Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 905-908, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

THE SYNTHESES AND BINDING AFFINITIES OF TOOLS FOR THE STUDY OF ANGIOTENSIN AT_2 RECEPTORS

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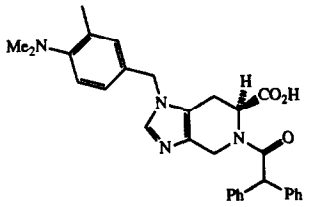
(*Received* 9 *June* 1992; *accepted* 15 *July* 1992)

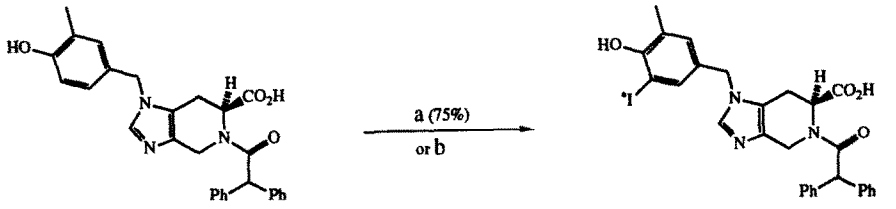
Abstract: Syntheses of radiolabeled, fluorescent and biotinyl probes with highly selective affinity for the angiotensin AT_2 receptor are described. The binding affinities at AT_2 and AT_1 receptors are reported.

A large body of information has been published in the recent scientific literature regarding the characterization and anatomical location of subclasses of angiotensin II receptors[1]. Much of this work has relied upon the use of radiolabeled angiotensin II (Ang II) or one of its analogues and displacement with subtype-specific Ang II receptor binding inhibitors. One AT_1 selective probe, ^{3}H-DUP 753[2], and one AT_2 selective probe, ^{125}I-CGP 42112A[3] have also been reported. We have previously published on nonpeptide, AT_2 selective agents[4,5]. These agents are readily modified to give a variety of AT_2 selective probes. Schemes I-III show the syntheses of ^{125}I-labeled, fluorescent and biotinyl analogues of PD 123319[6]. As shown in Table 1, each of these agents retains excellent AT_2 affinity and selectivity compared to the parent compound. Compounds **2**, **7**, and **10** have potential applications in the study of AT_2 receptors, including receptor binding assays, autoradiography, fluorescent imaging, histochemical staining and affinity chromatography.



PD 123319

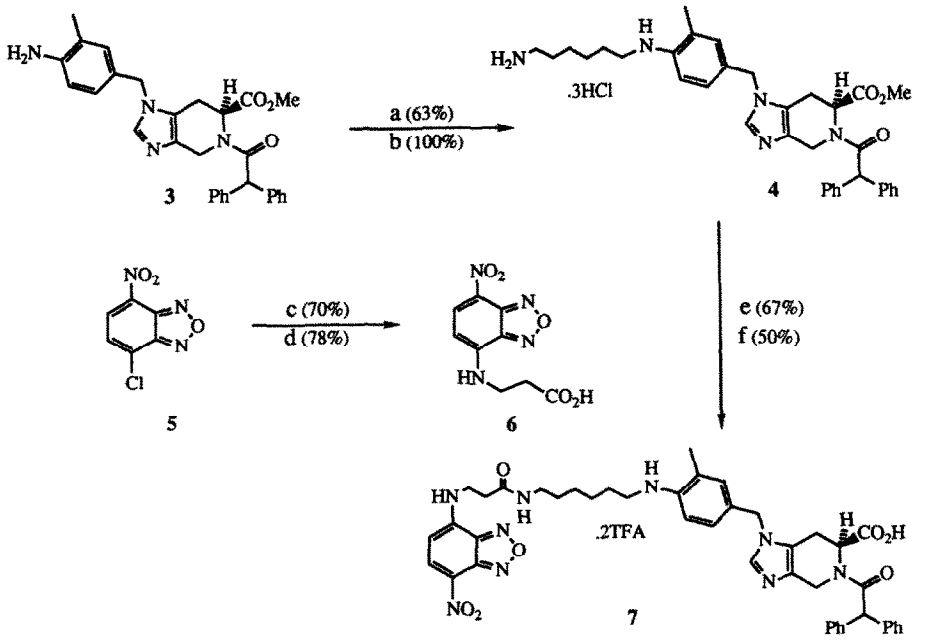
Iodination of the phenolic analogue, **1**, proceeds smoothly on a preparative scale using excess sodium iodide and Chloramine-T, followed by reversed phase chromatography to afford **2**. The synthesis was readily adapted for ^{125}I-labeling by using an excess of **1** and Chloramine-T.



1 2

Scheme I: Radiolabeled AT_2 Probe. a) Cold synthesis: 3.3eq NaI, 1.5eq Chloramine-T, MeOH-pH 7.4 phosphate buffer (0.1M), 15°C. C_{18}, MeCN-0.1%TFA_{aq} (30:70 → 40:60). b) Hot synthesis: **1** (2μg, 4.1nmol in 2μL MeOH + 40μL 0.5M pH 7.4 PO_4), $Na^{125}I$ (2.0 mCi, in 5.1μL 0.1M NaOH), Chloramine-T (30μg, 132 nmol in 30 μL 0.05M pH 7.4 PO_4). 1 min at 25° then quench with $NaHSO_3$ (10μg in 10 uL 0.05M pH 7.4 PO_4). Altech Econosil C_{18} 10μ (0.46 X 25cm), MeCN-0.1%TFA_{aq} (30:70, 1.5mL/min), RT: 3 min ($Na^{125}I$), 21 min (**1**), 39-42 min (**2**). Yield 1 mCi.

