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SUBSTITUTED PIPERIDINES - HIGHLY POTENT RENIN INHIBITORS DUE TO INDUCED FIT ADAPTATION OF THE ACTIVE SITE

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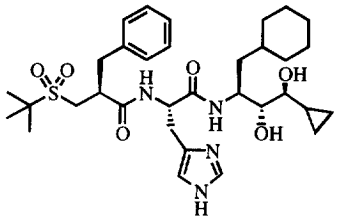
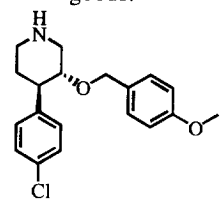
Abstract: The identification, synthesis and activity of a novel class of piperidine renin inhibitors is presented. The most active compounds show activities in the picomolar range and are among the most potent renin inhibitors ever identified.

The renin-angiotensin system (RAS) is widely accepted as a major regulator of cardiovascular and renal function.[1, 2] It consists of a two-step cascade which generates the biologically active angiotensin (Ang) II from angiotensinogen by the aspartic proteinase renin, followed by angiotensin-converting enzyme (ACE). Renin inhibition results in a total blockage of the RAS, since the cleavage of angiotensinogen by renin represents its rate limiting step and is highly specific. Thus, renin inhibition produces an antihypertensive effect comparable to that seen with ACE inhibitors and angiotensin II receptor antagonists,[3-5] but free of side effects due to insufficient specificity. The expected improved efficacy in the tissular systems of the heart[6, 7] and kidney[8-10] gives renin inhibitors the potential for improved prevention and treatment of end organ damage.[11] A significant number of peptidomimetic inhibitors of human renin (e.g. Remikiren **1**) designed as stable transition state analogues of the scissile Leu-Val moiety in human angiotensinogen have been developed up to clinical phase II.[12] Although clinical efficacy was established for several of them,[3] all development compounds were finally dropped. The main causes were insufficient bioavailability and too high cost of goods.



1
Remikiren



***rac*-2**
screening lead

A new structural class of renin inhibitors without resemblance to the renin substrate might therefore be the only chance to find a true drug candidate. Screening of the Roche Compound Library led to the identification of trans-4-(4-chlorophenyl)-3-(4-methoxybenzyl)-piperidine, ***rac*-2**. It is a weak inhibitor of human renin (IC_{50} = 50 μM) devoid of any inhibitory activity against HIV protease, porcine pepsin or bovine cathepsin D. Resolution of the racemate followed by X-ray analysis[13] clearly showed the R,R antipode (***(R,R)*-**

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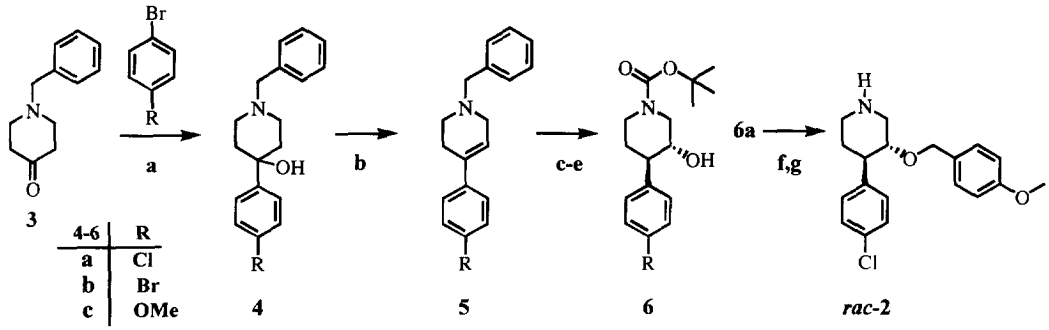
2, 99.4% ee, IC_{50} = 26 μM) to be responsible for the inhibitory activity (***(S,S)*-2**, 96.4% ee, IC_{50} = 1200 μM). The synthesis of racemic 3,4-disubstituted piperidine analogs of **2** is outlined in Scheme 1.

