Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 1, pp. 17-22, 1994
Printed in Great Britain

0960-894X/94 $6.00 + .00

IMIDAZO[4,5-b]PYRIDINE-BASED AT_1 / AT_2 ANGIOTENSIN II RECEPTOR ANTAGONISTS

Nathan B. Mantlo,*§ Dooseop Kim, Debra Ondeyka, Raymond S. L. Chang,†
Salah D Kivlighn,† Peter K. S. Siegl,† William J. Greenlee

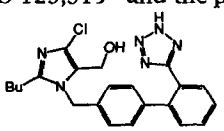
Exploratory Chemistry, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065
†Department of Pharmacology, Merck Research Laboratories, West Point, Pennsylvania, 19486

§Current Address: Pfizer Central Research, Groton, Connecticut 06340

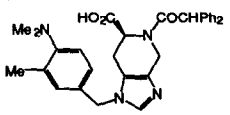
Abstract: The structure-activity relationships of 6-amido-imidazo[4,5-b]pyridine-based angiotensin II antagonists (V) demonstrate that high affinity for the AT_1 and AT_2 receptors is largely dependent upon the R^1 and R^4 substituents. Of this series, **L-162,441** and **L-162,620** exhibits subnanomolar (IC_{50}) binding affinities to both AT_1 and AT_2 receptors and potent antihypertensive effects in animals upon oral administration.

Introduction:

Angiotensin II receptor antagonists such as losartan[1] are being investigated as alternatives to angiotensin converting enzyme (ACE) inhibitors for treatment of hypertension in man. Several other non-peptide AII antagonists are being evaluated at the clinical or preclinical level.[2] All of the reported compounds in this class undergoing clinical trials are selective for the AT_1 receptor, which is largely responsible for the immediate pressor response brought about by AII.[3] A second AII receptor subtype, the AT_2 receptor, has been identified in various tissues in man. This receptor does not produce a pressor response after interaction with AII.[3,4] In fact, the physiological action of the AT_2 receptor has not been well defined apart from investigations which show a correlation with renal free water clearance,[5] restenosis following vascular injury,[6] collagen synthesis in cardiac fibroblasts,[7] and a hypotensive response to AIII binding in rats.[8] These investigations have been facilitated by discovery of non-peptidic AT_2 selective ligands such as PD-123,319[4] and the peptidic ligand CGP-42112A.[3b]



losartan

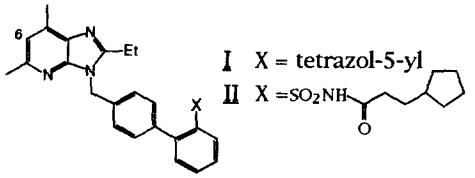


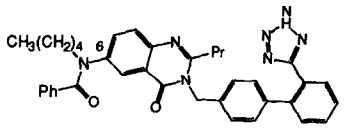
PD-123,319

Neither AT_1 selective AII antagonists nor ACE inhibitors decrease exposure of AT_2 receptors to AII. AT_1 selective AII antagonists, such as losartan, increase plasma renin activity[9] and chronic ACE inhibitor therapy does not decrease plasma AII levels compared to placebo.[10] Because the effects of chronic AT_2 stimulation have not yet been fully elucidated, blockade of both receptor subtypes with a balanced antagonist may provide benefits in addition to the treatment of hypertension compared to AT_1 receptor antagonists and ACE inhibitors.

Our approach to balanced antagonists of the AT_1 and AT_2 receptors was based on two previous discoveries made at Merck. The 6-amido substituent of the quinazolinone biphenyl tetrazole balanced antagonist III (L-159,689) is responsible for its increased binding affinity for the AT_2

receptor.[12] Molecular models suggested that substituents at the 6-position of the imidazo[4,5-b]pyridine I could be superimposed upon substituents at the 6-position of the quinazolinone III. The acylsulfonamide II exhibits a marked increase in AT_2 binding affinity over the tetrazole counterpart I. Substitution of the acylsulfonamide is critical to this enhanced activity, as long chain alkyl groups at the terminus exert a pronounced increase in AT_2 affinity over smaller, or less hydrophobic substituents.[13] We have previously described AT_1 selective imidazo[4,5-b]pyridine biphenyl tetrazole AII antagonists.[11] Herein we describe the modification of this structural motif by incorporating features of II and III to arrive at AII antagonists with balanced affinity for the AT_1 and AT_2 receptors.



