H-Val-OH, 72-18-4; BOC-Val-OH, 13734-41-3; p-$HOC_6H_4CH_2CH_2COOH$, 501-97-3; BOC-Tyr(Cl_2Bn)-OH, 40298-71-3; BOC-Arg(Tos)-OH, 13836-37-8; BOC-Sar-OH, 13734-36-6; angiotensin II, 11128-99-7.

Supplementary Material Available: Tables 1-5 containing final fractional coordinates, temperature parameters, bond distances, and bond angles (9 pages). Ordering information is given on any current masthead page.

Conformationally Restricted Polysubstituted Biphenyl Derivatives with Angiotensin II Receptors Antagonist Properties

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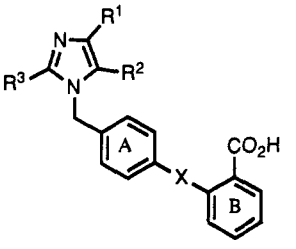
The synthesis and in vitro activity of new nonpeptide angiotensin II antagonists is presented. Compared to previously reported biphenyl compounds, the new analogues **8** and **9** have reduced conformational freedom derived from steric hindrance. Methyl 4'-methy-2',6'-dimethoxy[1,1'-biphenyl]-2-carboxylate **4** has been synthesized by a Von Pechmann condensation of orcinol with oxocyclohexane-2-carboxylate followed by dehydrogenation. This scheme provided the carbon skeleton of the biphenyl potentially substituted on the 2-, 2'-, 4'-, and 6'-positions. Elaboration of the subsituents led to a biphenyl derivative used to alkylate a 2-*n*-butyl-4-chloro-5-(hydroxymethyl)imidazole. After coupling with the imidazole two regioisomers were separated and identified by ^{1}H NMR. NOESY experiments were useful to establish regiochemistry of the final products that have angiotensin II blocking activity. Their affinity for angiotensin II receptors was established in a binding assay experiment and in an isolated organ test. The presence of 2',6'-dimethoxy substituent on the biphenyl moiety of the antagonist was found to significantly decrease affinity for the receptor.

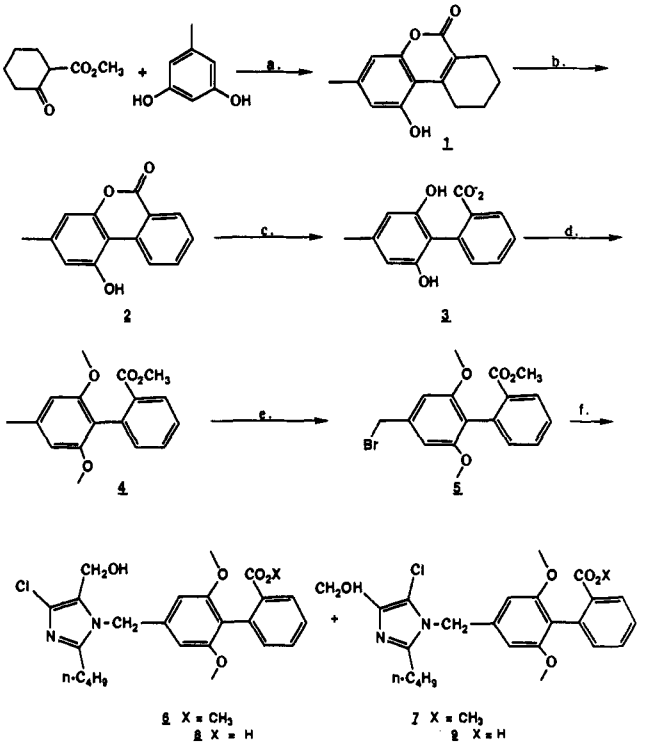
Recently, a series of *N*-(benzamidobenzyl)imidazole (formula I; X = CONH) and related compounds endowed with AII receptor antagonistic properties have been reported.[1] SAR studies revealed that the presence of ortho