Scheme 1.

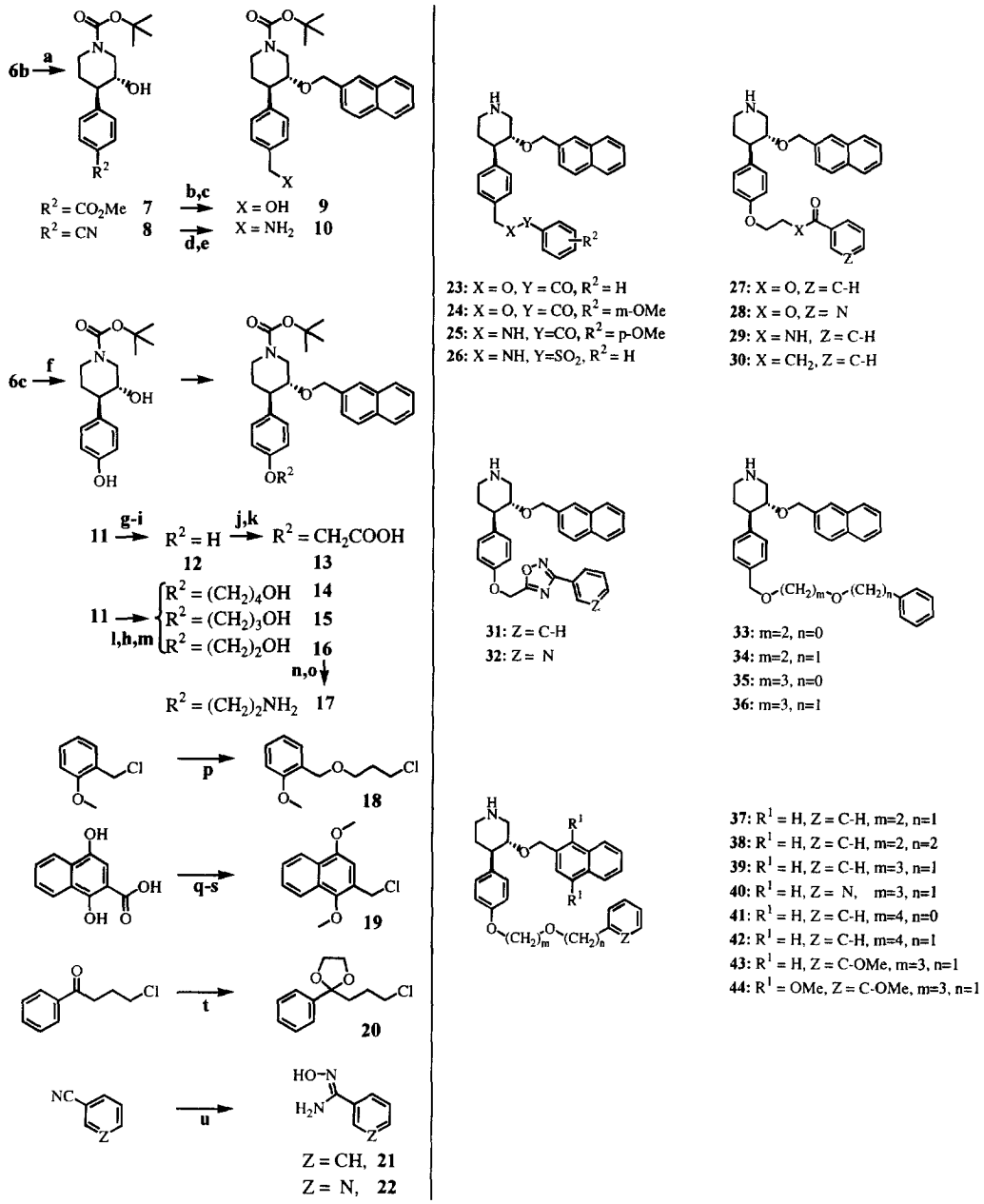


Reagents: (a) *n*-BuLi, THF, -70°C (54-90%); (b) p-TsOHx1H_2O, toluene, reflux (78%-99%); (c) i. $NaBH_4$, BF_3xEt_2O, DME, 15°C, ii. KOH aq., 15°C, iii. H_2O_2 aq. (30 wt-%), 70°C (57-92%); (d) **5a, 5b:** i. 2,2,2-trichloroethyl-chloroformate, Li_2CO_3, toluene, 105°C, 18 h (79%, 76%), ii. Zn, AcOH, r.t., 18 h (80%, 46%), **5c:** H_2-Pd/C, MeOH, r.t. (97%); (e) Boc_2O, $NaHCO_3$, dioxane/H_2O, r.t. (82-97%); (f) 4-methoxybenzyl chloride, NaH, DMF (85%); (g) HCl/MeOH, 50°C (80%).

X-ray structural data obtained for recombinant human renin complexed with ***(R,R)*-2** at low resolution (ca. 3.5 Å, data not shown) was interpreted as follows (compare Ref. 14 describing X-ray findings obtained with an analogue of ***(R,R)*-2**): The protonated nitrogen was positioned close to the two catalytic aspartic acid residues. One hydrogen bond was formed with the O-δ2 of Asp_{215}, a second with the carbonyl oxygen of Gly_{219}. The lipophilic chlorophenyl residue was directed towards the large hydrophobic subsite S_1/S_3 which normally accommodates the Leu and Phe side chains of angiotensinogen. The methoxybenzyl moiety occupied the space normally filled by the sulfonyl group of Remikiren (see Figure 1). The chlorine atom in position 4' did not use the space available in the S_1/S_3 subsite of the enzyme. This observation indicated structural modifications in the 4'-position. Introduction of functional groups in this position allowed the use of such intermediates as templates for the elaboration of a variety of extended 4'-substituents. The synthesis of racemic analogues of **2** with elongated 4' substituents is outlined in Scheme 2.

