Design and Synthesis of New Tetrazolyl- and Carboxy-biphenylylmethyl-quinazolin-4-one Derivatives as Angiotensin II AT_1 Receptor Antagonists

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A series of novel quinazolin-4-ones was designed and their molecular modeling simulation fitting to a new HipHop 3D pharmacophore model using CATALYST was examined. Several compounds showed significant high simulation fit values. The designed compounds were synthesized and eight of them were biologically evaluated in vitro using an AT_1 receptor binding assay, where compound **XX** competed weakly against radiolabeled Sar[1]Ile[8]-angiotensin II (Ang II) binding, compounds **XIV** and **XXII** showed moderate competition, and compound **XXV** showed almost equal ability to displace radiolabeled Sar[1]Ile[8]-Ang II binding to AT_1 receptors as losartan. In vivo biological evaluation study of compounds **XIV**, **XXII**, and **XXV** on both normotensive and hypertensive rats revealed that compound **XXV** demonstrated higher hypotensive and antihypertensive activity than the reference compound losartan. To obtain a highly active compound from a candidate set of only eight tested compounds illustrates the power and utility of our pharmacophore model.

1. Introduction

The octapeptide angiotensin II (Ang II) produced by the renin-angiotensin system (RAS) is a potent vasoconstrictor and thus plays an integral role in the pathophysiology of hypertension.[1] This directed many researchers toward designing drugs to block the effects of Ang II either by inhibiting the angiotensin-converting enzyme (ACE) or renin or by blocking the Ang II receptors. Ang II receptor antagonists have proved to lower blood pressure effectively,[2] and they are better tolerated than other classes of drugs.[3,4] The development of non-peptide Ang II receptor antagonists was sparked by patents of Takeda Chemical Industries in Japan in 1982, when Furukawa et al. disclosed a series of imidazole-5-acetic acid derivatives.[5,6] Further optimization of these compounds[7] gave rise to the discovery of the first lead compound losartan, which was launched in 1994 to treat hypertension.

Carini et al.[8] proposed a structure−activity relationship (SAR) of AT_1 receptor antagonists that suggested activity is improved by the presence of (i) a biphenyl group (The presence of a linker chain between the two phenyl moieties reduces the activity. The biphenyl moiety, which acts as a spacer connecting the acidic group with other features, could be replaced by other similar spacers.[9]); (ii) a tetrazole group, or an acidic isostere,[10,11] at the ortho position of the biphenyl group; (iii) a heterocyclic ring, to act as an acceptor in a hydrogen-bonding interaction with the receptor; and (iv) a short alkyl chain at the 2-position of the heterocycle, for efficient binding to the receptor.

It appears that all the Ang II antagonists designed to study AT_1 receptor antagonistic activity are based on the above model as all the active molecules have these pharmacophores.[11−13] Our current investigation is based on molecular modeling studies involving the synthesis of new tetrazolyl- and carboxy-biphenylylmethyl quinazoline derivatives by the replacement of the imidazole ring of losartan with 4-quinazolinones, a pharmacophore that has proven to be a rich source of exceptionally potent Ang II antagonists.[13] The 4-quinazolinone ring system has the same 1,3-arrangement of nitrogens found in the imidazole ring and can accommodate the requisite lipophilic side chain at position 2.[13h] Additionally, the carbonyl group of 4-quinazolinone can serve as a mimic of the hydroxymethyl group of losartan.[14] *tert*-Butyl ester analogues were designed as they can be considered as potential prodrugs that could be easily hydrolyzed in vivo, giving the carboxylic acid.

In this project, molecular modeling simulation studies were performed in order to predict the biological activity of the proposed compounds. The hypothesis generation was performed using the HipHop module of CATALYST software. This method is applicable when the 3D structure of the receptor is unknown and a series of compounds has been identified to have similar activity with dissimilar and/or flexible structures. The main idea of this approach is to identify the ideal hypothesis, which is a template derived from the structures of these compounds and representing the geometry of the receptor sites as a collection of functional groups in space. The program gave 10 hypotheses by default as a result of each run. All the 10 generated hypotheses had all the required features and gave good fitting with losartan and the target compounds, and we chose the hypothesis ranked number one. Retrospectively, we were pleased to note that this hypothesis gave the most consistent fit values with the experimental biological results discussed below.

The generated AT_1 receptor antagonists hypothesis was subjected to simulation compare/fit studies, using the best fit algorithm, with the conformational model of a test set of the proposed compounds [**XI**, **XIV**, **XVII**, **XVIII**, **XX**, **XXI**, **XXII**, **XXIII**, and **XXV** (Scheme 2)] in order to predict their antagonistic activity. The simulated fitting values of the best fit conformer may be a guide for estimating relative affinities of these compounds with their receptors. The designed com-

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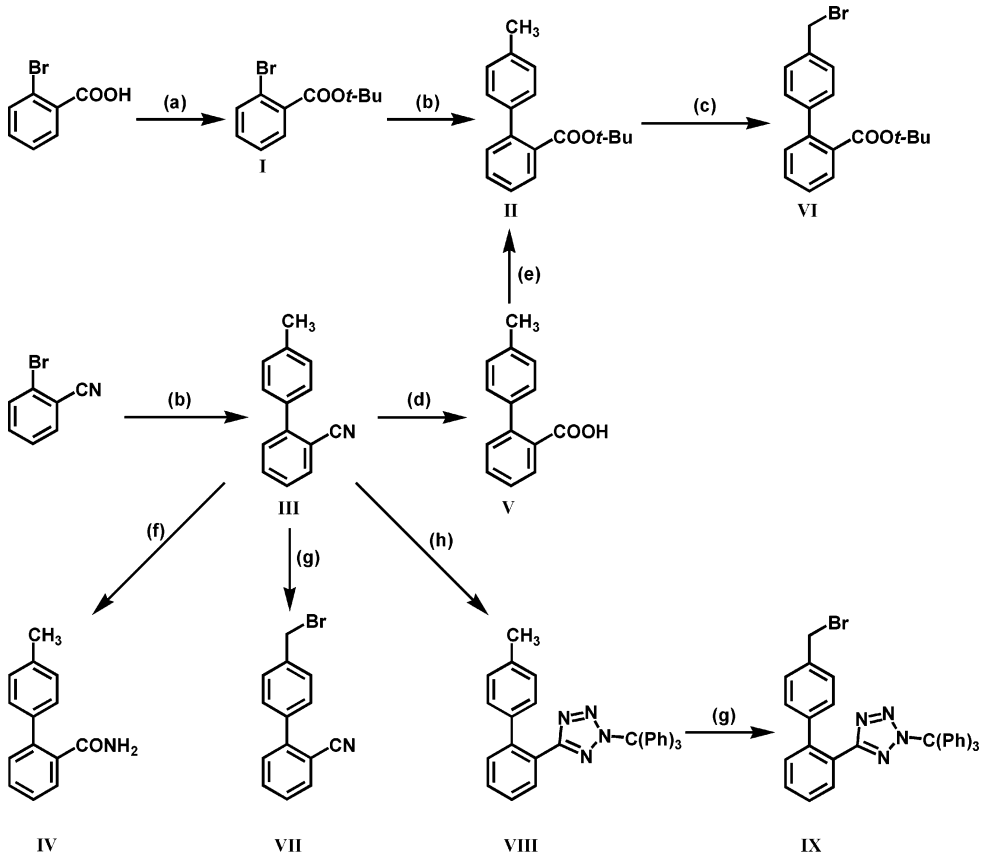
Scheme 1[a]



[a] (a) *t*-BuOH, DBU, *N*,*N*′-carbonyldiimidazole, (b) *p*-tolylboronic acid, Pd $(PPh_3)_4$, $NaCO_3$, (c) NBS, AIBN (or dibenzoyl peroxide), (d) $KOH/(CH_2OH)_2$, or $AcOH/H_2SO_4/H_2O$, (e) oxalyl chloride/CH_2Cl_2, t-BuOK/dry THF, (f) 10% NaOH, ethanol, (g) NBS, dibenzoyl peroxide, (h) Bu_3SnCl, NaN_3, $ClC(Ph)_3$.

pounds **XIV**, **XX**, **XXII**, and **XXV** showed the highest fit values in comparison to losartan. Accordingly, we were motivated to synthesize these compounds following the sequence outlined in Schemes 1 and 2, predicting that they would have a potent AT_1 antagonistic activity.

