room temperature and filtered to give a product that was washed with toluene, dissolved in hot acetone, refiltered, isolated by evaporation, and then purified by chromatography on silica gel. A fraction eluting in dichloromethane–methanol (10:1) was further purified by precipitation from acetone with petroleum ether to give *N*-(4-ethoxyphenyl)[(methoxycarbonyl)carbonyl]glycinamide (28): 0.29 g, 49%; mp 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 9.8 (br s, 1 H), 9.12 (br s, 1 H), 7.43 (d, J = 9 Hz, 2 H), 6.86 (d, J = 9 Hz, 2 H), 3.95 (m, 4 H), 3.80 (s, 3 H), 1.30 (q, J = 7 Hz, 3 H).

(iv) *N*-(4-Ethoxyphenyl)oxaloglycinamide (6). A solution of *N*-(4-ethoxyphenyl)[(methoxycarbonyl)carbonyl]glycinamide (28) (0.28 g, 1 mmol) in THF (20 mL) was diluted with water (10 mL) and then treated with aqueous sodium hydroxide (1 M; 1.5 mL, 1.5 mmol). The solution was stirred at room temperature for 2 h, filtered, and then acidified to pH 2 with hydrochloric acid (2 M). The solution was refrigerated for 1 h and the product that crystallized was isolated by filtration, washed with water, and dried to give *N*-(4-ethoxyphenyl)oxaloglycinamide (6): 0.23 g, 85%; mp 207 °C dec; ^{1}H NMR (DMSO-d_6) δ 13.80 (br s, 1 H), 9.80 (br s, 1 H), 8.85 (br m, 1 H), 7.45 (d, J = 9 Hz, 2 H), 6.85 (d, J = 9 Hz, 2 H), 3.95 (m, 4 H), 1.31 (t, J = 7 Hz, 3 H). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

Acknowledgment. We gratefully acknowledge the contributions of Dr. H. Tucker and R. I. Dowell to many useful discussions. We thank M. Hitchen for providing additional biological data and we thank Dr. L. Furlong for detailed guidance in its statistical evaluation.

Nonpeptide Angiotensin II Receptor Antagonists: Synthetic and Computational Chemistry of *N*-[[4-[2-(2*H*-Tetrazol-5-yl)-1-cycloalken-1-yl]phenyl]methyl]imidazole Derivatives and Their in Vitro Activity

Ho-Shen Lin,* Ashraff A. Rampersaud, Karen Zimmerman, Mitchell I. Steinberg, and Donald B. Boyd*

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received February 28, 1992*

A series of nonpeptide angiotensin II receptor antagonists was synthesized and tested in vitro to investigate requirements for recognition by and binding to AT_1 receptors. Compared to a known series of *N*-(biphenylylmethyl)imidazoles, including losartan (DuP 753), which has a more rigid conformation in the 2′-tetrazolylbiphenyl moiety, the new series replaces the terminal phenyl with cycloalkenyls. Compounds were made with five- to seven-membered rings and with either a hydroxymethyl (3) or carboxyl (4) group at the 5 position on the imidazole ring. The effects of the lipophilicity and steric bulk of the terminal ring system, the amount of π-electron density in the terminal ring, and the relative spatial proximity of the tetrazolyl and the middle phenyl are explored in terms of binding affinity to AT_1 receptors in rat adrenal glomerulosa and rabbit aorta. The physicochemical variables of the new compounds were quantitated by computational chemistry and compared to those of losartan and its carboxyl metabolite. Potency at the AT_1 receptors is maximized when the terminal ring is six-membered; an aromatic ring binds better than a cycloalkenyl ring. The 5-carboxyimidazole compounds show higher affinity than the 5-hydroxymethyl series.

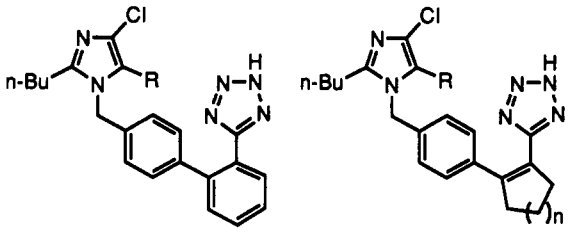
The renin–angiotensin system (RAS) is known to play an important role in the regulation of blood pressure and electrolyte and fluid balance under normal and a variety of pathophysiological conditions.[1,2] Angiotensin II (AII), the end product of the RAS cascade, has a powerful constricting action on arterioles and immediately elevates blood pressure. AII is one of the most studied hormones since it was isolated and crystallized 50 years ago by Lilly clinicians in Indianapolis.[3] The potent vasopressive action of AII is mediated through membrane-bound receptors coupled to G proteins in smooth muscle and other cells.[4] Although the receptors have been cloned, there is as yet no detailed three-dimensional structural information on them. In fact, there is not even a consensus on the most probable conformations adopted by the AII ligand itself. Being a linear octapeptide, AII is highly flexible and exists as populations of many conformations in polar and nonpolar environments. Hypotheses about its conformation at the receptor sites have been promoted.[5] However, the hypotheses have not been proven.

Over the last several of decades the RAS has been the target of therapeutic intervention in control of hypertension and related complications. Angiotensin-converting enzyme (ACE) inhibitors, such as captopril and enalapril, are useful in the treatment of hypertension, but they suffer from adverse side effects, such as hypotension, angioedema, and dry cough. Also, it is difficult to differentiate the ACE inhibitors clinically.[6] Antagonism of the AII receptors offers the prospect of a better intervention point in the RAS. There has been much work on saralasin, [Sar^1,Ala^8]AII, and other peptide AII receptor antagonists, but they have shortcomings of no oral bioavailability, poor in vivo stability, and partial agonist activity at high concentrations.[7]

(1) Peach, M. J. Renin-Angiotensin System: Biochemistry and Mechanisms of Action. *Physiol. Rev.* **1977**, *57*, 313–370.
(2) Vallotton, M. B. The Renin-Angiotensin System. *Trends Pharmacol. Sci.* **1987**, *8*, 69–74.
(3) Page, I. H.; Helmer, O. M. A Crystalline Pressor Substance (Angiotonin) Resulting from the Reaction between Renin and Renin-Activator. *J. Exp. Med.* **1940**, *71*, 29–42.
(4) Weber, V.; Monrot, C.; Bihoreau, C.; Corvol, P.; Clauser, E. The Difficult Challenge of Cloning the Angiotensin II Receptor. *Horm. Res.* **1990**, *34*, 101–104. Vallotton, M. B.; Capponi, A. M.; Johnson, E. I. M.; Lang, U. Mode of Action of Angiotensin II and Vasopressin on Their Target Cells. *Horm. Res.* **1990**, *34*, 105–110. Catt, K.; Abbott, A. Molecular Cloning of Angiotensin II Receptors May Presage Further Receptor Subtypes. *Trends Pharmacol. Sci.* **1991**, *12*, 279–281. Siemens, I. R.; Swanson, G. N.; Fluharty, S. J.; Harding, J. W. Solubilization and Partial Characterization of Angiotensin II Receptors from Rat Brain. *J. Neurochem.* **1991**, *57*, 690–700.
(5) For reviews, see, e.g., Smeby, R. R.; Fermandjian, S. Conformation of Angiotensin II. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins: A Survey of Recent Developments*; Weinstein, B., Ed.; Marcel Dekker: New York, 1978, Vol. 5, pp 117–162. Moore, G. J. Angiotensin and Bradykinin Receptors. In *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. G., Taylor, J. B., Emmett, J. C., Eds.; Pergamon Press: Oxford, UK, 1990; Vol. 3, pp 961–980; and references therein.
(6) O'Malley, K.; Kelly, J. G. New Developments in Antihypertensive Drugs. *Trends Cardiovasc. Med.* **1991**, *1*, 201–204.

0022-2623/92/1835-2658$03.00/0

About a decade ago, *N*-benzylimidazole-5-acetic acids were discovered to be weak, but selective nonpeptide AII antagonists.[8] More recently the disclosure of nonpeptide AII receptor antagonists that are not only specific, but also highly potent and orally bioavailable has stimulated a profusion of research.[9] These compounds are exemplified by losartan (DuP 753, 1), which is undergoing clinical evaluation as a potential antihypertensive.[10] Losartan is metabolized in vivo in rats, the hydroxymethyl group being oxidized to a carboxylic acid. The metabolite EXP3174 (2) is even more potent than the parent.[11]



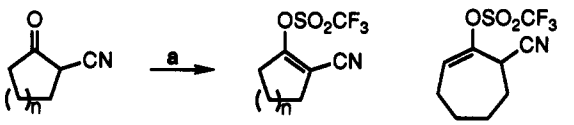
		R
1	losartan (DuP 753)	CH_2OH
2	EXP3174	CO_2H

	R	n
3a	CH_2OH	1
3b	CH_2OH	2
3c	CH_2OH	3
4a	CO_2H	1
4b	CO_2H	2
4c	CO_2H	3

The nonpeptide antagonists have also allowed identification of at least two receptor subtypes.[12,13] The AT_1 receptor is the one to which losartan binds strongly and which mediates the blood pressure response to AII. The function of the AT_2 receptor has not yet been identified.

Transport and receptor binding properties of a drug are often directly related to its lipophilic and steric characteristics. Computational chemistry[14,15] is an effective way

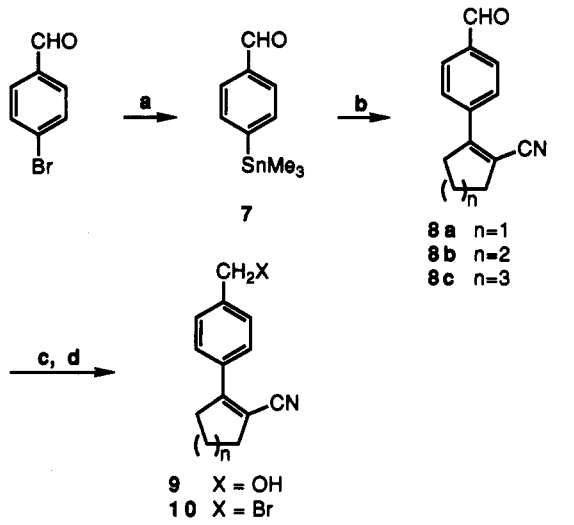
Scheme I[a]



5a n=1 **5b** n=2 **5c** n=3 **6** (n=1-3) **6c'**

[a] Reagents: (a) triflic anhydride, *N,N*-diisopropylethylamine, $ClCH_2CH_2Cl$ (for n=1, 2); or $KN(SiMe_3)_2$, *N*-phenyltriflimide, THF (for n=3).

Scheme II[a]



7 **8a** n=1 **8b** n=2 **8c** n=3 **9** X = OH **10** X = Br

[a] Reagents: (a) hexamethylditin, LiCl, $Pd(PPh_3)_4$, dioxane; (b) **6**, LiCl, $Pd(PPh_3)_4$, dioxane; (c) $NaBH_4$, C_2H_5OH; (d) CBr_4, PPh_3, CH_3CN.

(7) Bovy, P. R.; Blaine, E. H. Peptidic and Non-peptidic Angiotensin II Competitive Antagonists. *Curr. Cardiovasc. Patents* **1989**, *1*, 2044–2056.

(8) Furukawa, Y.; Kishimoto, S.; Nishikawa, K. 4-Chloro-2-phenylimidazole-5-acetic Acid Derivatives and Use as Diuretics and Hypotensives. U.S. Patent 4,355,040, 1982.

