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Synthesis and Biological Evaluation of the Potent Isoxazolidinyl Angiotensin II Receptor Antagonist CL332,877 and Its Enantiomers.

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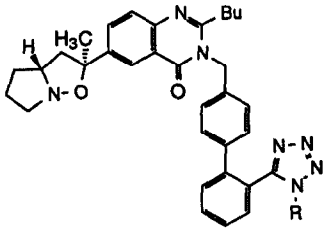
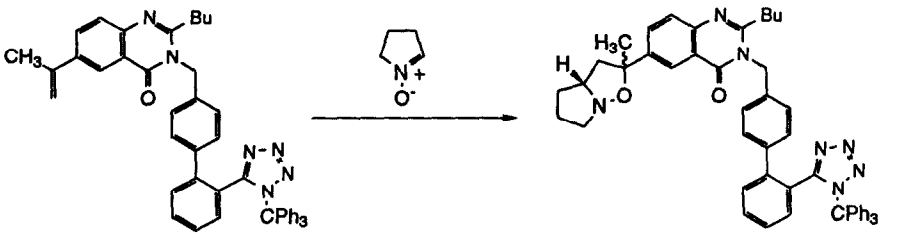
Abstract: An alternative synthesis of CL332,877, a potent isoxazolidinyl-quinazolinone angiotensin II receptor antagonist, is described. In addition, the enantiomers of CL332,877 were separated and evaluated both *in vitro* and *in vivo*.

In the course of our work on quinazolinone based angiotensin II (A II) receptor antagonists we prepared a series of 6-isoxazolidinyl derivatives, from which CL332,877 (1a) was chosen for pre-clinical development as an agent for the treatment of hypertension.[1] CL332,877 was found to be an exceptionally potent, long-acting, orally active, non-competitive A II antagonist. However, two major



1a R = Na CL332,877
1b R = H CL190,133

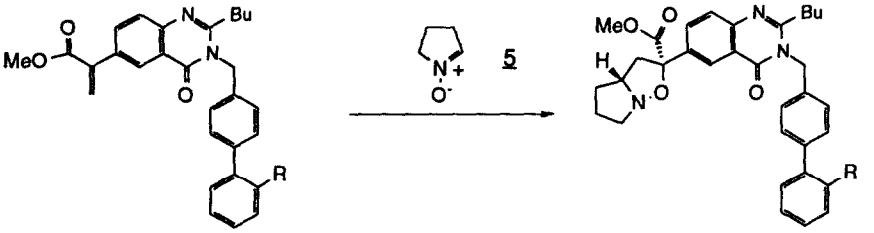
problems presented barriers to the timely development of this compound. First, the isoxazolidine moiety of CL332,877 is derived from the minor product of the diastereomeric pair produced in a nitrone cycloaddition with alkenyl-quinazolinone 2 (Scheme 1). The desired diastereomer 3a must be separated by a difficult, tedious chromatography from the undesired major isomer 3b and is obtained in only 20-25% yield under optimum conditions. Second, since CL332,877 is synthesized in racemic form, a route was needed to gain access to each enantiomer in order to evaluate their individual biological properties. We now report solutions to both of these problems along with the results of *in vitro* and *in vivo* screens assessing the activity of each of the enantiomers of CL332,877 as A II receptor antagonists.



2 3a α-CH_3, minor
3b β-CH_3, major

Scheme 1

In order to improve the synthesis of CL332,877 we required a method to increase the diastereoselectivity of the nitrone 1,3-dipolar cycloaddition reaction to the point where a chromatographic separation of the isomeric products was unnecessary. Since these reactions are known to be largely unaffected by changes in solvent polarity or reaction temperature,[2] it was decided to change the dipolarophile in the cycloaddition. Thus, we had previously found that Δ^1-pyrroline-N-oxide, 5, underwent cycloaddition with quinazolinone-acrylate ester 4a to provide good yields of a single diastereomeric adduct 6a with the same relative stereochemistry as in CL332,877 (Scheme 2).[1] Unfortunately, we were unable to make use of this reaction due to difficulty in obtaining 4a in acceptable yield. The subsequent development, in our laboratories,[3] of an efficient palladium(0)/CuI catalyzed coupling of α-stannylacrylate 7 to aryl iodides allowed us to investigate the practicality of converting aryl iodide 8 into CL332,877 via 6 (Scheme 3).