FORMULA I

substituent on the benzamide ring (ring B) produces an enhancement of affinity toward the receptor. This observation has been related to steric hindrance resulting in added conformational rigidity favoring the active conformation.[1] The report that imidazoles alkylated with a biphenyl-2-carboxylic acid[2] (formula I; X = single bond) were also receptor antagonists of the peptidic hormone angiotensin II prompted us to explore the effect of ortho substituents at positions 2' and 6' of the A ring in biphenyl derivatives. Such substitution would introduce steric interactions between the 2,2'- and 6,6'-positions, which would hinder rotation of the phenyl rings with respect to each other and favor a twisted conformation for the biphenyl moiety. This phenomenon has been amply documented in many related cases.[3] We decided to probe the effect of such structural changes on the biological activity of the AII antagonist series. Many syntheses of biaryls rely on the coupling of two aromatic precursors.[4] Widely useful procedures involve, i.a., Ullman,[5] Gomberg-Bachmann,[6] Meyers,[7] and Snieckus[8] reactions and each of these have advantages and limitations. Other methods have also been

Scheme I[a]



[a] Reagents and conditions: (a) $H_2SO_4/POCl_3$, 25 °C, 24 h; (b) Pd/C, 300 °C, 40 min; (c) $NaOH/H_2O$, MeOH, reflux, 3 h; (d) Dimethyl sulfate, 25 °C, 20 h (32% yield); (e) NBS, AIBN, CCl_4, reflux, 3 H; (f) imidazole; tButOK, DMF; 2.5 N NaOH.

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(1) 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.

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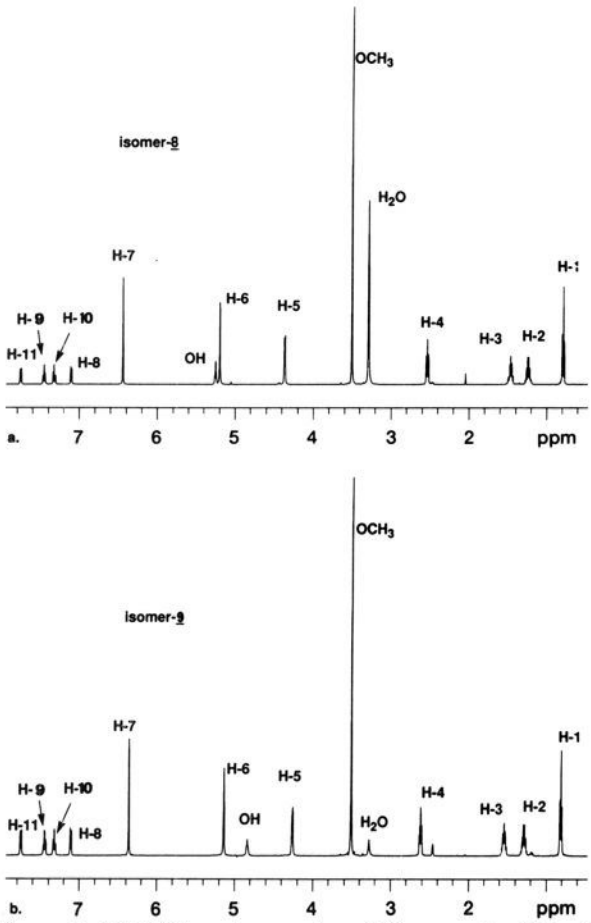

Figure 1. 500-MHz proton spectra of (a) a sample of 50 mM 4′-[[2-butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazolyl]-methyl]-2′,6′-dimethoxy-2-biphenylcarboxylic acid (**8**) and (b) a sample of 50 mM 4′-[[2-butyl-5-chloro-4-(hydroxymethyl)-1*H*-imidazolyl]methyl]-2′,6′-dimethoxy-2-biphenylcarboxylic acid (**9**), both in deuterated dimethyl sulfoxide.

