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Solid-Phase Synthesis of Endothelin Receptor Antagonists

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Abstract—A new solid-phase synthesis for ET receptor antagonists suitable for automation is presented. A support bound 2-hydroxybutyric acid derivative was converted to the corresponding ether derivatives using 4-halo-2-methylsulfonylpyrimidines. Subsequent Suzuki coupling with various aryl boronic acids gave the desired antagonists in good yields and purities. Highly potent antagonists with excellent selectivity for ET_A were obtained.
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The endothelins (ET-1, ET-2, ET-3)[1] are 21 amino acid peptides which constitute a family of highly potent endogenous vasoactive agents acting as modulators of the vascular tone, cell proliferation, and hormone production.[2] Their actions are mediated by the ET_A and the ET_B receptors, which are seven transmembrane domain receptors that couple to different intracellular signalling pathways via heterotrimeric G proteins.[3] The ET-1 selective ET_A receptor is mainly expressed on vascular smooth muscle cells and causes vasoconstriction.[4] On the other hand, the ET_B receptor on endothelial cells causes vasodilatation through the production of endothelium-derived relaxing factors (EDRF). The ET_B receptor is also expressed on vascular smooth muscle cells and mediates, like the ET_A receptor, vasoconstriction.[5] However, the roles of the ET_B receptor have not been fully characterized.

Due to the diversity of physiological effects elicited by the endothelins and because elevated levels of ET-1 have been found in a number of disease states, ET is considered to be relevant in the pathogenesis of several diseases such as myocardial infarction,[6] hypertension,[7] congestive heart failure,[8] atherosclerosis,[9] cerebral vasospasm,[10] renal failure,[11] prostate hyperplasia,[12] and prostate cancer.[13] With regard to the different localizations and functions of ET receptor subtypes, it might be beneficial to block specifically only one receptor or both receptors at the same time. Since it remains unclear which type of antagonists is more suitable for clinical purposes, a number of non-peptide endothelin receptor antagonists including ET_A selective,[14] ET_B selective[15] and mixed ET_A/ET_B[16] receptor antagonists have been published to date.

As part of our work on ET_A receptor antagonists[17] and mixed ET_A/ET_B receptor antagonists[18] we report in the present article our efforts towards potent ET_A receptor antagonists with increased selectivity. ET receptor antagonists with the core structure depicted in Figure 1 exhibited good selectivity for the ET_A receptor, in particular compounds with a methyl group as R^4-moiety. We envisaged that systematic modification of the substituents R^1 to R^3 might lead to ET_A receptor antagonists with enhanced selectivity.

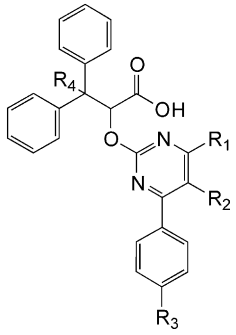

Figure 1. Class of ET_A receptor antagonists with good selectivity.