2. Results and Discussion

2.i. Synthesis. The intermediates (**I**, **II**, and **VI**−**IX**) that were used to introduce the biphenylmethyl moieties to the quinazoline ring systems (**X**, **XV**, and **XVI**) were synthesized according to reported methods (Scheme 1, see Experimental Section and Supporting Information). Intermediates **III**−**V** were prepared by new methods. Thus compound **III** was obtained with a Suzuki cross coupling reaction on *o*-bromobenzonitrile and *p*-tolylboronic acid. Attempts to synthesize the acid (**V**) by alkaline hydrolysis of **III** in refluxing ethanol for 20 h[15a] did not give the acid, but the amide (**IV**) was isolated as the sole product. Trying to push the latter reaction forward by increasing the reaction time gave a mixture of the acid (**V**) and the amide (**IV**). When the hydrolysis was performed using ethylene glycol,[15b] the acid (**V**) was isolated in good yield. Additionally, acid-catalyzed hydrolysis of the nitrile (**III**) resulted in the formation of **V** as the sole product in good yield.[15c] The spectral and analytical data of **III**−**V** matched the reported data for these compounds.

When quinazoline-2,4-(1*H*,3*H*)-dione (**X**) was alkylated with 4′-bromomethyl-biphenyl-2-carbonitrile (**VII**) in the presence of potassium carbonate and DMF, both the 3-substituted (**XI**) and the 1,3-disubstituted quinazolinediones (**XII**) were isolated (Scheme 2). The regioselectivity of the monoalkylation was confirmed by nuclear Overhauser effect spectroscopy (NOESY);

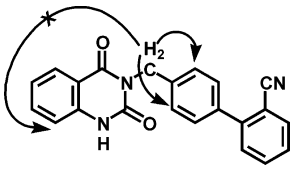
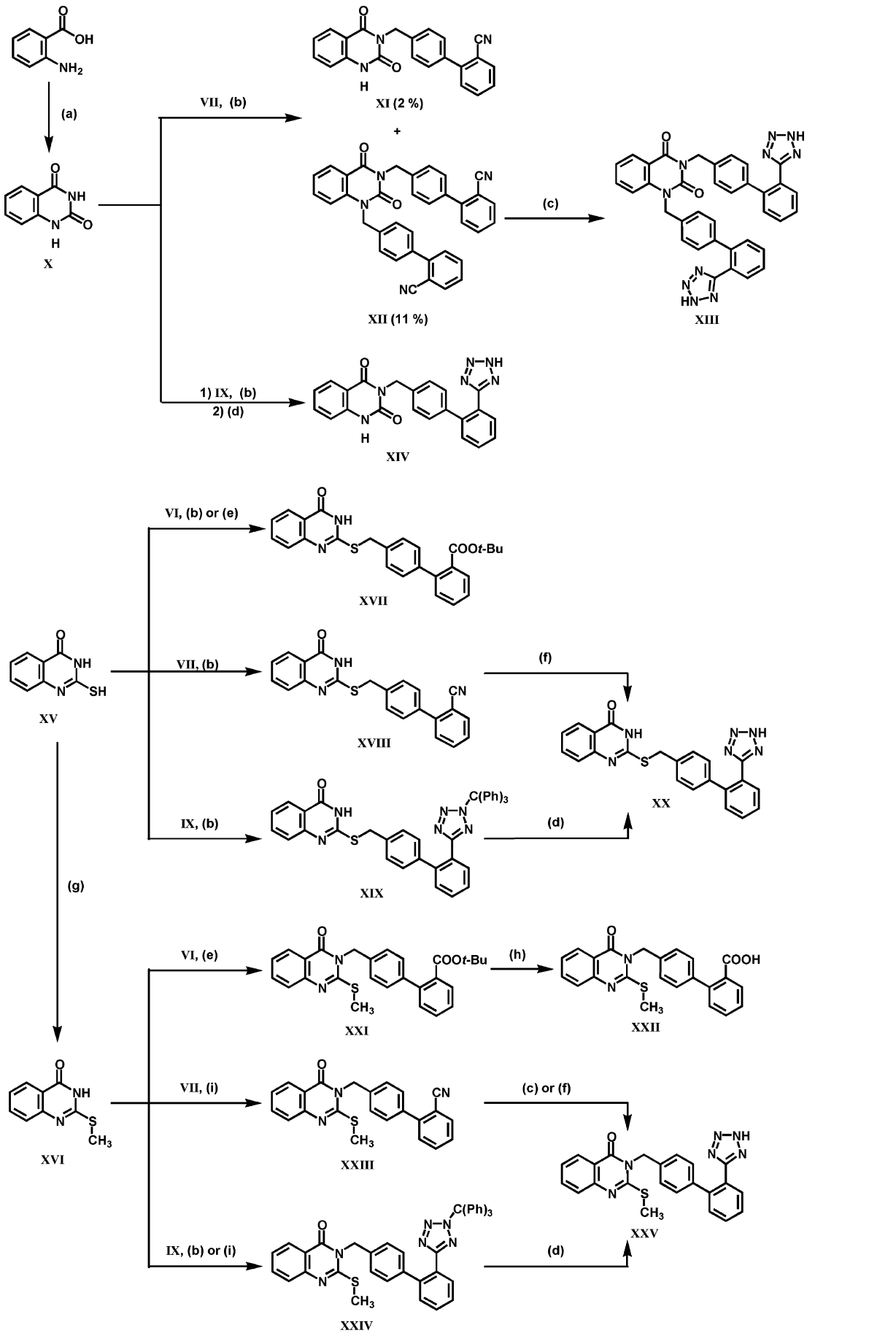


Figure 1. NOESY of compound **XI**.

irradiation of the benzylic CH_2 gave enhancement of the aromatic protons at positions 3 and 5 of the biphenyl moiety (at δ 7.57 ppm) and not the aromatic quinazoline protons (Figure 1). This indicates that N_3-alkylation occurred rather than the N_1-alkylation.

