Synthesis and Biological Study of 3-Butyl-1-(2,6-dichlorophenyl)-1H-[1,2,4]triazol-5(4H)-one Derivatives as Anti-hypertension Drugs

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Abstract: A series of nitric oxide-donating derivatives of [1,2,4]triazol-5(4H)-one (**9a-f** and **15a-f**) as a novel class of angiotensin II receptor AT_1 antagonists have been designed and synthesized by coupling furoxan and nitric oxide with lead compound **1**. The synthesized compounds were evaluated for their antagonism of AT_1 receptor with induced contraction in the rabbit thoracic aortic ring and the results showed that compounds **9b**, **15b** and **15d** exhibited potent antagonistic activity of AT_1 receptor. Moreover **9b, 15b, 15d, 15e** had good maximum NO release amount of this series.

Keywords: AT1 antagonist, Nitric oxide-donating, Anti-hypertension, Synthesis.

INTRODUCTION

The renin-angiotensin system (RAS) is a complex, highly regulated pathway that is integral in the regulation of blood volume, electrolyte balance, and arterial blood pressure. The primary purpose of angiotensin converting enzyme (ACE), one of the main enzymes of RAS, is to release angiotensin II from its endogenous precursor [1]. Angiotensin II, a potent vasoconstrictor, produces the majority of the effects contributing to the RAS pathway, such as direct vasoconstriction, enhancement of both catecholamine release and neurotransmission within the peripheral nervous system, and regulation of renal function [2].

ACE inhibitors (ACEI) are the first class of marketing drugs targeting to the RAS system, yet all ACEI can cause hypotension, hyperkalemia, and dry cough. Afterwards, research efforts on angiotensin receptor blocks which can replace ACE inhibitors as anti-hypertension with less adverse effects have led to the discovery of angiotensin II antagonists [3].

Angiotensin receptor blocks (ARBs), the second class to treat hypertension and related cardiovascular disorders, are non-peptide compounds that specifically block the binding of angiotensin II to the AT1 receptor, by occupying the space among the seven transmembrane helices of the receptor protein and interacting with the amino-acid residues in this region of the receptor molecule. The ARBs effectively prevent or reverse all of the known effects of angiorensin II, including rapid and slow pressor responses, stimulatory effects on the peripheral sympathetic nervous system, CNS effects, release of catechol amines, secrestion of aldosterone, both lower blood pressure and protect tissues from oxidative stress and resultant CVD [4, 5]. Eight compounds have been marketed, including losartan, valsartan, candesartan, irbesartan, olmesartan and telmisartan [6].

Compared with ACEI, ARBs have fewer side effects, as they do not inhibit the catabolism of bradykinin carried out by ACE enzyme and, therefore, do not induce cough and angioedema. However, this biopharmacological characteristic is also responsible for the weaker effectiveness of this class of drugs, due to their inability to increase the bradykinin level, leading to the decline of NO-induced vasorelaxing activity. The importance of NO not only lies in its vasorelaxing action, but also in its potent inhibition of platelet and neutrophil aggregation in the endothelium [7].

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The physiological levels of endogenous NO mediate multiple fundamental processes in the cardiovascular system, such as dilating blood vessels, inhibiting platelet adhesion and aggregation, and attenuating leukocyte adhesion and activation. NO donors are pharmacologically active substances that release NO spontaneously or through enzymatic pathways [8]. Organic nitrate and nitrite esters represent a class of NO-donor agents used in cardiovascular diseases since the 19th century. The effectiveness of treatment with these conventional esters is limited by their therapeutic half-life, systematic absorption with potentially adverse hemodynamic effects, and problems of drug tolerance. To overcome these limitations, novel NO donors have been developed, offering selectivity, a prolonged half-life, and a reduced incidence of drug intolerance. In the past few years, we have witnessed the flourishing of studies on several hybrid drugs, in which a well-known molecule with a particular pharmacological pattern has been linked to an NO-donor group, pursuing to improve the pharmacological profile or reduce the adverse effects [9-11]. Calderon reported that adding NO-donor side chain to losartan may improve its antiischemic cardio-protective properties and antiplatelet effects [12, 13]. It was also found that the NO-donor derivative of telmisartan may be a potent anti-hypertensive drug for treatment of hypertension and diabetes-related cardiovascular diseases in the clinic [14].