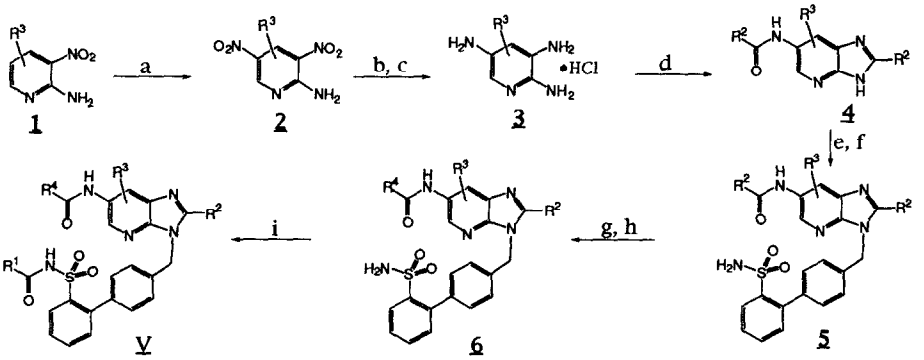


III

Synthesis:

The preparation of analogs of type V is described in Scheme I. Nitration of 2-amino-3-nitropyridine derivatives 1 proceeds smoothly to provide 2-amino-3,5-dinitropyridine analogs 2. Exhaustive reduction of the nitro groups was accomplished by the action of H_2 and Raney nickel catalysis. The resulting triaminopyridines are unstable upon exposure to atmospheric oxygen and are isolated as the hydrochloride salts 3. Treatment of 3 with R^2CO_2H in polyphosphoric acid results in imidazopyridine formation with concomitant amidation to afford 4 in high yield. Alkylation with 4-bromomethyl-2'-*tert*-butylamino-sulfonyl[1,1']biphenyl[13,14] followed by deprotection with trifluoroacetic acid provides 5. Differentiation of the R^2 groups of 5 is accomplished by hydrolysis to the free amine (not shown) followed by treatment with one equivalent of an acid chloride (R^4COCl) to yield 6. Reaction of 5 or 6 with an acid chloride or an isocyanate (in the case of example Vm, Table II) affords V.

SCHEME I

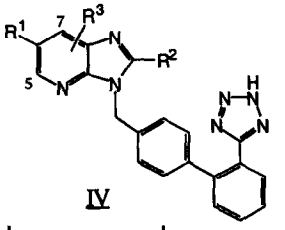


Reagents: a. HNO_3 (1 equiv.), H_2SO_4; 0°C to rt, 24 h; b. H_2 (1 atm.), Ra-Ni (5 %), 1:1 THF-MeOH; c. filter (under N_2) into 3 equiv. of conc. HCl, then concentrate *in vacuo*; d. R^2CO_2H (3 equiv.), polyphosphoric acid, 80 °C, 8 h; e. $CsCO_3$, 4-bromomethyl-2'-*tert*-butylamino-sulfonyl[1,1']biphenyl, DMF, rt, 3 - 8 h; f. trifluoroacetic acid, 24 h, rt; g. 3:1 conc. aqueous HCl / MeOH, 60 °C, 12 h; h. R^4COCl (1 equiv.), triethylamine (2 equiv.), 5:1 THF-DMF, -20 to 0 °C; i. R^1COCl (3 equiv.), DMAP (3 equiv.), pyridine, rt, 2 -12 h.

Results and Discussion:

The *in vitro* binding affinities of the compounds described in this paper (Table I and II) were determined by their ability to displace the specific binding ligand ^{125}I-Sar^1,Ile^8-AII from AT_1 receptors in rabbit aorta membranes or AT_2 receptors in rat midbrain membranes, and are expressed as IC_{50} values.[11b] As shown in Table I, moderate progress towards improvement of AT_2 binding affinity was obtained with 6-amido-imidazopyridine biphenyltetrazole analogs IVb-e compared to the 6-unsubstituted analogs I and IVa. The observed increase in activity is not of sufficient magnitude, as the AT_2/AT_1 selectivity is still greater than 200. This is in direct contrast to the remarkable gains in AT_2 affinity made by introduction of similar amide groups at the 6-position of III.[12] Several amide substituents were incorporated at the 5-position of the imidazopyridine (not shown) but AT_2 binding affinity was only slightly increased (2- to 4-fold) over analogs I and IVa. Substitution at the 7-position was not attempted because a bulky group at this site dramatically reduces AT_1 affinity.[15]

TABLE I AT_1 and AT_2 affinities of tetrazole analogs IV and compounds I, II, and III.