Reaction of **XII** with ammonium chloride and sodium azide resulted in the corresponding tetrazole analogue (**XIII**). When **X** was alkylated with 2-trityl-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole (**IX**) and refluxed in methanol, only the 3-substituted tetrazolylbiphenylyl derivative (**XIV**) could be isolated. Alkylation of 2-mercaptoquinazolin-4(3*H*)-one (**XV**) with the biphenylylmethyl bromide derivatives **VI**, **VII**, or **IX** gave the corresponding *S*-alkyl derivatives **XVII**, **XVIII**, and **XIX**, respectively. This agreed with literature reports that alkylation reactions of 2-mercaptoquinazolin-4(3*H*)-one occur preferentially on sulfur.[16,17] Tetrazole **XX** could be obtained either from the corresponding nitrile derivative **XVIII** via tetrazole ring formation or from the deprotection of the trityl tetrazolylbiphenylylmethyl quinazolinone (**XIX**). The latter method was more reproducible and gave higher yields. Similarly, alkylation reactions of 2-methylsulfanyl-3*H*-quinazolin-4-one (**XVI**) with **VI**, **VII**, or **IX** gave the corresponding 3-substituted derivatives **XXI**, **XXIII**, and **XXIV**. As mentioned before, the regioselectivity of such monoalkylation was also substantiated by NOESY

Scheme 2[a]



[a] (a) KCNO, (b) K_2CO_3, DMF, (c) NH_4Cl, NaN_3, (d) MeOH, reflux, (e) *t*-BuOK, DMSO, (f) Bu_3SnCl, NaN_3, (g) CH_3I, NaOH, (h) TFA, CH_2Cl_2, (i) K_2CO_3, CH_3CN.

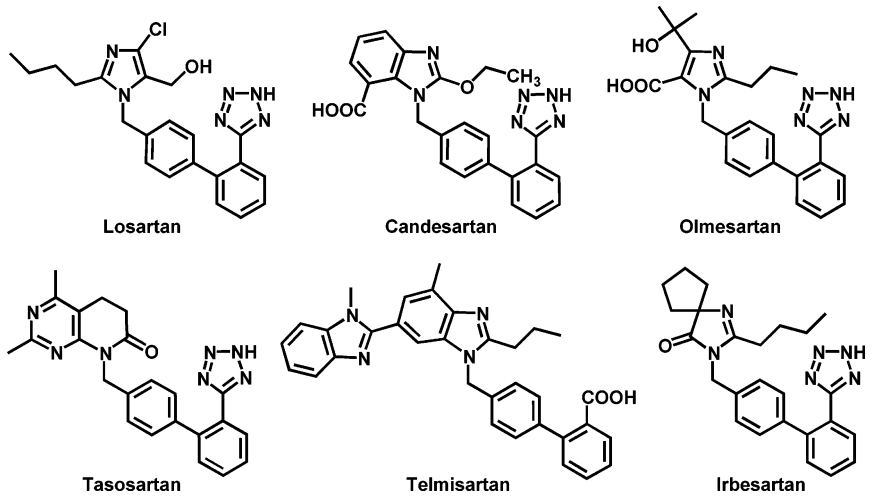


Figure 2. The structures of the lead AT_1 receptor antagonists representing the training set.

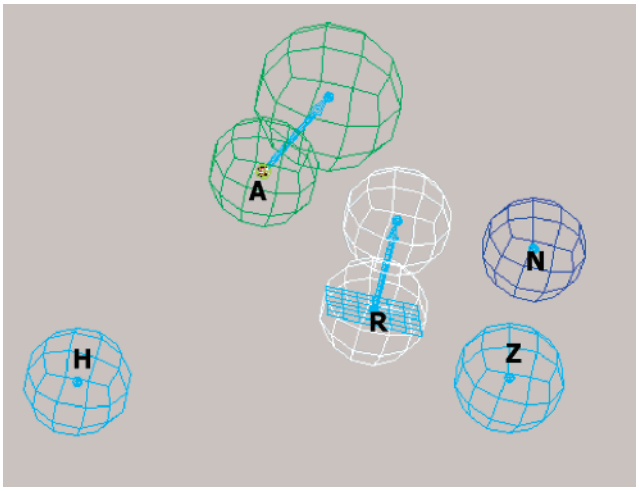


Figure 3. Hypothesis of AT_1 receptor antagonists: A, hydrogen-bond acceptor; H, hydrophobic aliphatic; N, negative ionizable; R, ring aromatic; Z, hydrophobic aromatic.

for compound **XXIII**, where it proved to occur at position 3 rather than 1. Hydrolysis of thioquinazolinone **XXI** using trifluoroacetic acid (TFA) gave the carboxylic acid (**XXII**). The tetrazole derivative **XXV** was prepared either by reacting the cyano derivative **XXIII** with the azide or by hydrolysis of the trityl derivative **XXIV** (Scheme 2).

2.ii. Molecular Modeling. Generation of AT_1 Receptor Antagonist Hypothesis. Many lead compounds reported to have selective AT_1 antagonistic activity have been clinically introduced for the treatment of hypertension. In this research, the sets of conformational models of six leads, namely, losartan (Cozaar),[8,18] telmisartan (Micardis, Pritor),[11] tasosartan (Verdia), irbesartan (Avapro, Aproval, and Karvea),[19] candesartan (Atacand),[20] and olmesartan (Benicar)[21] (Figure 2), were used to generate a HipHop common feature hypothesis of the AT_1 antagonists.

We assessed the generated 10 hypotheses, and that ranked number 1 was chosen as the valid ideal hypothesis. The choice of the ideal hypothesis among those generated was based on the following: The hypothesis showed full mapping of all its features with the lead compound (losartan) without any steric clashes (vide infra, Figure 4a) and high fit values with the rest of the training set. Retrospectively, the simulated fit value of **XXV** with hypothesis 1 was more consistent with the experimental results than with hypotheses 2−10. The ideal hypothesis encompassed five features, namely, hydrogen-bond acceptor, hydrophobic aliphatic, negative ionizable, ring aromatic, and hydrophobic aromatic (Figure 3).

The literature contains other reported Ang II pharmacophore models.[11,22] In 1993, Ries et al.[11] reported a model having five pharmacophoric groups generated by conformational analysis of a large number of benzimidazole Ang II antagonists, and these groups (a hydrogen-bond acceptor, a small alkyl residue, an acidic group, a lipophilic aromatic group, and a positive ionizable heteroatom) represented the receptor-bound conformation of Ang II antagonists. In 1994, Prendergast et al.[22a] reported another Ang II antagonist model, defined by four features on a chosen ligand, L-15809.[22b] Those features comprised an acidic group, an aromatic nitrogen for hydrogen bonding, an alkyl side chain with a minimum of two carbon atoms, and a supplementary point in the biphenyl group. The authors also mentioned the distances between those features. Recently, Krovat and Langer[22c] reported a HipHop common features hypothesis comprising seven features (two aromatic rings, two hydrogen

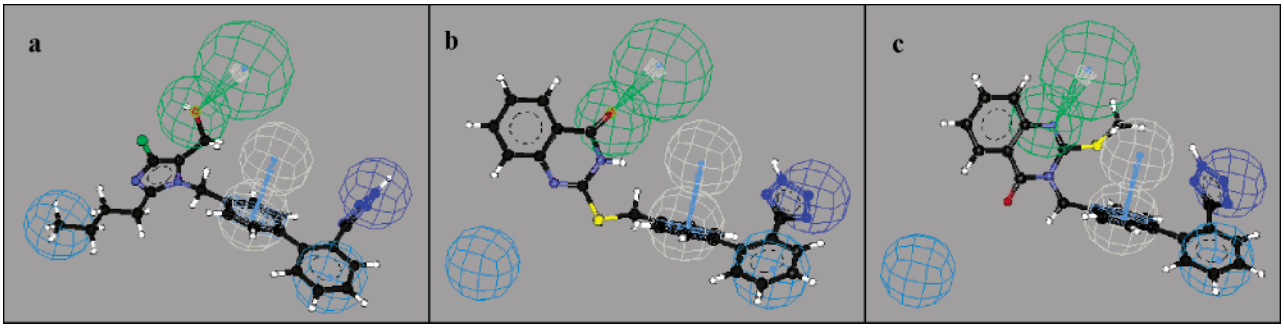


Figure 4. Mapping of (a) losartan, (b) compound **XX**, and (c) compound **XXV** with the generated AT_1 hypothesis.