(9) Duncia, J. V.; Chiu, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. M. The Discovery of Potent Nonpeptide Angiotensin II Receptor Antagonists: A New Class of Potent Antihypertensives. *J. Med. Chem.* **1990**, *33*, 1312–1329. Carini, D. J.; Duncia, J. V.; Johnson, A. L.; Chiu, A. T.; Price, W. A.; Wong, P. C.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists: N-[(Benzyloxy)benzyl]imidazoles and Related Compounds as Potent Antihypertensives. *J. Med. Chem.* **1990**, *33*, 1330–1336. Carini, D. J.; Duncia, J. V.; Aldrich, P. E.; Chiu, A. T.; Johnson, A. L.; Pierce, M. E.; Price, W. A.; Santella, J. B. III; Wells, G. J.; Wexler, R. R.; Wong, P. C.; Yoo, S.-E.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists: The Discovery of a Series of *N*-(Biphenylylmethyl)imidazoles as Potent, Orally Active Antihypertensives. *J. Med. Chem.* **1991**, *34*, 2525–2547.

(10) Christen, Y.; Waeber, B.; Nussberger, J.; Porchet, M.; Borland, R. M.; Lee, R. J.; Maggon, K.; Shum, L.; Timmermans, P. B. M. W. M.; Brunner, H. R. Oral Administration of DuP 753, a Specific Angiotensin II Receptor Antagonist, to Normal Male Volunteers. *Circulation* **1991**, *83*, 1333–1342. Christen, Y.; Waeber, B.; Nussberger, J.; Lee, R. J.; Timmermans, P. B. M. W. M.; Brunner, H. R. Dose-Response Relationships Following Oral Administration to DuP 753 to Normal Humans. *Am. J. Hypertens.* **1991**, *83*, 350s–353s.

(11) Wong, P. C.; Price, W. A., Jr.; Chiu, A. T.; Duncia, J. V.; Carini, D. J.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists. XI. Pharmacology of EXP3174: An Active Metabolite of DuP 753, An Orally Active Antihypertensive Agent. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 211–217. Chiu, A. T.; Carini, D. J.; Duncia, J. V.; Leung, K. H.; McCall, D. E.; Price, W. A., Jr.; Wong, P. C.; Smith, R. D.; Wexler, R. R.; Timmermans, P. B. M. W. M. DuP 532: A Second Generation of Nonpeptide Angiotensin II Receptor Antagonists. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 209–217.

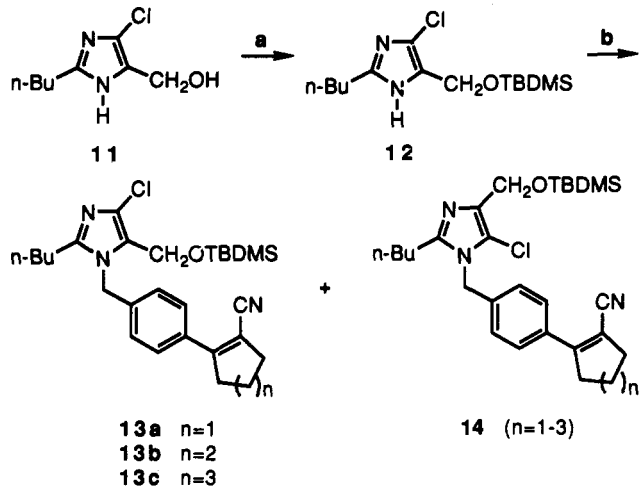
(12) Whitebread, S.; Mele, M.; Kamber, B.; de Gasparo, M. Preliminary Biochemical Characterization of Two Angiotensin II Receptor Subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 284–291. Chiu, A. T.; Herblin, W. F.; McCall, D. E.; Ardecky, R. J.; Carini, D. J.; Duncia, J. V.; Pease, L. J.; Wong, P. C.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Identification of Angiotensin II Receptor Subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 196–203. Wong, P. C.; Hart, S. D.; Zaspel, A. M.; Chiu, A. T.; Ardecky, R. J.; Smith, R. D.; Timmermans, P. B. M. W. M. Functional Studies of Nonpeptide Angiotensin II Receptor Subtype-Specific Ligands: DuP 753 (AII-1) and PD123177 (AII-2). *J. Pharmacol. Exp. Ther.* **1990**, *255*, 584–592.

(13) Wiest, S. A.; Rampersaud, A.; Zimmerman, K.; Steinberg, M. I. Characterization of Distinct Angiotensin II Binding Sites in Rat Adrenal Gland and Bovine Cerebellum Using Selective Nonpeptide Antagonists. *J. Cardiovasc. Pharmacol.* **1991**, *17*, 177–184.

(14) Lipkowitz, K. B.; Boyd, D. B., Eds.; *Reviews in Computational Chemistry*; VCH Publishers: New York, 1990, Vol. 1. Lipkowitz, K. B.; Boyd, D. B., Eds.; *Reviews in Computational Chemistry*; VCH Publishers: New York, 1991; Vol. 2.

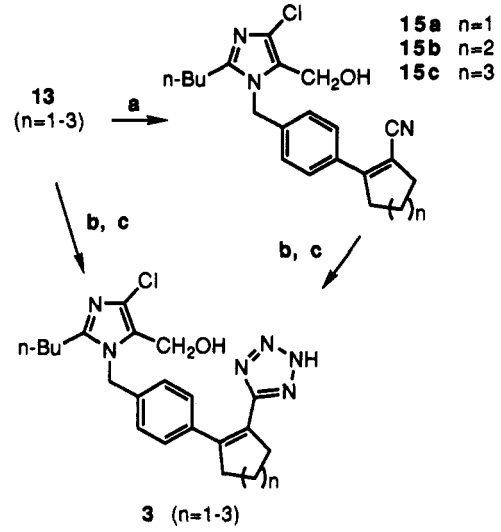
(15) Boyd, D. B. Successes of Computer-Assisted Molecular Design. In *Reviews in Computational Chemistry*; Lipkowitz, K. B., Boyd, D. B., Eds.; VCH Publishers: New York, 1990; Vol. 1, pp 355–371.

Scheme III[a]



[a] Reagents: (a) tert-butyldimethylsilyl chloride, imidazole, DMF; (b) NaH, **10**, DMF.

Scheme IV[a]



[a] Reagents: (a) tetrabutylammonium fluoride, THF; (b) tributyltin azide, 80 °C; (c) HCl, CH_3OH, or Et_2O.

to determine these characteristics even for molecular structures that have not previously been synthesized. Relative lipophilicity is commonly expressed in terms of the 1-octanol/water partition coefficient. The log $P_{o/w}$ values can be calculated based on the near additivity of parameters that give the contribution of each component structural fragment.[16,17] Likewise the relative steric bulk of a molecule can be expressed as the molecular refractivity (MR), which is a constitutive, additive property of a molecule.[16-18] Receptor binding will depend in part on the molecular shape of the ligand. Energy minimization by the molecular mechanisms method can be used to yield optimized bond lengths, bond angles, and conformations.[19-24]

Here we explore structural and physical properties of analogues of losartan in which the terminal phenyl is re-

(16) The methodologies of the CLOGP and CMR computer programs are described by Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley-Interscience: New York, 1979. Medchem Software Manual, Version 3, 1984, Pomona College, Claremont, California. See also, Leo, A. J. Methods of Calculating Partition Coefficients. In *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. G., Taylor, J. B., Ramsden, C. A., Eds.; Pergamon Press: Oxford, UK, 1990; Vol. 4, pp 295–319.

(17) CLOGP and CMR were interfaced to the Lilly corporate chemical structure database and developed into an interactive system with on-line help designed for both novice and expert users (D. B. Boyd, unpublished work). Experience with the CLOGP algorithm (Version 3.42) showed that calculated and experimental values of log $P_{o/w}$ values correlate well ($r^2 \approx$ 0.6–0.9) for a variety of drug series. The most recent version (Version 3.63, Daylight Chemical Information System, Irvine, CA) further refines and extends the parameterization and was used for generating the data in Table I. This version uses a fragment value of −0.81 for the acid fragment 2*H*-tetrazol-5-yl and −0.82 for 1*H*-tetrazol-5-yl (personal communication, C. Hansch and A. Leo, Pomona College, Claremont, CA).

(18) Dearden, J. C.; Bradburne, S. J. A.; Abraham, M. H. The Nature of Molar Refractivity. In *QSAR: Rational Approaches to the Design of Bioactive Compounds*, Pharmacochemistry Library Series; Silipo, C., Vittoria, A., Eds.; Elsevier: Amsterdam, 1991; Vol. 16, pp 167–170.

(19) Boyd, D. B. Aspects of Molecular Modeling. In *Reviews in Computational Chemistry*; Lipkowitz, K. B.; Boyd, D. B., Eds.; VCH Publishers: New York, 1990; Vol. 1, pp 321–354.

(20) Leach, A. R. A Survey of Methods for Searching Conformational Space of Small and Medium-Sized Molecules. In *Reviews in Computational Chemistry*; Lipkowitz, K. B.; Boyd, D. B., Ed.; VCH Publishers: New York, 1991; Vol. 2, pp 1–55.

(21) Motoc, I.; Dammkoehler, R. A.; Mayer, D.; Labanowski, J. Three-Dimensional Quantitative Structure-Activity Relationships. I. General Approach to the Pharmacophore Model Validation. *Quant. Struct.-Act. Relat.* **1986**, *5*, 99–105. Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. Three-Dimensional Quantitative Structure-Activity Relationships. II. Conformational Mimicry and Topographical Similarity of Flexible Molecules. *Quant. Struct.-Act. Relat.* **1986**, *5*, 138–152.

(22) Clark, M.; Cramer, R. D., III; Van Opdenbosch, N. Validation of the General Purpose TRIPOS 5.2 Force Field. *J. Comput. Chem.* **1989**, *10*, 982–1012.

(23) Molecular modeling was performed with the SYBYL software, Version 5.41c. Van Opdenbosch, N.; Cramer, R., III; Giarrusso, F. F. SYBYL: The Integrated Molecular Modeling System. *J. Mol. Graphics* **1985**, *3*, 110. Manual to SYBYL Molecular Modeling Software, Tripos Associates, 1699 Hanley Road, Suite 303, St. Louis, Missouri 63144. Computer experiments were done using a Macintosh II workstation with the NITRO interactive graphical front end to SYBYL running on VAX 8830 and 6000-540 superminicomputers. Energy minimization was done with the MAXIMIN2 routine using the Tripos force field and defaults. Electrostatics were ignored in the latter calculations because the Tripos force field performs optimally under these conditions (ref 22). Optimized geometries were used to systematically search conformational space. SYBYL's SEARCH routine varied the torsional angles at the rotatable bonds through 360° in 30° increments. Molecular mechanics energies were computed for allowable conformers so that the global minimum conformation could be found. SYBYL's MULTIFIT routine was used to overlap structures assuming a force constant of 5 kcal/mol-Å^2 between matched atoms (ref 24). The final step in obtaining the data in Table I was to do a further MAXIMIN2 optimization to relax the fitted structures to their nearest energy minimum.

(24) Experience with other molecules has shown that SYBYL's default value of 20 kcal/mol-Å for force constant in the flexible fitting procedure often introduces unrealistic structural distortions. Hence a lower value is used. Jungheim, L. J.; Boyd, D. B.; Indelicato, J. M.; Pasini, C. E.; Preston, D. A.; Alborn, W. E., Jr. Synthesis, Hydrolysis Rates, Supercomputer Modeling, and Antibacterial Activity of Bicyclic Tetrahydropyridazinones. *J. Med. Chem.* **1991**, *34*, 1732–1739. Robertson, D. W.; Boyd, D. B. Structural Requirements for Potent and Selective Inhibition of Low K_m, Cyclic-AMP-Specific Phosphodiesterases. In *Advances in Second Messengers and Phosphoprotein Research*; Strada, S. J.; Hidaka, H., Eds.; Raven Press: New York, 1991; Vol. 25, pp 321–340.

