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Studies on 1,2,4-Benzothiadiazine 1,1-Dioxide IX.[1] Synthesis and Pharmacological Evaluation of 1,2,4-Benzothiadiazine 1,1-Dioxide Biphenyl Tetrazoles as Angiotensin II Antagonists

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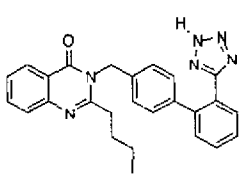
In the course of our investigations on the development of cardiovascular agents, 3-butyl-2-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**2**) was considered as a potential angiotensin II antagonist on the basis of bioisosteric replacement of the quinazoline ring of compound **1** with a 1,2,4-benzothiadiazine 1,1-dioxide ring system. Alkylation of **6** with **4** afforded **7** and **8** in 24% and 28% yields, respectively. An attempt to remove the trityl group of compounds **7** and **8** under acidic condition gave the ring opened products **9** and **11** in 28% and 36% yields, respectively. However, compounds **2** and **10** were obtained in 46% and 85% yields when compounds **7** and **8** were refluxed in methanol. Preliminary assays of compounds **9** and **11** against angiotensin II receptors revealed weak activity with IC_{50} values of 3.6 μM and 5.4 μM, respectively. Compound **10** (IC_{50} = 87 nM) exhibited stronger binding affinity than compound **2** (IC_{50} = 750 nM).

INTRODUCTION

The renin-angiotensin system remains an important target for the development of potential antihypertensive agents.[2] Previous attention has focused on the development of angiotensin converting enzyme (ACE) inhibitors.[3] Although ACE inhibitors are effective for the therapy of a variety of cardiovascular indications such as heart failure and myocardial hypertrophy,[4-5] they also possess side effects such as producing angioneurotic edema and dry coughing, presumably due to bradykinin which is a taregt of ACE as well.[2,6] It is generally believed that compounds which prevent intervention between the primary effector hormone angiotensin II (A II) and its receptor may possess fewer side effects.[2] The recent discovery of losartan as a potent and orally effective angiotensin AT_1-selective A II antagonist has generated significant interest in the search for other nonpeptide A II antagonists bearing novel heterocyclic elements.[7] Thus, a wide variety of A II antagonists have been described, most of which retain the biphenyl-tetrazole substructure present in losartan.[8] A series of quinazolin-4(3*H*)-one derivatives, such as 2-butyl-3-{[2′-(2*H*-tetrazol-5-yl)biphenyl-2-yl]methyl}quinazolin-4(3*H*)-one (**1**), has been recently synthesized and shown to be potent A II antagonists.[9] Since the biphenyl-tetrazole moiety is considered to be an essential acidic functional group for antagonism, in the course of our investigations on the development of cardiovascular agents, 3-butyl-2-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**2**) was considered as a potential angiotensin II antagonist on the basis of bioisosteric replacement of the quinazoline ring of compound **1** with 1,2,4-benzothiadiazine 1,1-dioxide. During the course of this investigation, compound **2** was proposed as an A II antagonist.[10] However, it has never been synthesized and evaluated because of the ex-



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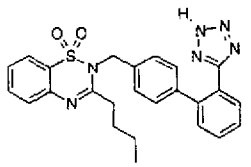

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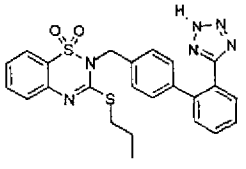

