

## Novel 4,5-dihydro-4-oxo-3*H*-imidazo[4,5-*c*]pyridines. Potent angiotensin II receptor antagonists with high affinity for both the AT<sub>1</sub> and AT<sub>2</sub> subtypes\*

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**Summary** — The synthesis and pharmacological activity of balanced high affinity non-peptide angiotensin II antagonists of the AT<sub>1</sub> and AT<sub>2</sub> subtype receptors have been presented. A series of previously prepared AT<sub>1</sub> selective 4,5-dihydro-4-oxo-3*H*-imidazo[4,5-*c*]pyridines were modified at four different positions in order to increase the AT<sub>2</sub> binding affinity by maintaining the nanomolar activity for the AT<sub>1</sub> receptor. The targeted AT<sub>2</sub>/AT<sub>1</sub> IC<sub>50</sub> binding ratio of ~ 1 was achieved with a number of compounds possessing a small alkyl chain at C-2, different acetamide groups at N-5 and a 3-fluoro and 2'-carboxamidodisulfonyl substituent at the biphenylmethyl moiety. These modifications led to analogue **12s**, which exhibited an AT<sub>2</sub>/AT<sub>1</sub> ratio of 0.74, a subnanomolar AT<sub>1</sub> antagonistic potency (0.18 nM) and a high metabolic stability in rat and monkey liver microsomes *in vitro*. After oral administration of 3 mg/kg to cynomolgus monkeys, EMD 90423 (potassium salt of **12s**) demonstrated good efficacy and a long duration of action as an antihypertensive agent.

**4,5-dihydro-4-oxo-3*H*-imidazo[4,5-*c*]pyridine / biphenylsulfonamide / angiotensin II receptor antagonist / AT<sub>1</sub>/AT<sub>2</sub> receptor subtype / antihypertensive activity**

### Introduction

The vasoactive hormone angiotensin II (ang II) produced by the renin-angiotensin system (RAS) is a potent regulator of blood pressure homeostasis, fluid volume and electrolyte balance in mammals [1]. The clinical success achieved by angiotensin converting enzyme (ACE) inhibitors in the treatment of hypertension and congestive heart failure has made the RAS a major focus for the discovery of novel antihypertensive agents [2]. However, ACE also has kininase activity, and this lack of specificity has been implicated in the occasional side effects of ACE inhibitors such as dry cough and angioedema [3]. With the development of ang II receptor antagonists, a more specific attempt to inhibit the activity of the RAS has become the main pharmacological approach.

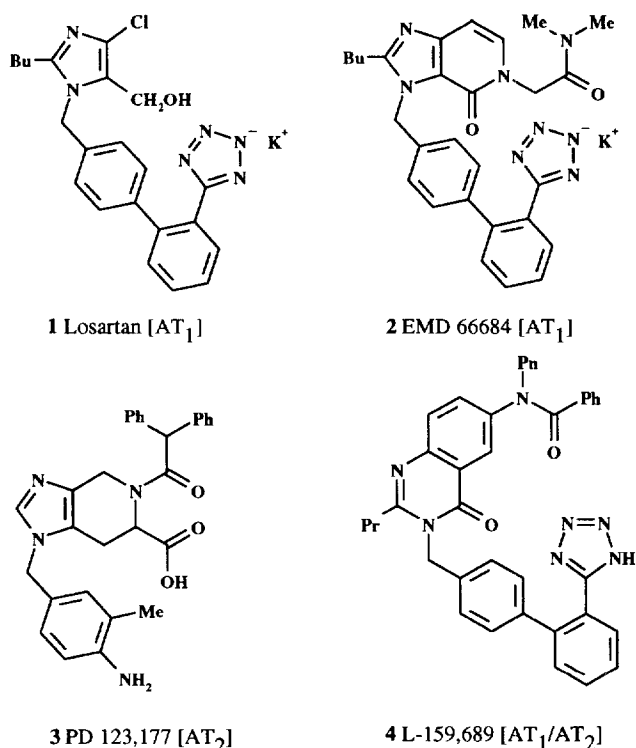
There are at least two distinct ang II receptor subtypes, designated as AT<sub>1</sub> and AT<sub>2</sub> [4]. Losartan **1**

(scheme 1), the most advanced non-peptide ang II receptor antagonist, mediates its effects by blocking the ang II AT<sub>1</sub> receptor subtype [5]. The AT<sub>1</sub> receptor is G-protein coupled [6] and mediates most of the known physiological effects of ang II, including the maintenance of blood pressure [7]. In recent years, a number of highly active non-peptide AT<sub>1</sub> selective ang II antagonists have been described [8]. In our group, 1,2-dihydropyridin-2-ones [9, 10], (6-oxo-3-pyrazinyl)-benzimidazoles [11], 4,5-dihydro-3*H*-imidazo[4,5-*c*]pyridin-4-ones [12], 7-ethyl-1,2-dihydroquinolin-2-ones [13] and 1,2,3,4-tetrahydropyrimidin-2,4-diones [14] have been studied as selective AT<sub>1</sub> antagonists.

With the discovery of the non-peptide ligand PD 123,177 **3** (scheme 1) the AT<sub>2</sub> receptor subtype has been identified in various tissues [15]. The AT<sub>2</sub> receptor also has a 7-transmembrane domain and is linked to phosphotyrosine phosphatase activity [16, 17]. The physiological role of this receptor has not yet been clearly defined, but recent studies have indicated that it may play a role in wound healing, cardiac remodeling and cerebral blood flow [7]. However, it has been shown that in the presence of a AT<sub>1</sub> selective antago-

\*Dedicated to Professor Dr Dr hc Ekkehard Winterfeldt, Hannover, on the occasion of his 65th birthday.

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**Scheme 1.** Selected structures of angiotensin II receptor antagonists.

nist (eg, losartan) the plasma concentration of ang II increases considerably [18]. To avoid an unopposed stimulation of the AT<sub>2</sub> receptor by the increased ang II plasma concentration, the development of balanced AT<sub>1</sub>/AT<sub>2</sub> receptor antagonists seemed to be justified due to safety considerations, even though a clear physiological response to AT<sub>2</sub> receptor stimulation has not been shown yet.

Thus, it seemed reasonable to develop compounds which would bind to both ang II subtypes. High affinity non-peptide AT<sub>1</sub>/AT<sub>2</sub>-balanced ang II ligands were first realized by 6-quinazolinones such as L-159,689 **4** (scheme 1) [19]. More recently, several other compounds with high affinity for both the AT<sub>1</sub> and AT<sub>2</sub> receptors have been described [20–24]. We previously reported the identification of the highly potent AT<sub>1</sub> receptor antagonist EMD 66684 **2** (scheme 1) [12]. During further studies of the structure–activity relationship (SAR) of **2**, we discovered structural modifications that enhanced the binding affinity of the imidazo[4,5-*c*]pyridine class of antagonists to the AT<sub>2</sub> receptor. Replacement of the dimethylacetamide in **2** by the phenylacetic acid isopropylester moiety led to a compound with a pronounced affinity

for this subtype (AT<sub>1</sub> = 6.5 nM, AT<sub>2</sub> = 32.0 nM; AT<sub>2</sub>/AT<sub>1</sub> = 5) [25]. Our next goal was to identify compounds with low nanomolar IC<sub>50</sub> values for both the AT<sub>1</sub> and the AT<sub>2</sub> receptors and to achieve an AT<sub>2</sub>/AT<sub>1</sub> IC<sub>50</sub> ratio of ~ 1.

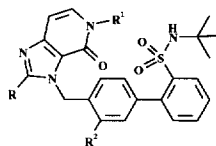
In this article, we report the synthesis and SAR studies of imidazo[4,5-*c*]pyridin-2-one derivatives as highly potent AT<sub>1</sub> receptor antagonists with AT<sub>2</sub>/AT<sub>1</sub> binding ratios close to unity.

## Chemistry

Two synthetic routes were used to generate the new compounds **11**, **12** and **13** shown in tables II–IV. The *N*-*tert*-butylsulfonamide intermediates **8**, **9** and **10** prepared during the course of this work are listed in table I.