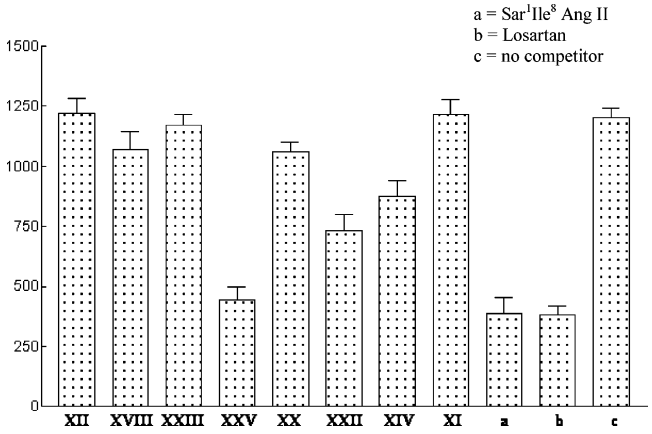


Figure 5. Screening for competition to binding of ^{125}I-Sar^{1}Ile^{8}-Ang II to membrane fraction of CHO.hAT$_1$ cells.

bond acceptors, two hydrophobic functions, and a negative ionizable function), generated by only two Ang II antagonists (**3k** and **4u**)[22a] which are not clinically used. In the same paper, the authors reported a Hypogen-derived hypothesis generated from a set of Ang II antagonists covering a wide activity range. Such a reported Hypogen hypothesis consisted of a hydrophobic aliphatic group, a hydrophobic aromatic group, a hydrogen-bond acceptor, a negative ionizable function, and an aromatic plane. However, the dimensions between the features in these HipHop and the Hypogen hypotheses were not published.

Herein, we report a new HipHop five features hypothesis (Figure 3) that could be generated from highly biologically active and clinically used Ang II antagonists in order to qualitatively prioritize the biological activity of the test set of Ang II antagonists. Such an hypothesis has the same types of features such as the reported Hypogen hypothesis.[22b] Additionally, we recorded the constraint angles and distances between the different features of our hypothesis (see Supporting Information). Crucially, we exploited our pharmacophore model to design a small number of novel AT$_1$ receptor antagonists that were evaluated in vitro and in vivo and found to be extremely potent.

Molecular Simulation Fitting of the AT$_1$ Antagonists Hypothesis (Pharmacophore) and the Test Set of the Target Biphenylylmethylquinazolinone Derivatives. The structures of the test set of the target quinazolinones (**XI**, **XIV**, **XVII**, **XVIII**, **XX**, **XXI**, **XXII**, **XXIII**, and **XXV**) were built using CATALYST software,[23] and their conformational models were generated[24] (in the energy range of 20 kcal/mol above the estimated global energy minimum). The fitting of the tested compounds was determined on the selected hypothesis of the AT$_1$ antagonists for predicting their activity, and this was performed using Best Fit during the Compare/Fit process. Different mappings for all the conformers of each compound of the test set to the selected hypothesis were visualized and the fit values of the best-fitting conformers were found (Table 1). This molecular modeling simulation study revealed that

Table 1. Best Fit Conformer for Each Compound in the Test Set When Mapped to the AT$_1$ Antagonists Hypothesis

compd	no. of conformers	rank of the best fit conformer	fit value
XI	198	15	2.86
XIV	96	5	3.80
XVII	96	9	2.93
XVIII	85	19	2.96
XX	126	48	3.91
XXI	65	16	2.87
XXII	94	26	3.29
XXIII	58	43	2.94
XXV	99	9	3.89

Table 2. Mean Count per Minute (cpm) and Standard Error Mean (SEM) for the Tested Compounds

compd	mean cpm	SEM	compd	mean cpm	SEM
a	385.0	70.5	**XVIII**	1068.3	77.4
b	383.7	36.1	**XX**	1061.5	39.4
c	1205.5	37.3	**XXII**	734.5	65.3
XI	1217.5	62.1	**XXIII**	1172.0	43.6
XII	1221.8	62.8	**XXV**	442.5	57.0
XIV	875.5	63.9			

compounds **XIV**, **XX**, **XXII**, and **XXV** could be considered as promising candidates, due to their high fit values to the hypothesis. (Figure 4 demonstrates the mapping of the generated hypothesis with losartan and compounds **XX** and **XXV**). Accordingly, we synthesized these compounds and examined their biological activity.

2.iii. Biological Screening. 2.iii.a. Receptor Binding Assay. A receptor binding assay was carried out by competitive displacement of the binding of ^{125}I-Sar^{1}Ile^{8}-Ang II to the membrane fraction of Chinese hamster ovary cells stably transfected with human AT$_1$ receptor cDNA (CHO.hAT$_1$ cells).[25] The experiments were performed on the candidate compounds and selected intermediates (Table 2 and Figure 5). The study showed that compounds **XIV**, **XXII**, and **XXV** were all able to displace radiolabeled Ang II. Compound **XXV** was found to be the most potent, showing displacement similar to that of losartan. Compound **XX** was shown not to compete well in the receptor binding assay, despite showing a high fit value in the molecular modeling studies. A similar disparity was reported for α_1 adrenoceptor ligands,[26] where it was found that the existence of an electron-rich edge on the molecule may cause a detrimental interaction between the compound and its receptor. Accordingly, we determined the atomic charge distribution for compound **XX** (Figure 6). We found a high electron density on the fused benzene ring of the quinazolinone nucleus and on the carbonyl oxygen at position 4 that was not observed with other tested biologically active molecules such as compound **XXV** (Figure 7). Such a high electron density might prevent binding of compound **XX** with the AT$_1$ receptors.

2.iii.a. In Vivo Study of Hypotensive and Antihypertensive Activity. Compounds **XIV**, **XXII**, and **XXV** were subjected to

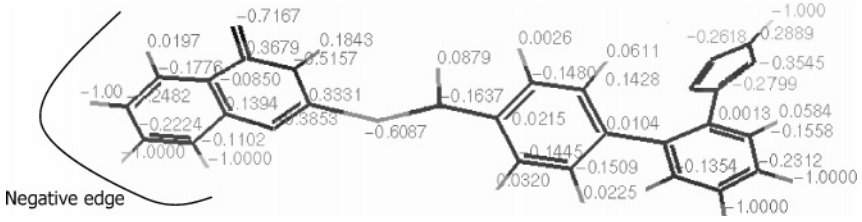


Figure 6. Atomic charge distribution on compound **XX**.

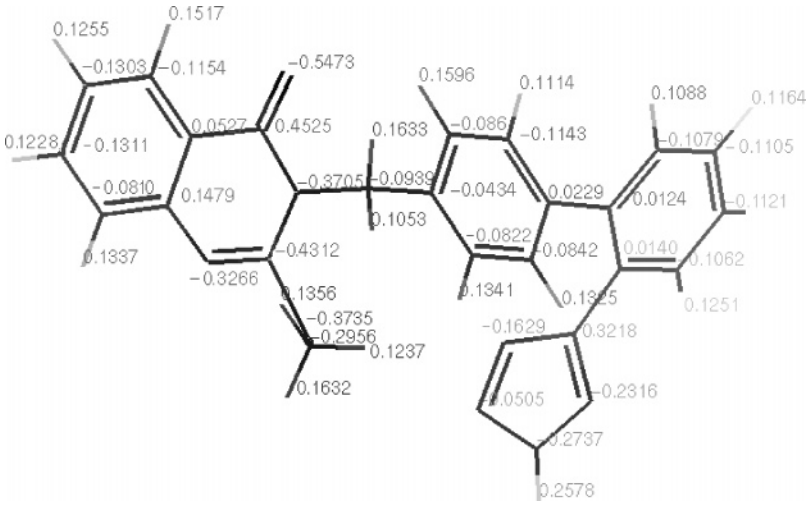


Figure 7. Atomic charge distribution on compound **XXV**.