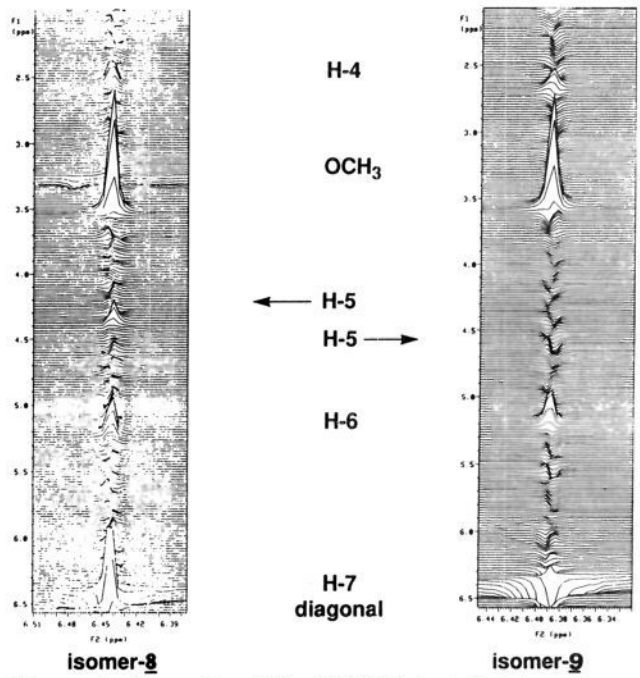

Figure 2. Expansion of the NOESY stacked-plot spectrum showing the cross-peaks for the H-7 protons of both isomers. The cross-relaxation with the hydroxymethyl protons (H-5) establishes the regiochemistry of the substituents 4,5 on the imidazolyl ring of isomer **9**. This particular cross peak is missing for isomer **8**, while all other cross-relaxation for H-7 are very similar. The cross-peaks are anti-phase with respect to the diagonal peak at 6.4 ppm as in expected for small molecules where $\omega\tau_c \ll 1$.[16] The mixing time was 1.5 s with a relaxation delay of 10.5 s ($\sim 5 \times T_1$). Similar cross-relaxation interactions were observed with a 0.5-s mixing time.

reported that are more limited in scope, including the addition of aryl carbanions to aryne[9] and electrochemical and photochemical coupling.[10] The choice of reaction is severely limited when one wishes to obtain unsymmetrical biphenyl.

We focused our effort on exploring a short and new approach to a 2′,6′-dimethoxybiphenyl-2-carboxylic acid as described below.

Chemistry

The 1-hydroxy-3-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (**2**) was obtained as previously described via a Von Pechmann condensation of orcinol with oxocyclohexane-2-carboxylate followed by aromatization.[11,12] Opening of the pyrone ring

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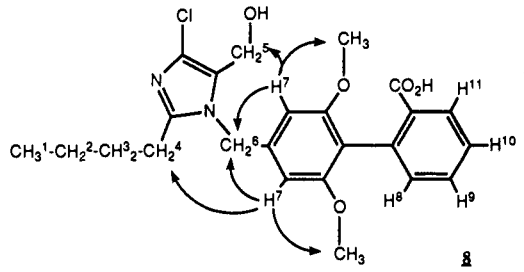
Table I. Affinities of the Biphenyl Derivatives 8–10 for Angiotensin II Receptors As Measured by Their IC_{50} (Binding to Rat Uterine Membranes) and pA_2 Values (Rabbit Aorta Rings)

no	R^1	R^2	formula,[a] MW	mp, °C	IC_{50},[b,c] μM	pA_2[d]
8	Cl	CH_2OH	$C_{24}H_{27}N_2O_5Cl$, 459.2	201–202	1.9, 120	nd
9	CH_2OH	Cl	$C_{24}H_{27}N_2O_5Cl$, 459.2	175–177	1.6, 340	5.5
10	Cl	CH_2OH	$C_{22}H_{23}N_2O_3Cl$, 398.9	168–170[e]	0.17,[f] 33	7.1