The in-vitro potencies[15] observed are shown in Table 1. Replacement of the chlorine atom in the 4'-position by substituents consisting of aromatic moieties attached by chains of variable length gave compounds of at least equal potency to ***rac*-2** in all cases with the exception of amide ***rac*-25**. The benzoate esters ***rac*-24** and ***rac*-27** showed potencies of 87 nM and 26 nM respectively. This represents an about 100 fold increase in affinity with respect to ***rac*-2**. This improvement was lost, whenever polar functionalities were introduced, such as pyridine rings, amide or sulfonamide functionalities. Hydrolytically labile ester functions could be replaced by oxadiazol moieties, thus ***rac*-31** was found to be equipotent to ***rac*-27**. Polyether links to the phenyl ring in the extended 4'-substituent finally gave compounds at least as potent as the ester- and oxadiazol-analogues. Propylene-dioxy benzyl ethers were clearly the most potent compounds. The unsubstituted ***rac*-39** showed an IC_{50} value of 8 nM. Derivatisation of alcohols **14-16** (Scheme 2) with variously substituted benzyl halides using parallel synthesis techniques led to the o-methoxy compounds ***rac*-43** and ***rac*-44**, which showed IC_{50} values of 1.5 nM and 0.060 nM respectively.

Scheme 2.