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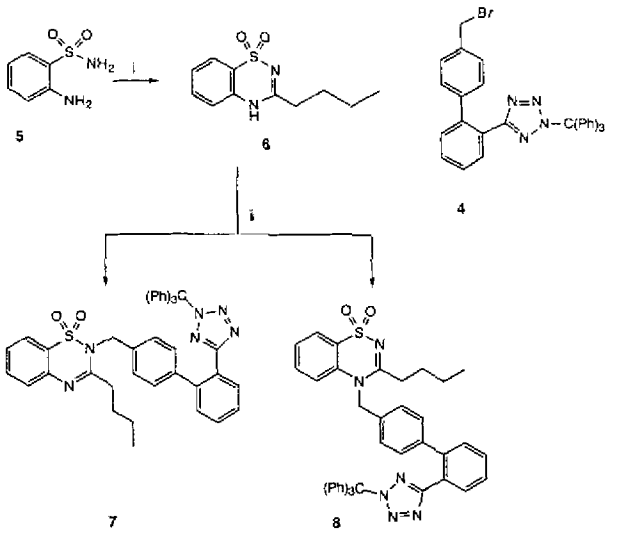
pected hydrolytic decomposition of the 1,2,4-benzothiadiazine 1,1-dioxides ring. Instead, compound **3** was synthesized and found to be a potent A II antagonist.[10] To provide a better understanding of the SAR among the A II antagonists, we synthesized and evaluated compound **2** and its derivatives.

RESULTS AND DISCUSSION

1,2,4-Benzothiadiazine 1,1-dioxide has drawn much attention since the discovery of the clinically useful diuretic activity of chlorothiazide[11] and the antihypertensive activity of diazoxide.[12] The presence of more than one nitrogen atom on this ring system results in a complicated prototropic tautomerism which has been intensively studied by UV,[13] ^{13}C-NMR spectroscopy[14] and Huckel MO calculations.[15] To synthesize compound **2** for the evaluation of its angiotensin AII receptor binding affinity, we were directed toward the synthesis of *N*-(triphenylmethyl)-5-[4′-(bromomethyl)-biphenyl-2-yl]tetrazole (**4**) *via* the approach described by Carini et al.[7] 3-Butyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**6**) was prepared in 68% yield by the condensation of 2-aminobenzenesulfonamide (**5**) with pentanoic acid in the presence of toluenesulfonic acid. Although it is generally accepted that the 4*H*-tautomer of 1,2,4-benzothiadiazine 1,1-dioxide ring system is prefered, alkylation of **6** with **4** in the presence of potassium carbonate at the refluxing temperature of acetone afforded not only the N-2 alkylated product 3-butyl-2-[[2′-(*N*-tritylmethyl)tetrazol-5-yl]-biphenyl-4-yl]methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**7**) in 24% yield but also the N-4 alkylated product 3-butyl-4-[[2′-(*N*-tritylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**8**) in 28% yield. The structural assignment of these two compounds was primarily based on the previous report by Jakobsen and Treppendahl [14] which demonstrated that the chemical shift of the carbon attached to the N-4 position is more deshielded than that of the N-2 position. Thus, the compound with a chemical shift of the benzylic proton of δ 5.08 in the ^{1}H-NMR spectrum and carbon absorption in the ^{13}C-NMR spectra located at δ 50.95 was assigned as compound **8**, whereas the compound with these peaks appearing at δ_H 4.99 and δ_C 46.33 was assigned as compound **7**.

To deprotect the trityl group, compound **7** was treated with 10% hydrochloric acid at room temperature. A single product was isolated, but was found to not be the desired compound **2** on the basis of Mass and NMR spectral data. The ^{1}H-NMR spectrum of the product revealed one set of

Scheme I

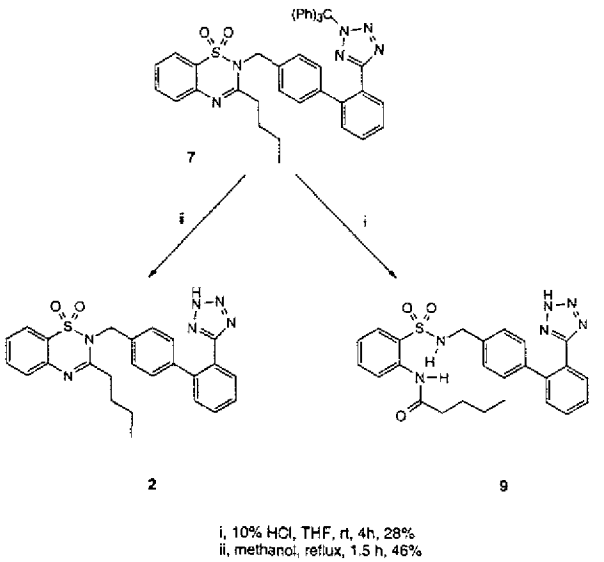


i, pentanoic acid, $CH_3(C_6H_4)SO_3H$, toluene, reflux, 96h (68 %)
ii, 4, K_2CO_3, KI, acetone, reflux, 72h, (7, 24 %), (8, 28%).

