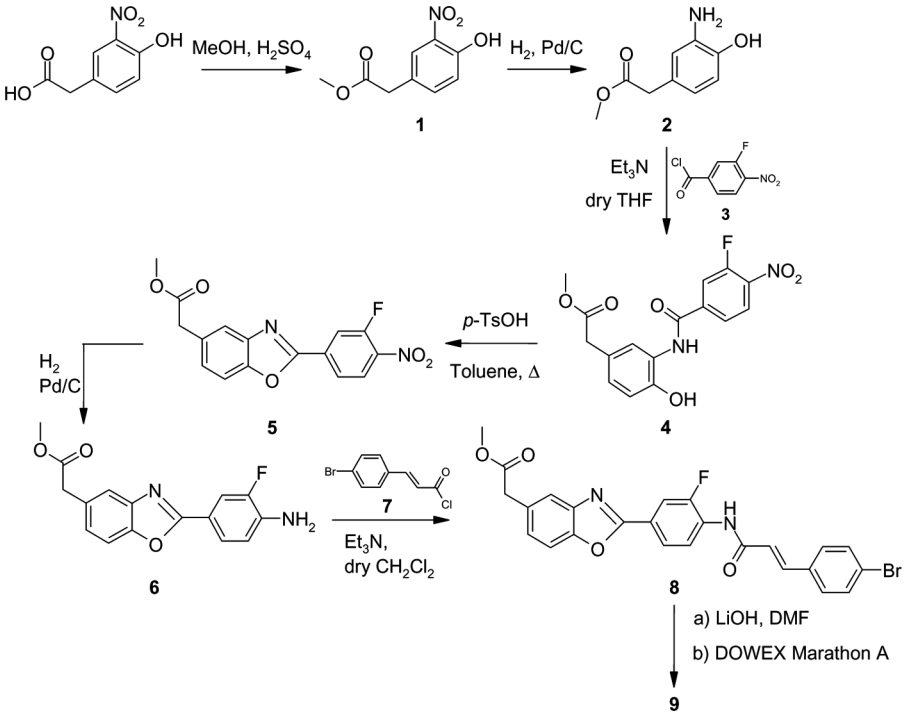


Figure 1. (2-{4-[(E)-3-(4-Bromophenyl)acryloylamino]-3-fluoro-phenyl}benzooxazol-5-yl) acetic acid.



Scheme 1. Synthesis of (2-{4-[(E)-3-(4-bromophenyl)acryloylamino]-3-fluoro-phenyl}benzooxazol-5-yl) acetic acid (**9**).

of the methyl ester was performed using LiOH in *N,N*-dimethylformamide. The final product (**9**) was obtained as the free benzooxazolyl acetic acid and purified by cation exchange column chromatography using a mixture of methanol and formic acid as eluent. Other similar compounds, having different functional groups, can be obtained by the same synthetic route.

EXPERIMENTAL

All chemicals were purchased from Sigma Aldrich (Gillingham, Dorset, UK) and used without further purification. Thionyl chloride was distilled before use.

4-Hydroxy-3-nitrophenylacetic acid, 3-fluoro-4-nitrobenzoic acid, and 4-bromocinnammic acid were purchased from Apollo Scientific Limited (Stockport, UK). 4-Bromocinnamoyl chloride (**7**) and compounds **1** and **2** were synthesised according to literature.[8,10] Thin-layer chromatography (TLC) was carried out on 0.25-mm silica gel on polyethylene terephthalate (PET) foils (Fluka) with a fluorescent indicator visualized using ultraviolet (UV) light or Ce-P-Mo staining solution. Column chromatography was performed on silica gel (particle size 0.035–0.070 mm). Anion exchange chromatography was performed on Dowex Marathon A (Sigma Aldrich). NMR spectra were recorded on Jeol 300-MHz or Jeol 400-MHz spectrometers. The chemical shifts are relative to residual solvent protons as reference. Electrospray ionization (ESI) mass spectra (MS) were recorded on a VG Quattro spectrometer (Fisons Instruments). Elemental analyses were performed by Medac Ltd. (Egham, UK).