Intermediates: *Reagents:* (a) $PdCl_2(CH_3CN)_2$, 1,3-bis(diphenylphosphino)propane, Et_3N, MeOH, CO (10 bar), 100°C (70%) or $NiBr_2(P(Ph)_3)_2$, $P(Ph)_3$, KCN, CH_3CN, 60°C (56%); (b) 2-naphthylmethyl bromide, NaH, DMF, r.t. (51%); (c) $LiBH_4$, THF, 65°C (96%); (d) 2-naphthylmethyl bromide, NaH, DMF, r.t. (97%); (e) BH_3xTHF, reflux (55%); (f) i. BBr_3, CH_2Cl_2, r.t., ii. Boc_2O, $NaHCO_3$, dioxane, H_2O, r.t. (84%); (g) allyl bromide, 2-butanone, K_2CO_3, reflux (100%); (h) 2-naphthylmethyl bromide, NaH, DMF, 50°C (70-90%); (i) $(P(Ph)_3)_2Pd(OAc)_2$, DABCO, H_2O, EtOH, reflux (73%); (j) ethyl bromoacetate, K_2CO_3, 2-butanone, reflux (90%); (k) NaOH, H_2O, dioxane, r.t. (quant.); (l) rac-2-(4-chloro-butoxy)-tetrahydropyran (Ref. 16), rac-2-(3-bromo-propoxy)-tetrahydropyran (Ref. 17), or rac-2-(2-iodo-ethoxy)-tetrahydropyran (Ref. 18), K_2CO_3, 2-butanone, reflux (68%, 78%, 67%); (m) 2N HCl/MeOH, r.t. (59%, 70%, 86%); (n) i. MsCl, CH_2Cl_2, Et_3N, 0°C-r.t., ii. NaN_3, DMSO, 80°C (54%); (o) $P(Ph)_3$, H_2O, AcOH, THF, r.t. (65%); (p) 3-chloro-1-propanol, NaH, DMF, r.t. (74%); (q) Me_2SO_4, CH_2Cl_2, NaOH, H_2O, NBu_4Br, r.t. (82%); (r) $LiAlH_4$, THF, r.t. (87%); (s) MsCl, CH_2Cl_2,

Et_3N, 0°C-r.t. (86%); (t) ethylene glycol, TsOH, triethyl orthoformate, r.t. (85%); (u) **21:** $NH_2OHxHCl$, MeOH, NaOH, reflux (quant), **22:** $NH_2OHxHCl$, MeONa, MeOH, reflux (quant.).

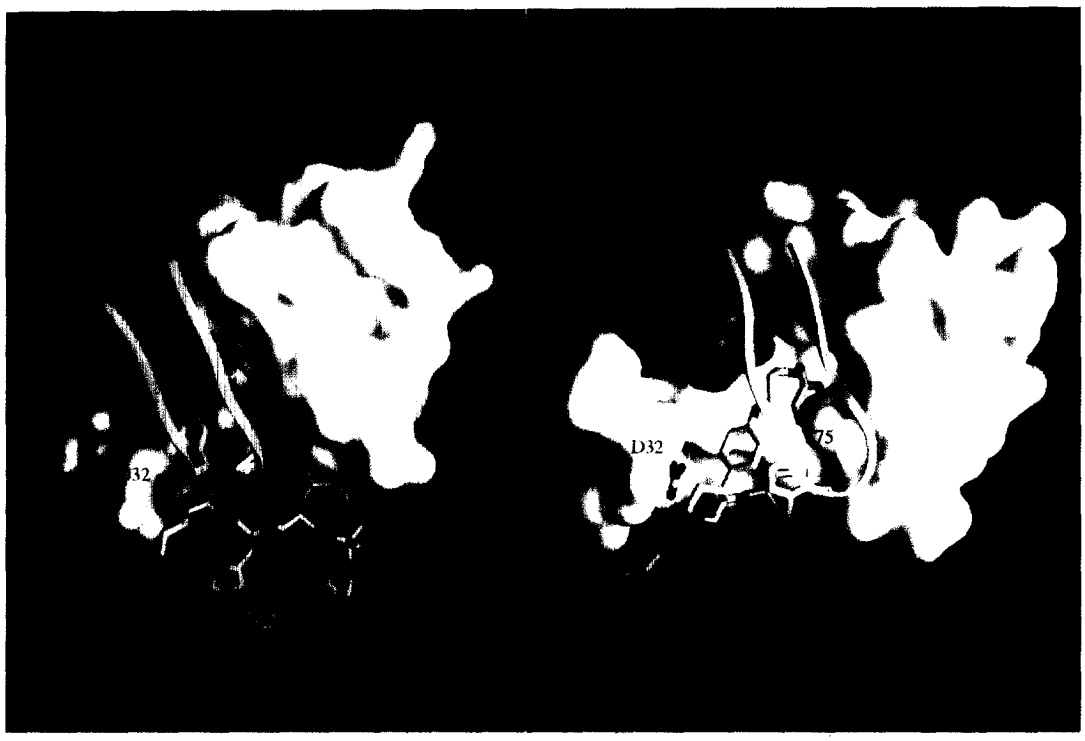
Boc-Cleavage conditions: (aa): HCl/MeOH, 50°C; (bb): $ZnBr_2$, CH_2Cl_2, r.t. (Ref. 19); (cc): TFA, CH_2Cl_2, r.t.