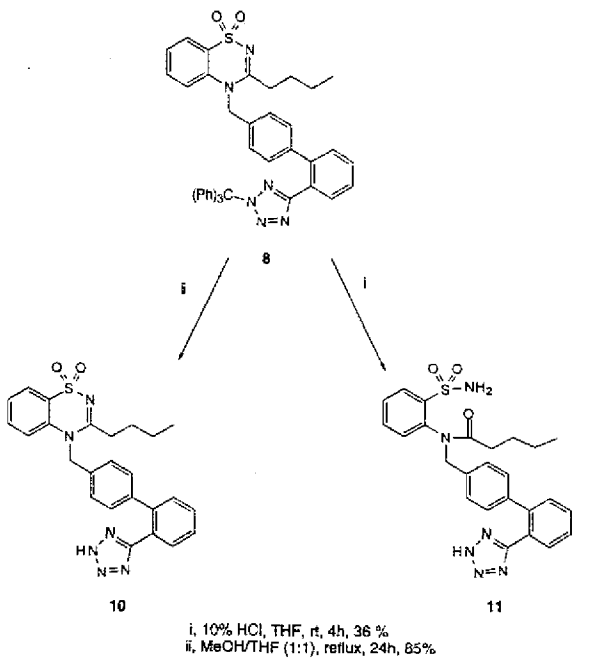
doublet peaks centered at δ 4.01 which corresponded to two benzylic protons. The doublet peaks were coupled to the triplet at δ 8.54 which was assigned to a SO_2NH moiety. Another singlet and D_2O exchangeable proton at δ 9.72 was assigned to (C=O)-NH. The mass spectrum of the product produced a molecular ion peak (M^+) at 491 which was 18 more than the desired compound **2**. This helps explain why an attempt to deprotect the trityl group of the compound under acidic condition not only removed the trityl group but also opened the 1,2,4-benzothiadiazine 1,1-dioxide ring system. The structure of the isolated product (28% yield) was therefore assigned as *N*-(2-butyryl-aminobenzenesulfonyl)-*N*-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methylamine (**9**). Under similar conditions, compound **8** was converted to 2-{*N*-butyryl-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl}aminobenzenesulfonamide (**11**) in 36% yield. On the basis of the ^{1}H-NMR spectrum, the structure of compound **11** was confirmed by the appearance of an exchangeable D_2O and broad singlet centered at δ 7.72 corresponding to two protons, indicating the existence of a SO_2NH_2 moiety. In the IR spectra of compound **11**, the strong carbonyl group absorption at 1635 cm^{-1} was at a lower frequency than in compound **9** (at 1665 cm^{-1}), indicative of a tertiary amide moiety in compound **11**. This lends some support to the structural assignment of compounds **9** and **11**. Interestingly, compounds **2** and **10** were obtained in 46% and 85% yield, respectively, when compounds **7** and **8** were refluxed in methanol.

The target compounds were tested in a radioligand assay using rabbit adrenal glands prepared as described by

Scheme II



Scheme III



Chiu et al.[16] Binding experiments were performed as previously described, using either [^{3}H] Losartan to the AT_1 receptor [16] or [^{125}I] CGP-42112A to the AT_2 receptor as the angiotensin-II radioligands.[17] The results (IC_{50} values) are shown in Table 1. A preliminary assay of compounds **9** and **11** against the angiotensin AT_1 receptor revealed weak activity with IC_{50} values of 3.6 μM and 5.4 μM, respectively. Compound **10** (IC_{50} = 87 nM) exhibited stronger binding affinity to AT_1 receptors compared to compound **2** (IC_{50} = 750 nM). Analysis of the functional antagonism of angiotensin-II induced contraction of the rat aorta by compound **10** will be published elsewhere.