Table I. Physical and Biological Properties of Compounds

compd	ring system	log $P_{o/w}$[a]	MR, cc/mol	distance, Å[b]	IC_{50}, μM[c]	pA_2[d]
1, losartan	phenyl	4.45	116.6	4.21	0.019[e]	8.42 (26)
2, EXP3174[g]	phenyl	4.92	117.0	4.21	0.011[f]	9.63 (5)
3a	cyclopentenyl	3.78	111.2	4.39	3.5 ● 0.9	6.45 (3)
3b	cyclohexenyl	4.34	115.8	4.27	3.2 ● 0.8	6.89 (4)
3c	cycloheptenyl	4.90	120.4	4.18	2.8 ● 0.3	6.33 (3)
4a	cyclopentenyl	4.25	111.5	4.39	0.31 ± 0.10	7.84 (3)
4b	cyclohexenyl	4.81	116.2	4.27	0.40 ± 0.12	8.39 (4)
4c	cycloheptenyl	5.37	120.8	4.18	0.39 ● 0.07	7.92 (3)

[a] Calculated for the 2*H*-tetrazol-5-yl tautomer. The partition coefficient corresponds to un-ionized species, whereas a distribution coefficient, which takes into account the pK_a of a compound, would show much more of the diacids in the aqueous phase. The degree of ionization at the receptors is unknown. [b] Distance between the centroid of tetrazole ring and the centroid of the penultimate phenyl ring in energy-minimized structures. On the basis of the modeling, it is assumed that distances are the same in the hydroxymethyl (3) series as in the carboxylic acid series (4). [c] Concentration to inhibit binding of radiolabeled AII to rat adrenal glomerulosa tissue by 50%. For each compound, $n = 3$. [d] Determined on isolated rabbit aorta rings (ref 13); in parentheses is given the number of experiments, n. [e] See ref 9. [f] See ref 11. [g] Metabolite of losartan.

placed with a cyclopentenyl, cyclohexenyl, or cycloheptenyl ring. The new compounds will probe effects of lipophilicity, steric bulk, and π-electron density on binding to the AT_1 receptors. One geometrical variable explored by this series is the distance between the acidic 2-tetrazolyl on the terminal cycloalkenyl and the rest of the molecule. Any of these finely tuned physical properties may affect the ability of these compounds to antagonize AII receptors. Thereby the compounds in our series will contribute to the understanding of the requirements for optimum binding.

Results and Discussion

Synthesis. It was envisioned that the synthesis of the target *N*-[[4-[2-(2*H*-tetrazol-5-yl)-1-cycloalken-1-yl]-phenyl]methyl]imidazoles, **3** and **4**, could proceed through alkylation of silyl-protected imidazole **12** with 4-(2-cyanocycloalkenyl)benzyl bromides **10**. The latter in turn could be derived from the palladium-assisted cross coupling of aryl stannane **7** and cycloalkenyl triflates **6**.[25]

2-Cyanocycloalkanones **5** were chosen as starting materials. Initial attempts to synthesize **5** from the corresponding bisnitriles via ultrasonic irradiation[26] at ca. 10 °C in the presence of potassium appeared problematic; the reactions proceeded very slugglishly at 10–30-g scale. Heating up to ca. 95 °C was needed to speed the reaction to a more acceptable rate.[27] Enol triflates **6a** and **6b** were readily obtained by treatment of **5a** and **5b** with triflic anhydride in the presence of diisopropylethylamine,[25] whereas an isomeric mixture of **6c** and **6c'** were obtained from **5c**. This bond shift problem was solved by reacting the potassium enolate of **5c** with *N*-phenyltriflimide to give a pure **6c** (Scheme I).[28]

Aryl stannane **7**, which was readily derived from palladium-assisted cross coupling of 4-bromobenzaldehyde and hexamethylditin,[29] smoothly underwent another round of palladium-assisted cross coupling with cycloalkenyl triflates **6** to provide 4-(2-cyanocycloalkenyl)benzaldehydes **8**,[25] which were reduced to alcohols **9** and subsequently converted to bromides **10** in good overall yields (Scheme II).

At this point, we were ready to perform the key alkylation reaction of silyl-protected imidazole **12** with bromides **10** so as to assemble the requisite framework of target molecules **3** and **4**. To avoid the unwanted O-alkylation that we had experienced, known imidazole **11**[8] was protected as the O-silylated derivative **12**. Subsequent alkylation of **12** with bromides **10** furnished **13** in good yields. A small amount of regioisomers **14** was obtained in less than 15% yield (Scheme III). It has been well-documented that compounds structurally similar to **13** are less polar and travel faster than their respective regioisomers on a silica gel TLC plate.[9,30]

With requisite framework in place, cycloalkenyls **13** were desilylated by treating with tetrabutylammonium fluoride to provide alcohols **15**, which in turn were treated with tributyltin azide[31] and then with either methanolic or ethereal hydrochloric acid solution to provide target tetrazoles **3** (Scheme IV). In a more straightforward manner, **3** were also obtained by direct treatment of **13** with tributyltin azide, followed by hydrolysis in either methanolic or ethereal hydrochloric acid solution to break both the oxygen–silicon and nitrogen–tin bonds. With regard to the conditions used for bond fission, HCl/Et_2O tended to

(25) Scott, W. J.; Stille, J. K. Palladium-Catalyzed Coupling of Vinyl Triflates with Organostannanes. Synthetic and Mechanistic Studies. *J. Am. Chem. Soc.* **1986**, *108*, 3033–3040. Wattanasin, S. Novel Route to 4-Aryl Coumarins. *Synth. Commun.* **1988**, *18*, 1919–1925.

(26) Luche, J. L.; Petrier, C.; Dupuy, C. Ultrasonic in Organic Synthesis. 5. Preparation and Some Reactions of Colloidal Potassium. *Tetrahedron Lett.* **1984**, *25*, 753–756.

(27) The experimental procedure of ref 26 was modified as follows: 1,4-dicyanobutane (18.4 mL, 162 mmol) was added dropwise to a stirred suspension of potassium (12.7 g, 326 mmol) in 900 mL of dry toluene at ambient temperature under argon atmosphere; the resulting mixture was heated in an oil bath at ca. 95 °C. After ca. 8 h, potassium was almost consumed, and the reaction mixture was allowed to cool to ambient temperature. Following the workup procedure as described in ref 26, 2-cyanopentanone (13.0 g, 74%) was obtained as an oil.

(28) McMurry, J. E.; Scott, W. J. A Method for the Regiospecific Synthesis of Enol Triflates by Enolate Trapping. *Tetrahedron Lett.* **1983**, *24*, 979–982.

(29) Compound **7** was prepared following the procedure established by N. G. Stevens and L. C. Blaszczak (Lilly Research Laboratories): A stirred suspension of 4-bromobenzaldehyde (2.00 g, 10.8 mmol), hexamethylditin (7.08 g, 21.6 mmol), dry LiCl (1.37 g, 32.4 mmol), and $Pd(PPh_3)_4$ (40 mg, 0.030 mmol) in 20 mL of dry dioxane was heated to reflux under atmosphere for 24 h. At ambient temperature, the mixture was diluted with 50 mL of EtOAc and filtered through Celite. The filtrate was washed with 1 N KF (20 mL), pH 7.0 phosphate solution (20 mL), and saturated aqueous NaCl (20 mL), and then dried over $MgSO_4$. After filtration and concentration, the residue was subjected to flash chromatography (gradient hexane to 10% EtOAc/hexane) to provide **7** (2.68 g, 92%) as an oil: ^1H NMR ($CDCl_3$) δ 0.35 (s, 9 H, CH_3), 7.69 (d, 2 H, J = 7.8 Hz, Ar), 7.82 (d, 2 H, J = 7.8 Hz, Ar), and 10.01 (s, 1 H, CHO).

(30) Bovy, P. R.; Collins, J. T.; Olins, G. M.; McMahon, E. G.; Hutton, W. C. Conformationally Restricted Polysubstituted Biphenyl Derivatives with Angiotensin II Receptors Antagonist Properties. *J. Med. Chem.* **1991**, *34*, 2410–2414.

(31) Duncia, J. V.; Pierce, M. E.; Santella, J. B., III. Three Synthetic Routes to a Sterically Hindered Tetrazole. A New One-Step Mild Conversion of an Amide into a Tetrazole. *J. Org. Chem.* **1991**, *56*, 2395–2400.

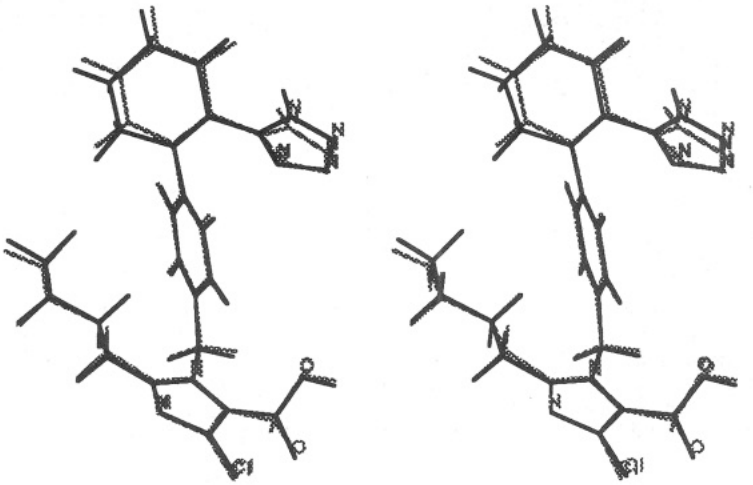


Figure 1. Stereoscopic molecular graphics shows the close similarity in conformation of losartan and that of the cyclohexenyl analogue. Alignment was achieved by flexibility fitting the four butyl carbons, the three atoms of the C–O–H moiety, and the seven atoms of the C=C-tetrazole moiety.

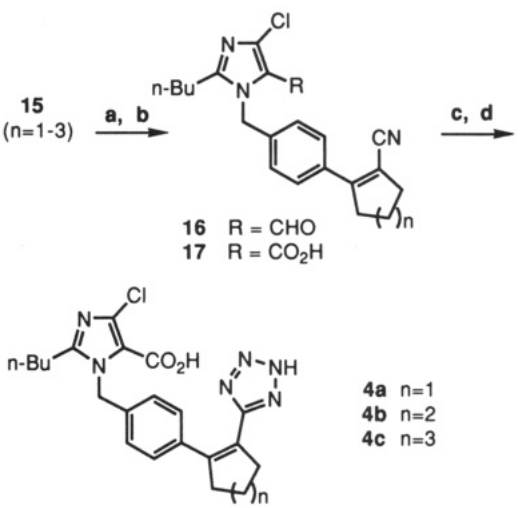
precipitate the desired product as well as offer easier isolation and improved yield.

In addition to the hydroxymethyl compounds **3**, we also synthesized the potentially more active carboxylates **4**, which are the presumed metabolites of the hydroxymethyl compounds in analogy to losartan.[11] Initial attempts to oxidize alcohols **15** with Jones reagent or pyridinium chlorochromate (PCC) met with only an intractable mixture. However, a mild, consecutive two-step oxidation using active manganese dioxide[32] and sodium chlorite[33] served just well to deliver carboxylic acids **17** in good yields. The carboxylic acids were subsequently converted to target tetrazoles **4** by treating with tributyltin azide and then hydrogen chloride gas, following the same protocol as described above (Scheme V).

Biology. Compounds **3** and **4** were evaluated for their binding affinities for the AT_1 subtype AII receptors[12,13] (IC_{50}) and for antagonism of AII-induced contraction (pA_2). Compounds **1** and **2** were tested in the latter assay for comparison. The IC_{50} values (Table I)were determined by measuring the ability of a compound to displace ^{125}I-labeled AII from its receptor on a rat adrenal glomerulosa preparation.[13] The pA_2 values were determined by a compound's ability to antagonize the AII-induced contraction of rabbit aorta ring.[13] In binding, all compounds produced a biphasic dose–response curve indicating the presence of high-affinity AT_1 and low-affinity AT_2 binding sites. Only the pA_2 values for the high-affinity AT_1 receptor are reported in Table I, because this receptor is associated with AII-induced smooth muscle contractions. In aorta, the antagonistic effect was surmountable and reversible, and no agonistic effect was observed.