In the previous work of our group, we have synthesized a series of [1,2,4]triazole derivatives based on the SAR of losartan and found that the compound biphenylmethyl [1,2,4]triazole (**1**) showed potent AT1 antagonist activity (IC_{50} = 1.99 nM) [15]. On the basis of the above study, compound **1** was chosen as the template in the attempt to find new satisfactory anti-hypertension drugs. By conjugating **1** with NO-donor group, such as nitrooxy and substituted furoxan moieties, a series of hybrid drugs were designed. As a result, the NO-sartan analogs **9a-f** and **15a-f** were synthesized and their activities were evaluated.

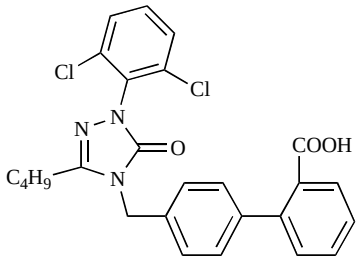

Fig. (1). The structure of compound **1.**

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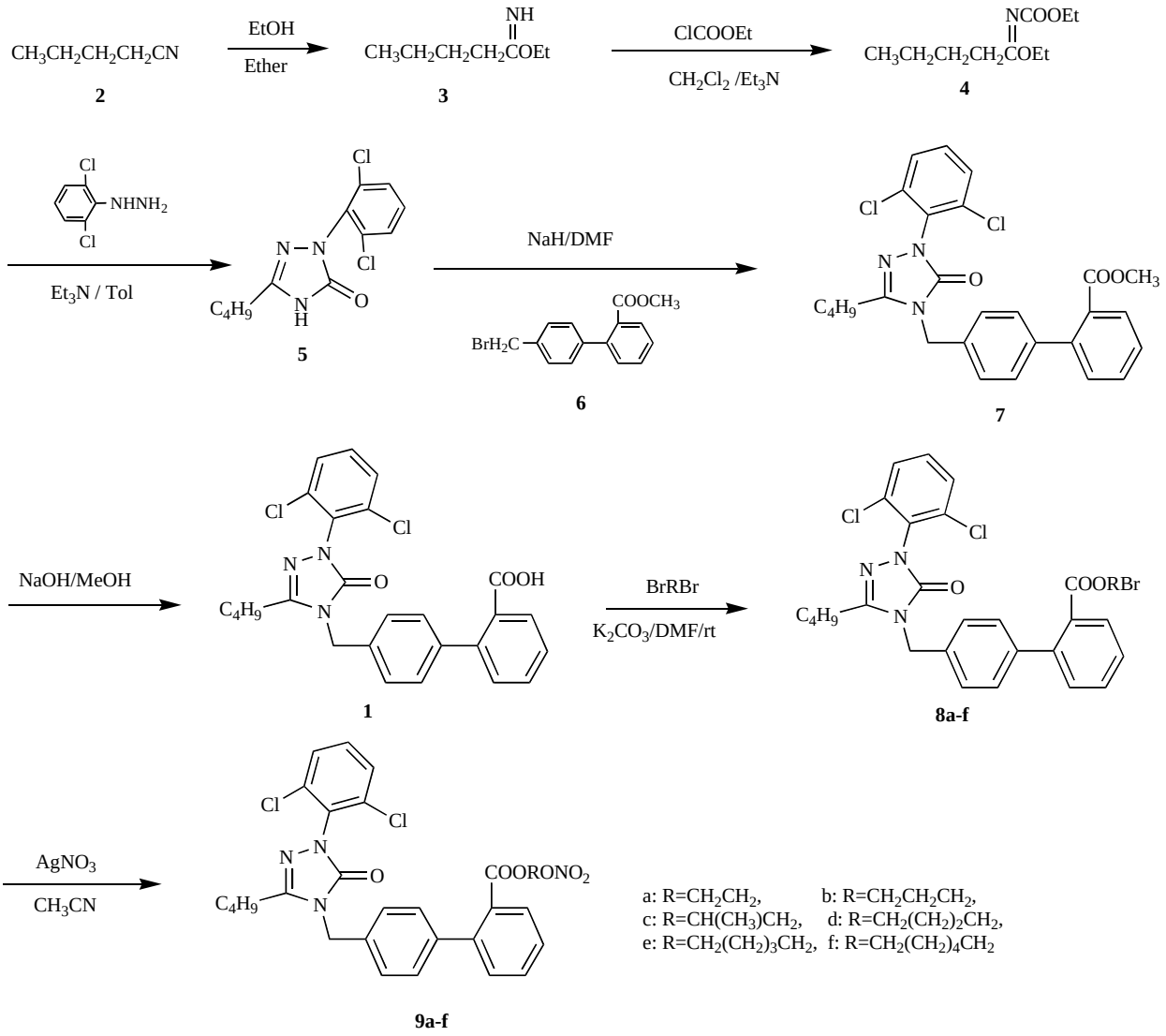