Table 1. *In Vitro* Activity of Target Compounds Against Angiotension II

Compounds	IC_{50} (μM) AT_1[a]	AT_2[b]
1[c]	0.13	NT[d]
2	0.75	19
9	3.6	NT
10	0.087	6.80
11	5.4	NT
Losartan	0.02	>10

[a] Inhibition of specific binding of [^{3}H]Losartan to the AT_1 receptor.
[b] Inhibition of specific binding of [^{125}I]CGP-42112A to the AT_2 receptor.
[c] *See Ref. 18.*
[d] Not tested.

EXPERIMENTAL

General Methods

Analytical samples were homogeneous by thin-layer chromatography (TLC) and afforded spectroscopic data which were consistent with the assigned structures. Melting points were obtained on a capillar Electrothermal apparatus and were uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance spectra were obtained using either a Varian AM-300 or Brucker AMX-400 spectrometer. Chemical shifts were reported in parts per million (δ, ppm) using $CHCl_3$ (δ_H 7.26) or DMSO (δ_H 2.49) as internal standards. EI mass spectrum were recorded on a JEOL JMS-D300 mass spectrometer from National Taiwan University, Taipei. Elemental analyses for C, H, and N were carried out on a Perkin-Elmer 240 Elemental Analyzer at National Taiwan University, Taipei and were within ±0.4% of the theoretical values. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60F-254, Merck) and spots were visualized under UV light and/or phosphomolybdic acid-ethanol. Column chromatography was performed with Kieselgel 60 (70-230 mesh) silica gel (Merck). All nonaqueus reactions were performed in oven-dried glassware and under an atmosphere of dry nitrogen or argon. All starting materials were obtained from commercial suppliers (Aldrich, Jannsen, Merck Fluka) and used without purification. HPLC grade solvents were purchased from Baker Analysed, Lab-scan and Alphs Chem Co. Solvents were dried as pre-

viously described.[19]

3-Butyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (6)

A mixture of 2-aminobenzenesulfonamide (2 g, 11.6 mmol), toluene-4-sulfonic acid and pentanoic acid in toluene (40 mL) was refluxed for 96 h. After the mixture was cooled to 0 °C, the solid was collected and recrystallized from ethyl acetate to give compound **6** (1.88 g, 68%), mp 158-160 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.86 (t, J = 7.3 Hz, 3H, CH_3), 1.34 (sext, J = 7.6 Hz, 2H, CH_2), 1.69 (quint, J = 7.7 Hz, 2H, CH_2), 2.52 (t, J = 7.7 Hz, 2H, CH_2), 7.32 (d, J = 8.0 Hz, 1H, Ar-H), 7.39 (t, J = 7.2 Hz, 1H, Ar-H), 7.50-7.60 (m, 1H, Ar-H), 9.88 (d, J = 7.0 Hz, 1H, Ar-H), 10.05 (br. 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 13.67, 22.04, 28.58, 36.07, 117.72, 120.78, 123.76, 126.67, 133.29, 135.24, 161.36; MS: m/z 239 (M^++1); Anal. Calcd for $C_{11}H_{14}N_2O_2S$ (238.30): C, 55.44; H, 5.92; N, 11.75. Found: C, 55.43; H, 5.71; N, 11.74.