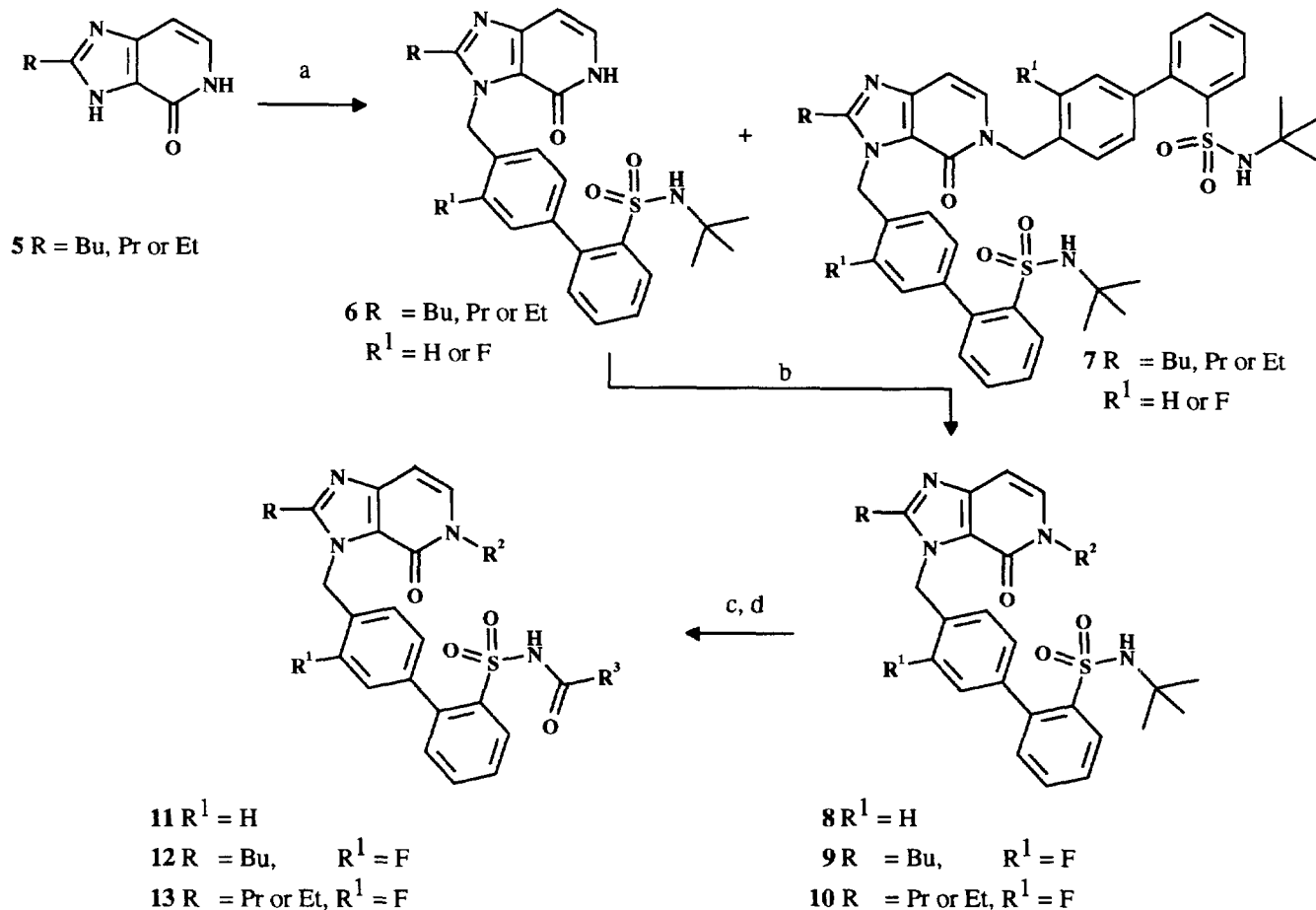
For the majority of these compounds, the key intermediates **8**, **9** and **10** were prepared in two steps by alkylation of the parent 4,5-dihydro-4-oxo-3*H*-imidazo[4,5-*c*]pyridines **5** [12] with the appropriate 4'-(bromomethyl)-*N*-*tert*-butyl-2-biphenylsulfonamide [26] or the corresponding 3-fluoro analogue [22] in the presence of potassium carbonate in dimethylformamide (scheme 2). This gave rise to a mixture of the desired compounds **6** and the bisalkylated products **7**, which were easily separated by silica gel chromatography. The regiochemistry of the alkylation could be verified by observation of nuclear Overhauser effects on compounds **6**. Deprotonation of the N-5 hydrogen in **6** with potassium *tert*-butoxide in dimethylformamide and reaction of the resulting anion with the appropriate electrophiles exclusively gave the *N*-alkylated derivatives **8**, **9** or **10**. Removal of the *tert*-butyl group via trifluoroacetic acid led to the free sulfonamides, which were treated with acid chlorides or chloroformates in pyridine in the presence of 4-dimethylaminopyridine to give the targeted sulfonylamides or sulfonylcarbamates **11**, **12** or **13** respectively.

In order to circumvent the bisalkylation problem in scheme 2, we sought a pathway which could be regiospecific and unequivocal. Several sulfonylamides and sulfonylcarbamates **13** were prepared by the alternate route shown in scheme 3. This general method is well illustrated by the preparation of compound **13i**. *N*-(4-Amino-2-chloro-3-pyridyl)butyramide **12**, which is readily available from 3,4-diamino-2-chloropyridine [12], was converted in six steps to the desired sulfonylamide **13i**. Alkylation of **12** with 4-bromo-2-fluorobenzylbromide provided a mixture of **13** and **14** in a regiospecific manner. Compounds **13** and **14** can be identified after separation by chromatography on silica gel, but heating this crude reaction mixture with hydrochloric acid directly afforded the required com-

**Table I.** Physical and chemical data of intermediates **8**, **9** and **10**.

Compound	R	R <sup>1</sup>	R <sup>2</sup>	Empirical formula <sup>a</sup>	Mp (°C)
<b>8a</b>	Bu	CH <sub>2</sub> Ph	H	C <sub>34</sub> H <sub>38</sub> N <sub>4</sub> O <sub>3</sub> S•0.5 H <sub>2</sub> O	134–135
<b>8b</b>	Bu	CH <sub>2</sub> CONPh <sub>2</sub>	H	C <sub>41</sub> H <sub>43</sub> N <sub>5</sub> O <sub>4</sub> S	178–179
<b>8c</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	H	C <sub>31</sub> H <sub>39</sub> N <sub>5</sub> O <sub>4</sub> S	90–91
<b>9a</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	F	C <sub>31</sub> H <sub>38</sub> FN <sub>5</sub> O <sub>4</sub> S	177–178
<b>10a</b>	Pr	CH <sub>2</sub> CONMe <sub>2</sub>	F	C <sub>30</sub> H <sub>36</sub> FN <sub>5</sub> O <sub>4</sub> S	82–83
<b>10b</b>	Et	CH <sub>2</sub> CONMe <sub>2</sub>	F	C <sub>29</sub> H <sub>34</sub> FN <sub>5</sub> O <sub>4</sub> S•1.5 H <sub>2</sub> O	81–82
<b>9b</b>	Bu	CH <sub>2</sub> Ph	F	C <sub>34</sub> H <sub>37</sub> FN <sub>4</sub> O <sub>3</sub> S	103–104
<b>9c</b>	Bu	CH <sub>2</sub> COPh	F	C <sub>35</sub> H <sub>38</sub> N <sub>4</sub> O <sub>4</sub> S•1.7 H <sub>2</sub> O	127–128
<b>9d</b>	Bu	CH <sub>2</sub> CH <sub>2</sub> COPh	F	C <sub>36</sub> H <sub>39</sub> FN <sub>4</sub> O <sub>4</sub> S•0.5 H <sub>2</sub> O	70–71
<b>9e</b>	Bu	CH <sub>2</sub> COMe	F	C <sub>30</sub> H <sub>35</sub> FN <sub>4</sub> O <sub>4</sub> S•1.0 H <sub>2</sub> O	103–104
<b>9f</b>	Bu	CH <sub>2</sub> CO <i>t</i> -Bu	F	C <sub>33</sub> H <sub>41</sub> FN <sub>4</sub> O <sub>4</sub> S•1.0 H <sub>2</sub> O	87–88
<b>9g</b>	Bu	CH <sub>2</sub> CON <i>n</i> -Bu	F	C <sub>33</sub> H <sub>41</sub> FN <sub>4</sub> O <sub>4</sub> S	57–58
<b>9h</b>	Bu	CH <sub>2</sub> COOEt	F	C <sub>31</sub> H <sub>37</sub> FN <sub>4</sub> O <sub>5</sub> S	72–73
<b>9i</b>	Bu	CH <sub>2</sub> CONHCH <sub>2</sub> <i>i</i> -Pr	F	C <sub>33</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>4</sub> S	131–132
<b>9k</b>	Bu	CH <sub>2</sub> CONH <i>n</i> -Pr	F	C <sub>32</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>4</sub> S	168–169
<b>9m</b>	Bu	CH <sub>2</sub> CONH <i>t</i> -Bu	F	C <sub>33</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>4</sub> S•1.0 H <sub>2</sub> O	83–84
<b>9n</b>	Bu	CH <sub>2</sub> CONHCH <sub>2</sub> Ph	F	C <sub>36</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>4</sub> S	178–179
<b>9o</b>	Bu	CH <sub>2</sub> COpip <sup>b</sup>	F	C <sub>34</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>4</sub> S	145–146
<b>9p</b>	Bu	CH <sub>2</sub> CONEt <sub>2</sub>	F	C <sub>33</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>4</sub> S	83–84
<b>9q</b>	Bu	CH <sub>2</sub> CONH <sub>2</sub>	F	C <sub>29</sub> H <sub>34</sub> FN <sub>5</sub> O <sub>4</sub> S	94–95
<b>10c</b>	Pr	CH <sub>2</sub> CONH <i>n</i> -Pn	F	C <sub>33</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>4</sub> S	144–145
<b>10d</b>	Pr	CH <sub>2</sub> CONH <i>n</i> -Bu	F	C <sub>32</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>4</sub> S	99–100
<b>10e</b>	Pr	CH <sub>2</sub> CONH <i>n</i> -Pr	F	C <sub>31</sub> H <sub>38</sub> FN <sub>5</sub> O <sub>4</sub> S	172–173
<b>10f</b>	Pr	CH <sub>2</sub> CONH(CH <sub>2</sub> ) <sub>2</sub> <i>i</i> -Pr	F	C <sub>33</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>4</sub> S	167–168
<b>10g</b>	Pr	CH <sub>2</sub> CONH(CH <sub>2</sub> ) <sub>3</sub> <i>i</i> -Pr	F	C <sub>34</sub> H <sub>44</sub> FN <sub>5</sub> O <sub>4</sub> S•1.0 H <sub>2</sub> O	105–106
<b>10h</b>	Pr	CH <sub>2</sub> CONH <i>t</i> -Bu	F	C <sub>32</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>4</sub> S•0.5 H <sub>2</sub> O	74–75
<b>10i</b>	Pr	CH <sub>2</sub> COpip <sup>b</sup>	F	C <sub>33</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>4</sub> S	154–155

<sup>a</sup>Analyses for C, H, N were correct to within ± 0.4% of theoretical values unless otherwise stated; <sup>b</sup>pip: piperidinyl.