5 **6** **7** **1** **8a-f** **9a-f**

Scheme 1. Synthetic route of nitrooxy derivative **9a-f**.

RESULTS AND DISCUSSION

Chemistry

Compounds **9a-f**, **15a-f** were prepared as shown in Scheme **1** and Scheme **2**. The intermediate **5** was synthesized according to reported methods [15]. Then **5** was converted to **7** with methyl 4'-(bromomethyl)biphenyl-2-carboxylate **6** and NaH in DMF. Through saponification of **7** with NaOH, we obtained the key compound **1**. Then **1** was esterized with alkyl dibromide to give **8a-f** which were further converted into their corresponding target nitrooxy derivatives **9a-f** by reaction with silver nitrate in acetonitrile under refluxing and in the darkness. In Scheme 2, the furoxans **14a-f** were synthesized as described previously in which thiophenol **11** was used as the starting material. Then **1** was condensed with **14a-f** respectively in CH_2Cl_2 in the presence of N, N-dicyclohexylcarbodiimide (DCC) and a catalytic amount of N, N-(dimethylamino)pyridine (DMAP) to afford the corresponding target compounds **15a-f**.

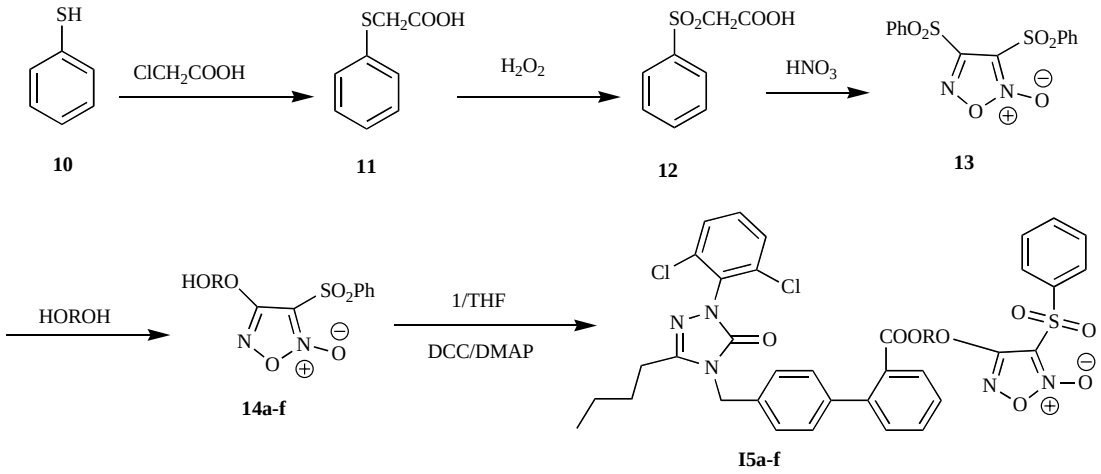
Biological Activity

The synthesized compounds were evaluated for their antagonism of Ang II induced contraction in the rabbit thoracic aortic ring with isolated rabbit aortic strips. Losartan was taken as a positive control drug in the assays. The target compounds and losartan inhibited Ang II (10^{-10} mol/L)-induced contraction in a contraction-related. The results reported in Table **1** showed that the compounds **9b** (IC_{50} =1.98nM), **15b** (IC_{50} =1.58nM) and **15d** (IC_{50} =2.04nM) exhibited potent antagonistic activity of AT1 receptor, which were similar to the marketed AT1 receptor antagonist losartan (IC_{50} = 1.99 nM). The NO production *in vitro* were determined by Greiss assay. The result showed that these compounds have different levels of NO release. **9b, 15b**, **15d, 15e** had good maximum NO release amount of this series.

EXPERIMENTAL SECTION

General