3-Butyl-2-[[2′-(*N*-triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (7) and 3-Butyl-4-[[2′-(*N*-triphenylmethyl)tetrazol-5-yl]-biphenyl-4-yl]methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (8)

To a mixture of compound **6** (1.0 g, 4.3 mmol), compound **7** (1.95 g, 3.5 mmol), potassium carbonate (0.58 g, 4.2 mmol) and potassium iodide (0.01 g, 0.06 mmol) in acetonitrile (60 mL) were refluxed under argon for 72 h. The solvent was then removed *in vacuo* and the residue was dissolved in water (100 mL). The resulting solution was extracted with ethyl acetate (2 × 100 mL) and the organic layer was collected and dried over magnesium sulfate. After evaporation, the residue was chromatographied on silica gel (4 × 15 cm; solvent systen: *n*-hexane/ethyl acetate = 4/1). The R_f = 0.36 fraction was collected and evaporated to give compound **7** (0.6 g, 24%). An analytical sample was recrystallized from dichloromethane and ethyl acetate, mp 162-164 °C; IR (KBr): 3050, 2875, 1600, 1580, 1330 (SO_2), 1180, 880, 850, 760, 700 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 7.4 Hz, 3H, CH_3), 1.21-1.33 (m, 2H, CH_2), 1.65 (quint, J = 7.6 Hz, 2H, CH_2), 2.45 (t, J = 7.9 Hz, 2H, CH_2), 4.99 (s, 2H, CH_2), 6.90 (m, 8H, Ar-H), 7.04 (d, J = 8.1 Hz, 2H, Ar-H), 7.21-7.27 (m, 3H, Ar-H), 7.28-7.32 (m, 4H, Ar-H), 7.41-7.48 (m, 4H, Ar-H), 7.53 (d, J = 7.9 Hz, 1H, Ar-H), 7.91 (t, J = 7.6 Hz, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.79, 22.16, 28.57, 35.24, 46.33, 121.26, 126.12, 126.78, 127.62, 128.24, 129.83, 130.20, 130.61, 133.52, 140.99, 141.17, 141.36, 142.31, 147.72, 157.53, 163.86; Anal. Calcd for $C_{44}H_{38}N_6O_2S$ (714.9): C, 73.92; H, 5.36; N, 11.76. Found: C, 73.70; H, 5.40; N, 11.70. The mother liquid was then extracted with dichloromethane (3 × 50 mL) and the organic layer was collected. After the mixture was dried over magnesium sulfate, the solvent was evaporated *in vacuo* to give a solid which was recrystallized from ether to afford compound **8** (0.7 g, 28%), mp 208-209 °C; IR (KBr): 2950, 2925, 1600, 1580, 1550, 1400, 1310 (SO_2), 1220, 760, 700 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.83 (t, J = 7.4 Hz, 3H, CH_3), 1.16-1.31 (m, 2H, CH_2), 1.73 (quint., J = 6.7 Hz, 2H, CH_2), 2.51 (t, J = 7.9 Hz, 2H, CH_2), 5.08 (s, 2H, CH_2), 6.80 (d, J = 8.6 Hz, 1H, Ar-H), 6.86-6.92 (m, 8H, Ar-H), 7.15 (d, J = 8.0 Hz, 1H, Ar-H), 7.17-7.24 (m, 8H, Ar-H), 7.30-7.45 (m, 5H, Ar-H), 7.46-7.51 (m, 2H, Ar-H), 7.92 (d, J = 6.0 Hz, 1H, Ar-H), 7.99 (d, J = 6.7 Hz, 1H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 13.75, 22.05, 28.38, 35.50, 50.95, 116.33, 123.70, 124.54, 124.99, 126.31, 127.29, 127.68, 127.92, 128.35, 130.04, 130.19, 130.31, 130.63, 132.21, 133.04, 137.51, 139.99, 140.98, 141.26, 162.51; Anal. Calcd for $C_{44}H_{38}N_6O_2S$ (714.9): C, 73.92; H, 5.36; N, 11.76. Found: C, 73.67; H, 5.31; N, 11.55.