Table 3. Mean Percentage Reduction of the Systolic Blood Pressure of Normotensive Rats Treated with 3 mg/kg (ip) of Different Compounds

group	mean systolic blood pressure ± SEM	mean % reduction
control	107.17 ± 0.79	
losartan	91.33 ± 1.28[a]	14.78
XIV	92.67 ± 2.36[a]	13.53
XXII	97.00 ± 1.67[a]	9.49
XXV	85.83 ± 2.21[a]	19.91

[a] Statistically significant difference from the control group at $p < 0.05$.

Table 4. Mean Percentage Reduction of the Systolic Blood Pressure of Hypertensive Rats Treated with 3 mg/kg (ip) of Different Compounds

group	mean systolic blood pressure ± SEM	mean % reduction
control	106.67 ± 0.95	
hypertensive rats	136.67 ± 1.43[a]	
losartan	105.50 ± 0.99[b]	22.81
XIV	110.50 ± 0.89[b]	19.15
XXII	113.83 ± 1.01[b]	16.71
XXV	101.83 ± 1.11[b]	25.49

[a] Statistically significant difference from the control group at $p < 0.05$. [b] Statistically significant difference from the HTR group at $p < 0.05$.

in vivo biological evaluation for their effects on systolic blood pressure (BP) of normotensive and hypertensive male Sprague−Dawley rats.[27] The BP was recorded 2 h after treatment with the test compounds. All compounds tested demonstrated hypotensive and/or antihypertensive activity for both normotensive and hypertensive rats, respectively (Tables3 and 4). Compounds **XIV** and **XXII** caused significant reduction in BP in normotensive and hypertensive rats. Compound **XIV** had a hypotensive activity equal to the standard drug losartan. Compound **XXV** was the most active compound and decreased the BP of both normotensive and hypertensive rats better than losartan. This suggests that compound **XXV** may have advantages over losartan.

It was reported that candesartan has more pronounced antagonistic activity than losartan against the AT_1 receptor, because it exhibits insurmountable antagonism, while that of losartan is surmountable.[28,29] Therefore, we used CATALYST software to carry out a 3D alignment of the two compounds **XXV** and candersartan (Figure 8). The study revealed good alignment between the two compounds as follows: (i) the sulfur of the SCH_3 side chain with the oxygen of the ethoxy side chain

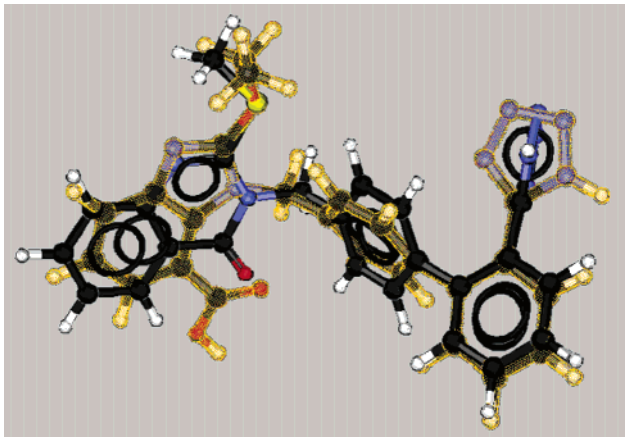


Figure 8. Alignment of compound **XXV** and candesartan (highlighted).

of candesartan; (ii) the keto group with the carboxylic acid group in candesartan (this carboxylic acid group is key to its sustained H-bonding interaction with a specific glutamic acid residue in the AT_1 receptor[30]); and (iii) N_1 and N_3 are perfectly aligned with those of candesartan. This means that compound **XXV** structurally resembles candesartan.

Conclusion

In this project we were able to design new ligands having Ang II (AT_1) receptor antagonistic activity using CATALYST software. The ideal pharmacophore model (hypothesis) was generated by the common feature hypothesis generation using a training set of known antihypertensive compounds (Figure 2). Compounds **XIV**, **XX**, **XXII**, and **XXV** showed high fit values to the generated AT_1 antagonists hypothesis. Eight of the synthesized compounds were biologically evaluated in vitro using an AT_1 receptor binding assay. Compound **XX** did not significantly compete with radiolabeled Ang II binding; compounds **XIV** and **XXII** showed moderate displacement, whereas compound **XXV** competed for radiolabeled Ang II to the same extent as losartan. The failure of compound **XX** to displace binding to the AT_1 receptors could be due to the unusually high electron density along an edge of the molecule. This suggests future directions for tuning of the pharmacophore model. In vivo biological evaluation studies of compounds **XIV**, **XXII**, and **XXV** on both normotensive and hypertensive rats revealed that compound **XIV** had equal hypotensive and moderate antihypertensive activity in comparison to losartan, whereas com-

pounds **XXII** showed moderate hypotensive and antihypertensive activity. Compound **XXV** demonstrated higher hypotensive and antihypertensive activity than losartan. These results indicate that such compounds had higher activity in the in vivo study than in the in vitro studies. We obtained highly active compounds from a set of only eight candidate molecules, which illustrates how useful the pharmacophore model is in the design of novel AT_1 antagonists.

Experimental Section

All reagents were supplied by Acros Organics, Aldrich, Avocado Scientific, BDH, Fischer, Lancaster, Merck, and VWR and were used with no further purification. TLC was performed on silica gel 60 F_{254} plates (Merck). Melting points were uncorrected and were determined on a Stuart Scientific apparatus or hot stage microscope (Reichert-Austria). The IR spectra were recorded on a Perkin-Elmer spectrum BX FT-IR spectrometer using KBr disks or a Shimadzu FT-IR 8300 spectrometer using Nujol. The NMR spectra were run in $CDCl_3$ unless otherwise stated with a JEOL JNM-EX270 DELTA spectrometer, at 270 MHz for ^{1}H NMR and 67.9 MHz for ^{13}C NMR, or on Bruker AV400 spectrometer, at 400 MHz for ^{1}H NMR and 100.58 MHz for ^{13}C NMR. *J* values are given in hertz. Mass spectra were carried out at the EPSRC National Mass Spectrometry Service Centre, Chemistry Department, University of Wales, Swansea, UK or The Microanalytical Centre, Cairo University, Egypt. Preparation of 2-bromobenzoic acid *tert*-butyl ester (**I**),[31] 4′-methylbiphenyl-2-carboxylic acid *tert*-butyl ester (**II**),[8,31−2] 4′-bromomethyl-biphenyl-2-carboxylic acid *tert*-butyl ester (**VI**),[8,31−2] 4′-bromomethyl-biphenyl-2-carbonitrile (**VII**),[8,32] 5-(4′-methylbiphenyl-2-yl)-2-trityl-2*H*-tetrazole (**VIII**),[8,32−3] 5-(4′-bromomethyl-biphenyl-2-yl)-2-trityl-2*H*-tetrazole (**IX**),[8,32] 1*H*-quinazoline-2,4-dione (**X**),[34−5] and 2-methylsulfanyl-3*H*-quinazolin-4-one (**XVI**)[35] were performed according to reported procedures. Compound **XV** was purchased from Aldrich. Purities for compounds **XI**, **XIV** and **XX** were found to be >95% by ^{1}H NMR spectroscopy and gave sharp melting points.