Melting points were determined using a capillary apparatus (RDCSY-I) and are reported directly. All of the compounds synthesized were purified by column chromatography (CC) on silica gel 60 (200-300 mesh) and thin-layer chromatography (TLC) on silica gel 60 F254 plates (250 μm; Qingdao Ocean Chemical Company, China). Subsequently, they were routinely analyzed by IR (Shimadzu FTIR-8400S), 1H-NMR (Bruker ACF-300Q, 300 MHz), MS (Hewlett-Packard 1100 LC/MSD spectrometer), and elemental analysis (Elementar Vario EL III instrument).



a: R=CH_2CH_2, b: R=$CH_2CH_2CH_2$, c: R=$CH(CH_3)CH_2$,

d: R=$CH_2(CH_2)_2CH_2$, e: R=$CH(CH_3)CH_2CH_2$, f: R=$CH_2CH{=}CHCH_2$

Scheme 2. Synthetic route of substituted furoxan derivatives **15a-f**.

Table 1. Inhibitory Activities of Ang II (10^{-10} mol/L)-Induced Contraction in a Contraction-Related and Maximum NO Release Amount *in vitro* of the New Compounds

Compd.	R	IC_{50}(nM)	NO_{max}(μM)
9a	CH_2CH_2	3.79	5.6×10^{-2}
9b	$CH_2CH_2CH_2$	1.98	6.2×10^{-2}
9c	$CH(CH_3)CH_2$	4.93	4.7×10^{-2}
9d	$CH_2(CH_2)_2CH_2$	6.55	3.8×10^{-2}
9e	$CH_2(CH_2)_3CH_2$	12.02	2.3×10^{-2}
9f	$CH_2(CH_2)_4CH_2$	13.13	1.5×10^{-2}
15a	CH_2CH_2	5.97	5.8×10^{-2}
15b	$CH_2CH_2CH_2$	1.58	6.3×10^{-2}
15c	$CH(CH_3)CH_2$	7.65	4.0×10^{-2}
15d	$CH_2(CH_2)_2CH_2$	2.04	6.0×10^{-2}
15e	$CH(CH_3)CH_2CH_2$	4.53	6.1×10^{-2}
15f	$CH_2CH{=}CHCH_2$	8.31	5.2×10^{-2}
losartan		1.99	NT
1		1.99	NT

The intermediates **5** that were used to introduce the [1,2,4]triazole moieties were synthesized according to reported methods [15].

Methyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**7**).

Sodium hydride (0.03g, 1.25mmol) was added to a stirred solution of **5** (0. 97g, 5 mmol) in dry DMF (10 mL) under nitrogen. After 30 min the bromo derivative **6** (0.315g, 1.1mmol) was added, and the resulting mixture was stirred for 3.5 h at 30℃. The solvent was removed in vacuo and the residue taken up in ethyl acetate and water. The organic phase was separated, washed with brine, dried (Na_2SO_4), and then concentrated. The residue was chromatographed over silica gel in 4:1 petroleum ether-ethyl acetate to give an oil which crystallized (0.43g, yield: 85.0%), white solid, mp: 128-130℃. ESI-MS: 510$[M+1]^+$