Compounds 25-44: Reagents: **23:** i. **9**, benzoyl chloride, Et_3N, CH_2Cl_2, r.t. (97%), ii. aa (70%); **24:** i. **9**, 3-methoxybenzoyl chloride, Et_3N, CH_2Cl_2, r.t. (99%), ii. aa (79%); **25:** i. **10**, *p*-methoxybenzoyl chloride, Et_3N, CH_2Cl_2, r.t., ii. aa (2 steps, 84%); **26:** i. **10**, phenylsulfonyl chloride, Et_3N, CH_2Cl_2, r.t., ii. aa (2 steps, 74%); **27:** i. **16**, benzoyl chloride, Et_3N, CH_2Cl_2, r.t., ii. aa (2 steps, 79%); **28:** i. **16**, nicotinic acid, EDC, Et_3N, CH_2Cl_2, r.t. (quant.), ii. aa (97%); **29:** i. **17**, benzoyl chloride, Et_3N, CH_2Cl_2, r.t. (34%), ii. aa (57%); **30:** i. **11**, **20**, K_2CO_3, 2-butanone, reflux (72%), ii. 2-naphthylmethyl bromide, NaH, DMF, r.t. (90%), iii. HCl 2N/THF 1:1, r.t. (30%); **31, 32:** i. **13**, **21 or 22**, HBTU, Et_3N, CH_2Cl_2, r.t., ii. benzene, reflux (34%, 53%), iii. cc (90%, 83%); **33:** i. **9**, 2-bromoethyl phenyl ether, KI, NaH, DMF, 60°C (19%), ii. aa (72%); **34:** i. **9**, benzyl-2-iodoethyl ether (Ref. 20), NaH, DMF, r.t. (49%), ii. aa (83%); **35:** **9**, 3-bromopropyl phenyl ether, NaH, DMF, r.t. (76%), ii. aa (89%); **36:** i. **9**, benzyl-3-bromopropyl ether, NaH, NMP, r.t. (53%), ii. aa (78%); **37:** i. **16**, benzyl bromide, NaH, DMF, r.t., ii. aa (2 steps 73%); **38:** i. **11**, 2-phenethyloxyethyl-methane-sulfonate (Ref. 21), K_2CO_3, 2-butanone, reflux (95%), ii. 2-naphthylmethyl bromide, NaH, DMF, r.t. (82%), iii. cc (76%); **39:** i. **15**, benzyl bromide, NaH, DMF, r.t., ii. aa (2 steps, 77%); **40:** i. **15**, 2-pyridylmethyl chloride, NaH, DMF, r.t., ii. aa (2 steps, 42%); **41:** i. **11**, (E)-(4-bromo-but-2-enyloxy)-benzene (Ref. 22), K_2CO_3, 2-butanone, reflux (99%), ii. H_2-Pd/C, MeOH, r.t. (quant.), iii. 2-naphthylmethyl bromide, NaH, DMF, r.t. (88%), iv. cc (95%); **42:** i. **14**, benzyl bromide, NaH, DMF, r.t., ii. aa (2 steps, 64%); **43:** i. **15**, 2-methoxybenzyl chloride, NaH, DMF, r.t., ii. aa (2 steps, 25%); **44:** i. **11**, **18**, K_2CO_3, DMF, 120°C (88%), ii. **19**, NaH, DMF, r.t. (85%), iii. bb (78%).