Our results show that variation of the ring size of the cycloalkenyl moiety of **3a–c** does not significantly affect antagonistic activity against AT_1 receptors; neither does it in the series **4a–c**. The cyclohexenyl **4b** has a slightly higher binding affinity than do **4a** and **4c**, but not to a significant extent. Nevertheless **4a–c** do show stronger binding affinity relative to **3a–c**, which parallels what has been observed of losartan (**1**) vs its metabolite (**2**). The data clearly demonstrate the importance of having a carboxylic acid at the 5 position of the imidazole ring in order to enhance binding affinity to the AT_1 receptors.

Scheme V[a]



[a] Reagents: (a) MnO_2, $ClCH_2CH_2Cl$, ultrasonic sound; (b) $NaClO_2$, NaH_2PO_4, t-BuOH, H_2O; (c) tributyltin azide, 80 °C; (d) HCl, CH_3OH, or Et_2O.

Computations. The biological activity of a compound will depend on its shape, size, and electronic distribution because these must be compatible with the corresponding features of the target receptor site. It is known that losartan has high affinity and specificity for the AT_1 receptor. Therefore, this ligand can serve as a template with which to compare our compounds. Figures 1 and 2 compare the three-dimensional structures of losartan and a representative member of our series, the cyclohexenyl analogue. In terms of molecular skeleton, the two molecules are practically identical. The most noticeable difference is that the cycloalkenyl ring is bulkier and nonplanar at its distal end.

The receptor binding properties of a drug are often directly related to its lipophilic and steric characteristics. The calculated log $P_{o/w}$ values in Table I show that all the compounds, including losartan, are rather lipophilic. The values for the cycloalkenyls are of the same order of magnitude as compounds with the terminal phenyl ring. Molar refractivity, MR, values (Table I) show that the molecular volumes of all the compounds are comparable. Regarding trends, we see that lipophilicity and steric bulk increase while the distance between the phenyl and tetrazole rings decreases as the cycloalkenyl group is enlarged

(32) Kimura, T.; Fujita, M.; Ando, T. Sonochemical Activation of Manganese Dioxide. *Chem. Lett.* **1988**, 1387–1388.
(33) Bal, B. S.; Childers, W. E., Jr.; Pinnick, H. W. Oxidation of α,β-Unsaturated Aldehydes. *Tetrahedron* **1981**, *37*, 2091–2096.

Figure 2. Stereoscopic molecular grpahics of losartan and the cyclohexenyl analogue show the only major difference in molecular volumes is in the region near the terminal phenyl (vs cyclohexenyl) ring. Contours circumscribe regions where the partially reduced analogue has greater bulk.

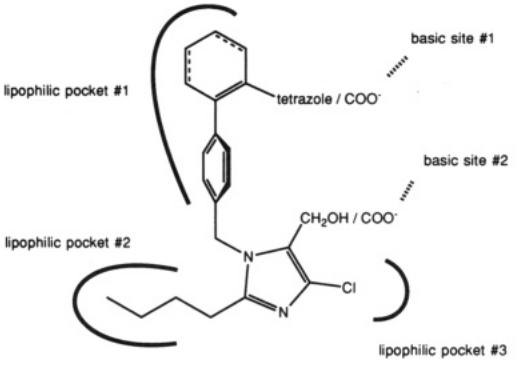


Figure 3. Schematic drawing of the AII receptor. Lipophilic pocket #1, which is occupied by the acid-bearing terminal ring, presumably does not interact as well with ligands that are partially reduced.

from a five- through a seven-membered ring. Smaller rings project the tetrazolyl group at slightly larger angles with respect to the inter-ring axis. Biological activity maximizes at the six-membered ring, but is lower with the cycloalkenyl rings compared to the compounds with an aromatic system.

In conclusion, our results show that compounds with a cycloalkenyl ring are unable to interact with AT_1 receptor sites as well as compounds with a phenyl ring. Thus, having the terminal ring system planar and rich in π electrons increases affinity to AT_1 receptors. This finding, in turn, implies that the end of the molecule bearing the ortho acidic group fits in a receptor pocket of limited width (Figure 3). It also should be noted that the antagonists are highly lipophilic, which implies that the receptor site environment is generally lipophilic. This result is consistent with the assumptions of other investigators.[34]

Experimental Section

Materials. Reagents were used as supplied unless otherwise noted. Reactions were run under dry nitrogen or argon atmosphere unless otherwise noted. Silica gel (E. Merck, 230–400 mesh ASTM) was used for flash column chromatography. LiChroprep RP-18 (E. Merck, 40-63 μm) was used for reverse-phase column chromatography. ^{1}H NMR spectra were recorded on a General Electric QE-300 instrument. Infrared (IR) spectra were determined on a Nicolet MX-1 FT-IR. Mass spectral data (MS) were obtained on either a Varian MAT-731 or a Zab 3F-VG spectrometer. Melting points are uncorrected.

Angiotensin II AT_1 Receptor Binding Assay. IC_{50} Measurement. Male Sprague–Dawley CD rats (280–320 g) were obtained from Harlan Breeding Laboratories (Greenfield, IN) and kept on standard laboratory chow. Adrenal membranes were prepared from the capsular portion (glomerulosal layer attached) of rat adrenal glands by differential centrifugation. Briefly, capsules were homogenized in a solution containing sucrose (250 mM), $MgCl_2$ (1 mM), and Tris (5 mM) at pH 7.5 and using a polytron at setting 5 for 20 s. The homogenate was stirred gently for 15 min and then centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 30000*g* for 30 min and the resulting pellet resuspended in 50 mM Tris. All the previous steps were carried out at 4 °C. Membrane preparations were stored in aliquots at −70 °C until used. Binding of [I^{125}]AII to adrenal membranes was performed at ambient temperature for 90 min in 96-well filtration plates containing a hydrophilic polyvinylidene fluoride membrane (0.45 μm, Millipore-GV Multiscreen). Each 250-μL incubate contained the following: Tris (50 mM), NaCl (120 mM), $MgCl_2$ (5 mM), dithiothreitol (1 mM), [I^{125}]AII (0.1 nM), 0.05% bovine serum albumin (BSA), and adrenal membrane protein (8–15 μg). Antagonists were added in concentrations from 10 nM to 100 μM. Nonspecific binding was measured in the presence of 0.1 μM [Sar^1,Ile^8]AII. Binding was terminated by vacuum filtration. Receptor–ligand complex trapped on filters was washed three times with 300 μL of ice-cold solution (Tris, 50 mM; NaCl, 120 mM; $MgCl_2$, 5 mM; dithiothreitol, 1 mM). Filter discs were dried, punched out, and counted in a gamma counter at 52% efficiency. Specific binding represented 96% of total binding (approximately 150 fmol AII/mg protein). Assays were performed in triplicate. The inhibitory concentration (IC_{50}) of an inhibitor that gave 50% displacement of the specific binding of [I^{125}]AII was estimated from the linear portion of the displacement curve.

pA_2 Measurement. New Zealand white rabbits (Hazelton, 2–3 kg) were sacrificed by cervical dislocation and the thoracic aortas were removed and cleaned of excess fat and connective tissue. Rings of tissue (3 mm wide) were mounted in 10-mL tissue baths between two L-shaped stainless steel hooks. The lower hook was attached to a stationary rod and the upper hook to a force displacement transducer (Grass Model FT.03). The bath chambers were maintained at 37 °C, aerated with 95% O_2/5% CO_2, and contained physiological solution of the following composition: NaCl (117 mM), glucose (5.6 mM), NaH_2PO_4 (1.0 mM), $MgSO_4$ (0.7 mM), KCl (5.2 mM), $CaCl_2$ (1.8 mM), $NaHCO_3$ (26 mM), and phentolamine hydrochloride (0.003 mM). Rings were equilibrated for 1 h with 2 g of tension. During the equilibration period, the tissues were washed by overflow every 15 min. Rings were then exposed to 10^{-8} M AII and were allowed to contract until a steady

(34) Matsoukas, J. M.; Bigam, G.; Zhou, N.; Moore, G. J. ^{1}H-NMR Studies of [Sar^1]Angiotensin II Conformation by Nuclear Overhauser Effect Spectroscopy in the Rotating Frame (ROESY): Clustering of the Aromatic Rings in Dimethylsulfoxide. *Peptides* **1990**, *11*, 359–366. Matsoukas, J. M.; Yamdagni, R.; Moore, G. J. ^{1}H-NMR Studies of Sarmesin and [Des^1]Sarmesin Conformation by Nuclear Overhauser Effect (NOE) Enhancement Spectroscopy: Folding of the N- and C-Terminal Domains. *Peptides* **1990**, *11*, 367–374.

state was reached. Tissues were then washed every 15 min for 1 h. This was repeated every hour until the AII response stabilized. A cumulative concentration–response curve to AII (10^{-10} to 10^{-7} M) was then obtained. At the conclusion of the concentration–response curve, tissues were washed every 2 min until baseline tension was reached, then every 15 min for 30 min. Compounds were added in a volume of 10 μL of DMSO and allowed to incubate for 30 min before repeating the concentration–response curve to AII. Contractions to AII were expressed as a percent of the maximum contraction obtained in the control curve (the first AII concentration–response curve). EC_{50}s (concentration that contracted the tissue to half the control maximum) for each curve were calculated using a four-parameter logistics model (NLIN, SAS Institute, Cary, NC). Potency data for each compound tested are expressed as the pA_2 (defined as $-\log K_B$, where K_B = (molar concentration of antagonist)/[(EC_{50} with antagonist/EC_{50} without antagonist) − 1].

Trifluoromethanesulfonic Acid 2-Cyano-1-cyclohexen-1-yl Ester (6b). Triflic anhydride (5.90 mL, 35.0 mmol) was added to a stirred, ice-cold solution of 2-cyanocyclohexanone (**5b**) (3.60 g, 29.2 mmol) and diisopropylethylamine (6.12 mL, 35.1 mmol) in 36 mL of dry 1,2-dichloroethane. The mixture was stirred at 0 °C for 2 h. Forty milliliters of EtOAc was added to the mixture, and the resulting suspension was filtered through a short pad of silica gel (eluted with EtOAc). The filtrate was concentrated in vacuo to give an oily residue. Flash chromatography of the residue (gradient 15% EtOAc/hexane to 25% EtOAc/hexane) afforded 5.66 g (75%) of the enol triflate **6b** as an oil: ^{1}H NMR ($CDCl_3$) δ 1.65–1.77 (m, 2 H, CH_2), 1.78–1.90 (m, 2 H, CH_2), 2.38–2.47 (m, 2 H, CH_2), and 2.48–2.57 (m, 2 H, CH_2); IR (neat) 2230, 1669, 1430, 1220, and 900 cm^{-1}; FABMS: calcd for $C_8H_9F_3NO_3S$ 256.0255, found 256.0253, M^+ + 1.

Trifluoromethanesulfonic Acid 2-Cyano-1-cyclopenten-1-yl Ester (6a). The procedure used for the preparation of **6b** was repeated with 2-cyanocyclopentanone **5a** to give **6a** (77%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.08–2.23 (m, 2 H, CH_2), 2.60–2.73 (m, 2 H, CH_2), and 2.76–2.90 (m, 2 H, CH_2): IR (neat) 2234, 1660, 1435, 1224, and 1137 cm^{-1}; FABMS calcd for $C_7H_6F_3NO_3S$ 242.0099, found 242.0126, M^+.

Trifluoromethanesulfonic Acid 2-Cyano-1-cyclohepten-1-yl Ester (6c). A solution of $KN(SiMe_3)_2$ (0.5 M in toluene, 14.0 mL, 7.01 mmol) was added dropwise to a stirred solution of 2-cyanoheptanone (**5c**) (800 mg, 5.84 mmol) in 8.0 mL of dry THF at −78 °C under argon atmosphere. The resulting suspension was stirred for 20 min before it was treated dropwise with a solution of *N*-phenyltriflimide (2.30 g, 6.42 mmol) in 23.0 mL of dry THF. The cold bath was removed, and the mixture was stirred for 3 h. After dilution with 8.0 mL of CH_2Cl_2, the mixture was filtered through a short pad of silica gel (eluted with 30% EtOAc/hexane) and concentrated in vacuo to give an oily residue. Flash chromatography of the residue (gradient toluene to 20% EtOAc/hexane) afforded **6c** (1.34 g, 85%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.63–1.83 (m, 6 H, $CH_2CH_2CH_2$), 2.41–2.50 (m, 2 H, CH_2), and 2.65–2.74 (m, 2 H, CH_2); IR (neat) 2220, 1424, 1216, 1137, and 860 cm^{-1}; FABMS calcd for $C_9H_{11}F_3NO_3S$ 270.0412, found 270.0383, M^+ + 1.