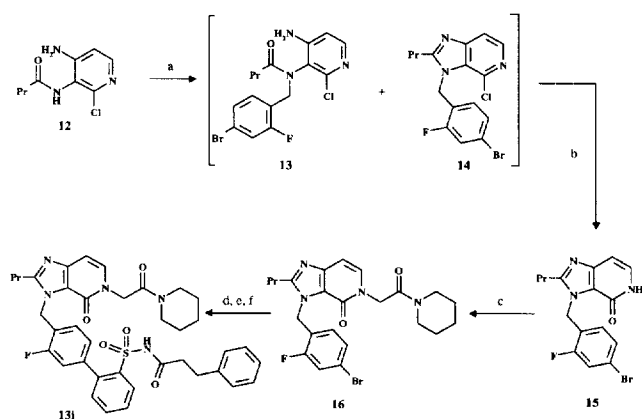
**Scheme 2.** Reagents: a) K<sub>2</sub>CO<sub>3</sub>, 4'-(bromomethyl)-*N*-*tert*-butyl-2-biphenylsulfonamide or 4'-(bromomethyl)-3'-fluoro-*N*-*tert*-butyl-2-biphenylsulfonamide, DMF, rt; b) KO<sup>t</sup>-Bu, R<sup>2</sup>-[Br,Cl], DMF, rt; c) TFA, rt; d) pyridine, DMAP, R<sup>3</sup>-COCl, rt.

pound **15**. Following this, **15** was reacted with 1-chloroacetyl piperidine in dimethylformamide in the presence of potassium *tert*-butoxide to give the alkylated derivative **16**. This product was then cross-coupled with [2-(*N*-*tert*-butylsulfamoyl)phenyl]boronic acid [22] using [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) [27] to obtain the key intermediate **10i** (table I). The bidentate ligand of the palladium catalyst speeded up the reductive elimination in the catalytic cycle and was essential for a high turnover in this reaction step. The use of tetrakis-(triphenylphosphine)palladium (0) predominantly led to debromination of compound **16**. The *tert*-butyl group in **10i** was subsequently removed by treatment with trifluoroacetic acid, and the free sulfonamide was acylated with 3-phenylpropionyl chloride in pyridine to give the final compound **13i**.

## Results and discussion

All compounds in tables II–IV were evaluated in vitro for their binding affinities for the AT<sub>1</sub> subtype ang II receptor (IC<sub>50</sub>), determined in rat adrenal cortical membranes, and for their binding affinities for ang II AT<sub>2</sub> receptors (IC<sub>50</sub>) in a rat adrenal medulla preparation using [<sup>125</sup>I] ang II as the radioligand. With selected acylsulfonamides in table VI, compound-mediated AT<sub>1</sub> antagonism of angiotensin II in vitro was determined in isolated rabbit thoracic aorta. Losartan **1**, EMD 66684 **2**, PD 123,177 **3** and L-159,689 **4** were used as reference standards in these assays.

To finally obtain balanced AT<sub>1</sub>/AT<sub>2</sub> receptor antagonists, our intention was to construct AT<sub>2</sub> affinity by structural modifications of our AT<sub>1</sub> selective imidazo[4,5-*c*]pyridin-2-ones such as EMD 66684 **2**.



**Scheme 3.** Reagents: **a**) KO<sup>t</sup>-Bu, 4-bromo-2-fluorobenzylbromide, NMP, rt; **b**) HCl, 105 °C; **c**) KO<sup>t</sup>-Bu, 1-chloroacetyl piperidine, DMF, rt; **d**) Na<sub>2</sub>CO<sub>3</sub>, Pd (dppf) Cl<sub>2</sub>, [2-(*N*-*tert*-butylsulfamoyl)phenyl]boronic acid, MeOCH<sub>2</sub>CH<sub>2</sub>OMe, 84 °C; **e**) TFA, rt; **f**) pyridine, DMAP, PhCH<sub>2</sub>CH<sub>2</sub>COCl, rt.

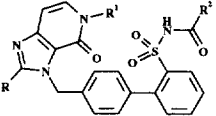
In a previous paper we described that the introduction of an isopropyl phenylacetate in the N-5 position of compound **2** resulted in the discovery of our first compound with an improved affinity for the AT<sub>2</sub> receptor (IC<sub>50</sub>(AT<sub>2</sub>) = 32.0 nM) [25]. However, the ester functionality was required for AT<sub>2</sub> activity since the corresponding acid showed markedly less AT<sub>2</sub> affinity, and the pronounced hydrolysis of the isopropylester in vivo hindered further advancement of these compounds [25].

We began our SAR studies by examining the effects of various modifications of the biphenyltetrazole moiety (table II). Initially we replaced the tetrazole unit by other bioisosters such as oxadiazoles or acyl-sulfonamides. The butoxycarbonylsulfonamide **11c**, the first compound prepared in the sulfonamide series, gave a 30-fold improvement in AT<sub>2</sub> affinity over that obtained for **2** while retaining nanomolar binding affinity for the AT<sub>1</sub> receptor. Maintaining the butoxycarbonyl group and introducing more lipophilic (benzyl) or bulky (diphenylacetamide) substituents at the N-5 position delivered two compounds, **11a** and **11b**, which were only moderately active concerning binding affinity for the AT<sub>1</sub> receptor.

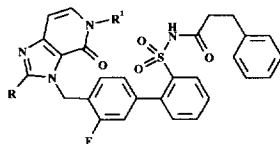
Next, a number of dimethylacetamide analogues of **11c** were prepared to optimize the sulfonamide substituent (R<sup>2</sup> in table II). In this series, the phenylethylcarboxamidossulfonyl derivative **11e** tended to be somewhat more potent at the AT<sub>2</sub> receptor than the other acylsulfonamides **11c**, **11d**, **11f** and **11g**.

In the course of that study, it became clear that the sulfonamide groups in these compounds were nearly optimized for AT<sub>2</sub> binding. Any significant additional enhancement regarding this affinity would need to be derived from changes made elsewhere in the molecule. When DuPont Merck scientists discovered that biphenyl 'ortho' substitution could increase the AT<sub>2</sub> affinity [28], we combined this 'ortho' substitution with the phenylethylcarboxamidossulfonyl acid isoster in **11e**. Introducing a 3-fluoro group in the biphenylmethyl moiety in **11e**, we were pleased to find that the biological activity was largely enhanced in compound **12a** (table III). The 3-fluoro analogue **12a** displayed a 22-fold improvement in AT<sub>2</sub> potency relative to the

**Table II.** Physical, chemical and biological data of target compounds **11**.

Cmpd				Empirical formula <sup>a</sup>	Mp (°C)	AT <sub>1</sub> <sup>b</sup>	AT <sub>2</sub> <sup>b</sup>	Ratio AT <sub>2</sub> /AT <sub>1</sub>
	R	R <sup>1</sup>	R <sup>2</sup>					
<b>11a</b>	Bu	CH <sub>2</sub> Ph	OBu	C <sub>35</sub> H <sub>38</sub> N <sub>4</sub> O <sub>5</sub> S•2.0 H <sub>2</sub> O	118–119	34.0	135.0	4.0
<b>11b</b>	Bu	CH <sub>2</sub> CONPh <sub>2</sub>	OBu	C <sub>42</sub> H <sub>43</sub> N <sub>5</sub> O <sub>6</sub> S	214–215	40.0	580.0	14.5
<b>11c</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	OBu	C <sub>32</sub> H <sub>39</sub> N <sub>5</sub> O <sub>6</sub> S	91–92	4.4	345.0	78.4
<b>11d</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	Ph	C <sub>34</sub> H <sub>35</sub> N <sub>5</sub> O <sub>5</sub> S•0.5 H <sub>2</sub> O	224–225	2.9	1500.0	517.2
<b>11e</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> Ph	C <sub>36</sub> H <sub>39</sub> N <sub>5</sub> O <sub>5</sub> S	195–196	4.7	210.0	44.7
<b>11f</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	CH <sub>2</sub> <i>i</i> -Pr	C <sub>32</sub> H <sub>39</sub> N <sub>5</sub> O <sub>5</sub> S•0.5 H <sub>2</sub> O	212–213	4.2	1300.0	309.5
<b>11g</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	CH <sub>2</sub> <i>t</i> -Bu	C <sub>33</sub> H <sub>41</sub> N <sub>5</sub> O <sub>5</sub> S	149–150	10.0	4300.0	430.0

<sup>a</sup>Analyses for C, H, N were correct to within ± 0.4% of theoretical values unless otherwise stated; <sup>b</sup>IC<sub>50</sub> values in nM. AT<sub>1</sub> receptor affinity was determined using rat adrenal cortical microsomes; AT<sub>2</sub> receptor affinities were determined using rat adrenal medulla microsomes (see *Experimental protocols*). In both cases [<sup>125</sup>I] ang II was used as radioligand.

**Table III.** Physical, chemical and biological data of target compounds **12** and **13**.

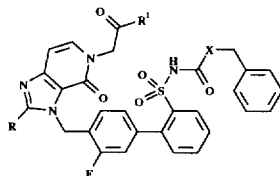
<i>Cmpd</i>	<i>R</i>	<i>R</i> <sup>1</sup>	<i>Empirical formula</i> <sup>a</sup>	<i>Mp</i> (°C)	<i>AT</i> <sub>1</sub> <sup>b</sup>	<i>AT</i> <sub>2</sub> <sup>b</sup>	<i>Ratio</i> <i>AT</i> <sub>2</sub> / <i>AT</i> <sub>1</sub>
<b>12a</b>	Bu	CH <sub>2</sub> CONMe <sub>2</sub>	C <sub>36</sub> H <sub>38</sub> FN <sub>5</sub> O <sub>5</sub> S•1.5 H <sub>2</sub> O	201–202	3.1	9.3	3.0
<b>13a</b>	Pr	CH <sub>2</sub> CONMe <sub>2</sub>	C <sub>35</sub> H <sub>36</sub> FN <sub>5</sub> O <sub>5</sub> S•1.0 H <sub>2</sub> O	202–203	2.7	6.5	2.4
<b>13b</b>	Et	CH <sub>2</sub> CONMe <sub>2</sub>	C <sub>33</sub> H <sub>32</sub> FN <sub>5</sub> O <sub>5</sub> S	195–196	3.8	18.0	4.7
<b>12b</b>	Bu	CH <sub>2</sub> Ph	C <sub>39</sub> H <sub>37</sub> FN <sub>4</sub> O <sub>4</sub> S	121–122	35.0	16.5	0.5
<b>12c</b>	Bu	CH <sub>2</sub> COPh	C <sub>40</sub> H <sub>37</sub> FN <sub>4</sub> O <sub>5</sub> S	144–145	30.0	24.0	0.8
<b>12d</b>	Bu	CH <sub>2</sub> CH <sub>2</sub> COPh	C <sub>41</sub> H <sub>39</sub> FN <sub>4</sub> O <sub>5</sub> S	67–68	39.0	26.0	0.7
<b>12e</b>	Bu	CH <sub>2</sub> COMe	C <sub>35</sub> H <sub>35</sub> FN <sub>4</sub> O <sub>5</sub> S•1.0 H <sub>2</sub> O	Oil	87.0	46.0	0.5
<b>12f</b>	Bu	CH <sub>2</sub> CO <i>t</i> -Bu	C <sub>38</sub> H <sub>41</sub> FN <sub>4</sub> O <sub>5</sub> S•1.7 H <sub>2</sub> O	126–127	17.0	32.0	1.9
<b>12g</b>	Bu	CH <sub>2</sub> CO <i>n</i> -Bu	C <sub>38</sub> H <sub>41</sub> FN <sub>4</sub> O <sub>5</sub> S•1.3 H <sub>2</sub> O	89–90	8.3	46.0	5.5
<b>12h</b>	Bu	CH <sub>2</sub> COOEt	C <sub>36</sub> H <sub>37</sub> FN <sub>4</sub> O <sub>6</sub> S	125–126	4.4	9.0	2.1
<b>12i</b>	Bu	CH <sub>2</sub> COOH	C <sub>34</sub> H <sub>33</sub> FN <sub>4</sub> O <sub>6</sub> S	131–132	34.0	210.0	6.2