The X-ray structural data obtained for renin complexed with ***(R,R)-39*** (Note 23) at 2.9 Å resolution demonstrated a new and entirely unexpected binding mode for this class of inhibitors (Figure 1).

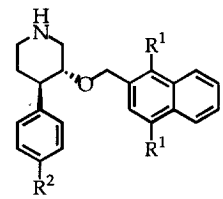
The protonated nitrogen was found to be symmetrically positioned between the two catalytic aspartic acid residues forming one hydrogen bond with each of the two carboxylates. The lipophilic naphthyl residue

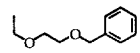
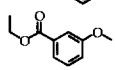
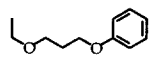
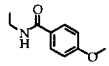
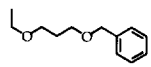
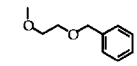
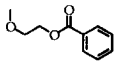
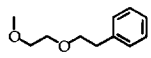
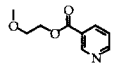
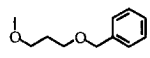
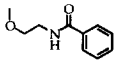
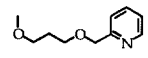
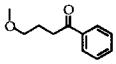
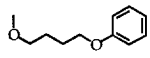
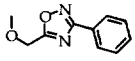
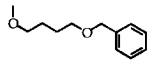
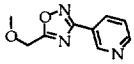
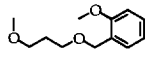
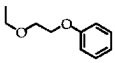
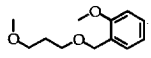
Figure 1. Molecular surface of the binding pockets in a complex of human recombinant renin with the peptidomimetic inhibitor Remikiren (Ref. 24) (left picture) and with the piperidine inhibitor ***(R,R)-39*** (right picture). The side-chain of Trp_{39} is shown in cyan. The two catalytic residues Asp_{32} and Asp_{215} are indicated. The trace of the flap region ranging from residue Thr_{72} to Ser_{81} including the side-chain of Tyr_{75} is indicated in yellow.



occupied the large hydrophobic S_1/S_3 subsite of renin. The 4'-substituted 4-phenyl moiety however induced the following substantial structural changes: 1) side chain rotations around the χ-1 (Cα-Cβ, 120° clockwise) and χ-2 (Cβ-Cγ, 180°) bonds of Trp_{39}; 2) lifting of the whole flap region ranging from residue Thr_{72} to Ser_{81}; 3) rotations of the χ-1 (100°, anticlockwise) bond of Tyr_{75} together with a side chain rotation of Leu_{73} (not shown in Figure 1). The 4-phenyl ring of the inhibitor was found to occupy a position close to that formerly occupied by the aromatic side chain of Tyr_{75}. These structural changes disrupt a hydrogen bond between Tyr_{75} and Trp_{39}, a motive so far conserved in known structures of aspartic proteinases with peptidomimetic inhibitors. Additionally, the rotation of the indolylmethyl side chain of Trp_{45} opened up a deep hydrophobic pocket, ideally

Table 1. IC_{50} values of piperidine renin inhibitors against purified recombinant human renin in comparison to Remikiren 1[15]