***N*-(2-Butyryl-aminobenzenesulfonyl)-*N*-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methylamine (9)**

To a mixture of compound **7** (220 mg, 0.31 mmol) in THF (6 mL) was added 10% HCl (3 mL). The mixture was stirred at room temperature for 4 hr. The pH of the mixture was adjusted to 8.0 by addition of 10% sodium hydroxide solution (3.5 mL). The solid was then collected by filtration and the solvent was removed *in vacuo*. The residue was dissolved in water (10 mL) and the solution was acidified with 10% HCl to pH 3 to produce a precipitate which was applied to a silica gel column [1.5 × 22 cm, 20 g; solvent system: ethyl acetate:ethanol = 9:1, R_f = 0.53] furnishing **9** (40 mg, 28%), mp 76-77 °C; IR (KBr): 3350 (NH), 1665 (C=O), 1580, 1520, 1480, 1440, 1420, 1330, 1150, 1130, 1090, 1070 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.89 (t, J = 7.5 Hz, 3H, CH_3), 1.27-1.39 (m, 2H, CH_2), 1.53-1.63 (m, 2H, CH_2), 2.40 (t, J = 7.5 Hz, 2H, CH_2), 4.01 (d, J = 6.2 Hz, 2H, CH_2), 6.99 (d, J = 8.6 Hz, 2H, Ar-H), 7.15 (d, J = 8.1 Hz, 2H, Ar-H), 7.24 (t, J = 7.7 Hz, 1H, Ar-H), 7.48-7.80 (m, 5H, Ar-H), 8.18 (d, J = 7.2 Hz, 1H, Ar-H), 8.54 (t, J = 6.3 Hz, 1H, Ar-H), 9.27 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 14.10, 22.10, 27.17, 37.03, 45.81, 123.59, 123.80, 124.15, 127.74, 128.12, 128.96, 129.09, 129.26, 130.97, 131.44, 131.44, 133.75, 136.03, 136.89, 138.63, 141.55, 171.65; MS: m/z 491 (M^++1); Anal. Calcd for $C_{25}H_{26}N_6O_3S$ (490.56): C, 61.21; H, 5.34; N, 17.13.

Found: C, 60.93; H, 5.34; N, 16.76.

2-{*N*-butyryl-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-aminobenzenesulfonamide (11)

Was prepared from **8** in 36% yield in the same manner which afforded compound **9**. mp 121-122 °C. IR (KBr): 1635 (C=O), 1580, 1480, 1440, 1400, 1330, 1160 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.74 (t, J = 7.4 Hz, 3H, CH_3), 1.12 (sext., J = 7.8 Hz, 2H, CH_2), 1.43 (quin, J = 8.0 Hz, 2H, CH_2), 1.90 (m, 2H, CH_2), 3.95 (d, J = 15 Hz, 1H, CH_aCH_b), 5.68 (d, J = 15 Hz, 1H, CH_aCH_b), 6.66 (dd, J = 1.2 Hz, 9.0 Hz, 1H, Ar-H), 7.00 (d, J = 8.1 Hz, 2H, Ar-H), 7.09 (d, J = 8.2 Hz, 2H, Ar-H), 7.48-7.61 (m, 4H, Ar-H), 7.65-7.71 (m, 2H, Ar-H), 7.77 (s, 2H, NH_2, D_2O exchangeable), 8.00 (dd, J = 5.1 Hz, 9.6 Hz, 1H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 14.15, 22.11, 27.13, 34.05, 51.47, 123.97, 128.15, 128.80, 128.99, 129.18, 129.37, 130.76, 130.90, 131.42, 132.55, 133.13, 137.47, 138.53, 138.69, 141.59, 141.63, 155.39, 172.31; MS: *m/z* 491 (M^++1); Anal. Calcd for $C_{25}H_{26}N_6O_3S$ (490.56): C, 61.21; H, 5.34; N, 17.13. Found: C, 61.19; H, 5.40; N, 17.10.