4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylic acid (**1**)

7(0.205g, 0.5mmol) was stirred for 40 min in alcohol (10 mL) and 10%NaOH (8 mL). The mixture was stirred for 3h under refluxed. Then the solvent was evaporated in vacuo and the residue taken up in diethyl ether. The resulting white solid was filtered, washed with diethyl ether, and dried (0.139g, yield: 56.6%), white solid, mp: 172～174℃; MS(ESI, m/z): 496.2$[M+1]^+$; IR(KBr, cm^{-1}): 2925.27, 1716.11, 1629.36, 1568.36, 1480.17, 1218.01, 1082.94; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.80(t, 3H, CH_3), 1.26-1.32(m, 2H, CH_2), 1.48-1.53(m, 2H, CH_2), 2.54(t, 2H, CH_2), 5.00(s, 2H, CH_2), 7.27-7.30(d, 2H, ArH), 7.35-7.39(m, 3H, ArH), 7.45-7.48(m, 1H, ArH), 7.54-7.62(m, 2H, ArH), 7.69-7.75(m, 3H, ArH), 12.51(s, 1H, COOH).

2-(nitrooxy)ethyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9a**)

A solution of **8a** (0.271g, 0.45mmol) in a small amount of CH_3CN (10 mL) was added to a stirred solution of $AgNO_3$ (0.1g, 0.6mmol) in CH_3CN (19 mL). Stirring was continued over 2 h at room temperature in the dark, and then the precipitate was filtered off, and the solvent was evaporated. The crude product was triturated with $CHCl_3$ (20 mL) and filtered off to remove the unreacted silver nitrate and AgBr. The solvent was evaporated to give **9a** (0.233g, yield: 89%).

Compound **9b-f** was synthesized from following the same procedure described above for the preparation of **9a**.

4-(2-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)ethoxy)-3-(phenylsulfonyl)- [1,2,5]oxadiazole 2-oxide (**15a**)

A solution of **1** (0.228g, 0.5mmol), DCC (0.103g, 0.5mmol) and DMAP (5.1 mg, 0.042mmol) in dry tetrahydrofuran (20 mL) was stirred at room temperature for 10 h. After filtration, the filtrate was evaporated to dryness in vacuo, and the crude product was purified by column chromatography (petroleum ether /EtOAc 4:1 to 3:1) to yield the compounds (0.11g, yield: 38%).

Compound **15b-f** was synthesized from **1** following the same procedure described above for the preparation of **15a**.

Spectra Data

2-(nitrooxy)ethyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9a**)

Colorless oil, MS(ESI, m/z): 583.89[M-1]$^-$; IR (KBr, cm^{-1}): 2958.34, 1720.62, 1636.94, 1566.36, 1479.85, 1441.25, 1270.05, 1242.96, 1132.75, 1090.12, 858.53, 791.71; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.81(t,3H,CH_3), 1.28-1.32(m, 2H, CH_2), 1.49-1.54(m, 2H, CH_2), 2.54(t, 2H, CH_2), 3.42(t, J=5.14Hz, 2H, CH_2), 4.00(t, J=5.14Hz, 2H, CH_2), 5.00(s, 2H, CH_2), 7.27-7.30(q, 4H, ArH), 7.36-7.41(d, 1H, ArH), 7.43-7.49(t, 1H, ArH), 7.56-7.62(m, 2H, ArH), 7.69-7.72(d, 2H, ArH), 7.77-7.79(d, 1H, ArH).

3-(nitrooxy)propyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9b**)

Colorless oil, MS(ESI, m/z): 621.1[M+Na]$^+$; IR(KBr, cm^{-1}): 2960.85, 1719.70, 1630.27, 1477.49, 1441.31, 1276.92, 1125.59, 862.56; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.82(t, 3H, CH_3), 1.30-1.34(m, 2H, CH_2), 1.49-1.55(m, 2H, CH_2), 1.74-1.79(m, 2H, CH_2), 2.54(t, 2H, CH_2), 4.07(t, J=6.17Hz, 2H, CH_2), 4.27(t, J=6.35Hz, 2H, CH_2), 4.99(s, 2H, CH_2), 7.27-7.30(q, 4H, ArH), 7.40(d, 1H, ArH), 7.47(t, 1H, ArH), 7.56-7.62(m, 2H, ArH), 7.70(d, 2H, ArH), 7.78(d, 1H, ArH).