<sup>a</sup>Analyses for C, H, N were correct to within  $\pm 0.4\%$  of theoretical values unless otherwise stated; <sup>b</sup>IC<sub>50</sub> values in nM.

unsubstituted derivative **11e**. For the first time, a compound with < 10 nM binding affinities for both receptor subtypes was obtained within this series.

We continued our studies by examining the effect of the alkyl substituent at C-2 of the imidazo[4,5-*c*]pyridin-2-one nucleus. Previous investigations in the AT<sub>1</sub> selective tetrazole series showed [12] that replacement of a butyl group by a propyl or ethyl side chain slightly increased AT<sub>1</sub> potency. Taking this into account, we synthesized compounds **13a** and **13b**. The data in table III illustrate that a propyl chain in this position was the best substitution for achieving high affinities for both subtypes with a nearly mixed binding profile (**13a**: AT<sub>2</sub>/AT<sub>1</sub> = 2.4). Although the 3-fluoro substituent turned out to be essential in increasing AT<sub>2</sub> potency, the degree of improvement depended on substituents R and R<sup>1</sup> (table III). Replacement of the N-5 dimethylacetamide (= R<sup>1</sup>) in **12a** with benzyl (**12b**), 2-oxoethyl (**12c**, **12e**, **12f** and **12g**) or 3-oxopropyl (**12d**) substituents led to compounds which retained the low AT<sub>2</sub>/AT<sub>1</sub> IC<sub>50</sub> ratios (5.5–0.5) but unfortunately showed a slight loss in binding to both receptor sites (up to almost 1 order of magnitude). The ethyl acetate analogue **12h** provided no apparent advantage over the acetamide **12a** in this respect. On the contrary, a possible in vivo hydrolysis to the acid **12i** would lead to a compound with diminished AT<sub>2</sub> potency.

After optimizing the acylsulfonamide (table II) and the residues R and R<sup>1</sup> (table III) in the 3-fluoro series, the most balanced compounds with high potency were butyl derivative **12a** and propyl derivative **13a**. Both analogues still had an AT<sub>2</sub>/AT<sub>1</sub> IC<sub>50</sub> ratio of 3.0 or 2.4, respectively. Therefore, we decided to focus on the acetamide function in position N-5. Variations in the length and/or bulk of the amide side chain were investigated. Several compounds with a butyl or propyl group at C-2 of the imidazo[4,5-*c*]pyridin-2-one nucleus (R in table IV) were prepared with different primary and secondary amines as R<sup>1</sup> (table IV). We were gratified to see that, compared to **12a** and **13a**, most of the analogues in table IV retained lower nanomolar binding affinities for both the AT<sub>1</sub> and AT<sub>2</sub> receptor, but displayed reduced IC<sub>50</sub> ratios of 1.4–0.5. When R<sup>1</sup> was lengthened to the *N*-benzyl derivative **12o** (butyl series) or the *N*-isohexyl compound **13g** (propyl series), there was a loss in potency for the AT<sub>1</sub> receptor (**12o** and **13g**) and the AT<sub>2</sub> receptor (**13g**). This loss in potency could be due to steric (or conformational) factors. In general, propyl derivatives were slightly more potent at both receptor sites compared to their butyl analogues. This can be seen with propylamides **12m** and **13e**. As shown in table IV, compared to **12a**, an NH<sub>2</sub> group (**12r**) was slightly diminished to AT<sub>2</sub> binding but had no negative effect on AT<sub>1</sub> potency. Propylamide **13e** was one of the most active

**Table IV.** Physical, chemical and biological data of target compounds **12** and **13**.

<i>Cmpd</i>	<i>R</i>	<i>R</i> <sup>1</sup>	<i>X</i>	<i>Empirical formula</i> <sup>a</sup>	<i>Mp</i> (°C)	<i>AT</i> <sub>1</sub> <sup>b</sup>	<i>AT</i> <sub>2</sub> <sup>b</sup>	<i>Ratio</i> <i>AT</i> <sub>2</sub> / <i>AT</i> <sub>1</sub>
<b>12k</b>	Bu	NHCH <sub>2</sub> <i>i</i> -Pr	CH <sub>2</sub>	C <sub>38</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>5</sub> S•4.0 H <sub>2</sub> O	127–128	5.8	3.3	0.57
<b>12m</b>	Bu	NH <i>n</i> -Pr	CH <sub>2</sub>	C <sub>37</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>5</sub> S•2.3 H <sub>2</sub> O	125–126	3.6	3.0	0.83
<b>12n</b>	Bu	NH <i>t</i> -Bu	CH <sub>2</sub>	C <sub>38</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>5</sub> S•2.2 H <sub>2</sub> O	116–117	3.9	3.0	0.77
<b>12o</b>	Bu	NHCH <sub>2</sub> Ph	CH <sub>2</sub>	C <sub>41</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>5</sub> S•1.0 H <sub>2</sub> O	128–129	11.5	7.0	0.61
<b>12p</b>	Bu	Pip <sup>c</sup>	CH <sub>2</sub>	C <sub>39</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>5</sub> S	204–205	4.0	3.8	0.95
<b>12q</b>	Bu	NEt <sub>2</sub>	CH <sub>2</sub>	C <sub>38</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>5</sub> S	157–158	5.0	7.0	1.40
<b>12r</b>	Bu	NH <sub>2</sub>	CH <sub>2</sub>	C <sub>34</sub> H <sub>34</sub> FN <sub>5</sub> O <sub>5</sub> S•2.0 H <sub>2</sub> O	101–102	2.9	13.0	4.48
<b>13c</b>	Pr	NH <i>n</i> -Pn	CH <sub>2</sub>	C <sub>38</sub> H <sub>41</sub> FN <sub>5</sub> O <sub>5</sub> S	131–132	8.7	8.4	0.97
<b>13d</b>	Pr	NH <i>n</i> -Bu	CH <sub>2</sub>	C <sub>37</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>5</sub> S•0.7 H <sub>2</sub> O	121–122	4.6	5.6	1.22
<b>13e</b>	Pr	NH <i>n</i> -Pr	CH <sub>2</sub>	C <sub>36</sub> H <sub>38</sub> FN <sub>5</sub> O <sub>5</sub> S	167–168	2.7	1.8	0.67
<b>13f</b>	Pr	NH(CH <sub>2</sub> ) <sub>2</sub> <i>i</i> -Pr	CH <sub>2</sub>	C <sub>38</sub> H <sub>42</sub> FN <sub>5</sub> O <sub>5</sub> S	104–105	6.3	6.5	1.03
<b>13g</b>	Pr	NH(CH <sub>2</sub> ) <sub>3</sub> <i>i</i> -Pr	CH <sub>2</sub>	C <sub>39</sub> H <sub>44</sub> FN <sub>5</sub> O <sub>5</sub> S•1.5 H <sub>2</sub> O	107–108	14.5	18.0	1.24
<b>13h</b>	Pr	NH <i>t</i> -Bu	CH <sub>2</sub>	C <sub>37</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>5</sub> S•1.5 H <sub>2</sub> O	109–110	3.7	2.1	0.57
<b>13i</b>	Pr	Pip <sup>c</sup>	CH <sub>2</sub>	C <sub>38</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>5</sub> S	206–207	3.6	2.6	0.72
<b>12s</b>	Bu	Pip <sup>c</sup>	O	C <sub>38</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>6</sub> S	173–174	4.7	3.5	0.74
<b>13k</b>	Pr	Pip <sup>c</sup>	O	C <sub>37</sub> H <sub>38</sub> FN <sub>5</sub> O <sub>6</sub> S	157–158	2.9	4.2	1.45

<sup>a</sup>Analyses for C, H, N were correct to within  $\pm 0.4\%$  of theoretical values unless otherwise stated; <sup>b</sup>IC<sub>50</sub> values in nM; <sup>c</sup>pip: piperidinyI.

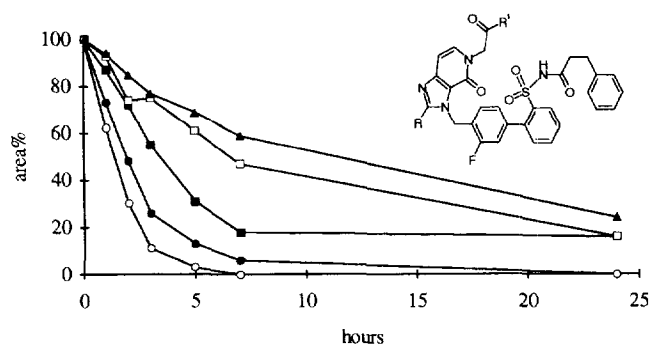
derivatives at both the AT<sub>1</sub> and AT<sub>2</sub> receptor with IC<sub>50</sub> values of 2.7 and 1.8 nM, respectively. Regarding tertiary amides there was no loss in AT<sub>1</sub> and AT<sub>2</sub> potency, as illustrated by the IC<sub>50</sub> values of compounds **12p**, **12q** and **13i**. Apparently there was no need for the N-H as a hydrogen-bond donor on the receptor.