2-(4-Formylphenyl)-1-cyclohexene-1-carbonitrile (8b). Palladium tetrakis(triphenylphosphine) (750 mg, 0.640 mmol) was added to a stirred suspension of enol triflate **6b** (5.42 g, 21.2 mmol), dry LiCl (2.74 g, 63.8 mmol), and aryl stannane **7** (6.00 g, 22.3 mmol) in 115 mL of dry dioxane, and the mixture was heated to reflux under argon atmosphere for 16 h. At ambient temperature, the mixture was diluted with 150 mL of EtOAc, washed with water and saturated aqueous NaCl solution, dried over $MgSO_4$, filtered, and concentrated in vacuo to give an oily residue. Flash chromatography of the residue (gradient 10% EtOAc/hexane to 15% EtOAc/hexane) and subsequent crystallization from CH_2Cl_2/hexane afforded **8b** (3.59 g, 80%) as a white crystal: mp 57.0–58.0 °C; ^{1}H NMR ($CDCl_3$) δ 1.73–1.85 (m, 4 H, CH_2CH_2), 2.40–2.53 (m, 4 H, CH_2), 7.52 (d, 2 H, J = 8.1 Hz, Ar), 7.90 (d, 2 H, J = 8.1 Hz, Ar), and 10.02 (s, 1 H, CHO); IR (KBr) 2192, 1688, 1605, 1214, and 1176 cm^{-1}; FABMS m/e 212 (M^+ + 1). Anal. ($C_{14}H_{13}NO$) C, H, N.

2-(4-Formylphenyl)-1-cyclopentene-1-carbonitrile (8a). The procedure used for the preparation of **8b** was repeated with enol triflate **6a** to give **8a** (76%) as a white crystalline material after crystallization from CH_2Cl_2/hexane: mp 82.0–83.0 °C; ^{1}H NMR ($CDCl_3$) δ 2.06–2.19 (m, 2 H, CH_2), 2.83–2.91 (m, 2 H, CH_2), 2.94–3.03 (m, 2 H, CH_2), 7.85 (d, 2 H, J = 8.2 Hz, Ar), 7.92 (d, 2 H, J = 8.2 Hz, Ar), and 10.03 (s, 1 H, CHO); IR (KBr) 2203 and 1669 cm^{-1}; FABMS m/e 198 (M^+ + 1). Anal. ($C_{13}H_{11}NO$) C, H, N.

2-(4-Formylphenyl)-1-cycloheptene-1-carbonitrile (8c). The procedure used for the preparation of **8b** was repeated with enol triflate **6c** to give **8c** (88%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.63–1.77 (m, 4 H, CH_2CH_2), 1.82–1.94 (m, 2 H, CH_2), 2.55–2.76 (m, 4 H, CH_2), 7.52 (d, 2 H, J = 8.1 Hz, Ar), 7.89 (d, 2 H, J = 8.1 Hz, Ar), and 10.01 (s, 1 H, CHO); IR (neat) 2207, 1701, and 1604 cm^{-1}; FABMS calcd for $C_{15}H_{16}NO$ 226.1232, found 226.1225, M^+ + 1.

2-[4-(Hydroxymethyl)phenyl]-1-cyclohexene-1-carbonitrile (9b). $NaBH_4$ (104 mg, 2.75 mmol) was added to a stirred solution of **8b** (1.16 g, 5.49 mmol) in EtOH/CH_2Cl_2 (10 mL/2 mL) at 0 °C, and the resulting suspension was stirred for 2.5 h. The mixture was quenched with HOAc (0.75 mL, 13 mmol), diluted with 25 mL of EtOAc, and filtered through a short pad of silica gel (eluted with EtOAc). After concentration of the filtrate and subsequent flash chromatography (20% EtOAc/hexane), **9b** (1.00 g, 100%) was obtained as an oil; ^{1}H NMR ($CDCl_3$) δ 1.68 (t, 1 H, J = 5.8 Hz, OH), 1.70–1.83 (m, 4 H, CH_2CH_2), 2.37–2.53 (m, 4 H, CH_2), 4.70 (d, 2 H, J = 5.8 Hz, CH_2O), and 7.36–7.41 (m, 4 H, Ar); IR (neat) 3610 (br), 2209, 1230, and 1041 cm^{-1}; FABMS calcd for $C_{14}H_{15}NO$ 213.1153, found 213.1142, M^+.

2-[4-(Hydroxymethyl)phenyl]-1-cyclopentene-1-carbonitrile (9a). The procedure used for the preparation of **9b** was repeated with **8a** to give **9a** (98%) as a white crystalline material after crystallization from CH_2Cl_2/hexane: mp 42.0–43.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.76 (t, 1 H, J = 5.9 Hz, OH), 2.01–2.15 (m, 2 H, CH_2), 2.76–2.86 (m, 2 H, CH_2), 2.88–2.98 (m, 2 H, CH_2), 4.72 (d, 2 H, J = 5.9 Hz, CH_2O), 7.41 (d, 2 H, J = 8.3 Hz, Ar), and 7.72 (d, 2 H, J = 8.3 Hz, Ar); IR (KBr) 3314 (br), 2203, 1035, and 1016 cm^{-1}; FABMS m/e 199 (M^+). Anal. ($C_{13}H_{13}NO$) C, H, N.

2-[4-(Hydroxymethyl)phenyl]-1-cycloheptene-1-carbonitrile (9c). The procedure used for the preparation of **9b** was repeated with **8c** to give **9c** (97%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.55–1.80 (m, 5 H, CH_2CH_2 and OH), 1.80–1.91 (m, 2 H, CH_2), 2.53–2.61 (m, 2 H, CH_2), 2.64–2.72 (m, 2 H, CH_2), 4.70 (s, 2 H, CH_2O), and 7.37 (br s, 4 H, Ar); IR ($CHCl_3$) 3618 (br), 2208, and 1045 cm^{-1}; FABMS calcd for $C_{15}H_{17}NO$ 227.1310, found 227.1301, M^+.

2-[4-(Bromomethyl)phenyl]-1-cyclohexene-1-carbonitrile (10b). Carbon tetrabromide (1.86 g, 5.03 mmol) and triphenyl phosphine (1.23 g, 4.69 mmol) were added to a stirred solution of alcohol **9b** (1.00 g, 4.69 mmol) in 10 mL of dry CH_3CN at 10 °C under argon atmosphere, and the mixture was stirred for 20 h. Then the mixture was diluted with 15 mL of EtOAc, filtered, and concentrated in vacuo. The residue was subjected to flash chromatography (gradient 10% EtOAc/hexane to 30% EtOAc/hexane) and subsequent crystallization from CH_2Cl_2/hexane to afford **10b** (1.13 g, 87%) as a crystal: mp 82.0–83.0 °C; ^{1}H NMR ($CDCl_3$) δ 1.70–1.85 (m, 4 H, CH_2CH_2), 2.37–2.50 (m, 4 H, CH_2), 4.48 (s, 2 H, CH_2Br), 7.34 (d, 2 H, J = 8.2 Hz, Ar), and 7.41 (d, 2 H, J = 8.2 Hz, Ar); IR (KBr) 2202 and 817 cm^{-1}; FABMS m/e 276 (M^+, ^{79}Br) and 278 (M^+, ^{81}Br). Anal. ($C_{14}H_{14}BrN$) C, H, N.

2-[4-(Bromomethyl)phenyl]-1-cyclopentene-1-carbonitrile (10a). The procedure used for the preparation of **10b** was repeated with **9a** to give **10a** (100%) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.01–2.15 (m, 2 H, CH_2), 2.77–2.86 (m, 2 H, CH_2), 2.88–2.98 (m, 2 H, CH_2), 4.49 (s, 2 H, CH_2Br), 7.42 (d, 2 H, J = 8.2 Hz, Ar), and 7.69 (d, 2 H, J = 8.2 Hz, Ar); IR (neat) 2208 cm^{-1}; FABMS calcd for $C_{13}H_{13}{}^{79}BrN$ 262.0231, found 262.0254, M^+ + 1; m/e 264 (M^+ + 1, ^{81}Br).

2-[4-(Bromomethyl)phenyl]-1-cycloheptene-1-carbonitrile (10c). The procedure used for the preparation of **10b** was repeated with **9c** to give **10c** (95%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.60–1.75 (m, 4 H, CH_2CH_2), 1.80–1.93 (m, 2 H, CH_2), 2.52–2.72 (m, 4 H, CH_2), 4.48 (s, 2 H, CH_2Br), 7.35 (d, 2 H, J = 8.1 Hz, Ar), and 7.40 (d, 2 H, J = 8.1 Hz, Ar); IR (neat) 2205 cm^{-1}; FABMS calcd for $C_{15}H_{17}{}^{79}BrN$ 290.0544, found 290.0515, M^+ + 1; m/e 292 (M^+ + 1, ^{81}Br).

2-Butyl-4-chloro-5-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-1*H*-imidazole (12). A stirred solution of imidazole

11 (200 mg, 1.30 mmol), *tert*-butyldimethylsilyl chloride (411 mg, 2.73 mmol), and imidazole (152 mg, 2.23 mmol) in 2.0 mL of dry DMF was stirred at ambient temperature for 3.5 h. The mixture was filtered through a short pad of silica gel (eluted with 20% EtOAc/hexane), and the filtrate was concentrated in vacuo. Flash chromatography (eluted with 50% EtOAc/hexane) of the residue and subsequent crystallization from CH_2Cl_2/hexane afforded **12** (315 mg, 80%): mp 125.0–127.0 °C; ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $SiCH_3$), 0.91 (s, 9 H, $C(CH_3)_3$), 0.94 (t, 3 H, J = 7.3 Hz, CH_3), 1.33–1.46 (m, 2 H, CH_2), 1.66–1.77 (m, 2 H, CH_2), 2.71 (t, 2 H, J = 7.6 Hz, CH_2), 4.65 (s, 2 H, CH_2O), and 8.91 (br s, 1 H, NH); IR ($CHCl_3$) 3450, 3250 (br), 1257, 1068, and 838 cm^{-1}; FABMS m/e 303 (M^+ + 1, ^{35}Cl) and 305 (M^+ + 1, ^{37}Cl). Anal. ($C_{14}H_{27}ClN_2OSi$) C, H, N.