3-Butyl-4-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (10)

A mixture of compound **8** (0.5 g, 0.7 mmol) in THF and methanol (1:1, 25 mL) was refluxed for 24 h. After the solvent was removed *in vacuo*, the residue was applied to a silica gel column (4 × 15 cm, 60 g; solvent system: ethyl acetate). The R_f = 0.2 fraction was collected and evaporated *in vacuo* to give compound **10** (0.28 g, 85%). An analytical sample was recrystallized from dichloromethane and *n*-hexane, mp 136-140 °C; IR (KBr): 3500 (NH), 1700 (C=O), 1600, 1580, 1558, 1400, 1310 (SO_2), 1200, 1110, 760, 700 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.84 (t, J = 7.4 Hz, 3H, CH_3), 1.30 (sex, J = 7.2 Hz, 2H, CH_2), 1.61 (quin, J = 7.6 Hz, 2H, CH_2), 2.73 (t, J = 7.2 Hz, 2H, CH_2), 5.49 (s, 2H, CH_2), 6.62-6.67 (m, 3H, Ar-H), 7.10-7.14 (m, 4H, Ar-H), 7.47-7.57 (m, 3H, Ar-H), 7.87 (dd, J = 7.8, 1.4 Hz, 1H, Ar-H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.65, 21.30, 27.70, 34.58, 49.73, 117.47, 123.16, 123.84, 125.49, 126.45, 127.84, 129.41, 130.57, 131.05, 133.21, 134.56, 137.11, 138.52, 140.82, 163.04; Anal. Calcd for $C_{25}H_{24}N_6O_2S$ (472.57): C, 63.54; H, 5.12; N, 17.78. Found: C, 63.50; H, 5.18; N, 17.70.

3-Butyl-2-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (2)

Compound **7** (0.1 g, 0.14 mmol) was refluxed in methanol (5 mL) for 2 h. The solvent was then removed *in vacuo* to produce an oily residue. The residue was applied to a silica gel column (1 × 10 cm; solvent system: ethyl acetate/*n*-hexane = 3/2). The R_f = 0.42 fraction was collected and the solvent was removed by evaporation to afford **2** (30 mg, 46%). An analytical sample was recrystallized from ether, mp 74-76 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J = 6.1 Hz, 3H, CH_3), 1.39 (sex, J = 7.1 Hz, 2H, CH_2), 1.73 (q, J = 7.5 Hz, 2H, CH_2), 2.61 (t, J = 7.2 Hz, 2H, CH_2), 5.13 (s, 2H, CH_2), 7.12 (d, J = 6.8 Hz, 2H, Ar-H), 7.18 (d, J = 8.2 Hz, 2H, Ar-H), 7.2-7.70 (m, 7H, Ar-H), 7.92 (t, J = 8.1 Hz, 1H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 14.36, 22.80, 29.38, 35.64, 47.23, 121.89, 127.56, 127.82, 128.86, 128.92, 130.25, 130.32, 131.19, 131.30, 131.36, 131.64, 131.85, 134.44, 134.48; MS: *m/z* 473 (95, M^++1); 235 (100); 307 (13); Anal. Calcd for $C_{25}H_{24}N_6O_2$. 1/2 H_2O (481.57): C, 62.35; H, 5.23; N, 16.99. Found: C, 62.38; H, 5.09; N, 17.45.

Angiotensin II Receptor Binding Assay

A II was prepared by modification of the methods of Chiu et al.[16] and Witebread et al.[17] This modified assay measured binding of [^{3}H]Losartan to the angiotensin AT_1 receptor and [^{125}I]GCP-42112A to the antiogensin AT_2 receptor. Adrenal membranes of male or female New Zealand derived albino rabbits weighing 2.5-3.0 Kg were prepared in modified Tris-HCl pH 7.4 buffer using standard techniques. A 5 mg aliquot of membranes was incubated with 4.2 nM [^{3}H]Losartan or 25 pM [^{125}I]GCP-42112A for 45 minutes at 25 °C. Non-specific binding was estimated in the presence of 1 μM angiotensin II. Membranes were filtered, washed 3 times, and counted to determine [^{3}H]Losartan or [^{125}I]GCP-42112A binding. Assays were performed in duplicate. The inhibitory concentration (IC_{50}) of an inhibitor that produced 50% displacement of the specific binding of labeled A II was estimated from the linear portion of the displacement curve.

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Key Words

Angiotensin II receptor antagonist; 2*H*-1,2,4-Benzothiadiazine 1,1-dioxide; Biphenyltetrazole.

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