Two of the tertiary amides, butyl piperidide **12p** and propyl piperidide **13i**, met our requirements for in vitro balance by exhibiting AT<sub>2</sub>/AT<sub>1</sub> IC<sub>50</sub> ratios of 0.95 and 0.72, respectively, together with low nanomolar binding affinities for both subtypes.

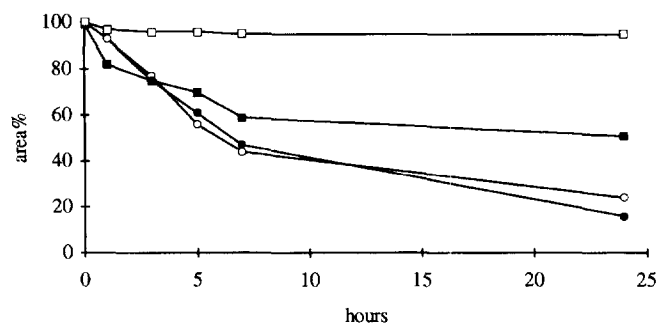
Orientating studies of metabolism were carried out in vitro in order to examine the stability of the 3-fluoro acylsulfonamides **12** and **13**. Incubation with microsomes of different species were carried out in buffer solution (pH 7.4), containing a NADPH regeneration system. Figure 1 shows the decrease in unchanged drug expressed as area % (HPLC). These

investigations on different acylsulfonamides with rat liver microsomes showed a fast cleavage to the free sulfonamide (the free sulfonamides of compounds **9** and **10** in table I), depending on the side chain at R<sup>1</sup> (fig 1). Other metabolites were not detected. Cleavage to the free sulfonamide was not influenced by the side chain at C-2 (butyl or propyl) of the imidazo[4,5-*c*]pyridin-2-one moiety. Among the acylsulfonamides tested, the butyl and propyl piperidides **12p** and **13i** showed the highest metabolic stability. The results of some derivatives **12p**, **12r**, **13c**, **13h** and **13i** have been summarized in figure 1.

In order to raise the metabolic stability the phenylethylcarbonyl group of the sulfonylamides, **12p** and **13i** were replaced by a benzyloxycarbonyl group. This isosteric replacement of a carbon with an oxygen (X = CH<sub>2</sub> or O in table IV) led to sulfonylcarbamates **12s** and **13k**, respectively. Both compounds showed comparable IC<sub>50</sub> values at the AT<sub>1</sub> as well as the AT<sub>2</sub>



**Fig 1.** In vitro metabolism of selected compounds during incubation with rat liver microsomes shown as decrease in unchanged drug expressed as area % (HPLC). R = Bu, R': (□) NHpip (**12p**), (○) NH<sub>2</sub> (**12r**). R = Pr, R': (■) NHt-Bu (**13h**), (●) NHn-Pn (**13c**), (▲) NHpip (**13i**).



**Fig 2.** Metabolic stability of butyl sulfonamide **12p** during incubation with rat (●) or monkey (○) and butyl carbamate **12s** with rat (■) or monkey (□) liver microsomes.

receptor relative to acyl derivatives **12p** and **13i** (table IV). The in vitro stability of butyl sulfonamide **12p** and butyl sulfonylcarbamate **12s** is shown in figure 2 by way of example. Incubations of **12p** with rat or monkey liver microsomes showed a cleavage of 84 and 75% within 24 h, respectively. Incubation of **12s** with liver microsomes of both species showed a higher stability. Only 48% of **12s** was cleaved by rat liver microsomes. In addition, butyl carbamate **12s** was nearly completely stable during incubation with monkey liver microsomes (fig 2).

A model of the AT<sub>1</sub> receptor–ligand interactions for 4,5-dihydro-4-oxo-3H-imidazo[4,5-c]pyridine-based AT<sub>1</sub> selective ang II antagonists has been discussed [12]. Possible roles have been proposed for substituents at C-2, N-5 and at the biphenyl moiety (cf, formula in table III), briefly, for interactions with the AT<sub>1</sub> receptor: (i) hydrophobic associations between a lipophilic pocket and an aliphatic chain at C-2; (ii) an ionic interaction between a cationic group on the receptor and the acidic substituent on the biphenyl chain; and (iii) a hydrogen bond between a proton donor on the receptor and an oxygen as a proton acceptor at N-5 (eg, the acetamides). The AT<sub>2</sub> receptor was quite sensitive to changes in the substituent of the acylsulfonamide. There was a slight preference for phenylethyl or benzyloxy over other acylsulfonamides. The acylsulfonamide could be involved in hydrogen-bonding with the AT<sub>2</sub> receptor, and the lipophilic tail (phenylethyl or benzyloxy) was needed to reach a hydrophobic pocket on the receptor. A propyl chain at C-2 was most effective in establishing an important hydrophobic contact to this receptor. More importantly, the substantial increase in AT<sub>2</sub> binding affinity attained by the 3-fluoro substituent on the biphenyl moiety strongly suggested that the fluoro

atom was involved in a discrete interaction with the AT<sub>2</sub> receptor. The requirement of a fluorine at that particular position implies that this atom is directly involved in binding, perhaps by interacting with a hydrophobic site. At the N-5 position, binding to the AT<sub>2</sub> receptor was sensitive to changes in the size and shape of the amide substituent (eg, piperidine). Again, the carbonyl function of the acetamides was involved in hydrogen-bonding whereas the piperidine was important in making a hydrophobic interaction with the AT<sub>2</sub> receptor.

Quan et al [21] had shown that the ratio of the half maximal inhibition constants (IC<sub>50</sub> values) for the AT<sub>1</sub> and AT<sub>2</sub> receptor binding affinities critically depend upon the radioligand used. They found that compounds which possessed a balanced AT<sub>2</sub>/AT<sub>1</sub> ratio when using [<sup>125</sup>I] ang II as the ligand, displayed a heavily deranged AT<sub>2</sub>/AT<sub>1</sub> ratio when using [<sup>125</sup>I] [Sar<sup>1</sup>, Ile<sup>8</sup>] ang II as the radioligand instead. This derangement was largely due to an improvement in the apparent AT<sub>1</sub> receptor affinity and a concomitantly occurring decrease in the AT<sub>2</sub> receptor affinity. While Quan et al were able to overcome this imbalance in the AT<sub>1</sub>/AT<sub>2</sub> receptor binding affinities by appropriate structural modifications of their compounds, the possibility of an only apparent balance in the AT<sub>1</sub>/AT<sub>2</sub> receptor affinity ratio of our compounds caused some concern. At least all our binding data had been obtained using [<sup>125</sup>I] ang II as the radioligand. We therefore carried out binding experiments with [<sup>125</sup>I] [Sar<sup>1</sup>, Ile<sup>8</sup>] ang II as the radioligand using selected compounds. The results obtained with **12k**, **12s** and **13f** are summarized in table V. While we observed a slight, but not unexpected, decrease in the IC<sub>50</sub> values of our test compounds when using [<sup>125</sup>I] [Sar<sup>1</sup>, Ile<sup>8</sup>] ang II as the ligand, the IC<sub>50</sub> ratios of the AT<sub>1</sub>/AT<sub>2</sub>



**Table V.** Comparison of IC<sub>50</sub> values of selected AT<sub>1</sub>/AT<sub>2</sub> receptor antagonists with regard to the displacement of [<sup>125</sup>I] ang II and [<sup>125</sup>I] [Sar<sup>I</sup>, Ile<sup>8</sup>] ang II.

<i>Cmpd</i>	<i>[<sup>125</sup>I] ang II</i>		<i>Ratio</i>	<i>[<sup>125</sup>I] [Sar<sup>I</sup>, Ile<sup>8</sup>] ang II</i>		<i>Ratio</i>
	<i>AT<sub>1</sub><sup>a</sup></i>	<i>AT<sub>2</sub><sup>a</sup></i>	<i>AT<sub>2</sub>/AT<sub>1</sub></i>	<i>AT<sub>1</sub><sup>a</sup></i>	<i>AT<sub>2</sub><sup>a</sup></i>	<i>AT<sub>2</sub>/AT<sub>1</sub></i>
<b>12k</b>	5.8	3.3	0.6	10	13.5	1.4
<b>12s</b>	4.7	3.5	0.7	6.0	20	3.3
<b>13f</b>	6.3	6.5	1.0	7.5	13.5	1.8

<sup>a</sup>IC<sub>50</sub> values in nM.

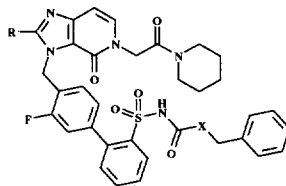
receptors were only minimally affected, indicating that our compounds were already optimized with regard to the AT<sub>1</sub> as well as the AT<sub>2</sub> receptor binding. Since in addition under in vivo conditions the natural ligand will be ang II, we decided not to elaborate further on improving our already well balanced ang II receptor antagonists.

The in vitro AT<sub>1</sub> antagonism of all tested acetamides in tables III and IV was in the nano- or subnanomolar range. Data of piperidides **12p**, **12s**, **13i** and **13k** are shown in table VI together with those of the reference compounds **1–4**. These data indicate that both sulfonylcarbamates **12s** and **13k** were superior to losartan **1**, L-159,689 **4** and equipotent to EMD 66684 **2** concerning their functional AT<sub>1</sub> potency in vitro.

Of all investigated sulfonamides, piperidide **12s** met best our stringent target for balanced in vitro binding affinity together with high AT<sub>1</sub> antagonism and in vitro metabolic stability. As a potent representative of this class of compounds, **12s** was selected as

its potassium salt EMD 90423 for in vivo evaluation as an antihypertensive drug.