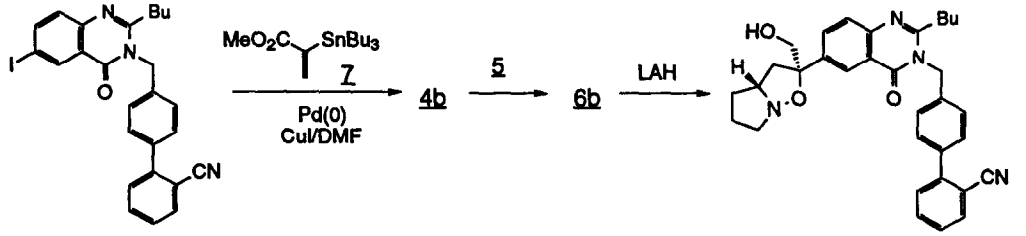
4a R = TetTr
4b R = CN

6a R = TetTr
6b R = CN

Scheme 2

In the event, 6-iodoquinazolinone 8 was coupled with α-stannylacrylate 7 to provide acrylate ester 4b in 80% yield (Scheme 3). 1,3-Dipolar cycloaddition of 4b with nitrone 5 proceeded at room temperature in acetone to give 6b as the only identifiable cycloadduct in 90% yield. This ester was next reduced with lithium aluminum hydride in THF at -78°C to give alcohol 9 in quantitative yield. Temperature control was extremely important during this reaction as competitive reduction of the quinazolinone ring to the dihydro-quinazolinone occurred at higher temperatures. With this efficient route to isoxazolidine-alcohol 9 in hand we then sought a method to convert the angular -CH_2OH

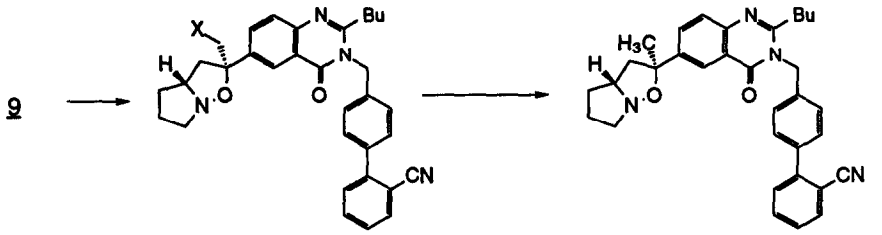
functionality into the methyl group of CL332,877 without cleaving the sensitive N-O bond of the isoxazolidine ring.



8 9

Scheme 3

Several methods were investigated for removal of the hydroxyl functionality from 9 (Scheme 4). Initially, alcohol 9 was converted into the corresponding neopentyl mesylate 10a or tosylate 10b, followed by attempted reduction to the alkane with various hydride reagents (e.g. LAH, $LiHBEt_3$, etc.).[4] These procedures returned starting material under normal conditions and, under more forcing conditions, gave products resulting from reduction of the quinazolinone or isoxazolidine rings. Even the triflate derivative 10c could not be converted into the alkane under an assortment of conditions (e.g. H_2, LAH/$TiCl_3$, $NaBH_3CN$/DMF, $NaBH_4$/t-BuOH, Li_5CuH_6, Na/NH_3, Ra Ni).[4] Next, alcohol 9 was converted into bromide 10d in 60% yield, using 1,2-dibromotetrachloroethane/Bu_3P,[5] and then subjected to a wide variety of reducing agents without success. Radical initiated reduction of 10d with tri-n-butyltin hydride did give a product resulting from reduction of the bromide, but the bicyclic isoxazolidine had apparently rearranged in the process, possibly by insertion of the methyl radical into the isoxazolidine ring. The same problem was encountered on attempted radical deoxygenation of thiocarbonyl derivatives of 9 using numerous procedures described by Barton.[6] Iodide 10e, synthesized in 30% yield from alcohol 9 (I_2, Bu_3P, $Bu_4N^+I^-$), proved to be similarly unproductive for preparing the desired alkane.