2-[4-[[2-Butyl-4-chloro-5-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-1*H*-imidazol-1-yl]methyl]phenyl]-1-cyclohexene-1-carbonitrile (13b) and Its Isomer 14b. Imidazole **12** (1.54 g, 5.10 mmol) was added to a stirred suspension of NaH (174 mg, 4.34 mmol; 60% dispersion in mineral oil) in 15 mL of dry DMF at 0 °C under argon atmosphere, and the mixture continued to stir until hydrogen gas evolution ceased. A solution of bromide **10b** (1.00 g, 3.64 mmol) in 10 mL of dry DMF was added dropwise to the mixture, and the resulting mixture was stirred at 0 °C for 1.5 h. The mixture was quenched with NH_4Cl (380 mg, 7.28 mmol), diluted with 50 mL of EtOAc, and filtered through a short pad of silica gel (eluted with EtOAc). The filtrate was concentrated in vacuo to give an oil. Flash chromatography (gradient 10% EtOAc/toluene to 20% EtOAc/toluene) afforded **13b** (1.55 g, 85%) as an oil and **14b** (175 mg, 10%) as a crystalline material after crystallization from CH_2Cl_2/hexane, mp 95.0–96.0 °C. **13b**: ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 6 H, $SiCH_3$), 0.84 (s, 9 H, $C(CH_3)_3$), 0.87 (t, 3 H, J = 7.3 Hz, CH_3), 1.25–1.40 (m, 2 H, CH_2), 1.60–1.71 (m, 2 H, CH_2), 1.75–1.85 (m, 4 H, CH_2CH_2), 2.40–2.50 (m, 4 H, CH_2), 2.54 (br t, 2 H, J = 7.8 Hz, CH_2), 4.53 (s, 2 H, CH_2O), 5.21 (s, 2 H, CH_2N), 7.02 (d, 2 H, J = 8.1 Hz, Ar), and 7.34 (d, 2 H, J = 8.1 Hz, Ar); IR (neat) 2209, 1253, 1057, and 838 cm^{-1}; FABMS calcd for $C_{28}H_{41}{}^{35}ClN_3OSi$ 498.2707, found 498.2678, M^+ + 1; m/e 500 (M^+ + 1, ^{37}Cl). Anal. ($C_{28}H_{40}ClN_3OSi$) C, H, N. **14b**: ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 6 H, $SiCH_3$), 0.84 (t, 3 H, J = 7.3 Hz, CH_3), 0.90 (s, 9 H, $C(CH_3)_3$), 1.24–1.37 (m, 2 H, CH_2), 1.55–1.65 (m, 2 H, CH_2), 1.70–1.82 (m, 4 H, CH_2CH_2), 2.37–2.49 (m, 4 H, CH_2), 2.55 (br t, 2 H, J = 7.8 Hz, CH_2), 4.64 (s, 2 H, CH_2O), 5.07 (s, 2 H, CH_2N), 7.03 (d, 2 H, J = 8.1 Hz, Ar), and 7.32 (d, 2 H, J = 8.1 Hz, Ar); IR ($CHCl_3$) 2200, 1255, 1048, and 838 cm^{-1}; FABMS m/e 498 (M^+ + 1, ^{35}Cl) and 500 (M^+ + 1, ^{37}Cl). Anal. ($C_{28}H_{40}ClN_3OSi$) C, H, N.

2-[4-[[2-Butyl-4-chloro-5-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-1*H*-imidazol-1-yl]methyl]phenyl]-1-cyclopentene-1-carbonitrile (13a) and Its Isomer 14a. The procedure used for the preparation of **13b** and **14b** was repeated with **10a** to give **13a** (85%) as an oil and **14a** (14%) as a crystalline material after crystallization from CH_2Cl_2/hexane, mp 125.0–126.5 °C. **13a**: ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6 H, $SiCH_3$), 0.81 (s, 9 H, $C(CH_3)_3$), 0.85 (br t, 3 H, J = 7.3 Hz, CH_3), 1.26–1.38 (m, 2 H, CH_2), 1.57–1.70 (m, 2 H, CH_2), 2.02–2.13 (m, 2 H, CH_2), 2.50 (t, 2 H, J = 7.8 Hz, CH_2), 2.81 (br t, 2 H, J = 7.5 Hz, CH_2), 2.90 (br t, 2 H, J = 7.5 Hz, CH_2), 4.50 (s, 2 H, CH_2O), 5.20 (s, 2 H, CH_2N), 7.02 (d, 2 H, J = 8.2 Hz, Ar), and 7.66 (d, 2 H, J = 8.2 Hz, Ar). IR (neat) 2209, 1257, and 838 cm^{-1}; FABMS calcd for $C_{27}H_{39}{}^{35}ClN_3OSi$ 484.2551, found 484.2528, M^+ + 1; m/e 486 (M^+ + 1, ^{37}Cl). Anal. ($C_{27}H_{38}ClN_3OSi$) C, H, N. **14a**: ^{1}H NMR ($CDCl_3$) δ 0.09 (s, 6 H, $SiCH_3$), 0.85 (t, 3 H, J = 7.3 Hz, CH_3), 0.90 (s, 9 H, $C(CH_3)_3$), 1.25–1.38 (m, 2 H, CH_2), 1.55–1.65 (m, 2 H, CH_2), 2.01–2.13 (m, 2 H, CH_2), 2.55 (br t, 2 H, J = 7.8 Hz, CH_2), 2.78–2.84 (m, 2 H, CH_2), 2.87–2.93 (m, 2 H, CH_2), 4.64 (s, 2 H, CH_2O), 5.09 (s, 2 H, CH_2N), 7.05 (d, 2 H, J = 8.2 Hz, Ar), and 7.67 (d, 2 H, J = 8.2 Hz, Ar); IR (KBr) 2201, 1256, 1044, and 852 cm^{-1}; FABMS m/e 484 (M^+ + 1, ^{35}Cl) and 486 (M^+ + 1, ^{37}Cl). Anal. ($C_{27}H_{38}ClN_3OSi$) C, H, N.

2-[4-[[2-Butyl-4-chloro-5-[[[(1,1-dimethylethyl)dimethylsilyl]oxy]methyl]-1*H*-imidazol-1-yl]methyl]phenyl]-1-cycloheptene-1-carbonitrile (13c) and Its Isomer 14c. The procedure used for the preparation of **13b** and **14b** was repeated with **10c** to give **13c** (85%) as an oil and **14c** (11%) as an oil. **13c**: ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6 H, $SiCH_3$), 0.82 (s, 9 H, $C(CH_3)_3$), 0.85 (t, 3 H, J = 7.3 Hz, CH_3), 1.22–1.38 (m, 2 H, CH_2), 1.55–1.73 (m, 6 H, CH_2), 1.80–1.90 (m, 2 H, CH_2), 2.48–2.69 (m, 6 H, CH_2), 4.50 (s, 2 H, CH_2O), 5.19 (s, 2 H, CH_2N), 6.99 (d, 2 H, J = 8.1 Hz, Ar), and 7.32 (d, 2 H, J = 8.2 Hz, Ar); IR (neat) 2206, 1461, 1251, 1056, and 837 cm^{-1}; FABMS calcd for $C_{29}H_{43}{}^{35}ClN_3OSi$ 512.2860, found 512.2865, M^+ + 1; m/e 514 (M^+ + 1, ^{37}Cl). Anal. ($C_{29}H_{42}ClN_3OSi$) C, H, N. **14c**: ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6 H, $SiCH_3$), 0.85 (t, 3 H, J = 7.3 Hz, CH_3), 0.90 (s, 9 H, $C(CH_3)_3$), 1.22–1.39 (m, 2 H, CH_2), 1.55–1.73 (m, 6 H, CH_2), 1.80–1.93 (m, 2 H, CH_2), 2.52–2.70 (m, 6 H, CH_2), 4.67 (s, 2 H, CH_2O), 5.09 (s, 2 H, CH_2N), 7.02 (d, 2 H, J = 8.1 Hz, Ar), and 7.34 (d, 2 H, J = 8.1 Hz, Ar); IR (neat) 2220, 1254, 1047, and 838 cm^{-1}; FABMS calcd for $C_{29}H_{43}{}^{35}ClN_3OSi$ 512.2860, found 512.2853, M^+ + 1; m/e 514 (M^+ + 1, ^{37}Cl).

2-[4-[[2-Butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazol-1-yl]methyl]phenyl]-1-cyclohexene-1-carbonitrile (15b). A solution of tetrabutylammonium fluoride (1.2 mL, 1.2 mmol; 1.0 M in THF) was added to a stirred solution of **13b** (500 mg, 1.00 mmol) in 8 mL of dry THF at 0 °C under argon atmosphere, and the mixture was stirred for 1 h. After dilution with 10 mL of CH_2Cl_2, the mixture was filtered through a short pad of silica gel (eluted with EtOAc) and concentrated in vacuo. The residue was purified by flash chromatography (gradient 15% EtOAc/toluene to 40% EtOAc/toluene) to yield **15b** (344 mg, 89%) as a foam: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.3 Hz, CH_3), 1.21–1.40 (m, 2 H, CH_2), 1.58–1.86 (m, 6 H, CH_2), 1.87 (br s, 1 H, OH), 2.35–2.50 (m, 4 H, CH_2), 2.55 (br t, 2 H, J = 7.8 Hz, CH_2), 4.48 (br s, 2 H, CH_2O), 5.21 (s, 2 H, CH_2N), 7.00 (d, 2 H, J = 8.1 Hz, Ar), and 7.33 (d, 2 H, J = 8.1 Hz, Ar); IR ($CHCl_3$) 3600 (br), 2209, 1459, and 1254 cm^{-1}; FABMS calcd for $C_{22}H_{27}{}^{35}ClN_3O$ 384.1843, found 384.1853, M^+ + 1; m/e 386 (M^+ + 1, ^{37}Cl). Anal. ($C_{22}H_{26}ClN_3O$) C, H, N.

2-[4-[[2-Butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazol-1-yl]methyl]phenyl]-1-cyclopentene-1-carbonitrile (15a). The procedure used for the preparation of **15b** was repeated with **13a** to give **15a** (91%) as a crystalline material after crystallization from CH_2Cl_2/hexane; mp 134.0–135.5 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7.3 Hz, CH_3), 1.25–1.40 (m, 2 H, CH_2), 1.58–1.71 (m, 2 H, CH_2), 1.78 (t, 1 H, J = 5.9 Hz, OH), 2.02–2.13 (m, 2 H, CH_2), 2.53 (br t, 2 H, J = 7.8 Hz, CH_2), 2.78–2.85 (m, 2 H, CH_2), 2.87–2.93 (m, 2 H, CH_2), 4.48 (d, 2 H, J = 5.9 Hz, CH_2O), 5.22 (s, 2 H, CH_2N), 7.02 (d, 2 H, J = 8.2 Hz, Ar), and 7.68 (d, 2 H, J = 8.2 Hz, Ar); IR (KBr) 3205 (br), 2206, and 1023 cm^{-1}; FABMS m/e 370 (M^+ + 1, ^{35}Cl) and 372 (M^+ + 1, ^{37}Cl). Anal. ($C_{21}H_{24}ClN_3O$) C, H, N.

2-[4-[[2-Butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazol-1-yl]methyl]phenyl]-1-cycloheptene-1-carbonitrile (15c). The procedure used for the preparation of **15b** was repeated with **13c** to give **15c** (86%) as a foam: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.3 Hz, CH_3), 1.24–1.40 (m, 2 H, CH_2), 1.58–1.72 (m, 7 H, CH_2 and OH), 1.80–1.90 (m, 2 H, CH_2), 2.51–2.68 (m, 6 H, CH_2), 4.46 (s, 1 H, CH_2O), 5.21 (s, 2 H, CH_2N), 6.98 (d, 2 H, J = 8.1 Hz, Ar), and 7.33 (d, 2 H, J = 8.1 Hz, Ar); IR ($CHCl_3$) 3606 (br), 2209, 1457, and 1253 cm^{-1}; FABMS calcd for $C_{23}H_{29}{}^{35}ClN_3O$ 398.1999, found 398.1988, M^+ + 1; m/e 400 (M^+ + 1, ^{37}Cl).