As shown in table VII, EMD 90423 and losartan were given orally at a dose of 3 mg/kg to conscious, sodium-depleted cynomolgus monkeys. Heart rate (HR), arterial blood pressure (AP) and plasma renin activity (PRA) were measured 1 and 3 h after administration. EMD 90423 produced a maximum hypotensive response of 26.3% in systolic, 27.5% in diastolic and 32.6% in mean arterial blood pressure accompanied by a maximum rise of 73.3% in plasma renin activity. However, the maximum blood pressure lowering effect of losartan amounted only to 11.7% in systolic, 9.5% in diastolic and 12.4% in mean arterial blood pressure, and the maximum rise of plasma renin activity was 52.4%. Heart rate was changed only to a small extent in both compounds. The observed fall of blood pressure of our compound lasted for more than 8 h (data not shown in table VII), which demonstrated the high efficacy of EMD 90423 after oral administration.

**Table VI.** In vitro binding (AT<sub>1</sub>, AT<sub>2</sub>) and antagonism (AT<sub>1</sub>) of selected acylsulfonamides **12**, **13** and reference compounds **1–4**.

<i>Cmpd</i>	<i>R</i>	<i>X</i>	<i>Binding AT<sub>1</sub><sup>a</sup></i>	<i>Binding AT<sub>2</sub><sup>a</sup></i>	<i>Ratio AT<sub>2</sub>/AT<sub>1</sub></i>	<i>Antagonism AT<sub>1</sub><sup>a</sup></i>
<b>12p</b>	Bu	CH <sub>2</sub>	4.0	3.8	0.95	0.30
<b>13i</b>	Pr	CH <sub>2</sub>	3.6	2.6	0.57	0.90
<b>12s</b>	Bu	O	4.7	3.5	0.74	0.18
<b>13k</b>	Pr	O	2.9	4.2	1.45	0.20
<b>1<sup>b</sup></b>	—	—	8.2	> 10 000	> 1220	3.00
<b>2<sup>c</sup></b>	—	—	0.7	> 10 000	> 14 286	0.20
<b>3<sup>d</sup></b>	—	—	> 10 000	30.0	< 0.003	—
<b>4<sup>e</sup></b>	—	—	2.3	1.3	0.57	0.80

<sup>a</sup>IC<sub>50</sub> values in nM; <sup>b</sup>losartan; <sup>c</sup>EMD 66684; <sup>d</sup>PD 123,177; <sup>e</sup>L-159,689.

**Table VII.** Effects of a 3 mg/kg oral dose of EMD 90423 (potassium salt of compound **12s**) and losartan in salt-depleted cynomolgus monkeys 1 and 3 h after administration. Results are shown as means  $\pm$  SEM of 3–4 animals.

Parameters	EMD 90423	$\Delta$ % baseline	Losartan	$\Delta$ % baseline
HR (bpm)				
Baseline	181.1 $\pm$ 19.6	–	168.2 $\pm$ 13.4	–
1 h	168.2 $\pm$ 24.7	– 7.1	154.6 $\pm$ 14.7	– 8.1
3 h	169.7 $\pm$ 25.7	– 6.3	168.5 $\pm$ 11.1	+ 0.2
AP syst (mmHg)				
Baseline	98.3 $\pm$ 10.7	–	93.5 $\pm$ 4.9	–
1 h	80.5 $\pm$ 7.4	– 18.1	82.6 $\pm$ 10.2	– 11.7
3 h	72.4 $\pm$ 5.6	– 26.3	86.3 $\pm$ 6.4	– 7.7
AP diast (mmHg)				
Baseline	61.4 $\pm$ 6.6	–	55.5 $\pm$ 4.3	–
1 h	45.1 $\pm$ 4.2	– 26.5	50.2 $\pm$ 8.1	– 9.5
3 h	44.5 $\pm$ 3.1	– 27.5	54.2 $\pm$ 7.3	– 2.3
AP mean (mmHg)				
Baseline	78.3 $\pm$ 8.6	–	70.3 $\pm$ 5.0	–
1 h	55.5 $\pm$ 3.1	– 29.1	61.6 $\pm$ 9.4	– 12.4
3 h	52.8 $\pm$ 3.4	– 32.6	66.6 $\pm$ 7.8	– 5.3
PRA (ng ang I/mL/h)				
Baseline	44.2 $\pm$ 19.3	–	39.5 $\pm$ 10.2	–
1 h	76.6 $\pm$ 24.8	+ 73.3	60.2 $\pm$ 16.8	+ 52.4
3 h	75.1 ( <i>n</i> = 2)	+ 69.9	58.8 $\pm$ 14.0	+ 48.9

Bpm: beats per minute; h: hour; AP syst: systolic arterial blood pressure; AP diast: diastolic arterial blood pressure; AP mean: mean arterial blood pressure; PRA: plasma renin activity; ang I: angiotensin I.

## Conclusions

This paper describes a novel series of potent non-peptide ang II AT<sub>1</sub> antagonists with balanced affinity for the AT<sub>1</sub> and AT<sub>2</sub> receptor subtypes derived from substitution of a 4,5-dihydro-4-oxo-3*H*-imidazo[4,5-*c*]-pyridine nucleus. Compounds with a propyl or a butyl group at C-2, different acetamides at N-5, a 3-fluoro and a 2'-carboxamidomethyl substituent at the biphenyl-methyl moiety exhibited nanomolar affinities for both the AT<sub>1</sub> and AT<sub>2</sub> receptor. Replacement of the phenylethylcarbonyl by a benzyloxycarbonyl group at the sulfonamide position gave rise to an improvement in metabolic stability in vitro. With acetylpipecide **12s**, one of the most potent AT<sub>1</sub>/AT<sub>2</sub> balanced affinity antagonists actually known related to in vitro properties was observed. After oral administration to cynomolgus monkeys, EMD 90423 demonstrated good efficacy and a long duration of action as an antihypertensive drug. These results make this ang II antagonist a promising candidate for the treatment of hypertension and congestive heart failure if AT<sub>2</sub> functions are proven to be physiologically significant.

## Experimental protocols

### Chemistry

Melting points were determined with a HWS Labortechnik SGV 500 Plus melting point apparatus and are uncorrected. IR, NMR, and mass spectra are in agreement with the structures cited and were recorded on a Bruker 85 IFS 48 IR spectrophotometer, a Bruker AC 200, WM 250 or AM 500 (TMS as internal standard), and a Fisons (formerly Vacuum Generator) VG 70-70E (electron-impact: ei) or 70-250SE (fast atom bombardment: fab) at 70 eV, respectively. Microanalyses were obtained with a Perkin-Elmer 240B CHN analyzer. Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F<sub>254</sub> plates with a layer thickness of 0.25 mm from Merck KGaA (Darmstadt, Germany). Visualization was performed with UV and I<sub>2</sub>. Yields were not optimized. The preparative chromatography was performed on Merck KGaA silica gel 60 (230–400 mesh) and all solvents were of Merck extra-pure grade. NADP, G6P, G6PDH, Na<sub>2</sub>-EDTA, MgCl<sub>2</sub>·6 H<sub>2</sub>O, K<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> were purchased from Merck KGaA (Darmstadt, Germany), the protein-assay reagent came from Pierce (Rockford, USA).

*General procedure for the preparation of 3-[[2'-tert-butylamino-sulfonyl-4-biphenyl]methyl]-4,5-dihydro-3*H*-imidazo[4,5-*c*]-pyridin-4-ones **6***

Compounds **6** (scheme 2) were synthesized by the representative procedure illustrated for **6** [R = Bu, R<sub>1</sub> = F].

**2-Butyl-3-[[3-fluoro-(2'-tert-butylaminosulfonyl)-4-biphenyl]methyl]-4,5-dihydro-3H-imidazo[4,5-c]pyridin-4-one** **6**. For the preparation of this compound [R = Bu, R<sub>1</sub> = F], the solution of 2-butyl-4,5-dihydro-4-oxo-3H-imidazo[4,5-c]pyridine **5a** (30.0 g, 157.0 mmol) in dimethylformamide (500 mL) was treated with finely-ground potassium carbonate (21.7 g, 157.0 mmol) and 4'-(bromomethyl)-3'-fluoro-N-tert-butyl-2-biphenylsulfonamide (62.8 g, 157.0 mmol). The reaction mixture was stirred at ambient temperature overnight. It was diluted with water (800 mL) and extracted with ethyl acetate (3 x 300 mL). After drying over anhydrous sodium sulfate, the solvent was evaporated to yield a yellow oil which was purified by flash chromatography on silica gel with ethyl acetate as eluent to provide 33.3 g (42%) of **6** [R = Bu, R<sub>1</sub> = F] as white crystals and 5.4 g (5.4%) of the bisadduct **7** [R = Bu, R<sub>1</sub> = F]. **6** [R = Bu, R<sub>1</sub> = F]: mp = 261–262 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 11.30 (sbr, 1H), 8.07 (dd, *J* = 1.1 Hz, *J* = 8.0 Hz, 1H), 7.65 (td, *J* = 1.2 Hz, *J* = 7.2 Hz, 1H), 7.61 (td, *J* = 1.5 Hz, *J* = 7.6 Hz, 1H), 7.32 (td, *J* = 1.2 Hz, *J* = 7.5 Hz, 1H), 7.16 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 7.14 (dd, *J* = 1.6 Hz, *J* = 6.9 Hz, 1H), 6.88 (t, *J* = 8.0 Hz, 1H), 6.84 (s, 1H), 6.59 (d, *J* = 7.1 Hz, 1H), 5.89 (s, 2H), 2.73 (t, *J* = 7.4 Hz, 2H), 1.72–1.66 (m, 2H), 1.40–1.32 (m, 2H), 1.01 (s, 9H), 0.89 (t, *J* = 7.4 Hz, 3H). Anal (C, H, N) C<sub>27</sub>H<sub>31</sub>FN<sub>4</sub>O<sub>3</sub>S. **7** [R = Bu, R<sub>1</sub> = F]: mp = 106–107 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 8.07 (d, *J* = 7.7 Hz, 1H), 7.66–7.55 (m, 5H), 7.35–7.26 (m, 4H), 7.20–7.14 (m, 3H), 6.92–6.85 (m, 3H), 6.74 (d, *J* = 7.2 Hz, 1H), 5.91 (s, 2H), 5.35 (s, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 1.74–1.66 (m, 2H), 1.41–1.34 (m, 2H), 1.04 (s, 9H), 1.00 (s, 9H), 0.90 (t, *J* = 7.4 Hz, 3H). Anal (C, H, N) C<sub>44</sub>H<sub>49</sub>F<sub>2</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>.