4′-(2,4-Dioxo-1,4-dihydro-2*H*-quinazolin-3-ylmethyl)biphenyl-2-carbonitrile (XI) and 1,3-Bis[2′-cyanobiphenyl-4-ylmethyl]-1*H*-quinazoline-2,4-dione (XII). 1*H*-Quinazoline-2,4-dione (**X**) (0.30 g, 1.85 mmol) was dissolved in dry DMF (30 mL). Anhydrous K_2CO_3 (0.76 g, 5.51 mmol, 2.98 equiv) was added portionwise to the reaction mixture, which was stirred for 1 h under nitrogen. 4′-Bromomethyl-biphenyl-2-carbonitrile (**VII**) (0.503 g, 1.85 mmol, 1 equiv) was added to the reaction mixture and stirring continued for 22 h. Water (150 mL) was added, and the resulting white suspension was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by flash column chromatography (1% methanol in chloroform) to give the 3-substituted quinazolinedione (**XI**) as a white solid (11 mg, 2%). 1,3-Bis[2′-cyanobiphenyl-4-ylmethyl]-1*H*-quinazoline-2,4-dione (**XII**) was also isolated as a white solid (110 mg, 11%). An analytical sample was recrystallized from methanol/chloroform to give the bis-substituted quinazolinedione (**XII**) as colorless needles.

Data for compound XI: mp 271−273 °C; ^{1}H NMR (270 MHz; DMSO-d_6) 5.17 (s, 2 H, CH_2), 7.23 (t, 2 H, *J* = 6.9, Ar-*H*), 7.44−7.80 (m, 7 H, Ar-*H*), 7.95 (t, 2 H, *J* = 7. 7, Ar-*H*), 8.32 (m, 1 H, Ar-*H*), 11.59 (br s, 1 H, N*H*); HRMS (ES) calcd for $C_{22}H_{19}N_4O_2$ (MNH_4^+) 371.1503, found 371.1501.

Data for compound XII: mp 140−142 °C; ^{1}H NMR (270 MHz) 5.40 (s, 2 H, CH_2), 5.45 (s, 2 H, CH_2), 7.14−7.75 (m, 18 H, Ar-*H*), 8.27 (dd, 2 H, *J* = 7.9, 1.5, Ar-*H*); HRMS (ES) calcd for $C_{36}H_{28}N_5O_2$ (MNH_4^+) 562.2238, found 562.2239. Anal. ($C_{36}H_{24}N_4O_2$) C, H, N.

3-[2′-(2*H*-Tetrazol-5-yl)biphenyl-4-ylmethyl]-1*H*-quinazoline-2,4-dione (XIV). To a solution of 1*H*-quinazoline-2,4-dione (**X**) (0.15 g, 0.9 mmol) in dry DMF (15 mL), anhydrous K_2CO_3 (0.37 g, 2.68 mmol, 2.98 equiv) was added portionwise and the reaction mixture stirred for 30 min under nitrogen. 5-(4′-Bromomethyl-biphenyl-2-yl)-2-trityl-2*H*-tetrazole (**IX**) (0.5 g, 0.9 mmol, 1 equiv) was added and stirring continued for 23 h. Water (75 mL) was added, and the resulting white suspension was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo giving the crude product as a thick yellow oil (476 mg, 83%). The crude reaction mixture was taken as such and refluxed in methanol (10 mL) for 6 h and then concentrated in vacuo. The crude product was recrystallized from chloroform/methanol to give the 3-substituted quinazolinone (**XIV**) (46 mg, 13% overall yield from **X**): mp 253−255 °C; ^{1}H NMR (270 MHz; DMSO-d_6) 5.09 (s, 2 H, CH_2), 7.04 (d, 2 H, *J* = 8.2, Ar-*H*), 7.23 (m, 4 H, Ar-*H*), 7.51−7.71 (m, 5 H, Ar-*H*), 7.95 (d, 1 H, *J* = 6.9, Ar-*H*), 11.56 (s, 1 H, N*H*); HRMS (ES) calcd for $C_{22}H_{20}N_7O_2$ (MNH_4^+) 414.1673, found 414.1669.

4′-(4-Oxo-3,4-dihydroquinazolin-2-ylsulfanylmethyl)biphenyl-2-carbonitrile (XVIII). To a solution of 2-mercapto-3*H*-quinazolin-4-one (**XV**) (0.33 g, 1.84 mmol) in dry DMF (30 mL) was added anhydrous K_2CO_3 (0.76 g, 5.48 mmol, 2.98 equiv) portionwise and the reaction mixture stirred for 1 h. 4′-Bromomethyl-biphenyl-2-carbonitrile (**VII**) (0.5 g, 1.84 mmol, 1 equiv) was added to the reaction mixture and stirring was continued for 24 h. Water (150 mL) was added, and the resulting white suspension was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was recrystallized from CH_2Cl_2/hexane/methanol giving **XVIII** as off-white needles (495 mg, 73%): mp 226−228 °C; ^{1}H NMR (270 MHz; DMSO-d_6) 4.58 (s, 2 H, CH_2), 7.42 (t, 1 H, *J* = 7.9, Ar-*H*), 7.52−7.66 (m, 7 H, Ar-*H*), 7.74−7.81 (m, 2 H, Ar-*H*), 7.92 (d, 1 H, *J* = 7.7, Ar-*H*), 8.04 (d, 1 H, *J* = 7.7, Ar-*H*); HRMS (ES) calcd for $C_{22}H_{16}N_3OS$ (MH^+) 370.1009, found 370.1010. Anal. ($C_{22}H_{15}N_3OS$) H, N; C: calcd 71.52; found 71.10.

2-[2′-(2*H*-Tetrazol-5-yl)biphenyl-4-ylmethylsulfanyl]-3*H*-quinazolin-4-one (XX). Method A. To a solution of 4′-(4-oxo-3,4-dihydroquinazolin-2-ylsulfanylmethyl)biphenyl-2-carbonitrile (**XVIII**) (0.5 g, 1.35 mmol, 1 equiv) in dry DMF (8.5 mL) were added tributyltin chloride (1.76 g, 5.41 mmol, 4 equiv) and sodium azide (0.35 g, 5.41 mmol, 4 equiv). The mixture was heated at 130 °C under nitrogen for 12 h, after which further tributyltin chloride (0.4 mL, 0.44 g, 1.35 mmol, 1 equiv) and sodium azide (88 mg, 1.35 mmol, 1 equiv) were added. After 26 h, the reaction mixture was cooled, poured into ice/water, and acidified with 6 N aqueous HCl. The resulting precipitate was extracted with CH_2Cl_2 (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by flash column chromatography (chloroform/methanol 19:1 ramping to chloroform/methanol 9:1), 57 mg (11%) of the starting material (**XVIII**) was recovered, and the biphenyltetrazole derivative (**XX**) was isolated as a white solid (356 mg, 64%). An analytical sample was recrystallized from chloroform/methanol to give colorless needles: mp 238−240 °C.