1-(nitrooxy)propan-2-yl4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9c**)

Colorless oil, MS(ESI, m/z): 621.3[M+Na]$^+$; IR(KBr, cm^{-1}): 2960.85, 1716.85, 1632.21, 1452.61, 1272.22, 1050.95, 1027.34, 1005.95, 763.71; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.82(t, 3H, CH_3), 1.07(dd, J=15.21, 6.55Hz, 3H, CH_3), 1.30-1.34(m, 2H, CH_2), 1.50-1.54(m, 2H, CH_2), 2.57(t, 2H, CH_2), 4.13-4.15(m, 1H, CH), 4.32-4.36(m, 1H, CH), 4.56-4.59(m, 1H, CH), 5.01(s, 2H, CH_2), 7.27-7.30(q, 4H, ArH), 7.39(d, 1H, ArH), 7.47(t, 1H, ArH), 7.56-7.62(m, 2H, ArH), 7.69-7.72(d, 2H, ArH), 7.77-7.79(d, 1H, ArH).

4-(nitrooxy)butyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9d**)

Colorless oil, MS(ESI, m/z): 613.11[M+H]$^+$; IR(KBr, cm^{-1}): 2953.74, 1719.62, 1626.48, 1438.39, 1278.52, 1278.52, 1129.15, 862.56, 791.47, 763.03; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.82(t, 3H, CH_3), 1.26-1.30(m, 2H, CH_2), 1.41-1.45(m, 4H, 2CH_2), 1.54-1.58(m, 2H, CH_2), 2.57(t, 2H, CH_2), 4.01(t, J=5.55Hz, 2H, CH_2), 4.40(t, J=5.22Hz,2H,CH_2), 5.02(s, 2H, CH_2), 7.34-7.40(q, 4H, ArH), 7.43-7.47(d, 1H, ArH), 7.47-7.52(t, 1H, ArH), 7.56-7.59(m, 2H, ArH), 7.68-7.75(d, 2H, ArH), 7.77-7.79(d, 1H, ArH).

5-(nitrooxy)pentyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9e**)

Colorless oil, MS(ESI, m/z): 649.1[M+K]$^+$; IR(KBr, cm^{-1}): 2960.85, 1720.12, 1627.23, 1438.39, 1278.34, 1050.95, 1027.34, 862.56, 791.47, 759.48; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.82(t, 3H, CH_3), 1.13-1.16(m, 2H, CH_2), 1.30-1.34(m, 4H, CH_2), 1.50-1.54(m, 4H, 2CH_2), 2.56(t, 2H, CH_2), 3.98(t, J=6.59Hz, 2H, CH_2),4.435(t, J=6.34Hz, 2H, CH_2), 5.02(s, 2H, CH_2), 7.32(s, 4H, ArH), 7.40-7.43(d, 1H, ArH), 7.46-7.52(t, 1H, ArH), 7.56-7.66(m, 2H, ArH), 7.68-7.75(m, 3H, ArH).

6-(nitrooxy)hexyl-4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenyl-2-carboxylate (**9f**)

Colorless oil, MS(ESI, m/z): 641.19[M+H]$^+$; IR(KBr, cm^{-1}): 2953.74, 1719.74, 1626.15, 1452.61, 1279.08, 1050.95, 1027.34, 866.11, 791.47, 763.03; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.82(t, 3H, CH_3), 1.13-1.16 (m, 2H, CH_2), 1.30-1.34 (m, 6H, 3CH_2), 1.50-1.54 (m, 4H, 2CH_2), 2.55(t, 2H, CH_2), 3.97(t, J=6.41Hz, 2H, CH_2), 4.44(t, J=6.59Hz, 2H, CH_2), 5.00(s, 2H, CH_2), 7.32(s, 4H, ArH), 7.40-7.43(d, 1H, ArH), 7.46-7.52(t, 1H, ArH), 7.56-7.66(m, 2H, ArH), 7.68-7.75(m, 3H, ArH).