Compound	R¹	R²	IC_{50} [nM]	Compound	R¹	R²	IC_{50} [nM]
Remikiren			0.025				
***rac*-23**	H		55	***rac*-34**	H		91
***rac*-24**	H		87	***rac*-35**	H		5.4×10^2
***rac*-25**	H		$>10^5$	***rac*-36**	H		7.4×10^2
***rac*-26**	H		4.0×10^4	***rac*-37**	H		53
***rac*-27**	H		26	***rac*-38**	H		1.6×10^2
***rac*-28**	H		5.8×10^2	***rac*-39**	H		8.0
***rac*-29**	H		2.6×10^3	***rac*-40**	H		2.1×10^2
***rac*-30**	H		2.2×10^2	***rac*-41**	H		2.2×10^2
***rac*-31**	H		41	***rac*-42**	H		1.0×10^2
***rac*-32**	H		8.8×10^2	***rac*-43**	H		1.5
***rac*-33**	H		1.6×10^2	***rac*-44**	OMe		0.060

made to host the extended lipophilic side chain in position 4' of the inhibitor. A small cavity can be identified close to the terminal aromatic ring of the extended 4'-substituent. It accommodates the o-methoxy function of compounds ***rac*-43** and ***rac*-44** which results in the improved affinity. In conclusion, high throughput screening, followed by extensive chemical modification of the lead compound guided by X-ray structure analysis and

computer assisted molecular modeling has led to the identification of a new class of highly potent renin inhibitors. Further structural modifications to realize the optimization of physicochemical properties and the pharmacological profile will be described in the following publication.

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13. Resolution was achieved by derivatization of the amino group of ***rac*-2** with (-)-(1S,4R)-camphanoyl chloride (CH_2Cl_2, 0°C, 99%). The diastereomeric camphanamides were separated by column chromatography on silicagel. The optically pure amines were obtained by reductive cleavage of the chiral auxiliary: ***(R,R)*-2** (Dibah, THF, 0°C, 24%, 99.4% ee); ***(S,S)*-2** (LAH, THF, r.t., 42%, 96.4% ee). Hydrogenation of the diastereomeric camphanamide corresponding to the inactive enantiomer yielded N-[(1S,4R)-camphanoyl]-3-hydroxy-4-phenyl-piperidine (H_2, 5%Pd/C, MeOH, r.t. (96%)). The absolute configuration of the chiral centers in positions 3 and 4 of the piperidine ring was determined by X-ray crystallographic analysis, and found to be (3S,4S) relative to the absolute (1S,4R) configuration of the camphanoyl moiety.
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15. ***Inhibition of recombinant human renin in vitro:*** The assay consisted of a two step procedure: in the first step the enzymatic reaction was performed in poly-propylene 96-well plates and in the second step the accumulated product angiotensin I was measured in immunoplates by an enzyme immunoassay (EIA). i) *Inhibition of recombinant human renin in vitro*: Assay buffer: 100 mM sodium phosphate, pH 7.4, including 0.1% BSA. The incubates were composed of 190 µL per well of an enzyme mixture and optionally 10 µL of DMSO with or without renin inhibitors for the inhibition experiments. The enzyme mixture was premixed in the assay buffer at 4°C and was composed of recombinant human renin (0.1 ng/mL), human tetradecapeptide substrate (0.5 µM) and hydroxyquinoline sulfate in assay buffer (1 mM). The plates were then incubated at 37°C for 3 hours and the angiotensin I accumulation measured by EIA. ii) *Enzyme immunoassay (EIA):* 100 µL of the incubates or angiotensin I standards were transferred to Nunc-Immuno plates (MaxiSorp™), coated with ANG(4-10)/BSA conjugate, and 100 µL of an angiotensin I rabbit antiserum added (AI-AS 1, Roche). Then, the plates were incubated at 4°C overnight and the EIA evaluated with a commercially available biotin-streptavidin system to estimate the bound angiotensin I antibodies (Amersham Life Science). The IC_{50} of the standard curve was 50 pg angiotensin I/well and not different from the one created by RIA. The IC_{50} values depicted are mean values of 2-5 independent determinations.
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