Method B. 2-[2′-(2-Trityl-2*H*-tetrazol-5-yl)biphenyl-4-ylmethylsulfanyl]-3*H*-quinazolin-4-one (**XIX**) (100 mg, 0.15 mmol) was heated at reflux in methanol (10 mL) for 20 h. The reaction mixture was concentrated in vacuo. The crude product was recrystallized from chloroform/methanol to give the biphenyltetrazole derivative (**XX**) as a white solid (53 mg, 86%): mp 245−246 °C; ^{1}H NMR-(270 MHz; DMSO-d_6) 4.48 (s, 2 H, CH_2), 7.04−7.07 (d, 2 H, Ar-*H*), 7.29−7.63 (m, 7 H, Ar-*H*), 7.74−7.80 (m, 2 H, Ar-*H*), 8.04 (d, 1 H, *J* = 7.9, Ar-*H*); HRMS (ES) calcd for $C_{22}H_{20}N_7OS$ (MH^+) 413.1179, found 413.1181.

4′-(2-Methylsulfanyl-4-oxo-4*H*-quinazolin-3-ylmethyl)biphenyl-2-carboxylic Acid (XXII). To a solution of 4′-(2-methylsulfanyl-4-oxo-4*H*-quinazolin-3-ylmethyl)biphenyl-2-carboxylic acid *tert*-butyl ester (**XXI**) (100 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL). The reaction mixture was stirred at room temperature for 13 h, concentrated in vacuo, and dissolved in CH_2Cl_2 (7 mL), and the pH was adjusted to 3 by addition of 10% NaOH. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and

the combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was washed with ether and dried in vacuo to give the acid (**XXII**) as a white solid (75 mg, 85%): mp 209−210 °C; ^{1}H NMR (270 MHz) 2.61 (s, 3 H, C*H*$_3$), 5.41 (s, 2 H, C*H*$_2$), 7.24−7.92 (m, 11 H, Ar-*H*). 8.24 (d, 1 H, J = 7.9, Ar-*H*); HRMS (ES) calcd for $C_{23}H_{19}N_2O_3S$ (MH^+) 403.1111, found 403.1115. Anal. ($C_{23}H_{18}N_2O_3S$) C, H, N, S.

4′-(2-Methylsulfanyl-4-oxo-4*H*-quinazolin-3-ylmethyl)biphenyl-2-carbonitrile (XXIII). 2-Methylsulfanyl-3*H*-quinazolin-4-one (**XVI**) (3.53 g, 18.37 mmol) was dissolved in warm acetonitrile (200 mL), and anhydrous K_2CO_3 (7.57 g, 54.74 mmol, 2.98 equiv) was added portionwise. 4′-Bromomethyl-biphenyl-2-carbonitrile (**VII**) (5.00 g, 18.37 mmol, 1 equiv) was added to the reaction mixture, which was heated at reflux for 4 h. After evaporation of the solvent, the residue was diluted with water (100 mL) and extracted with ethyl acetate (150 mL). The organic extract was washed with water (3 × 100 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was digested with hot ethyl acetate, filtered, and recrystallized from chloroform/methanol to give the biphenyl methyl quinazoline (**XXIII**) as colorless needles (5.82 g, 83%): mp 194−195 °C; ^{1}H NMR (270 MHz) 2.65 (s, 3 H, C*H*$_3$), 5.44 (s, 2 H, C*H*$_2$), 7.36−7.75 (m, 11 H, Ar-*H*), 8.24 (d, 1 H, J = 7.9, Ar-*H*); HRMS (ES) calcd for $C_{23}H_{18}N_3OS$ (MH^+) 384.1165, found 384.1165. Anal. ($C_{23}H_{17}N_3OS$) H, N; C: calcd 72.04; found 71.01.

2-Methylsulfanyl-3-[2′-(2*H*-tetrazol-5-yl)biphenyl-4-ylmethyl]-3*H*-quinazolin-4-one (XXV). Method A. Tributyltin chloride (3.13 g, 9.62 mmol, 4 equiv) and sodium azide (0.62 g, 9.62 mmol, 4 equiv) were added to a solution of 4′-(2-methylsulfanyl-4-oxo-4*H*-quinazolin-3-ylmethyl)biphenyl-2-carbonitrile (**XXIII**) (0.92 g, 2.4 mmol, 1 equiv) in dry DMF (15 mL). The mixture was heated at 130 °C under nitrogen for 12 h. Further tributyltin chloride (0.78 g, 2.4 mmol, 1 equiv) and sodium azide (0.16 g, 2.4 mmol, 1 equiv) were added and stirring resumed at the same temperature for a further 14 h. The reaction mixture was allowed to cool, poured into ice/water, and acidified with 6 N aqueous HCl. The resulting precipitate was extracted with CH_2Cl_2 (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic portions were washed with brine (15 mL), dried ($MgSO_4$), and concentrated in vacuo to yield an off white solid which was purified by flash column chromatography (chloroform/methanol 99:1 ramping to chloroform/methanol 9:1) to give the biphenyltetrazole (**XXV**) as a white solid (683 mg, 67%), and 100 mg (11%) of the starting material **XXIII** was recovered.

Method B. To a solution of 4′-(2-methylsulfanyl-4-oxo-4*H*-quinazolin-3-ylmethyl)biphenyl-2-carbonitrile (**XXIII**) (500 mg, 1.3 mmol, 1 equiv) in dry DMF (20 mL) were added sodium azide (84.5 mg, 1.3 mmol, 1 equiv) and ammonium chloride (69.5 mg, 1.3 mmol, 1 equiv), and the reaction mixture was heated at 140 °C under nitrogen for 16 h. Further sodium azide (84.5 mg, 1.3 mmol, 1 equiv) and ammonium chloride (69.5 mg, 1.3 mmol, 1 equiv) were added, and stirring at 140 °C was continued for another 26 h. The reaction mixture was allowed to cool and water (200 mL) was added. The reaction mixture was acidified to pH 2 with 10% HCl. Ethyl acetate (50 mL) was added, and the layers were separated. The aqueous portion was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with brine (20 mL), dried ($MgSO_4$), and concentrated in vacuo to give the crude product as an off white solid which was purified by flash column chromatography (chloroform/methanol 99:1 ramping to chloroform/methanol 9:1) to give the biphenyltetrazole (**XXV**) as a white solid (360 mg, 65%).

Method C. Method B used in the preparation of compound **XX** was followed, using 2-methylsulfanyl-3-[2′-(2-trityl-2*H*-tetrazol-5-yl)biphenyl-4-ylmethyl]-3*H*-quinazolin-4-one (**XXIV**) (100 mg, 0.15 mmol). The reaction mixture was heated at reflux for only 16 h. The crude product was recrystallized from chloroform/methanol to give the biphenyltetrazole (**XXV**) as a white solid (55 mg, 86%); R_f (chloroform/methanol 9:1) 0.39. An analytical sample was recrystallized from chloroform−methanol to give colorless needles: mp 235−236 °C; ^{1}H NMR (270 MHz; DMSO-d_6) 2.61 (s, 3 H, C*H*$_3$), 5.33 (s, 2 H, C*H*$_2$), 7.06 (d, 2 H, J = 8.2, Ar-*H*), 7.20 (d, 2 H, J = 8.2, Ar-*H*), 7.45−7.70 (m, 6 H, Ar-*H*), 7.82 (dt, 1 H, J = 7.7, 1.5, Ar-*H*), 8.11 (dd, 1 H, J = 7.9, 1.2, Ar-*H*); HRMS (ES) calcd for $C_{23}H_{19}N_6OS$ (MH^+) 427.1336, found 427.1335. Anal. ($C_{23}H_{18}N_6OS\cdot H_2O$) C, H, N.