[a] New compounds were identified by a combination of their spectroscopic data and confirmed by exact mass measurement and/or analysis for C, H, N within ±0.4% of the theoretical values. [b] Concentration of the test compound inhibiting specific binding of labeled AII by 50% was derived from analysis of plots of the percentage of specific binding vs the log concentration of the compound (at least 10 concentrations ranging over 5 orders of magnitude were used). Data are derived from one experiment performed with doses in duplicate. [c] The IC_{50} values for each receptor separately were obtained by the methodology described more in details in ref 18. [d] Antagonism of AII-induced contraction of rabbit aortic rings (ref 18). [e] Lit. 168–169.5 °C (see ref 18b). [f] An IC_{50} of 0.28 μM was reported for displacement of [^{3}H]AII from its specific binding sites in a rat adrenal cortical microsome preparation (see ref 18b).



8

Figure 3. In the depicted isomer 8, the arrows indicate the NOE's observed between the protons 7 at the 3′,5′-positions of the biphenyl and the various protons in close spatial proximity.[14] Not indicated on this scheme are the arrows indicating cross-relaxations between the protons at the benzylic hinge (H6) and those from the butyl chain (H4 at ~2.5 ppm), from the hydroxymethylene substituent (H5 at ~4.35 ppm).

followed by methylation in basic aqueous medium with dimethyl sulfate resulted in the expected biphenyl ester 4 along with some C-alkylation products on the 3′- and 5′-positions. The methyl 4′-methyl-2′,6′-dimethoxybiphenyl-2-carboxylate (4) was purified by chromatography and was obtained in 32% yield from 2. Selective radical bromination at the 4′-methyl was achieved with *N*-bromosuccinimide in refluxing carbon tetrachloride in the presence of AIBN to give 5. A small amount of geminal dibrominated compound (~10%) was also generated during this procedure. The coupling between the anion of 2-butyl-4(5)-chloro-5(4)-(hydroxymethylene)imidazole[13] and the bromide 5 produced two isomers 6 and 7.[1] These could be separated by chromatography on silica gel, and the structures of the corresponding regioisomeric acids 8 and 9, obtained by saponification, were elucidated on the basis of completely analyzed ^{1}H NMR (Figures 1a and 1b) and of 2D NOE (NOESY) experiments at 500 MHz.[14]

Figure 2 shows the cross-relaxation pathways[15] for the acids 8 and 9 derived from the two ester isomers. Examination of the NOESY spectrum for isomer 8 reveals strong cross-peaks between H-7 and H-6, H-4, H-5 and the methyl protons from the methoxy group (Figure 2).[16] Likewise, H-6 has significant cross-relaxation with H-4 and H-5 as indicated in Figure 3. We interpret these cross-relaxation interactions as establishing the regiochemistry of this isomer. The hydroxymethyl group is at the 5-position of the imidazole nucleus. In the NOESY spectrum of the second acid isomer, 9, the cross-peak corresponding to the cross-relaxation between H-5 and H-7 is missing while all the other cross-relaxation for H-7 are present with a similar intensity (Figure 2).

Biology

Compounds 8 and 9 were evaluated for specific binding to the AII receptors (IC_{50}) and antagonism of AII-induced contraction of rabbit aorta rings (pA_2).[17] Compound 10 (see ref 18a) was tested in the same assays for reference purpose. The first test is a binding assay determining the ability of a compound to displace ^{125}I-labeled angiotensin II from its receptor of a rat uterus membrane preparation.[18] The IC_{50} values resulting from that assay are listed in Table I. The IC_{50} value for AII was 2.2 nM. All compounds produced a biphasic displacement curve indicating the presence of high-affinity (80%) and low-affinity (20%) binding sites. Recent studies using selective AII receptor ligands have revealed that there are two AII receptor subtypes in various target tissues.[19,20] From structure–activity studies, binding to the high affinity site correlates with AII antagonist activity. Peptides[21] and nonpeptides[22] have been identified that bind selectively to the low-af-

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finity AII receptor population.