**General procedure for the preparation of 3-[[2'-tert-butylaminosulfonyl-4-biphenyl]methyl]-4,5-dihydro-3H-imidazo[4,5-c]pyridin-4-ones 8, 9 and 10**

Most compounds **8**, **9** and **10** (table I) were synthesized by the representative procedure illustrated for **9a**.

**2-Butyl-3-[[3-fluoro-(2'-tert-butylaminosulfonyl)-4-biphenyl]methyl]-4,5-dihydro-5-[(2-oxo-2-piperidino)ethyl]-3H-imidazo[4,5-c]pyridin-4-one** **9a**. Under a nitrogen atmosphere, **6** [R = Bu, R<sub>1</sub> = F] (1.15 g, 2.32 mmol) was dissolved in dry dimethylformamide (15 mL). At ambient temperature, potassium *tert*-butoxide (0.29 g, 2.55 mmol) was added to the solution. After the resulting mixture had been stirred at the same temperature for 15 min, 1-chloroacetyl-piperidine (0.41 g, 2.55 mmol) was added at once. The reaction mixture was stirred for 18 h at ambient temperature and concentrated in vacuo to an oil, which was partitioned between water (50 mL) and ethyl acetate (50 mL). The organic extract was dried on Na<sub>2</sub>SO<sub>4</sub>, concentrated in vacuo, and chromatographed on silica gel with 50% ethyl acetate in hexane as eluent. Recrystallization from 50% ethyl acetate in diethylether provided 1.28 g (89%) of **9a** as white crystals, mp = 145–146 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 8.03 (dd, *J* = 1.0 Hz, *J* = 8.0 Hz, 1H), 7.62 (td, *J* = 1.1 Hz, *J* = 7.4 Hz, 1H), 7.56 (td, *J* = 1.0 Hz, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 7.3 Hz, 1H), 7.27 (dd, *J* = 1.5 Hz, *J* = 8.0 Hz, 1H), 7.15 (dd, *J* = 1.6 Hz, *J* = 8.0 Hz, 1H), 6.80 (s, 1H), 6.77 (t, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 7.3 Hz, 1H), 5.84 (s, 2H), 4.86 (s, 2H), 3.49–3.40 (m, 4H), 2.70 (t, *J* = 7.4 Hz, 2H), 1.77–1.69 (m, 2H), 1.64–1.54 (m, 4H), 1.47–1.43 (m, 2H), 0.97 (s, 9H), 0.92 (t, *J* = 7.4 Hz, 3H). Anal (C, H, N) C<sub>34</sub>H<sub>42</sub>FN<sub>5</sub>O<sub>4</sub>S.

**General procedure for the preparation of 3-[[2'-carboxamidosulfonyl-4-biphenyl]methyl]-4,5-dihydro-3H-imidazo[4,5-c]pyridin-4-ones 11, 12 and 13**

Most compounds **11**, **12** and **13** (tables II–IV) were synthesized by the representative procedure illustrated for **12p**.

**2-Butyl-3-[[3-fluoro-2'-(3-phenylethylcarboxamidosulfonyl)-4-biphenyl]methyl]-4,5-dihydro-5-[(2-oxo-2-piperidino)ethyl]-3H-imidazo[4,5-c]pyridin-4-one** **12p**. A solution of **9a** (1.28 g, 2.06 mmol) in trifluoroacetic acid (TFA, 10 mL) was stirred overnight at ambient temperature. The excess TFA and volatiles were removed in vacuo to give the crude sulfamoyl intermediate. To a solution of the crude product in pyridine (20 mL) under a nitrogen atmosphere was added at ambient temperature 4-dimethylaminopyridine (0.50 g, 4.13 mmol). After stirring at ambient temperature for 0.5 h the mixture was cooled to 5 °C, 3-phenylpropionyl chloride (0.61 mL, 4.13 mmol) was added dropwise and the resulting mixture was stirred at ambient temperature for 48 h. The reaction mixture was quenched by addition of water (200 mL) and extracted with ethyl acetate (3 x 100 mL). The organic extract was dried on Na<sub>2</sub>SO<sub>4</sub>, concentrated in vacuo, and chromatographed on silica gel with ethyl acetate. Recrystallization from 50% ethyl acetate in diethylether provided 1.21 g (84%) of **12p** as white crystals, mp = 204–205 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 11.68 (s, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.27–7.16 (m, 4H), 7.11 (d, *J* = 7.4 Hz, 2H), 6.91 (d, *J* = 8.0 Hz, 1H), 6.76 (t, *J* = 7.9 Hz, 1H), 6.66 (d, *J* = 7.3 Hz, 1H), 5.88 (s, 2H), 4.90 (s, 2H), 3.52–3.44 (m, 4H), 2.71–2.65 (m, 4H), 2.55 (q, *J* = 7.4 Hz, 2H), 1.68–1.50 (m, 6H), 1.49–1.42 (m, 2H), 1.34–1.24 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). Anal (C, H, N) C<sub>39</sub>H<sub>42</sub>FN<sub>5</sub>O<sub>5</sub>S.

**Alternative procedure for the preparation of 3-[[2'-tert-butylaminosulfonyl-4-biphenyl]methyl]-4,5-dihydro-3H-imidazo[4,5-c]pyridin-4-one intermediates 9 and 10**

Some compounds **10** (scheme 3, table I) were synthesized by the representative procedure illustrated for **10i**.

**3-(4-Bromo-2-fluorobenzyl)-4,5-dihydro-4-oxo-2-propyl-3H-imidazo[4,5-c]pyridine** **15**. To a solution of *N*-(4-amino-2-chloro-3-pyridyl)butyramide **12** (65.3 g, 305.0 mmol) in 1-methyl-2-pyrrolidinone (NMP, 300 mL) was added a solution of potassium *tert*-butoxide (37.7 g, 336.0 mmol) in NMP (100 mL) under a nitrogen atmosphere, and the reaction mixture was stirred for 0.5 h. A solution of 4-bromo-2-fluorobenzylbromide (90.0 g, 336.0 mmol) in NMP (200 mL) was added dropwise, and the reaction mixture was stirred for 4 h. The reaction mixture of the intermediates **13** and **14** (which can be identified by usual workup) was treated with hydrochloric acid (32%, 600 mL) and heated at 105 °C for 18 h. The solution was cooled to ambient temperature, the resultant precipitate was filtered, washed with water, and dried in vacuo. Recrystallization of the residue from ethyl acetate yielded 80.3 g (72%) of **15** as white crystals, mp = 178–179 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 11.21 (sbr, 1H), 7.59 (dd, *J* = 2.0 Hz, *J* = 9.9 Hz, 1H), 7.37 (dd, *J* = 2.1 Hz, *J* = 8.2 Hz, 1H), 7.07 (d, *J* = 6.9 Hz, 1H), 6.74 (t, *J* = 8.2 Hz, 1H), 6.53 (d, *J* = 6.9 Hz, 1H), 5.73 (s, 2H), 2.65 (t, *J* = 7.3 Hz, 2H), 1.76–1.56 (m, 2H), 0.99 (t, *J* = 7.3 Hz, 3H). Anal (C, H, N) C<sub>16</sub>H<sub>15</sub>BrFN<sub>3</sub>O.

**3-(4-Bromo-2-fluorobenzyl)-4,5-dihydro-5-(2-oxo-2-(1-piperidyl)-ethyl)-2-propyl-3H-imidazo[4,5-c]pyridin-4-one** **16**. Under a nitrogen atmosphere a mixture of **15** (5.0 g, 12.5 mmol), potassium *tert*-butoxide (1.5 g, 13.5 mmol), and DMF (50.0 mL) was stirred at 5 °C for 0.5 h. Subsequently, 1-chloroacetyl-piperidine (1.4 g, 13.5 mmol) was added dropwise and the reaction mixture was stirred at ambient temperature for 3.0 h. The reaction mixture was concentrated in vacuo to an oil which was partitioned between water (200 mL) and ethyl acetate (200 mL). The organic extract was dried on Na<sub>2</sub>SO<sub>4</sub>, concen-

trated in vacuo, and chromatographed on silica gel with 50% ethyl acetate in hexane as eluent. Recrystallization from 50% ethyl acetate in diethylether provided 5.1 g (83 %) of **16** as white crystals, mp = 156–157 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 7.59 (dd, *J* = 1.9 Hz, *J* = 9.9 Hz, 1H), 7.34 (dd, *J* = 2.3 Hz, *J* = 8.3 Hz, 1H), 7.28 (d, *J* = 7.3 Hz, 1H), 6.67 (t, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 7.2 Hz, 1H), 5.72 (s, 2H), 4.83 (s, 2H), 3.49–3.36 (m, 4H), 2.68 (t, *J* = 7.3 Hz, 2H), 1.79–1.59 (m, 2H), 1.59–1.36 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H). Anal (C, H, N) C<sub>23</sub>H<sub>26</sub>BrFN<sub>4</sub>O<sub>2</sub>.