IV

Entry	R^1	R^2	R^3	AT_1 (nM)[a] IC_{50}	AT_2 (nM)[a] IC_{50}
I (L-158,809)[11]	H	-Et	5,7-$(CH_3)_2$	0.3	>10,000
II[13]				0.1	33
III[12]				1.0	0.7
IVa (L-158,338)[11a]	H	-Pr	7-CH_3	1.0	>10,000
IVb	-N(Bn)COBu	-Bu	5-CH_3	0.5	900
IVc	-N(Bu)COPh	-Bu	7-CH_3	0.6	400
IVd	-NHCONPh2	-Bu	5-CH_3	0.6	510
IVe	-NHCONPh2	-Bu	7-CH_3	0.6	140

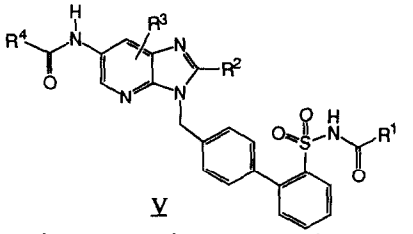
a. The standard error, expressed as percent of the mean IC_{50}'s, was determined to be 30 % or less.

In order to further increase activity at the AT_2 receptor, compounds which contained both the 6-amidoimidazopyridine and the sulfonamide substituents were prepared (Table II).[14] This combination provided potent antagonists V, many of which exhibit balanced binding affinity to the AT_1 and AT_2 receptors (AT_2/AT_1 IC_{50} ratio $\leq$ 10). By employing the sulfonamide moiety, we were able to use less bulky 6-amido substituents compared to those of structures IVb-e while retaining excellent AT_2 binding affinity. A 6-butyramido group (R^4 - COPr) is sufficient for good activity, but slightly larger groups do exhibit increased AT_2 affinity (Vh vs. Vi).

The AT_2 enhancing effect of longer chain alkyl groups on the acyl sulfonamide (R^1) is demonstrated in the series Va-c. Similarly, a propyl group at the 4-position of the benzoyl sulfonamide Vg affords a 15-fold improvement in AT_2 activity when compared to Vf. Extending the R^1 chain length and bulk past the effective size of a cyclopentylethyl (entries Vc-e), or benzyloxy (entry Vl) did not further increase AT_2 affinity. Depending on the disposition of R^3, even the less bulky butylsulfonylcarbamate (i.e., Vk) afforded excellent potency at AT_1 and AT_2. The sulfonylurea, Vm, was suboptimal at AT_2 possibly due to increased polarity. Other studies have shown that AT_2 affinity is not compatible with polar sulfonamide substituents.[13]

The effect of the 2-substituent (R^2) was also examined in this series. A butyl or propyl substituent gave similar results. Shortening the chain length resulted in a disproportionate dropoff in activity at both receptors, favoring AT_2 activity. As exemplified by entry Vj, the 2-ethyl analog is more selective for the AT_2 receptor, albeit the AT_1 affinity is 50 to 90 - fold lower than most of the other analogs in the class.

TABLE II AT_1 and AT_2 binding affinities of sulfonamide analogs V.



V

Entry	R^1	R^2	R^3	R^4	AT_1 (nM)[a] IC_{50}	AT_2 (nM)[a] IC_{50}
Va	-Bu	-Bu	H	-Bu	1.6	17.5
Vb	-Pn	-Bu	H	-Bu	0.37	2.7
Vc	-$(CH_2)_2$-cyclopentyl	-Bu	H	-Bu	0.12	0.45
Vd	-$(CH_2)_2$-cyclopentyl	-Bu	5-Me	-Bu	0.10	0.31
Ve	-$(CH_2)_2$-cyclopentyl	-Pr	5,7-$(Me)_2$	-Pr	0.26	0.25
Vf	-Ph	-Pr	7-Me	-Pr	4.6	59
Vg	-Ph-4-Pr	-Pr	7-Me	-Pr	3.3	3.9
Vh	-OBu	-Pr	7-Me	-Pr	0.51	5.6
Vi	-OBu	-Pr	7-Me	-Bu	0.27	2.23
Vj	-OBu	-Et	H	-Bu	11.5	4.2
Vk (L-162,441)	-OBu	-Bu	5-Me	-Bu	0.11	0.47
Vl (L-162,620)	-OCH_2Ph	-Pr	7-Me	-Pr	0.33	0.94
Vm	-NHBu	-Pr	7-Me	-Pr	4.3	28

a. The standard error, expressed as percent of the mean IC_{50}'s, was determined to be 30 % or less.