In designing the fluorescent probe we relied upon established structure-activity relationships (SAR) for PD 123319 analogues[4] which indicate a broad tolerance for N(1) substituents. Additionally, since N-alkyl anilines consistently give better AT_2 affinity than anilides, we chose an ω-aminoalkyl spacer between the fluorescent group and the AT_2 ligand rather than an ω-aminoacid



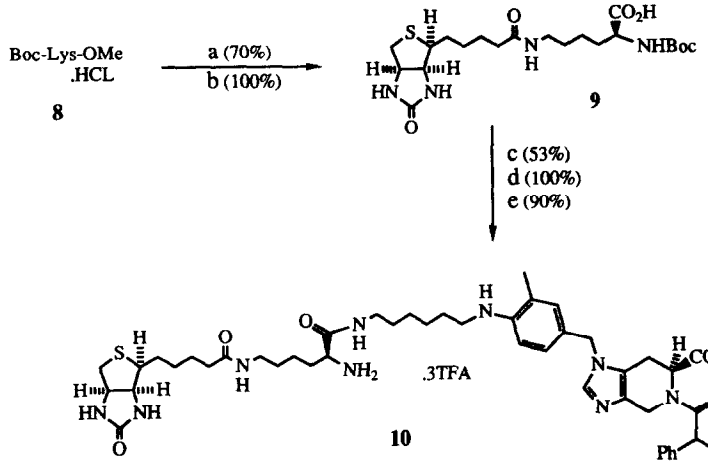
3 4 5 6 7

Scheme II: Fluorescent AT_2 Probe. a) 1.1eq BOC-NH$(CH_2)_5$CHO, 3Å Sieves, THF, 24 hr then $NaCNBH_3$, MeOH, Bromocresol Green indicator, HOAc to pH 4. Si Gel, $CHCl_3$-MeOH (99:1). b) MeOH-HCl, CH_2Cl_2, 3h. c) β-Ala-O*t*Bu-HCl, 2eq Et_3N, KI(cat), MeCN, 30 min. Si Gel, $CHCl_3$-MeCN (85:15). d) TFA-CH_2Cl_2 (2:1), 6 hr. Si Gel, $CHCl_3$-MeOH-HOAc (95:4:1). e) 5eq Et_3N, **6**, DCC, HOBT, DMF-CH_2Cl_2 (3:1), 24 hr. Si Gel, $CHCl_3$-MeOH (99:1 → 96:4). f) 3eq NaOH, MeOH, THF, H_2O, 8 hr. C_{18}, MeCN-O.1%TFA_{aq} (35:65 → 40:60).

spacer. Thus, reductive amination of **3** with N-BOC-6-aminohexanal and subsequent removal of the BOC protecting group affords **4**. Selective acylation of the more reactive amino group with the fluorescent aminoacid, **6** (prepared by the reaction of β-alanine *t*-butyl ester with **5**[7] followed by treatment with TFA) and saponification of the resulting product gives the fluorescent probe, **7**. Remarkably, **7** has an AT_2 IC_{50} of 3.6 nM which is slightly better than PD 123319 (8.3 nM). This compound is also quite AT_2 specific since it is inactive in the AT_1 binding assay at 1 uM concentration.

Given the AT_2 binding affinity of **7**, a similar strategy was used to prepare a biotinyl analogue of PD 123319. For the biotinyl compound we chose to extend the linker by insertion of a lysine residue. The longer linker was designed to both increase the likelihood of coincidental binding by avidin and the AT_2 receptor and improve water solubility of **10** relative to **7** by salt formation at the additional amino group. Thus, acylation of **4** with an N(α)BOC-N(ε)biotinyl-lysine residue (**9**) and subsequent removal of protecting groups yields **10**. This extended compound still binds well to the AT_2 receptor (IC_{50} = 7.0 nM) and has no detectable AT_1 binding at 0.1 μM. We are currently studying the AT_2 binding of the **10**-avidin complex and the effects of enzyme linked avidins and biotin antibodies on the AT_2 binding of **10**.

The receptor binding activities of **7** and **10** indicate that the group at the end of the 6-carbon linker has little effect upon AT_2 receptor affinity or upon the ratio of AT_2/AT_1 binding. Thus, substitution of other functional groups such as fluorescein and rhodamine B at the end of either the short or the long linker would also be expected to give useful AT_2 probes.



Scheme III: Biotinyl AT_2 Probe. a) 1.2eq(Biotin, DCC, HOBT), Et_3N, DMF, 24 hr. Si Gel, $CHCl_3$-MeOH (99:1 → 90:10). b) 2eq NaOH, MeOH, THF, H_2O, 12 hr. c) **4**, 5eq Et_3N, DCC, HOBT, DMF, 24 hr. Si Gel, $CHCl_3$-MeOH (99:1 → 92:8). d) MeOH-HCl, CH_2Cl_2, 3 hr. e) 10eq NaOH, MeOH, THF, H_2O, 90 min. C_{18}, MeCN-0.1%TFA_{aq} (75:25 → 65:35).

Table 1: AT_1 and AT_2 Receptor Binding Affinities[8]

Compound	FAB-MS[9]	Receptor Binding	
		AT_2 IC_{50} (nM)	AT_1
PD 123319	509 (M+1)	8.3	NA* at 0.1 μM
2	608 (M+1)	5.1	NA* at 0.1 μM
7	814 (M+1)	3.6	NA* at 1 μM
10	956 (M+Na) 934 (M+1)	7.0	NA* at 0.1 μM

* Not Active (≤10% Inhibition).

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8. The receptor binding assays are as described in reference 6 except that BSA was omitted from the assay buffer. Results are the average of two concurrent experiments.

9. The authors thank Dana DeJohn, Tracy Stevenson, and Leslie McMacken for FAB mass spectral data.