Methyl [3-(3-Fluoro-4-nitro benzoylamino)-4-hydroxy-phenyl] Acetate (4)

3-Fluoro-4-nitrobenzoic acid (2.19 g, 9.93 mmol) was suspended in dry CH_2Cl_2 (20 ml). $SOCl_2$ (2 ml) was added, followed by few drops of dimethylformamide (DMF). The mixture was refluxed overnight under N_2. The solvent was removed under vacuum, and the product (**3**) was used without further purification. Compound **2** (1.8 g, 9.93 mmol) and an equimolar quantity of **3** were dissolved in dry CH_2Cl_2 (45 ml) containing triethylamine (3.5 ml). The solution was stirred under N_2 overnight at room temperature and then washed with HCl 1 M (3×25 ml). The organic phase was dried ($MgSO_4$) and evaporated under vacuum. The product was recrystallized from CH_2Cl_2/methanol (100:1) as yellow needles. Yield 70%. R_f 0.23 (100:1 CH_2Cl_2/methanol). ^{1}H NMR (300 MHz, DMSO) δ 3.57 (s, 2H, CH_2), 3.59 (s, 3H, CH_3), 6.86 (d, 1H, aromatic, $J = 8.25$), 6.96 (dd, 1H, aromatic, $J = 8.25$, 2.01), 7.46 (d, 1H, aromatic, $J = 2.01$), 7.94–8.31 (m, 3H, aromatic), 9.80 (bs, NH + OH). ^{13}C (75 MHz, DMSO) δ 39.38, 51.63, 115.86, 117.67 (d, $J = 22.30$), 124.36 (d, $J = 4.37$), 124.60, 124.70, 126.20, 126.55 (d, $J = 2.41$), 127.54, 138.40 (d, $J = 7.39$), 141.44 (d, $J = 7.47$), 149.26, 154.29 (d, $J = 261$), 162.47, 171.89. MS (ESI): m/z (%) = 347 (100) $[M]^+$. Anal. calcd. for $C_{16}H_{13}FN_2O_6$: C, 55.18; H, 3.76; N, 8.04. Found: C, 55.03; H, 3.77; N, 7.88.

Methyl [2-(3-Fluoro-4-nitrophenyl)benzooxazol-5-yl] Acetate (5)

Compound **4** (2.3 g, 6.60 mmol) was added to a solution of *p*-toluenesulfonic acid monohydrate (0.95 g, 5 mmol) in dry toluene (200 ml). The mixture was refluxed overnight using a Dean–Stark apparatus. The solvent was removed under vacuum, and the product was purified by chromatography on silica gel. Elution was started with 100% hexane, and the polarity of the eluent was increased gradually to 3:1 hexane/ethyl acetate. Yield: 60%. $R_f = 0.64$ (3:1 hexane/ethyl acetate). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.65 (s, 3H, CH_3), 3.71 (s, 2H, CH_2), 7.31 (dd, 1H, aromatic, $J = 8.43$, 1.65), 7.51 (d, 1H, aromatic, $J = 8.43$), 7.66 (d, 1H, aromatic, $J = 1.65$), 8.08–8.17 (m, 3H, aromatic). ^{13}C (75 MHz, $CDCl_3$) δ 40.97, 52.21, 110.90, 117.31 (d, $J = 23.61$), 121.46, 123.27 (d, $J = 4.37$), 126.95 (d, $J = 2.49$), 128.06, 131.53, 133.86 (d, $J = 8.67$),

138.64 (d, $J = 9.88$), 142.08, 152.07 (d, $J = 278$), 157.43, 159.84, 171.75. MS (ESI): m/z (%) = 331 (100) $[M + H]^+$, 371 (37) $[M + H + K]^+$. Anal. calcd. for $C_{16}H_{11}FN_2O_5 \cdot 0.2H_2O$: C, 57.54; H, 3.44; N, 8.39. Found: C, 57.54; H, 3.48; N, 8.40.