Catalyst Molecular Modeling Experiments. All molecular modeling studies were performed using a Silicon Graphics desktop (SGI) Fuel workstation under an IRIX 6.8 operating system, at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt. The training set was selected as described above (Figure 2). The generation of the pharmacophore model for AT_1 receptor antagonists was accomplished using CATALYST, version 4.8, from Accelrys, Inc. (previously known as Molecular Simulations, Inc.) (San Diego, CA). Molecules were built within CATALYST[23] and conformational models for each compound were generated automatically using the poling algorithm.[24] This emphasizes representative coverage over a 20 kcal/mol energy range above the estimated global energy minimum and the best quality generation technique was chosen. The training set molecules, with their associated conformational models, were used for common features hypothesis generation using the HipHop module by default.[36] In this study, hydrophobic aliphatic regions, hydrophobic aromatic regions, hydrogen-bond acceptors, ring aromatic, and negative ionizable points were used as the chemical features, which were reported to be crucial for the AT_1 antagonistic activity.[22b] By this process we specified the crucial features required for binding with the Ang II receptor, in accordance with the literature.[22b]

In Vitro Receptor Binding Assay. Preparation of Membrane Fraction Containing Human AT_1 Receptor Protein. For screening of Ang II AT_1 receptor antagonistic activity, a particulate fraction (containing cell membranes) was obtained from 20 150 cm^2 culture flasks of Chinese hamster ovary cells, previously stably transfected with a plasmid containing full-length cDNA encoding the human AT_1 receptor open reading frame (CHO.hAT_1 cells).[25,37] CHO.hAT_1 cells were routinely cultured as monolayers in Ham's F12 medium containing 5% fetal bovine serum, l-glutamine, penicillin/streptomycin, sodium pyruvate, and nonessential amino acids (complete medium). After harvesting the cells using trypsin-EDTA, cell pellets after 1000 rpm centrifugation were resuspended in complete medium, recentrifuged, and then washed and recentrifuged once more in complete medium. After removal of the medium, cell pellets were stored at −70 °C until use. Cell pellets were then resuspended in ice-cold 50 mM Tris. HCl buffer, pH 7.4, containing the protease inhibitors aprotinin (1 μg/mL) and soy bean trypsin inhibitor (1 μg/mL) (resuspension buffer).[25,37] Cells were disrupted by passing several times through graded syringe needles (range between 19 and 25 gauge) while kept on ice. This suspension was then centrifuged at 100*g* for 5 min to remove nuclear debris. The resulting supernatant was then centrifuged at 100 000*g* for 45 min to obtain the membrane fraction. The pellet was then resuspended in resuspension buffer, aliquoted, and stored at −70 °C until use.

Procedure for Receptor Binding Assay. Membrane protein was diluted in assay buffer to give ∼50 μg/50 μL protein. Assay buffer consisted of resuspension buffer with the further addition of 100 mM NaCl, 6 mM $MgCl_2$, and 0.1% w/v bovine serum albumin. Incubations of membrane protein fractions known to contain human AT_1 receptor were carried out for 1 h at room temperature as follows. The total volume incubation volume was 150 μL comprising, 50 μL membrane fraction in assay buffer, 50 μL competitor at 10 μM (final concentration) or no competitor in assay buffer, and 50 μL ^{125}I-Sar^{1}Ile^8-angiotensin II (^{125}I-Ang II) at 0.15 nM (final concentration) in assay buffer. After the incubation period the tubes were placed on ice and 850 μL of ice-cold assay buffer added. Tubes were then centrifuged at 11 000*g* for 5 min at 4 °C. Supernatant containing unbound radioactivity was aspirated and discarded. The pellets were then washed once with a further 850 μL of ice-cold assay buffer and recentrifuged at 11 000*g* for 5 min at 4 °C. Following final removal of the supernatant by aspiration, tubes were counted in a Wallac γ-counter for 1 min per tube. Results

are expressed as counts per minute (cpm). Counts per minute were counted in the residual pellet after removing unbound soluble ^{125}I-Ang II from the tubes. The pellet contains AT_1 receptor still embedded in cellular membrane and as such can be spun down as a solid pellet. The control tube was the same assay buffer (including the same final concentration of DMSO, as in all the samples) so in this tube there was no unlabeled competitor to the ^{125}I-Ang II, hence having the highest cpm. The more efficient the competitor, the less ^{125}I-Ang II is able to bind to the AT1 receptor available, so there is less radioactivity in the pellet, as unlabeled competitor has displaced radiolabeled angiotensin in the binding equilibrium (N.B., competitors are in large excess over labeled Ang II).

In Vivo Pharmacological Testing. Male Sprague−Dawley rats (230−250 g) were obtained from the Animal House facility of The National Research Centre Laboratory, Cairo, Egypt. They were acclimatized for 1 week in the animal facility at a temperature controlled at 23−25 °C with alternating 12-h light and dark cycles and provided with chow and water ad libitum.

In Vivo Hypotensive Activity Testing: The Effect of Tested Compounds on Normotensive Rats. Thirty normal rats were used in this set of experiments; animals were divided into five groups ($n = 6$) as follows. Group 1 was kept as a normal control and were given a single dose of DMSO [0.1 mL, intraperitoneal (ip)]. Group 2 received 3 mg/kg ip injection of losartan in 0.1 mL of DMSO. Groups 3−5 were given ip injection of 3 mg/kg of the tested compounds **XIV**, **XXII**, and **XXV** dissolved in 0.1 mL of DMSO. Blood pressure (BP) was recorded after 2 h of treatment. BP was measured using the tail-cuff method in conscious rats.[27] To reduce spontaneous variation in blood pressure, animals were adjusted to the experimental cage by bringing them into the restraining cage, which was enclosed in a 31−32 °C measuring chamber three or four times before starting of the experiments for a period of 30−60 min. To measure blood pressure, a tubular inflatable cuff is placed around the base of the tail and an electronic pulse detector is positioned distal to the cuff. The cuff is inflated to approximately 300 mmHg. As the pressure in the cuff is slowly released, the systolic pressure is detected and subsequently recorded on a polygraph (BP-98A, Softron Co., Ltd., Tokyo, Japan).

In Vivo Antihypertensive Activity Testing: The Effect of Tested Compounds on the DOCA−Salt Hypertension Model. Six animals served as normal controls. Thirty rats were used for induction of experimental hypertension by daily subcutaneous injection of 10 mg/kg deoxycorticosterone acetate (DOCA) and animals were fed a high salt diet (8% NaCl) for 3−4 weeks. BP was recorded 24 h before starting the treatment using the tail-cuff method in conscious rats as previously mentioned. Normal control received ip injection of 0.1 mL of DMSO. Hypertensive animals were divided into five groups ($n = 6$). Group 1 received ip injection of 0.1 mL of DMSO and served as the hypertensive animals. Group 2 received losartan (standard drug: 3 mg/kg in 0.1 mL DMSO, ip). Groups 3−5 were given the same doses of the tested compounds. Two hours later, BP was recorded for all groups as mentioned before. Data are given as means ± SE; $p < 0.05$ was considered to be significant. Statistical analysis was performed by ANOVA and LSD.

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Supporting Information Available: Table of constraint angles and distances for the pharmacophore model and experimental details for the preparation of compounds **III**−**V**, **XIII**, **XVII**, **XIX**, **XXI**, and **XXIV**. This material is available free of charge via the Internet at http://pubs.acs.org.

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