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Chemistry

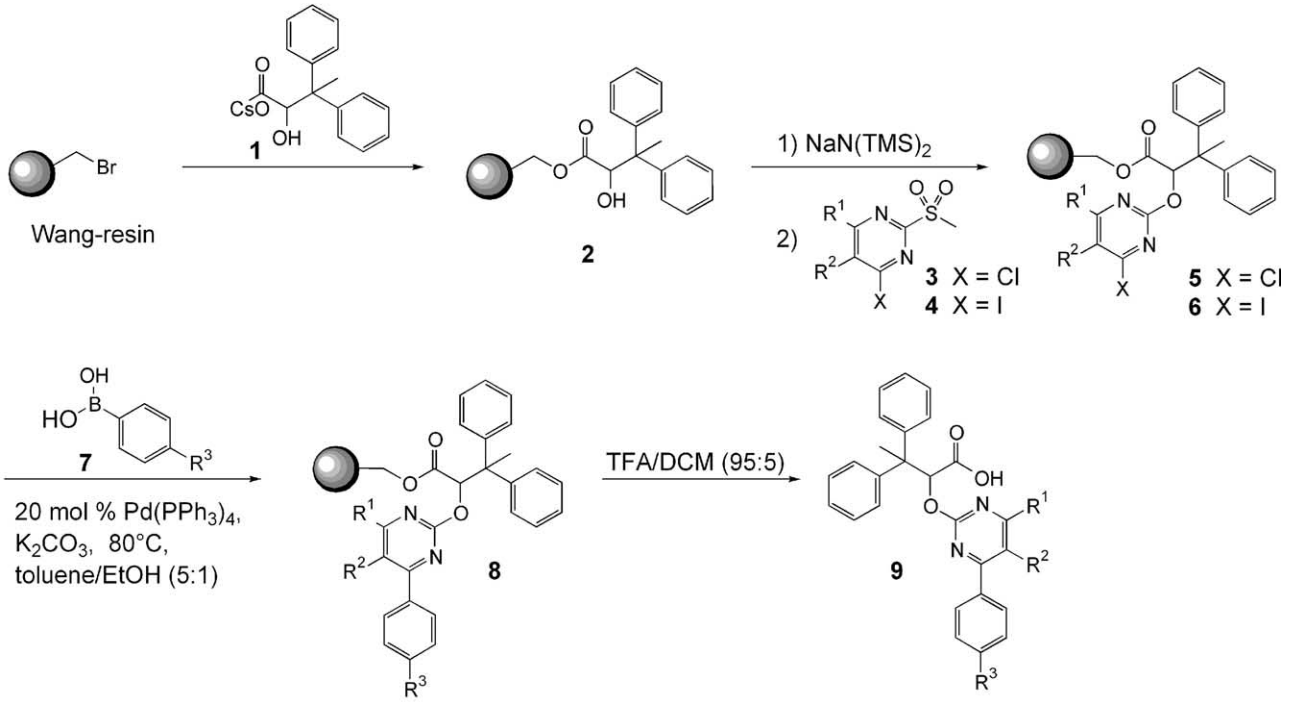
Initial attempts to synthesize the desired ET receptor antagonists in solution starting from 2-hydroxy-3,3-diphenyl-butyric acid and 2-methylsulfonylpyrimidines **3** or **4** using sodium hydride as base gave the corresponding ethers in less than 10% yield. Alternatively, protection of **1** as benzyl ester and subsequent reaction with pyrimidine building blocks **3** or **4** under basic conditions led to mixtures of mono- and bisether derivatives by substitution of both the methylsulfonyl group and halide (X = Cl, I). The yields of the etherification were typically in the range of 10–40% depending on reaction conditions with the undesired bisether as the main product.

We therefore developed the solid-phase synthesis depicted in Scheme 1, which avoided the problems encountered in the solution phase approach.

In the first step, Wang bromide resin was reacted with the cesium salt of *rac*-2-hydroxy-3,3-diphenyl-butyric acid (**1**). The thus protected α-hydroxy carboxylic acid[19] was deprotonated with a solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran which in an automated synthesis can be handled more conveniently than the often used sodium hydride suspension.[20] The alcoholate of **2** cleanly underwent the desired etherification when treated with excess pyrimidine **3** or **4**,[21] respectively. The bisetherification of the pyrimidine building blocks which gave rise to difficult to remove by-products in solution chemistry, was negligible in the solid-phase approach due to the pseudo-dilution effect.[22] As expected no substitution of the halide versus the methylsulfonyl group was observed. To achieve complete conversion of **2** to the ether derivatives **5** or **6**, respectively, the above sequence was repeated once.

For the subsequent Suzuki coupling a set of different palladium catalysts, solvents and bases was screened using 4-ethoxyphenyl boronic acid and support bound chloride **5a** or iodide **6a**, respectively, as substrates. Best results in terms of purity and conversion of starting material were obtained with tetrakis(triphenylphosphine) palladium, aqueous potassium carbonate and a mixture of dimethyl formamide/water or toluene/ethanol. Replacement of the carbonate base by triethylamine had a negative effect on the reaction. Hardly any difference in reactivity between the chloride **5a** and the iodide **6a** was observed in the coupling reaction when 20 mol% of the palladium catalyst were used. However, some iodides **6** were sensitive to hydrolysis leading to trace amounts of the corresponding by-product (X = OH) of the Suzuki coupling. Reaction with other phenyl boronic acid derivatives occasionally gave the desired coupling products in lower purities when the solvent system dimethyl formamide/water was used. In order to have a robust protocol for the envisaged library synthesis we therefore chose to use a 5:1 mixture of toluene/ethanol. For the same reason we kept the high catalyst loading of 20 mol%. The reaction of chloride **5a** and 4-ethoxyphenyl boronic acid using only 2 mol% of tetrakis(triphenylphosphine) palladium gave comparable results.

The desired antagonists **9** were obtained in good purities and yields after cleavage from the resin with trifluoro acetic acid. A small library of ET receptor antagonists was prepared following the above protocol using various pyrimidine and phenyl boronic acid building blocks. Some typical examples obtained from a set of pyrimidine building blocks and 4-ethoxyphenyl boronic acid are summarized in Table 1.[23] Several highly potent and selective ET_A receptor antagonists like **9a** (Fig. 2)



Scheme 1. Solid-phase synthesis of ET-receptor antagonists **9**.