The ability of compounds 8 and 10 to antagonize the contraction of smooth muscles stimulated by angiotensin II was tested on isolated rabbit aorta rings.[18] From this assay, pA_2 values are derived, which are listed in Table I. The antagonistic effect was competitive and reversible. No agonistic effect was observed.

Conclusion

We have reported an original approach of the conversion of a pyrone into a biphenyl derivative. Elaboration of the substituents allowed the preparation of conformationally restricted AII antagonist candidates.

The biological results obtained for the new compounds 8 and 9 indicate that they are selective ligands for the subclass of AII receptors involved in vascular contractile activity and blood pressure regulation. The 2',6'-dimethoxybiphenyl-2-carboxylic acid derivatives are moderately potent competitive antagonists. Their affinity for the AII receptor was significantly lower than that of the unsubstituted reference compound 10.

In the case reported for the benzamidobenzyl derivatives (formula I; X = CONH), ortho substitution to the amide bond was hypothesized to force ring B out of the plane of the amide as a consequence of steric hindrance and increase the amide bond's rotational barrier. This more rigid conformation was speculated to be the reason behind the increase in binding affinity of 1 order of magnitude. In contrast, forcing ring B out of the plane and increasing the rotational barrier between rings A and B of the biphenyl derivative (formula I; X = single bond) did result in a decrease in binding affinity of 1 order of magnitude. It is not known at this time whether this reflect the inability for 8 and 9 to achieve an ideal active conformation or if the extra volume associated to the methoxy substituents (positioned on the A ring) interfere with binding to the receptor.

Experimental Section

The reagents and solvents were commercially available (Aldrich Chemical Co.) and of synthetic grade. The 2-*n*-butyl-4-(hydroxymethyl)-5-chloroimidazole and 10 were obtained via known syntheses.[2,13] Analytical TLC plates and silicagel (230–400 mesh) were purchased from EM Reagents. Melting points were taken using a Mettler FP80/81 apparatus and are uncorrected. ^{1}H NMR spectra were routinely obtained on a Varian VXR-300 300 MHz in $CDCl_3$, DMSO-d_6, or CD_3OD. Mass spectra were obtained using a Finnigan MAT 90 or a VG Model 250T spectrometer with either DCI or FAB ionization. Phase-sensitive NOESY spectra were collected at 500 MHz on a Varian VXR-500S spectrometer. The sample (15 mg in 0.75 mL of DMSO-d_6) was degassed using the freeze–thaw method. The NOESY spectra were collected at 30 °C using a 340 × 4096 hypercomplex data set and were zero-filled to 4096 × 4096 data points. Elemental analysis for C, H, N was obtained from Galbraith Laboratories, Inc.

1-Hydroxy-3-methyl-7,8,9,10-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-6-one (1) and 1-hydroxy-3-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (2) were obtained as previously described[11] except for the aromatization step, which was carried on with Pd/C in a manner analogous to that described in ref 12. The overall yield was 25%.

4'-Methyl-2',6'-dimethoxy[1',1'-biphenyl]-2-carboxylic Acid, Methyl Ester (4). 1-Hydroxy-3-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (MW = 226; 3 g, 13.2 mmol) was dissolved in 50 mL of NaOH (30%) and 30 mL of methanol. The mixture was heated at reflux for 3 h. The methanol was evaporated under vacuum, and after the mixture was cooled to 25 °C, 10 mL of dimethyl sulfate was added at once, followed by 30 mL, dropwise. After the reaction mixture was stirred at 25 °C for 16 h, the pH was still basic. The reaction mixture was treated with an additional 10 mL of dimethyl sulfate and stirred at 25 °C for an additional 6 h. Sodium bicarbonate was added to pH 7, and the reaction mixture extracted with ethyl acetate. Examination of the extract by TLC (silica gel, 10% ethyl acetate in hexane) indicated two major products and one minor, which were separated and purified on a silica gel column. The desired product (1.24 g, 32% yield), as identified by NMR and MS, eluted last (R_f = 0.4) and had a melting point of 66–68 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): 7.95 (d, 1 H, J = 8 Hz), 7.55 (t, 1 H, J = 8 Hz), 7.35 (m, 2 H), 6.5 (s, 2 H), 3.7 (s, 6 H), 3.65 (s, 3 H), 2.4 (s, 3 H). FABMS ($C_{17}H_{18}O_4$, calcd M + H$^+$, found): 286.3, 286. Elemental Anal. (C, H).