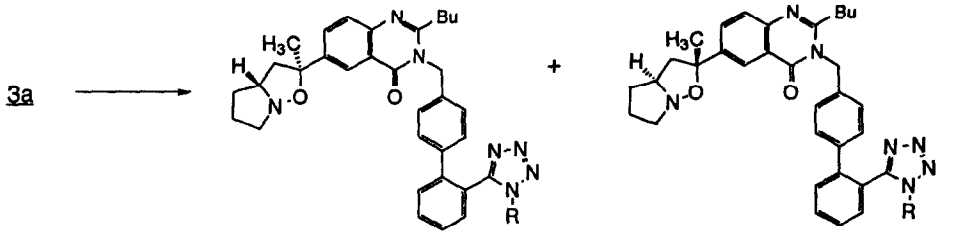
10a X = OMs 10d X = Br
10b X = OTs 10e X = I
10c X = OTf 10f X = SBT

Scheme 4

Finally it was determined that alcohol 9 could be converted into sulfide 10f (SBT = 2-S-benzothiazole) efficiently by reaction with 2,2'-dithiobis(benzothiazole) and tributylphosphine in

refluxing toluene.[7] Careful room temperature desulfurization of 10f using activated Raney nickel in DMF, with a small amount of added pyridine, gave the desired angular methyl compound 11, with minimal cleavage of the isoxazolidine ring (Scheme 4).[8] Numerous other desulfurization techniques were attempted with no improvement over this procedure. The overall yield for conversion of isoxazolidine-ester 8 to compound 11 was 30%, and no chromatography was required. Biphenyl nitrile 11 was then converted into trityl tetrazole 3a by the procedure of Duncia[9], followed by deprotection and formation of the tetrazole sodium salt to give 1a in 74% yield.

Although the synthetic sequence described above could conceivably be adapted to provide an asymmetric synthesis of either enantiomer of CL332,877 by using a chiral acrylamide derivative rather than acrylate ester 4b, a more expedient method was pursued. Thus, chromatography of trityl tetrazole derivative 3a on a chiral column (Chiracel OD-R) eluting with hexanes/isopropanol (7:3) gave each of the enantiomers, 12 and 13, in greater than 99% e.e. (Scheme 5). This technique was successfully used to prepare ~100mg of each of the two enantiomers which were then detritylated and converted into their respective sodium salts, 14 and 15 (absolute configurations have been assigned arbitrarily).



3a → 12 R = CPh_3 + 13 R = CPh_3

14 R = Na 15 R = Na

Scheme 5

Biological evaluation of the enantiomers was performed both *in vitro* and *in vivo*. Binding affinities of the enantiomers were measured using both bovine adrenal cortex and rat adrenal cortex.[10] IC_{50} values for racemate 1a , the enantiomers 14 and 15, and DuP753 (losartan) are shown in Table 1. Thus, the racemic parent compound, 1a, and enantiomer 14 are approximately two-fold more potent than enantiomer 15, and at least three-fold more potent than DuP753.

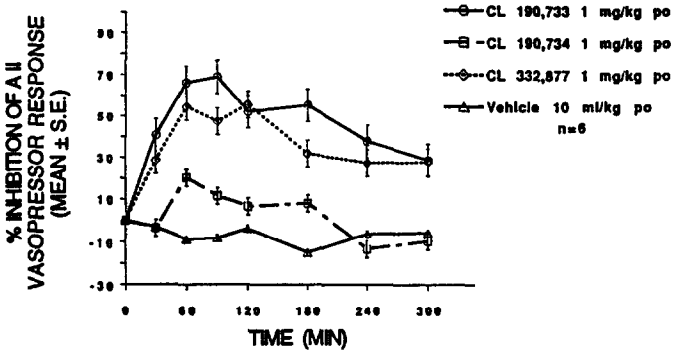
Table1: Relative IC_{50} Values for 1a, 14, 15 and DuP753.