**3-[[3-Fluoro-(2'-tert-butylaminosulfonyl)-4-biphenyl]-methyl]-4,5-dihydro-5-(2-oxo-2-(1-piperidyl)ethyl)-2-propyl-3H-imidazo[4,5-c]pyridin-4-one 10i.** A solution of **16** (1.0 g, 2.04 mmol) in dimethoxyethane (50.0 mL) was treated with 2 N sodium carbonate (10.0 mL), [2-(*N*-tert-butylsulfamoyl)-phenyl]boronic acid (2.63 g, 10.2 mmol) and [1,1'-bis-(diphenylphosphino)ferrocene]dichloropalladium (II) (0.2 g, 0.17 mmol) successively. The mixture was stirred at 84 °C for 1.5 h, cooled to room temperature, quenched with water (150 mL) and extracted with ethyl acetate (3 x 100 mL). The organic extract was dried on Na<sub>2</sub>SO<sub>4</sub>, concentrated in vacuo, and chromatographed on silica gel with ethyl acetate. Recrystallization from 50% ethyl acetate in *n*-heptane provided 1.16 g (94%) of **10i** as light yellow crystals, mp = 154–155 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 8.03 (dd, *J* = 1.5 Hz, *J* = 7.3 Hz, 1H), 7.62 (td, *J* = 1.5 Hz, *J* = 7.4 Hz, 1H), 7.57 (td, *J* = 1.6 Hz, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 7.3 Hz, 1H), 7.28 (dd, *J* = 1.6 Hz, *J* = 7.7 Hz, 1H), 7.11 (dd, *J* = 1.6 Hz, *J* = 7.9 Hz, 1H), 6.82 (s, 1H), 6.77 (t, *J* = 7.9 Hz, 1H), 6.60 (d, *J* = 7.3 Hz, 1H), 5.85 (s, 2H), 4.87 (s, 2H), 3.49–3.40 (m, 4H), 2.69 (t, *J* = 7.4 Hz, 2H), 1.77–1.69 (m, 2H), 1.64–1.53 (m, 4H), 1.48–1.42 (m, 2H), 1.00 (s, 9H), 0.92 (t, *J* = 7.4 Hz, 3H). Anal (C, H, N) C<sub>33</sub>H<sub>40</sub>FN<sub>5</sub>O<sub>4</sub>S.

**3-[[3-Fluoro-2'(3-phenylethylcarboxamidulosulfonyl)-4-biphenyl]-methyl]-4,5-dihydro-5-[(2-oxo-2-piperidino)ethyl]-2-propyl-3H-imidazo[4,5-c]pyridin-4-one 13i.** This compound was prepared from **10i** (1.0 g, 1.61 mmol) according to the synthesis of compound **12p** and provided 0.91 g (81%) of **13i** as white crystals, mp = 206–207 °C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ ppm: 11.64 (s, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 7.3 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.23–7.12 (m, 4H), 7.07 (d, *J* = 7.3 Hz, 2H), 6.87 (d, *J* = 7.9 Hz, 1H), 6.61 (t, *J* = 7.9 Hz, 1H), 6.61 (d, *J* = 7.2 Hz, 1H), 5.84 (s, 2H), 4.86 (s, 2H), 3.48–3.40 (m, 4H), 2.66–2.61 (m, 4H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.73–1.64 (m, 2H), 1.63–1.53 (m, 4H), 1.47–1.41 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). Anal (C, H, N) C<sub>38</sub>H<sub>40</sub>FN<sub>5</sub>O<sub>6</sub>S.

## Biology

### Angiotensin II receptor binding assay

Wistar rats were killed by decapitation and the adrenal glands removed. By applying a slight positive pressure onto the glands the capsular layer was separated from the medulla. All subsequent steps were carried out at 4 °C. The tissues were collected separately in 200 mM sucrose, 1.0 mM EDTA, 10 mM Tris/HCl, pH 7.2. The glomerulosa cell layers were homogenized using a Polytron PT 10/35 followed by three strokes in a glass/teflon homogenizer. The homogenate was centrifuged for 10 min at 3000 *g*. The supernatant was filtered through gauze; the filtrate was centrifuged for 13 min at 12 000 *g*. The membrane vesicles contained in the supernatant were sedimented by centrifugation for 60 min at 102 000 *g*. The supernatant was discarded; the pellet was resuspended in 0.25%

BSA, 5 mM MgCl<sub>2</sub>, 50 mM Tris/HCl, pH 7.2, and was stored frozen in 1–2-mL aliquots in liquid N<sub>2</sub>. Plasma membrane vesicles from the medullary portion were prepared in an identical fashion.

Before carrying out the binding assays, the frozen stored membrane solutions were thawed and appropriately diluted in 0.25% BSA, 5 mM MgCl<sub>2</sub>, 50 mM Tris/HCl, pH 7.2. The medulla plasma membrane solution was incubated in the presence of 5 mM DTT for 20 min at 30 °C to inactivate the residual AT<sub>1</sub>-receptors in this preparation.

The binding assay was carried out in a total of 500 µL; 400 µL of the membrane suspension (appropriately diluted with 0.25 or 0.0067% BSA (to achieve 0.25 or 0.01% BSA in the membrane suspension), 5 mM MgCl<sub>2</sub>, 50 mM Tris/HCl, pH 7.2), 50 µL of [<sup>125</sup>I]-ang II (conc between 0.06 and 0.12 nM), and 50 µL of a 10% DMSO solution containing various amounts of unlabelled ang II or competitors. Each concentration was determined in triplicate. The incubation was carried out for 60 min at room temperature. The incubation was terminated by rapidly filtering the incubation volume through Whatman GF/C filters, which were rinsed immediately with 2 x 4 mL ice-cold 0.9% NaCl solution. The radioactivity trapped on the filter were counted in a g-counter (Packard, Cobra 5010). Non specific binding was determined in the presence of 1 µM unlabelled ang II. The effect of the competitors were determined by estimating the concentration at which they displayed the bound [<sup>125</sup>I]-ang II half maximally.

### Antagonism of angiotensin II-contracted rabbit aortic rings

New Zealand white rabbits were stunned by a blow to the head and exsanguinated. The aorta was excised and placed in an oxygenated O<sub>2</sub>/CO<sub>2</sub> (95%:5%) physiological salt solution consisting of (mM) NaCl, 118.1; KCl, 4.7; MgSO<sub>4</sub>, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25; and glucose, 11.1.

The aorta was dissected free from fat and connective tissue and cut into rings of approximately 2 mm. The rings were mounted into 50-mL organ baths and were allowed to equilibrate for 60 to 90 min under a resting tension of 2 g. After equilibration, a cumulative concentration–contractile response curve for ang II was obtained. After a washout period, the rings were contracted with ang II at a concentration which induced 50% of the maximal response (3–8 nM). After a stable value was obtained, the preparations were washed three times and the tension was allowed to return to the baseline. This procedure was repeated every 40 min for a total of three or four cycles. The level of the concentration observed during the last cycle was used as the control (predrug) value. Subsequent contractions were obtained in the presence of increasing concentrations of the test compound, which was added 15 min prior to the agonist. One out of four vessel rings was exposed to the solvent without the test compound and served as time and solvent control in each experiment. The reduction in contractile force in the presence of the test compound was expressed as a percentage of the predrug value. Data were expressed as mean values. IC<sub>50</sub> values (concentrations required to inhibit predrug responses by 50%) were determined graphically.

### Preparation of liver microsomes

Male Wistar rats were used to prepare liver microsomes. The rats were killed by decapitation and the livers homogenized with 2 vol phosphate buffer, 0.1 M, pH 7.4. Microsomes were prepared according to the method of Kremers et al [29]. The final microsomal pellets were resuspended in phosphate buffer and stored at –80 °C. The protein concentration of the microsomal preparation was determined by the method of Bradford [30]. Monkey livers were obtained from Corning Hazeleton

GmbH, Münster, Germany. Monkey liver microsomes were prepared by the same way as described above for rat liver microsomes.

#### Incubation procedures

The metabolism of  $AT_1/AT_2$  antagonists was measured in standard assay mixtures that contained the following in a final vol of 10 mL: 0.1 M phosphate buffer (pH 7.4), 15 mg microsomal proteins, 1.77 mM G6P, 0.42 mM NADP, 1 mM  $MgCl_2 \cdot 6H_2O$  and 0.25 mg drug, solved in 0.25 mL ethanol. After 1 min preincubation at 37 °C the reaction was initiated by the addition of 0.23 U/mL G6PDH. At the appropriate times, samples of 1 mL were taken, then the reaction was stopped by the addition of 3 mL ethanol. Upon cooling on ice and subsequent centrifugation (2.5 min, 8000 g) to pellet the denaturated proteins, the supernatant was evaporated to dryness and resolved in acetonitrile (40%), sterile-filtered (0.45  $\mu$ m) and analyzed by HPLC.

#### Chromatographic analysis

For the HPLC a Merck-Hitachi pump 655A-12 was employed with a L 5000 LC controller, 655-A-40 autosampler, L 4200 UV/visible light detector, a D-2500 chromato-integrator. Separation of drug and metabolites was performed on a Li-Chrosorb RP 8, 7- $\mu$ m, 250-column with an acetonitrile/water gradient from 40/60 to 25/75 (+ 0.015% TFA) as elution solvent at a flow rate of 1 mL/min, and the eluate was monitored at 230 nm. Products were identified by EI-MS.

#### In vivo activity

Female cynomolgus monkeys (*Macaca fascicularis*) weighing 3–8 kg were used. The animals were housed under constant temperature and lighting conditions and provided with food consisting of a commercial pellet diet for monkeys (Sniff p no 2/81013), cereal mixture, barley germ, fruit and vegetables. The animals were treated daily with furosemide, 4 mg/kg im, beginning on the fourth day before an experiment. On the day of the experiment the animals were treated with the final dose of furosemide. Ten min before each measurement the animals were treated with ketamine (Ketavet®, Parke Davis), 0.4 mg/kg im, for sedation. About 1.5 h after the last furosemide treatment, arterial blood pressure (AP) and heart rate (HR) were measured by the cuff method (Dinamap®, Vital-Daten-Monitor 1846, Critikon). In detail, a pneumatic cuff (Critikon Disposacuff®, no 2500, range 8–13 cm) was positioned on the upper arm of the monkeys and AP and HR were measured every minute and were allowed to stabilize before drug administration. Following this, test substances were applied orally and AP and HR were measured after 0, 1 and 3 h. Blood samples for the measurement of PRA were collected before and after administration of the compounds as indicated. The blood samples were taken by direct puncture of the saphenous vein.

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