4'-(Bromomethyl)-2',6'-dimethoxy[1,1'-biphenyl]-2-carboxylic Acid, Methyl Ester (5). An aliquot (1.24 g, 4.4 mmol) of the above material was dissolved in 125 mL of refluxing CCl_4 and treated with 266 mg of NBS and 65 mg of AIBN. After the reaction mixture was refluxed for 3 h, ^{1}H NMR analysis indicated bromination of material. After removal of succinimide by filtration, the product was crystallized from ethyl acetate/hexane. The first crop afforded 850 mg of material (mp 88.5–88.8 °C) that was 80% pure desired compound by NMR. No further attempts were made to remove the major contaminant, the 4',4'-dibrominated material. ^{1}H NMR ($CDCl_3$, 300 MHz): 7.98 (d, 1 H, J = 8 Hz), 7.55 (t, 1 H, J = 8 Hz), 7.4 (t, 1 H, J = 8 Hz), 7.3 (d, 1 H, J = 8 Hz), 6.65 (s, 2 H), 4.55 (s, 2 H), 3.75 (s, 6 H), 3.65 (s, 3 H).

4'-[[2-Butyl-4(5)-chloro-5(4)-(hydroxymethyl)-1*H*-imidazolyl]methyl]-2',6'-dimethoxy[1,1'-biphenyl]-2-carboxylic Acid, Methyl Ester. In a flask under a nitrogen atmosphere was dissolved 500 mg of 2-butyl-4-(hydroxymethyl)-5-chloroimidazole (2.7 mmol) in 25 mL of dimethylformamide. To the solution at 25 °C was added 2.7 mL of potassium *tert*-butoxide, 1 N in THF. After the resulting mixture was stirred at the same temperature for 15 min, 1.2 g of the 4'-(bromomethyl)-2',6'-dimethoxy-2-biphenylcarboxylic acid methyl ester (80% pure, 2.7 mmol) was added at once. The reaction mixture was allowed to stir for 16 h at 25 °C and concentrated in vacuo to an oil that was partitioned between water and ethyl acetate. The organic phase after drying and concentration was chromatographed on a silica gel column (50 g of SiO_2, 0.040–0.063 particle size) eluted first with 10% ethyl acetate in chloroform to yield 6 (400 mg; 32%) and then with pure ethyl acetate to elute the second isomer 7 (190 mg; 17%). ^{1}H NMR (6) ($CDCl_3$, 300 MHz): 7.95 (d, 1 H, J = 8 Hz), 7.55 (t, 1 H, J = 8 Hz), 7.4 (t, 1 H, J = 8 Hz), 7.25 (d, 1 H, J = 8 Hz), 6.25 (s, 2 H), 5.25 (s, 2 H), 4.55 (s, 2 H), 3.65 (s, 3 H) 3.6 (s, 6 H), 2.65 (t, 2 H, J = 7 Hz), 1.7 (m, 2 H), 1.4 (m, 2 H), 0.9 (t, 3 H, J = 7 Hz). ^{1}H NMR (7) ($CDCl_3$, 300 MHz): 7.95 (d, 1 H, J = 8 Hz), 7.55 (t, 1 H, J = 8 Hz), 7.4 (t, 1 H, J = 8 Hz), 7.25 (d, 1 H, J = 8 Hz), 6.25 (s, 2 H), 5.15 (s, 2 H), 4.6 (s, 2 H), 3.6 (s, 6 + 3 H), 2.65 (t, 2 H, J = 7 Hz), 1.7 (m, 2 H), 1.35 (m, 2 H), 0.9 (t, 3 H, J = 7 Hz).