Tissue Type	DuP 753	1a	14	15
Rat adrenal	57 nM	17 nM	17 nM	26 nM
Bovine "	330 nM	26 nM	38 nM	77nM

The same two-fold difference in potency between the enantiomers is seen *in vivo* in an A II challenge screen measuring the antagonism of the vasopressor response to a 0.05 µg/kg i.v. dose

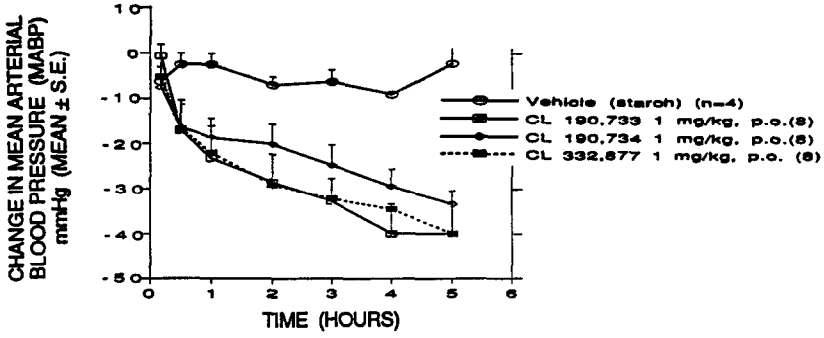
of A II in conscious spontaneously hypertensive rats (SHR) (Figure 1).[11] Enantiomer 14 is slightly more potent than racemate 1a in this assay.

Figure 1: Inhibition of A II Challenge in SHR by a 1mg/kg Oral Dose of 1a(CL332,877), 14(CL190,733) and 15(CL190,734).



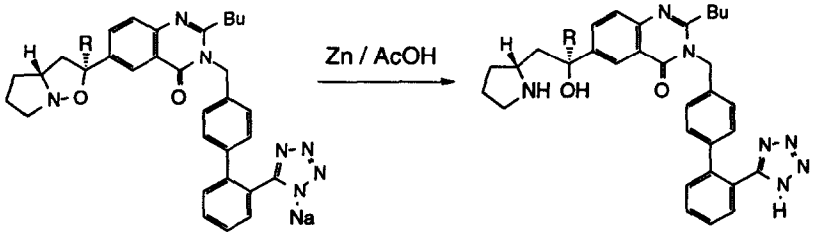
On the other hand, on oral dosing in an aorta-coarcted rat model[11] of renin dependent hypertension compounds 14 and racemate 1a were equipotent, both being approximately twice as active as enantiomer 15, (Figure 2). The enantiomers were further characterized by their effect on the A II-induced contraction of rabbit femoral artery. Schild plot analysis of this data determined that both 14 and 15 were non-competitive antagonists of A II with apparent pA_2's of 10.6 and 10.7 respectively (1a has previously been shown[1] to be a non-competitive antagonist with a $pA_2 = 10.9$).

Figure 2: Effect of Oral Administration of 1a(CL332,877), 14(CL190,733) and 15(CL190,734) (1mg/kg) on Mean Arterial Blood Pressure in Conscious Renin-Dependent Aorta-Coarcted Rats.



It should be noted at this point that 1a was also tested for its metabolic stability, since we had previously determined that compound 16[1] was rapidly and extensively metabolized on incubation with hepatic subcellular fractions from both rat and man.[12a] One of the metabolites was identified as the ring-opened amino-alcohol 17 (Scheme 6).[12b] The putative metabolite of 1a was synthesized by reaction with zinc in acetic acid to provide 18 in 41% yield. Amino-alcohol 18 proved to be an

extremely potent A II antagonist in vitro (IC_{50}= 3.5nM), ten times more potent than Merck's imidazo-[4,5-b]pyridine A II antagonist L-158,809[13] (IC_{50}= 27nM) in the same assay.[10] However, 18 was only moderately active on oral dosing (3mg/kg p.o.) in the aorta-coarcted rat model of hypertension, causing a maximum 32mm Hg fall in mean arterial blood pressure 4 hours after dosing.



16 R = H
1a R = CH_3

17 R = H
18 R = CH_3

Scheme 6

In summary, we have developed effective synthetic routes to the potent A II receptor antagonist CL332,877 (1a) each of its enantiomers (14 and 15), and a possible metabolite (18). Compounds 1a, 14, 15 and 18 were all found to be extremely potent *in vitro* while significant differences were found on oral dosing in two *in vivo* models for A II receptor antagonist activity.

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