Methyl [2-(4-Amino-3-fluorophenyl)benzooxazol-5-yl] Acetate (6)

Compound **5** (1.2 g, 3.63 mmol) was dissolved in ethyl acetate (20 ml), then 10% Pd/C (30 mg) was added and H_2 was bubbled through the stirred mixture for 5 h. The catalyst was removed by filtration, and the filtrate was evaporated under vacuum. Yield quantitative. R_f 0.55 (3:2 hexane/ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.07 (s, 3H, CH_3), 3.13 (s, 2H, CH_2), 4.79 (bs, NH_2), 6.27–7.24 (m, 6H, aromatic). ^{13}C (75 MHz, DMSO) δ 40.48, 51.53, 109.65, 113.73 (d, $J = 21.12$), 114.16, 115.59 (d, $J = 4.90$), 119.40, 124.21 (d, $J = 1.81$), 125.26, 130.07, 139.69 (d, $J = 13.05$), 142.13, 149.15, 149.98 (d, $J = 238.27$), 162.94 (d, $J = 3.16$), 171.39. MS (ESI): m/z (%) = 301 (100) $[M + H]^+$. Anal. calcd. for $C_{16}H_{13}FN_2O_3 \cdot H_2O$: C, 60.38; H, 4.75; N, 8.80. Found: C, 60.25; H, 4.52; N, 9.04.

Methyl (2-{4-[(E)-3-(4-Bromo-phenyl)acryloylamino]-3-fluorophenyl}benzooxazol-5-yl) Acetate (8)

A solution of **7** (817 mg, 3.33 mmol) in 10 ml of dry CH_2Cl_2 was added dropwise to a solution of **6** (1 g, 3.33 mmol) in dry CH_2Cl_2 (30 ml) and Et_3N (1 ml). The mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the residue was washed with THF/water (1:1). Product **8** was further purified by chromatography on silica gel starting with CH_2Cl_2 as eluent and then gradually increasing the polarity to 50:1 CH_2Cl_2/methanol. Yield 90%. R_f 0.31 (100:1 CH_2Cl_2/methanol). ^{1}H NMR (400 MHz, DMSO) δ 3.62 (s, 3H, CH_3), 3.83 (s, 2H, CH_2), 7.15 (s, 1H, CH), 7.20 (s, 1H, CH), 7.30–8.53 (m, 10H, aromatic), 10.24 (s, 1H, NHCO). ^{13}C (75 MHz, DMSO) δ 40.33, 51.70, 110.49, 114.03 (d, $J = 21.73$), 120.45, 122.17 (d, $J = 8.67$), 122.44, 122.81, 123.22, 123.84 (d, $J = 3.77$), 127.01, 129.74, 129.99 (d, $J = 11.16$), 131.36, 131.99, 133.88, 140.05, 141.58, 149.23, 152.39 (d, $J = 246.34$), 161.44 (d, $J = 2.49$), 164.00, 171.64. MS (ESI): m/z (%) = 507 (100) $[M]^+$. Anal. calcd. for $C_{25}H_{18}BrFN_2O_4$: C, 58.95; H, 3.56; N, 5.50. Found: C, 58.24; H, 3.56; N, 5.39.

(2-{4-[(E)-3-(4-Bromophenyl)acryloylamino]-3-fluorophenyl}benzooxazol-5-yl) Acetic Acid (9)

LiOH monohydrate (0.25 g, 5.9 mmol) was added to a solution of **8** (1 g, 1.96 mmol) in DMF (40 ml) and stirred overnight at room temperature. The solvent was removed under vacuum, and the residue was purified by ion-exchange chromatography over Dowex Marathon A previously washed with water and then methanol. The product was eluted with methanol/formic acid 3 M (8:2) and recrystallized from methanol.

Yield 28.3%. ^{1}H NMR (300 MHz, DMSO) δ 3.71 (s, 2H, CH_2), 7.08 (s, 1H, CH), 7.15 (s, 1H, CH), 7.20–8.53 (m, 10H, aromatic), 10.24 (s, 1H, NHCO), 12.40 (bs, COOH). ^{13}C (75 MHz, DMSO) δ 40.30, 110.38, 114.03 (d, $J = 21.73$), 120.46,

122.19 (d, $J = 7.99$), 122.46, 122.80, 123.26, 123.84 (d, $J = 2.41$), 127.11, 129.76, 129.99 (d, $J = 11.16$), 132.01, 132.07, 133.89, 140.07, 141.53, 149.13, 152.39 (d, $J = 245.74$), 161.36 (d, $J = 3.09$), 164.02, 172.76. MS (ESI): m/z (%) = 493 (100) $[M]^+$. Anal. calcd. for $C_{24}H_{16}BrFN_2O_4 \cdot 1.8H_2O$: C, 54.62; H, 3.74; N, 5.31. Found: C, 54.62; H, 3.74; N, 5.10.

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