4-(2-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)ethoxy)-3-(phenylsulfonyl)-[1,2,5]-oxadiazole 2-oxide (**15a**)

Colorless oil, MS(ESI, m/z): 763.91; IR(KBr, cm^{-1}):2953.74, 1721.34, 1616.56, 1552.30, 1450.41, 1360.19, 1246.45, 1169.77, 1086.49, 757.58, 597.43, 556.87; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.79(t, 3H, CH_3), 1.25-1.29(m, 2H, CH_2), 1.45-1.49(m, 2H, CH_2), 2.54(t, 2H, CH_2), 4.44(S, 4H, 2CH_2), 4.97(s, 2H, CH_2), 7.25-7.33(q, 4H, ArH), 7.43-7.51(d, 1H, ArH), 7.54-7.55(m, 4H, ArH), 7.65-7.71(m, 3H, ArH), 7.74-7.84(m, 2H, ArH), 7.93-7.96(m, 2H, ArH).

4-(3-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)propoxy)-3-(phenylsulfonyl)-[1,2,5]-oxadiazole 2-oxide (**15b**)

Colorless oil, MS(ESI, m/z): 800.3[M+Na]$^+$; IR(KBr, cm^{-1}): 2960.85, 1716.90, 1614.64, 1552.81, 1450.39, 1168.25, 1086.49, 759.79, 597.43, 556.87; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.79(t, 3H, CH_3), 1.28-1.33(m, 2H, CH_2), 1.48-1.53(m, 2H, CH_2), 1.82-1.86(m, 2H, CH_2), 2.54(t, 2H, CH_2), 4.11-4.16(m, 4H, 2CH_2), 4.98(s, 2H, CH_2), 7.25-7.33(q, 4H, ArH), 7.43-7.51(d, 1H, ArH), 7.54-7.55(m, 4H, ArH), 7.65-7.71(m, 3H, ArH), 7.74-7.84(m, 2H, ArH), 7.93-7.96(m, 2H, ArH).

4-(2-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)propoxy)-3-(phenylsulfonyl)-[1,2,5]oxadiazole 2-oxide (**15c**)

Colorless oil, MS(ESI, m/z): 800.4[M+Na]$^+$; IR(KBr, cm^{-1}): 2960.85, 1716.90, 1614.64, 1552.81, 1450.39, 1168.25, 1086.49, 759.79, 597.43, 556.87; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.79(t, 3H, CH_3), 1.11-1.13(m, 3H, CH_3), 1.30(m, 2H, CH_2), 1.56(m, 2H, CH_2), 2.54(t, 2H, CH_2), 4.23(d, 1H, CH), 4.38(d, 1H, CH), 4.99(s, 2H, CH_2), 5.25(m, 1H, CH), 7.25-7.33(q, 4H, ArH),7.43-7.51(d, 1H, ArH), 7.54-7.55(m, 4H, ArH), 7.65-7.71(m, 3H, ArH), 7.74-7.84(m, 2H, ArH), 7.93-7.96(m, 2H, ArH).

4-(4-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)butoxy)-3-(phenylsulfonyl)-[1,2,5]oxadiazole 2-oxide (**15d**)

Colorless oil, MS(ESI, m/z): 814.3[M+Na]$^+$; IR(KBr, cm^{-1}): 2960.85, 1716.96, 1613.79, 1552.23, 1449.56, 1168.25, 1085.33,

759.79, 597.43; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.79(t, 3H, CH_3), 1.05(d, 3H, CH_3), 1.28-1.33(m, 2H, CH_2), 1.47-1.51(m, 2H, CH_2), 1.80-1.84(m, 2H, CH_2), 2.54(t, 2H, CH_2), 4.06-4.28(m, 1H, CH), 5.00(s, 2H, CH_2), 7.25-7.33(q, 4H, ArH), 7.43-7.51(d, 1H, ArH), 7.54-7.55(m, 4H, ArH), 7.65-7.71(m, 3H, ArH), 7.74-7.84(m, 2H, ArH), 7.93-7.96(m, 2H, ArH).