Table 1. ET receptor antagonists **9**

Compd	R^1	R^2	R^3	Yield%[a]
9a	Me	H	OEt	46,[b] 65[c]
9b	Et	H	OEt	97[c]
9c	Me	Me	OEt	56[c]
9d	H	Me	OEt	34[b]
9e	H	OMe	OEt	58[b]
9f	H	Et	OEt	68[b]

[a]Overall yields, purity was generally >80% as determined by RP-HPLC at 214 nm.
[b]Pyrimidine chloride **3** used in the synthesis.
[c]Pyrimidine iodide **4** used in the synthesis.

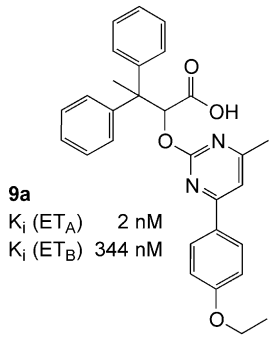


Figure 2. Potent and selective ET_A receptor antagonist.

were obtained.[24] Further results will be reported together with a detailed SAR study in due course.

In summary, a new solid-phase synthesis of highly potent and selective ET_A antagonists which avoids several shortcomings of the corresponding synthesis in solution was established. The developed protocol is suitable for automated synthesis and was used for the generation of a small library of ET receptor antagonists.

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23. The synthesis was carried out on the Myriad core system (Mettler-Toledo). All washing steps and reagent additions were performed in an automated manner. The palladium catalyst was added manually, but could in principle also be added as a suspension on the Myriad core system or as a solid using a dry pipette. All reaction steps were performed under an inert atmosphere of argon. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification.

Representative procedure: Resin **2** was prepared by standard procedures from Wang-bromide resin (Novabiochem) and cesium salt **1** (cf. ref 19).

Resin **2** (90 mg, 0.1 mmol) was washed with DMF (3 mL) and suspended in DMF (2 mL). Under stirring a 1M solution of sodium bistrimethylsilylamide in THF (120 μL, 0.12 mmol, Aldrich) was added to the suspension at room temperature. After 15 min the resin was filtered to remove excess sodium bistrimethylsilylamide, washed with DMF (3 mL) and subsequently suspended in DMF (2 mL). A 1M solution of pyrimidine **3** or **4** (see ref 21), respectively, in DMF was added (270 μL, 0.27 mmol). The mixture was incubated for 20 h under stirring and occasional gas agitation. The resin was washed successively with DMF (3×3 mL), DCM (3×3 mL), MeOH (3 mL) and DCM (2×3 mL). Deprotonation and etherification with pyrimidine **3** (or **4**), respectively, were repeated to achieve complete conversion to pyrimidine derivative **5** (or **6**).

Resin **5** (or **6**) was washed with DMF (3 mL) and suspended in toluene/ethanol (5:1, 2 mL). To this suspension a 1M solution of boronic acid derivative **7** in DMF (400 μL, 0.4 mmol) and a 2.6M solution of potassium carbonate in water (580 μL, 1.5 mmol) were added successively. After manual addition of tetrakis(triphenylphosphine)palladium (23 mg, 0.02 mmol, 20 mol%) the reaction mixture was stirred under occasional gas agitation at 80 °C for 24 h. At ambient temperature the solvents with the excess reagents were removed by filtration and the resin was washed successively with water (3 mL), DMF (3×3 mL), DCM (3×3 mL), MeOH (3 mL), DCM (3×3 mL). The desired products **9** were obtained after cleavage (1 h, rt) from resin **8** using trifluoro acetic acid/DCM (95:5). In selected cases the products which were generally obtained in >80% purity were purified by preparative RP-HPLC. **9a**, yield: 30.5 mg (0.065 mmol, 65% overall using iodide **4a**). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): δ 7.8 (d, 2H); 7.2 (m, 10H); 6.9 (s, 1H); 6.7 (d, 2H); 5.8 (s, 1H); 3.9 (m, 2H); 2.4 (s, 3H); 2.0 (s, 3H); 1.4 (t, 3H). MS (ESI): 469 ($M+H^+$).
24. Receptor Binding Studies. The binding studies were performed using CHO cells stably expressing human ET_A or ET_B receptors. Membrane protein (10–50 μg) was incubated for 30 min at 25 °C in 50 mM Tris–HCl, pH 7.4, containing 5 mM $MnCl_2$, 40 μg/mL bacitracin, and 0.2% BSA, with 25 pM [^{125}I]ET-1 (ET_A assay) or 25 pM [^{125}I]ET-3 (ET_B assay) in the presence or absence of the test compound. Nonspecific binding was measured with 0.1 μM ET-1.

After incubation, membranes were collected on GF/B glass fiber filters and radioactivity was determined by liquid scintillation counting.