4'-[[2-Butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazolyl]methyl]-2',6'-dimethoxy[1,1'-biphenyl]-2-carboxylic Acid (8). The isomer 6 methyl ester obtained above was stirred for 20 h at 25 °C in a mixture of 20 mL of methanol and 20 mL of 2.5 N sodium hydroxide. The methanol was eliminated in vacuo, and water (10 mL) was added. The pH was adjusted to 4 by addition of 1 N hydrochloric acid. The resulting white precipitate was collected by filtration and dried (330 mg; 80% yield; mp 175.7–176.6 °C). ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 12.3 (s, 1 H), 7.76 (d, 1 H, J = 8 Hz), 7.45 (t, 1 H, J = 8 Hz), 7.38 (t, 1 H, J = 8 Hz), 7.25 (d, 1 H, J = 8 Hz), 6.45 (s, 2 H), 5.25 (t, 1 H, J = 3 Hz), 5.2 (s, 2 H), 4.38 (d, 2 H, J = 3 Hz), 3.5 (s, 6 H), 2.55 (t, 2 H, J = 7 Hz), 1.5 (m, 2 H), 1.25 (m, 2 H), 0.8 (t, 3 H, J = 7 Hz). HRMS (calcd for $C_{24}H_{27}N_2O_5Cl$, found): 459.1687 (M + H monoisotropic), 459.1734. Elemental Anal. (C, H, N).

4'-[[2-Butyl-4-(hydroxymethyl)-5-chloro-1*H*-imidazolyl]methyl]-2',6'-dimethoxy[1,1'-biphenyl]-2-carboxylic Acid (9). By a similar procedure, compound 7 (90 mg) was hydrolyzed in a mixture of 5 mL of methanol and 5 mL of 2.5 N sodium hydroxide. The methanol was eliminated in vacuo, and water (5 mL) was added. The pH was adjusted to 4 by addition of 1 N hydrochloric acid. The resulting white precipitate was collected by filtration and dried (50 mg; 62% yield; mp 201.3–201.5 °C). ^{1}H NMR (DMSO-d_6, δ ppm): 12.1 (s, 1 H), 7.8 (d, 1 H, J = 8 Hz), 7.5 (t, 1 H, J = 8 Hz); 7.12 (t, 1 H, J = 8 Hz), 7.18 (d, 1 H, J = 8 Hz), 6.4 (s, 2 H), 5.2 (s, 2 H), 4.9 (t, 1 H, J_{HH} = 3 Hz), 4.3 (d, 2 H, J_{HH} = 3 Hz), 3.55 (s, 6 H), 2.55 (t, 2 H, J = 7 Hz), 1.6 (m, 2 H), 1.35 (m, 2 H), 0.85 (t, 3 H, J = 7 Hz). HRMS (calcd for

$C_{24}H_{27}N_2O_5Cl$, found): 459.1687 (M + H monoisotopic), 459.1725. Elemental Anal. (C, H, N).