4-(3-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-[1,2,4]triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)butoxy)-3-(phenylsulfonyl)-[1,2,5]oxadiazole 2-oxide (**15e**)

Colorless oil, MS(ESI, m/z): 814.3$[M+Na]^+$; IR(KBr, cm^{-1}): 2960.85, 1718.96, 1613.79, 1552.23, 1449.56, 1168.25, 759.79; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.79(t, 3H), 1.28-1.33(m, 2H, CH_2), 1.47-1.54(m, 6H, $3CH_2$), 2.54(t, 2H, CH_2), 4.06(t, 2H, CH_2), 4.28(t, 2H, CH_2), 5.00(s, 2H, CH_2), 7.25-7.33(q, 4H, ArH), 7.43-7.51(d, 1H, ArH), 7.54-7.55(m, 4H, ArH), 7.65-7.71(m, 3H, ArH), 7.74-7.84(m, 2H, ArH), 7.93-7.96(m, 2H, ArH).

4-(4-(4'-((3-butyl-1-(2,6-dichlorophenyl)-5-oxo-1H-1,2,4-triazol-4(5H)-yl)methyl)biphenylcarbonyloxy)but-2-enyloxy)-3-(phenylsulfonyl)-[1,2,5]oxadiazole 2-oxide (**15f**)

Colorless oil, MS(ESI, m/z): 805.3$[M+NH_4]^+$; IR(KBr, cm^{-1}): 2932.38, 1715.94, 1613.79, 1547.83, 1440.90, 1368.69, 1168.25, 1085.33, 759.79, 597.43; ^{1}H-NMR(DMSO-d_6), δ(ppm): 0.80(t, 3H, CH_3), 1.28-1.33 (m, 2H, CH_2), 1.47-1.54 (m, 2H, CH_2), 2.25-2.29(m, 2H, CH_2), 4.86(s, 2H, CH_2), 5.00(s, 2H, CH_2), 5.25(s, 2H, 2CH), 7.25-7.33(q, 4H, ArH), 7.43-7.51(d, 1H, ArH), 7.54-7.55(m, 4H, ArH), 7.65-7.71(m, 3H, ArH), 7.74-7.84(m, 2H, ArH), 7.93-7.96(m, 2H, ArH).

BIOLOGY

AT$_1$-Antagonist Activity

The compounds were tested for antagonist activity in rabbit aortic rings [16]. Male New Zealand white rabbits (2-2.5kg) 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. 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 values for AII were calculated from the AII concentration-response curves while IC_{50} were determined.

Measurement of NO Release

The different levels of NO production *in vitro* were determined by Greiss assay [17]. Culture medium, after the treatment period, was removed and the compound (100µl) was reacted with 50µl sulfanilamide (10mg/ml in 5% orthophosphoric acid) and 50µl N-(1-naphthyl)ethylenediamine (1mg/ml) in a multiwell microtiter plate. Similarly, different concentrations of sodium nitrite were reacted with sulfanilamide and N-(1-naphthyl)ethylenediamine to produce a standard curve for determining the nitrite (NO) concentration in each sample. The absorbance was measured at a wavelength of 570nm on a multiwell plate reader and the nitrite concentration in each sample was determined.

CONCLUSION

In conclusion, we have obtained a new series of oxide-donating derivatives of [1,2,4]triazol-5(4H)-one derivatives. These new compounds were confirmed that NO-sartans are actually pharmacodynamic hybrids possessing both the AT1-antagonist activity of sartans and a "slow NO donor". In addition, they seemed to possess antihypertensive properties not inferior to those of the "native" sartans. Further biological evaluations on these compounds, such as the beneficial roles played by NO in the cardiovascular system are currently in progress.

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