2-Butyl-4-chloro-1-[[4-[2-(2*H*-tetrazol-5-yl)-1-cyclohexen-1-yl]phenyl]methyl]-1*H*-imidazole-5-methanol (3b). A stirred mixture of **13b** (117 mg, 0.235 mmol) and tributyltin azide (3.0 mL) was heated at 80 °C under argon atmosphere for 3 days. After the mixture was cooled to ambient temperature, it was added with stirring to 10 mL of ice-cold, dry methanol saturated with HCl gas. The resulting mixture was stirred for 10 min before it was concentrated in vacuo. The light brown oily residue was filtered through a short pad of silica gel (eluted with EtOAc), and the filtrate was concentrated in vacuo to give an oil. Flash chromatography (gradient 20% EtOAc/$CHCl_3$ to 12% CH_3OH/$CHCl_3$) and subsequent crystallization from CH_2Cl_2/Et_2O afforded **3b** (35.0 mg, 35%) as a white solid: mp 125.0–128.0 °C; ^{1}H NMR (CD_3OD) δ 0.81 (t, 3 H, J = 7.3 Hz, CH_3), 1.17–1.32 (m, 2 H, CH_2), 1.38–1.52 (m, 2 H, CH_2), 1.80–1.90 (m, 4 H, CH_2), 2.43–2.54 (m, 6 H, CH_2), 4.39 (s, 2 H, CH_2O), 5.20 (s, 2 H, CH_2N), 6.89 (d, 2 H, J = 8.2 Hz, Ar), and 6.96 (d, 2 H, J = 8.2 Hz, Ar); IR (KBr) 3283 (br), 3100–2400 (br), 1462, 1259, and 1021 cm^{-1}; FABMS calcd for $C_{22}H_{28}{}^{35}ClN_6O$ 427.2013, found 427.2027, M^+ + 1; m/e 429 (M^+ + 1, ^{37}Cl).

2-Butyl-4-chloro-1-[[4-[2-(2*H*-tetrazol-5-yl)-1-cyclopenten-1-yl]phenyl]methyl]-1*H*-imidazole-5-methanol (3a).

The procedure used for the preparation of **3b** was repeated with **13a**; reverse-phase column chromatography (gradient 30% CH_3OH/H_2O to 60% CH_3OH/H_2O) was used for purification to afford **3a** (60%) as a white solid after crystallization from CH_2Cl_2/hexane: mp 100.0–103.0 °C; ^1H NMR (CD_3OD) δ 0.82 (t, 3 H, J = 7.3 Hz, CH_3), 1.20–1.35 (m, 2 H, CH_2), 1.44–1.55 (m, 2 H, CH_2), 2.07–2.18 (m, 2 H, CH_2), 2.54 (br t, 2 H, J = 7.7 Hz, CH_2), 2.90–3.01 (m, 4 H, CH_2), 4.44 (s, 2 H, CH_2O), 5.27 (s, 2 H, CH_2N), 6.99 (d, 2 H, J = 8.1 Hz, Ar), and 7.18 (d, 2 H, J = 8.1 Hz, Ar); IR (KBr) 3600–2400 (br), 1467, 1257, and 1020 cm^{-1}; FABMS calcd for $C_{21}H_{23}{}^{35}ClN_6O$ 413.1857, found 413.1848, M^+ + 1; m/e 415 (M^+ + 1, ^{37}Cl).

2-Butyl-4-chloro-1-[[4-[2-(2*H*-tetrazol-5-yl)-1-cyclohepten-1-yl]phenyl]methyl]-1*H*-imidazole-5-methanol (3c). The procedure used for the preparation of **3b** was repeated with **13c**; reverse-phase column chromatography (gradient 40% CH_3OH/H_2O to 80% CH_3OH/H_2O) was used for purification, to afford **3c** (56%) as a white solid after crystallization from CH_2Cl_2/hexane: mp 115.0–118.0 °C; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.1 Hz, CH_3), 1.20–1.38 (m, 2 H, CH_2), 1.54–1.80 (m, 6 H, CH_2), 1.86–1.97 (m, 2 H, CH_2), 2.56 (br t, 2 H, J = 7.7 Hz, CH_2), 2.65–2.74 (m, 2 H, CH_2), 2.86–2.95 (m, 2 H, CH_2), 4.44 (s, 2 H, CH_2O), 5.18 (s, 2 H, CH_2N), 6.91 (d, 2 H, J = 7.8 Hz, Ar), and 6.98 (d, 2 H, J = 7.8 Hz, Ar); IR (KBr) 3316 (br), 3200–2500 (br), 1458, 1255, and 1021 cm^{-1}; FABMS m/e 441 (M^+ + 1, ^{35}Cl) and 443 (M^+ + 1, ^{37}Cl). Anal. ($C_{23}H_{29}ClN_6O$) C, H, N.

2-[4-[(2-Butyl-4-chloro-5-formyl-1*H*-imidazol-1-yl)methyl]phenyl]-1-cyclohexene-1-carbonitrile (16b). Activated manganese dioxide (1.57 g, 18.1 mmol) was added to a solution of **15b** (1.38 g, 3.61 mmol) in 58 mL of dry 1,2-dichloroethane, and the resulting suspension was sonicated for 8 h under argon atmosphere. The mixture was filtered through a short pad of silica gel (eluted with 50% EtOAc/CH_2Cl_2), and the filtrate was concentrated in vacuo. Flash chromatography (gradient 30% EtOAc/Hexane to 80% EtOAc/hexane) gave **16b** (1.10 g, 80%) as an oil and recovered starting **15b** (200 mg, 14%): ^1H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7.3 Hz, CH_3), 1.22–1.40 (m, 2 H, CH_2), 1.55–1.85 (m, 6 H, CH_2), 2.30–2.50 (m, 4 H, CH_2), 2.62 (br t, 2 H, J = 7.7 Hz, CH_2), 5.54 (s, 2 H, CH_2N), 7.05 (d, 2 H, J = 8.1 Hz, Ar), 7.32 (d, 2 H, J = 8.1 Hz, Ar), and 9.73 (s, 1 H, CHO); IR (neat) 2201, 1666, and 1275 cm^{-1}; FABMS calcd for $C_{22}H_{25}{}^{35}ClN_3O$ 382.1686, found 382.1701, M^+ + 1; m/e 384 (M^+ + 1, ^{37}Cl).

2-[4-[(2-Butyl-4-chloro-5-formyl-1*H*-imidazol-1-yl)methyl]phenyl]-1-cyclopentene-1-carbonitrile (16a). The procedure used for the preparation of **16b** was repeated with **15a** to afford **16a** (80%) as an oil and recovered starting material **15a** (20%): ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7.3 Hz, CH_3), 1.30–1.42 (m, 2 H, CH_2), 1.62–1.74 (m, 2 H, CH_2), 2.00–2.13 (m, 2 H, CH_2), 2.62 (br t, 2 H, J = 7.7 Hz, CH_2), 2.75–2.95 (m, 4 H, CH_2), 5.56 (s, 2 H, CH_2N), 7.07 (d, 2 H, J = 8.2 Hz, Ar), 7.67 (d, 2 H, J = 8.2 Hz, Ar), and 9.74 (s, 1 H, CHO); IR (neat) 2210, 1666, and 1276 cm^{-1}; FABMS calcd for $C_{21}H_{23}{}^{35}ClN_3O$ 368.1530, found 368.1484, M^+ + 1; m/e 370 (M^+ + 1, ^{37}Cl). Anal. ($C_{21}H_{22}ClN_3O$) C, H, N.

2-[4-[(2-Butyl-4-chloro-5-formyl-1*H*-imidazol-1-yl)methyl]phenyl]-1-cycloheptene-1-carbonitrile (16c). The procedure used for the preparation of **16b** was repeated with **15c** to afford **16c** (87%) as an oil and recovered starting material **15c** (4%): ^1H NMR ($CDCl_3$) δ 0.87 (t, 3 H, J = 7.3 Hz, CH_3), 1.27–1.40 (m, 2 H, CH_2), 1.60–1.75 (m, 6 H, CH_2), 1.79–1.90 (m, 2 H, CH_2), 2.50–2.70 (m, 6 H, CH_2), 5.52 (s, 2 H, CH_2N), 7.03 (d, 2 H, J = 8.1 Hz, Ar), 7.32 (d, 2 H, J = 8.1 Hz, Ar), and 9.72 (s, 1 H, CHO); IR (neat) 2209, 1665, and 1278 cm^{-1}; FABMS calcd for $C_{23}H_{27}{}^{35}ClN_3O$ 396.1843, found 396.1828, M^+ + 1; m/e 398 (M^+ + 1, ^{37}Cl). Anal. ($C_{23}H_{26}ClN_3O$) C, H, N.

2-Butyl-4-chloro-1-[[4-(2-cyano-1-cyclohexen-1-yl)phenyl]methyl]-1*H*-imidazole-5-carboxylic Acid (17b). A solution of $NaClO_4$ (2.17 g, 24.0 mmol) and NaH_2PO_4 (2.49 g, 18.1 mmol) dissolved in 20 mL of water was added to a solution of **16b** (1.00 g, 2.62 mmol) and 2-methyl-2-butene (22.4 mL) in 20 mL of *tert*-butyl alcohol. The mixture was stirred vigorously at ambient temperature for 16 h. Twenty milliliters of EtOAc was added to the mixture, and the organic layer was separated, washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash chromatography of the residue (gradient 60% EtOAc/hexane to 5% HOAc/EtOAc) afforded **17b** (910 mg, 87%) as a white foam: ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 7.2 Hz, CH_3), 1.20–1.37 (m, 2 H, CH_2), 1.57–1.77 (m, 6 H, CH_2), 2.32–2.45 (m, 4 H, CH_2), 2.59 (br t, 2 H, J = 7.6 Hz, CH_2), 5.51 (s, 2 H, CH_2N), 6.98 (d, 2 H, J = 7.8 Hz, Ar), and 7.28 (d, 2 H, J = 7.8 Hz, Ar); IR (KBr) 3440 (br), 3300–2400 (br), 2206, 1696, 1265, and 1149 cm^{-1}; FABMS calcd for $C_{22}H_{25}{}^{35}ClN_3O_2$ 398.1635, found 398.1634, M^+ + 1; m/e 400 (M^+ + 1, ^{37}C).

2-Butyl-4-chloro-1-[[4-(2-cyano-1-cyclopenten-1-yl)phenyl]methyl]-1*H*-imidazole-5-carboxylic Acid (17a). The procedure used for the preparation of **17b** was repeated with **16a** to afford **17a** (93%) as a white crystalline material after crystallization from CH_2Cl_2/hexane: mp 158.0–160.0 °C; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3 H, J = 7.3 Hz, CH_3), 1.25–1.43 (m, 2 H, CH_2), 1.63–1.74 (m, 2 H, CH_2), 2.03–2.15 (m, 2 H, CH_2), 2.63 (br t, 2 H, J = 7.7 Hz, CH_2), 2.79–2.96 (m, 4 H, CH_2), 5.58 (s, 2 H, CH_2N), 7.04 (d, 2 H, J = 8.2 Hz, Ar), and 7.69 (d, 2 H, J = 8.2 Hz, Ar); IR (KBr) 3430 (br), 3300–2400 (br), 2202, 1663, and 1273 cm^{-1}; FABMS calcd for $C_{21}H_{23}{}^{35}ClN_3O_2$ 384.1479, found 384.1486, M^+ + 1; m/e 386 (M^+ + 1, ^{37}Cl). Anal. ($C_{21}H_{22}ClN_3O_2$) C, H, N.

2-Butyl-4-chloro-1-[[4-(2-cyano-1-cycloheptен-1-yl)phenyl]methyl]-1*H*-imidazole-5-carboxylic Acid (17c). The procedure used for the preparation of **17b** was repeated with **16c** to afford **17c** (90%) as a white crystalline material after crystallization from toluene/hexane: mp 74.0–75.5 °C; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7.3 Hz, CH_3), 1.25–1.41 (m, 2 H, CH_2), 1.58–1.73 (m, 6 H, CH_2), 1.80–1.91 (m, 2 H, CH_2), 2.51–2.69 (m, 6 H, CH_2), 5.54 (s, 2 H, CH_2N), 6.99 (d, 2 H, J = 8.1 Hz, Ar), and 7.32 (d, 2 H, J = 8.1 Hz, Ar); IR (KBr) 3425–2500 (br), 2206, 1698, 1265, and 1150 cm^{-1}; FABMS calcd for $C_{23}H_{27}{}^{35}ClN_3O_2$ 412.1792, found 412.1780, M^+ + 1; m/e 414 (M^+ + 1, ^{37}Cl). Anal. ($C_{23}H_{26}ClN_3O_2$) C, H, N.