Biology. Uteri were removed from Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, DE) weighing 200–250 g; the rate were anesthetized with 80 mg/kg i.p. sodium pentobarbital. The estrous cycles of rats were not controlled. All procedures were at 4 °C. Uteri were scraped free of mucosa and endometrium and homogenized in 20 volumes of ice-cold phosphate-buffered saline containing 5 mM EDTA. The homogenate was centrifuged at 1500*g* for 20 min. and the supernatant was recentrifuged at 100000*g* for 60 min. The pellet was resuspended in buffer consisting of 2 mM EGTA and 50 mM Tris-HCl, pH 7.5, to a final protein concentration of 4 mg/mL. Protein concentration was measured by using the Bio-Rad protein assay kit (Cambridge, MA). The assay for the receptor binding consisted of 0.25 mL of a solution containing 5 mM $MgCl_2$, 2 mM EGTA, 0.5% bovine serum albumin, 50 mM Tris-HCl, pH 7.5, and [^{125}I]Ang II (approximately 105 cpm) in the presence or absence of unlabeled peptide. The reaction was initiated by the addition of 50 μg of membrane protein, and the mixture was incubated at 25 °C for 30 min. The incubation was terminated with ice-cold 50 mM Tris-HCl, pH 7.5, and the mixture was filtered to separate membrane-bound labeled peptide from free ligand. The incubation tubes and filters were washed with ice-cold buffer. Filters were assayed for radioactivity in a Micromedic gamma counter (Micromedic Systems, Horsham, PA). Nonspecific binding was defined as binding in the presence of 10 μM unlabeled Agn II and was 15% or less of total binding. Specific binding was calculated as total binding minus nonspecific binding. Binding data were analyzed by a nonlinear least-squares curve-fitting program.

The compounds were tested for agonist and antagonist activity in rabbit aortic rings. Male New Zealand white rabbits (2–2.5 kg) were sacrificed using an overdose of pentobarbital and exsanguinated via the carotid arteries. The thoracic aorta was removed, cleaned of adherent fat and connective tissue, and then cut into 3-mm ring segments. The endothelium was removed from the rings by gently sliding a rolled-up piece of filter paper into the vessel lumen. The rings were then mounted in a water-jacketed tissue bath, maintained at 37 °C, between a moveable and a fixed stainless steel wire, with the moveable end attached to an FT03 Grass transducer coupled to a Model 7D Grass polygraph for recording isometric force responses. The bath was filled with 20 mL of oxygenated (95% oxygen/5% carbon dioxide) Krebs solution of the following composition (nM): 130 NaCl, 15 $NaHCO_3$, 15 KCl, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, and 11.4 glucose. The preparations were equilibrated for 1 h before approximately 1 g of passive tension was placed on the rings. For the agonist assay, the rings were exposed to increasing concentrations of the test compound, at 30-min intervals, during which time the tissue was washed three times with 20 mL of fresh Krebs solution. For the measurement of antagonistic activity, paired rings from the same rabbits were used: one was exposed to increasing concentrations of AII, (at 30-min intervals), and a second ring was exposed to increasing concentrations of AII in the presence of the test compound, which was added 5 min prior to the addition of AII. The concentration–response curves for AII in the presence of the antagonist were evaluated in terms of the percent of the maximal contraction of the control ring exposed only to AII. pD_2 values for AII were calculated from the AII concentration response curves while pA_2s were determined according to Schild.[18]

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Synthesis and DNA-Binding Properties of Polyamine Analogues

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The synthesis of a series of novel polyamine analogues is reported. The DNA binding of these compounds and a variety of other polyamines were compared with their IC_{50} values against HeLa cell. There seemed to be no apparent correlation between the DNA binding and toxicity against HeLa cells.

Introduction

Studies of the interactions between polyamines and nucleic acids in vitro have been reported,[1] but the biological relevance to in vivo situations is unclear. A growing amount of evidence suggests that polyamines do play a role in both modulation of cellular response to DNA reactive agents[2,3] and in maintenance of chromatin structure.[4] Recently, we reported the synthesis of polyamine analogues possessing antitumor[5,6] or antiprotozoal[7a] activity. The mechanism of action of these compounds is unknown; however, they may act through the displacement of the naturally occurring polyamines from DNA binding sites.

In this study the structure–activity relationships of a series of polyamine analogues toward binding to DNA is examined. If such an interaction is relevant to the antitumor activity of these compounds, one would expect some correlation between DNA-binding ability and growth inhibition, information useful in the design of more effective antitumor agents.

Chemistry

The synthesis of polyamine analogues 8 and 10 with hydroxyl or carbonyl substitution in the aminopropyl moiety followed the route outlined in Scheme I. Heating a mixture of the epoxide 4 and a *N,N'*-dibenzyldiamino-

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