In vivo potency of the compounds in Table II (dosed as the potassium salts) was determined by assessing the inhibition of pressor responses to 0.1 μg/kg i.v. AII in conscious normotensive animals. The duration of action is expressed as the time until the peak response falls below 30 % inhibition for a single bolus dose of the drug. Potency (ED_{50} value) is expressed as the dose required to elicit a 50% peak inhibition of AII. Compounds that distinguished themselves in the rat were further evaluated in the beagle dog. In general, the sulfonylcarbamate analogs exhibited better *in vivo* activity in the rat compared to acylsulfonamides where R^1 = alkyl (Va-e). In addition, the 5- or 7-methyl imidazopyridine analogs produced more favorable *in vivo* rat activity compared to their *des*-methyl counterparts (data not shown). The *in vivo* profile of two balanced compounds, L-162,441 (Vk) and L-162,620 (Vl), is shown in Table III. As can be seen both compounds exhibit excellent oral activity and good duration of action in both rats and dogs.

TABLE III Comparison of the iv and oral effects of **L-162,441** and **L-162,620** on the inhibition of AII-induced pressor responses in rats and beagle dogs.[11c]

- **L-162,441 (Vk), AT_2/AT_1 IC_{50} ratio = 4.3**

	Dose (mg/kg)	% Max. Inhibition	Duration[a]
Rat			
iv ED_{50} = 0.069 mg/kg (0.062 - 0.076)	0.3, iv	82 ±4	>6 hr, n=4
po ED_{50} = 0.014 mg/kg (0.001 - 0.167)	0.3, po	86 ±2	>6 hr, n=4
Beagle Dog	0.3, iv	100 ±0	>6 hr, n=4
	1.0, po	100 ±0	>6 hr, n=4

- **L-162,620 (Vl), AT_2/AT_1 IC_{50} ratio = 2.8**

	Dose (mg/kg)	% Max. Inhibition	Duration[a]
Rat			
iv ED_{50} = 0.13 mg/kg (0.12 - 0.15)	0.3, iv	85 ±1	>6 hr, n=4
po ED_{50} = 0.28 mg/kg (0.25 - 0.31)	0.3, po	58 ±12	>6 hr, n=6
Beagle Dog			
iv ED_{50} = 0.027 mg/kg (0.025 - 0.029)	0.3, iv	100 ±0	>6 hr, n=2
po ED_{50} = 0.58 mg/kg (0.52 - 0.64)	1.0, po	76 ±7	>6 hr, n=5

a. The duration of action was less than 24 hr at these doses.

Conclusion:

A new class of potent and balanced imidazopyridine AT_1 and AT_2 receptor antagonists has been developed. The balanced activity is largely determined by the nature of the 6-amidoimidazopyridine and acylsulfonamide substituents. Fine-tuning the relative AT_1 versus AT_2 binding affinity can be achieved by subtle variations at the 2-position and methylation at the 5- or 7-position of the imidazopyridine. Of the compounds in this paper, L-162,441 (Vk), and L-162,620 (Vl) exhibit AT_2/AT_1 IC_{50} ratios of 4.3 and 2.8, respectively, along with sub-nanomolar affinity to each receptor.[16] These antagonists show potent oral antihypertensive effects in rats and dogs along with good duration of action after a single bolus dose. Efforts are ongoing to determine the potential advantage of these new compounds over the conventional AT_1 selective

antagonists.[2] These results along with analysis of the compounds on human AT_1 and AT_2 receptors will be reported in due course.

Acknowledgment: We thank Tsing-Bau Chen, Kristie Faust and Stacey Scheck for the angiotensin II receptor assays and Gloria Zingaro and Terry Schorn for the conscious animal assays.

REFERENCES:

1. (a) Chiu, A.T.; McCall, D.E.; Price, W.A.; Wong, P.C.; Carini, D.J.; Duncia, J.V.; Johnson, A.L.; Wexler, R.R.; Yoo, S.E.; Timmermans, P.B.M.W.M. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 711-718. (b) Christen, Y.; Waeber, B.; Nussberg, J.; Porchet, M.; Borland, R.M.; Lee, R.J.; Maggon, K.; Shum, L.; Timmermans, P.B.M.W.M.; Brunner, H.R. *Circulation*, **1991**, *83*, 1333-1342.
2. For reviews on the topic of non-peptide AII antagonists see: a. Buhlmeyer, P., *Current Opinion in Therapeutic Patents*, **1992**. 1693-1718. b. Hodges, J. C., Hamby, J. M.,Blankley, C. J., *Drugs of the Future*, **1992**. *17*, 575-593.
3. a. Chang, R.S.L.; Lotti, V.J. Two Distinct Angiotensin II Receptor Binding Sites in Rat Adrenal Revealed by New Selective Nonpeptide Ligands. *Mol. Pharmacol.*, **1990**, *29*, 347-351. b. Whitebread, S.; Mele, M.; Kramer, B.; de Gasparo, M. *Biochem. Biophys. Res. Commun.*, **1989**, *163*, 284 - 291.
4. Blankley, C.J.; Hodges, J.C.; Klutchko, S.R.; Himmelsbach, R.J.; Chucholowski, A.; Connolly, C.J.; Neergaard, S.J.; Van Nieuwenhze, M.S.; Sebastian, A.; Quin, III, J.; Essenburg, A.D.; Cohen, D.M. *J. Med. Chem.*, 1991, *34*, 3248-3260.
5. Keiser, J.A.; Bjork, F.A.; Hodges, J.C.; Taylor Jr., D.G. *J. Pharmacol. Exp. Ther.* , **1992**, *262*, 1154 -1160.
6. Janiak, P.; Pillon, A.; Prost, J.F.; Vilaine, J.P. *Hypertension*, 1992. *20*, 737-745.
7. Brilla, C.G., *Circulation*, **1992**. *86*, Supplement I , 672.
8. Scheuer, D.A.; Perrone, M. H. *Am. J. Physiol.*, **1993**, *264*, R917-R923.
9. Munafo, A.; Chrite, Y.; Nussberger, J.; Shum, L.Y. Borland, M.; Lee, R.J.; Waeber, B.; Biollaz, J.; Brunner, H.R. *Clin. Pharmacol. Ther.* **1992**, *51*, 513 - 521.
10. Biollaz, J.; Brunner, H.R.; Gavras, I.; Waeber, B.; Gavras, H. *J. Cardiovasc. Pharmacol.* **1982**, *4*, 966-972.
11. a. Mantlo, N. B.; Chakravarty, P. K.; Ondeyka, D.; Siegl, P. K. S.; Chang, R. S. L.; Lotti, V. J.; Faust, A. K.; Chen, T. B. Schorn, T. W.; Sweet, C. S.; Emmert, S. E.; Patchett, A. A.; Greenlee, W. J. *J. Med. Chem.* **1991**, *34*, 2919. b. Chang, R.S.L.; Siegl, P.K.S.; Clineschmidt, B.V.; Mantlo, N.B.; Chakravarty, P.K; Greenlee, W.J.; Patchett, A.A.; Lotti, V.J. *J. Pharmacol. Exp. Ther.* , **1992**, *262 (1)*, 133 - 138. c. Siegl, P.K.S.; Chang, R.S.L.; Mantlo, N.B.; Chakravarty, P.K; Ondeyka, D.L.; Greenlee, W.J.; Patchett, A.A.; Sweet, C.S. Lotti, V.J. *J. Pharmacol. Exp. Ther.* , **1992**, *262 (1)*, 139 - 144.
12. de Laszlo, S.E.; Quagliato, C.S.; Greenlee, W.J.; Patchett, A.A.; Kilvlighn, S.D.; Chang, R.S.L.; Siegl, P.K.S.; Schorn, T.S.; Faust, K.A.; Chen, T.B.; Zingaro, G.J.; Lotti, V.J. *Book of Abstracts*, 206th National Meeting of the American Chemical Society, Chicago, Il. Aug. 22 -26, **1993**, MEDI # 64.
13. Naylor, E.M.; Chakravarty, P.K.; Chen, A.; Strelitz, R.A.; Chang, R.S.L.; Chen, T.B.; Faust, K.A.; Lotti, V.J.; Kilvlighn, S.D.; Zingaro, G.J.; Schorn, T.S.; Siegl, P.K.S.; Patchett, A.A.; Greenlee, W.J. *Book of Abstracts*, 206th National Meeting of the American Chemical Society, Chicago, Il. Aug. 22-26, **1993**, MEDI # 76.
14. Chakravarty, P.K.; Mantlo, N.B.; Greenlee, W.J.; Patchett, A.A.; Kim, D. *U.S. Patent No. 5,223,501*, 1993.
15. Mantlo, N.B.; Chakravarty, P.K.; Ondeyka, D.; Chen, A.; Camara, V.J.; Chang, R.S.L.; Lotti, V.J.; Siegl, P.K.S.; Patchett, A.A.; Greenlee. W.J. Potent, Orally Active Imidazo[4,5-b]pyridine Angiotensin II Receptor Antagonists. *Book of Abstracts*, 201st American Chemical Society National Meeting, Atlanta, GA April 14-19, **1991**, MEDI # 71.
16. Efforts are underway to determine the extent of agonism or antagonism of the AT_2 receptor with this structural class.

(*Received* 10 *August* 1993; *accepted* 20 *August* 1993)