2-Butyl-4-chloro-1-[[4-[2-(2*H*-tetrazol-5-yl)-1-cyclopenten-1-yl]phenyl]methyl]-1*H*-imidazole-5-carboxylic Acid (4a). A stirred mixture of **17a** (170 mg, 0.440 mmol) and tributyltin azide (4.8 mL) was heated at 85 °C under argon atmosphere for 5 days. After the mixture was cooled to ambient temperature, it was added with stirring to 30 mL of ice-cold, dry methanol saturated with HCl gas. The resulting mixture was stirred for 10 min before it was concentrated in vacuo. The residual oil was diluted in ca. 15 mL of EtOAc/hexane (1:1) to cause precipitation. The precipitate was filtered off and subjected to reverse-phase chromatography (gradient 40% CH_3CN/H_2O to 70% CH_3CN/H_2O) to give **4b** (71.0 mg, 37%) as a white solid after crystllization from THF/Et_2O: mp 123.0–126.0 °C; ^1H NMR (CD_3OD) δ 0.83 (t, 3 H, J = 7.3 Hz, CH_3), 1.21–1.35 (m, 2 H, CH_2), 1.49–1.60 (m, 2 H, CH_2), 2.07–2.18 (m, 2 H, CH_2), 2.62 (br t, 2 H, J = 7.7 Hz, CH_2), 2.92–3.02 (m, 4 H, CH_2), 5.61 (s, 2 H, CH_2N), 6.96 (d, 2 H, J = 8.1 Hz, Ar), and 7.15 (d, 2 H, J = 8.1 Hz, Ar); IR (KBr) 3600–2400 (br), 1700, 1596, 1523, 1411, and 1264 cm^{-1}; FABMS calcd for $C_{21}H_{24}{}^{35}ClN_6O_2$ 427.1649, found 427.1630, M^+ + 1; m/e 429 (M^+ + 1, ^{37}Cl).

2-Butyl-4-chloro-1-[[4-[2-(2*H*-tetrazol-5-yl)-1-cyclohexen-1-yl]phenyl]methyl]-1*H*-imidazole-5-carboxylic Acid (4b). A stirred mixture of **17b** (500 mg, 1.26 mmol) and tributyltin azide (1.7 mL) was heated at 80 °C under argon atmosphere for 5 days. The mixture was cooled to ambient temperature and added with stirring to 40 mL of ice-cold, dry Et_2O saturated with HCl gas, and the resulting suspension was stirred for 1 h. After filtration, the solid precipitate was taken up in triethylamine (237 μL, 1.70 mmol) and subjected to flash chromatography (gradient EtOAc to 5% HOAc/EtOAc) to give **4b** (405 mg, 73%) as a white solid after crystallization from THF/Et_2O/hexane: mp 126.0–129.0 °C; ^1H NMR (CD_3OD) δ 0.84 (t, 3 H, J = 7.2 Hz, CH_3), 1.20–1.35 (m, 2 H, CH_2), 1.43–1.58 (m, 2 H, CH_2), 1.86 (br s, 4 H, CH_2), 2.50 (br s, 4 H, CH_2), 2.58 (br t, 2 H, J = 7.7 Hz, CH_2), 5.59 (s, 2 H, CH_2N), 6.89 (d, 2 H, J = 7.9 Hz, Ar), and 6.97 (d, 2 H, J = 7.9 Hz, Ar); IR (KBr) 3600–2400 (br), 1703, and 1268 cm^{-1}; FABMS calcd for $C_{22}H_{26}{}^{35}ClN_6O_2$ 441.1806, found 441.1798, M^+ + 1; m/e 443 (M^+ + 1, ^{37}Cl).

2-Butyl-4-chloro-1-[[4-[2-(2*H*-tetrazol-5-yl)-1-cycloheptен-1-yl]phenyl]methyl]-1*H*-imidazole-5-carboxylic Acid (4c). The procedure used for the preparation of **4b** was repeated with **17c** to give **4c** (31%) as a white solid after crystallization from EtOAc/Et_2O: mp 203 °C dec; ^1H NMR (CD_3OD) δ 0.84 (t, 3 H, J = 7.3 Hz, CH_3), 1.20–1.35 (m, 2 H, CH_2), 1.40–1.55 (m, 2

H, CH_2), 1.64-1.80 (m, 4 H, CH_2), 1.86-2.00 (m, 2 H, CH_2), 2.53 (br t, 2 H, J = 7.6 Hz, CH_2), 2.63-2.78 (m, 4 H, CH_2), 5.63 (s, 2 H, CH_2N), and 6.84-6.92 (m, 4 H, Ar); IR (KBr) 3600-2400 (br), 1682, 1209, and 1145 cm^{-1}; FABMS calcd for $C_{23}H_{27}{}^{35}ClKN_6O_2$ 493.1521, found 493.1511, $M^+ + K$; FDMS m/e 455 ($M^+ + 1$, ^{35}Cl) and 457 ($M^+ + 1$, ^{37}Cl).

Acknowledgment. Physical chemistry data were furnished by the staff of our Chemistry and Biotechnology Department. Dr. Roger G. Harrison gave essential encouragement at the initiation of the chemistry for this project. For helpful discussion, we acknowledge Ms. N. G. Stevens, Dr. C. Hansch, and Dr. A. Leo.

Registry No. **3a**, 141754-22-5; **3b**, 141754-23-6; **3c**, 141754-24-7; **4a**, 141754-25-8; **4b**, 141754-26-9; **4c**, 141754-27-0; **5a**, 2941-29-9; **5b**, 4513-77-3; **5c**, 7391-45-9; **6a**, 141754-28-1; **6b**, 141754-29-2; **6c**, 141754-30-5; **8a**, 141754-31-6; **8b**, 141754-32-7; **8c**, 141754-33-8; **9a**, 141754-34-9; **9b**, 141754-35-0; **9c**, 141754-36-1; **10a**, 141754-37-2; **10b**, 141754-38-3; **10c**, 141754-39-4; **11**, 79047-41-9; **12**, 137582-52-6; **13a**, 141754-40-7; **13b**, 141754-41-8; **13c**, 141754-42-9; **14a**, 141754-43-0; **14b**, 141754-44-1; **14c**, 141754-45-2; **15a**, 141754-46-3; **15b**, 141754-47-4; **15c**, 141754-48-5; **16a**, 141754-49-6; **16b**, 141754-50-9; **16c**, 141754-51-0; **17a**, 141754-52-1; **17b**, 141754-53-2; **17c**, 141754-54-3.

Metabolism of 5-Hydroxytryptamine by Brain Synaptosomes and Microsomes in the Presence of Cysteine and Glutathione

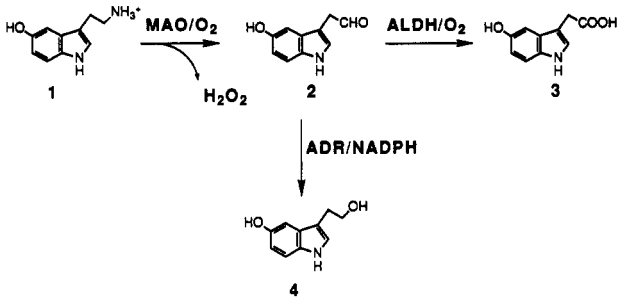
Satendra Singh and Glenn Dryhurst*

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019. *Received June 28, 1991*

Incubation of 5-hydroxytryptamine (1) with L-cysteine and pig or bovine brain microsomes and an NADPH-generating system or with synaptosomes results in the rapid formation of the (2*R*,4*R*)- and (2*S*,4*R*)-epimers of 2-[(5-hydroxy-1*H*-indol-3-yl)methyl]thiazolidine. Incubation of 1 and glutathione under the same experimental conditions yields the (2*R*,4*R*)- and (2*S*,4*R*)-epimers of α-amino-4-[[(carboxymethyl)amino]carbonyl]-2-[(5-hydroxy-1*H*-indol-3-yl)methyl]-δ-oxo-3-thiazolidinepentanoic acid. These various thiazolidine derivatives are formed by nucleophilic addition of the thiol residues of cysteine or glutathione to the aldehyde group of 5-hydroxyindole-3-acetaldehyde (2), the primary product of the monoamine oxidase-mediated oxidative deamination of 1. The facile reaction of cysteine and glutathione with 2 might represent a mechanism designed to scavenge the biogenic aldehyde and therefore to prevent its alkylation of key intraneuronal protein nucleophiles.

The major catabolic pathway for the indolic neurotransmitter 5-hydroxytryptamine (1; serotonin) in the central nervous system (CNS) derives from the action of monoamine oxidase (MAO; EC 1.4.3.4) which catalyzes the oxidative deamination of the indolamine to give 5-hydroxyindole-3-acetaldehyde (2) (Scheme I).[1] It appears to be generally accepted that 2 is further metabolized primarily by a reaction catalyzed by aldehyde dehydrogenase (ALDH; EC 1.2.1.3) to 5-hydroxyindole-3-acetic acid (3). A minor pathway involves reduction of 2 by aldehyde reductase (ADR; EC 1.1.1.2) to 5-hydroxytryptophol (4). The aldehyde residue of 2 is an electrophilic center which would be expected to undergo facile reactions with cellular nucleophiles. Indeed, suggestions have been made that 2 reacts with nucleophilic residues associated with neuronal macromolecules including membrane proteins,[2-4] although the resulting adducts have not been isolated and characterized. Nevertheless, such reactions in vivo might provide an explanation[3,5] for the low excretion of 3 following administration of 1.[6] However, defense mechanisms exist within neurons to protect against such electrophilic insult. For example, cysteine and glutathione are protective nucleophiles which occur in relatively high concentrations within neurons. Intraneuronal concentrations of glutathione have been estimated to be in the range 0.9-3.4 mM[7,8] whereas cysteine occurs at somewhat lower concentrations (ca. 0.1 mM).[9,10] Accordingly, under metabolic conditions where 2 is not rapidly converted into 3 and 4, it might be anticipated that the aldehyde is scavenged (conjugated) by glutathione and/or cysteine. Recently, Susilo et al.[11,12] reported that incubation of tryptamine with brain homogenates (pig, bovine, rat) resulted in the formation of a new metabolite, (4*R*)-2-(3′-indolylmethyl)-1,3-thiazolidine-4-carboxylic acid.

Scheme I



This compound was thought to be formed as a result of the reaction between indole-3-acetaldehyde, the MAO-

* Corresponding author.

(1) Udenfriend, S.; Titus, E.; Weissbach, H.; Peterson, R. E. Biogenesis and metabolism of 5-hydroxyindole compounds. *J. Biol. Chem.* **1956**, *219*, 335-344.
(2) Nilsson, G. E.; Tottmar, O. Biogenic aldehydes in brain: On their preparation and reactions with rat brain tissue. *J. Neurochem.* **1987**, *48*, 1566-1572.
(3) Alivisatos, S. G. A.; Ungar, F. Incorporation of radioactivity from labeled serotonin and tryptamine into acid-insoluble material from subcellular fractions of brain. I. The nature of the substrate. *Biochemistry* **1968**, *7*, 285-292.
(4) Ungar, F.; Alivisatos, S. G. A. Subsynaptosomal distribution, inhibition and characterization of the binding of [^{14}C]-5-hydroxyindoleacetaldehyde to brain preparation. *Res. Commun. Chem. Pathol. Pharmacol.* **1976**, *13*, 449-464.
(5) Nakai, K. Serotonin metabolism in herbivores. *Nature* **1958**, *181*, 1734-1735.
(6) Erspamer, V. Fate of indolealkylamines in the organism. *J. Physiol.* **1955**, *127*, 118-133.
(7) Mesina, J. E.; Page, R. H.; Hetzel, F. W.; Chopp, M. Administration of L-2-oxothiazolidine-4-carboxylate increases glutathione levels in rat brain. *Brain Res.* **1989**, *478*, 181-183.
(8) Slivka, A.; Mytilinou, C.; Cohen, G. Histochemical evaluation of glutathione in brain. *Brain Res.* **1987**, *409*, 275-284.

0022-2623/92/1835-2667$03.00/0