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Discovery and optimization of a new class of potent and non-chiral indole-3-carboxamide-based renin inhibitors

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ABSTRACT

Selective inhibition of the aspartyl protease renin has gained attraction as an interesting approach to control hypertension and associated cardiovascular risk factors given its unique position in the renin–angiotensin system. Using a combination of high-throughput screening, parallel synthesis, X-ray crystallography and structure-based design, we identified and optimized a novel series of potent and non-chiral indole-3-carboxamides with remarkable potency for renin. The most potent compound **5k** displays an IC_{50} value of 2 nM.

Hypertension is one of the major risk factors for cardiovascular diseases, which are the leading cause of mortality in the Western world.[1] Lowering blood-pressure can considerably reduce the risk of myocardial infarction, stroke, heart failure and end-stage kidney disease.[2] However, despite available therapies, approximately 70% of patients with hypertension do not reach their target blood pressure levels. Some of them yet do not respond fully to a combination of treatments. Consequently, opportunities remain for designing and developing well-tolerated effective medicines to control hypertension and associated cardiovascular diseases.

The renin–angiotensin system (RAS) is well-established as an endocrine system involved in regulation of blood pressure and fluid electrolytes.[3] Activation of the RAS is initiated by several signals including lowering of blood pressure, decrease in circulating volume or decrease in plasma-sodium concentration. These signals stimulate the release of the aspartyl protease renin, which cleaves angiotensinogen to produce angiotensin I. Since renin forms the rate-limiting step in this cascade and angiotensinogen is its only known substrate,[4] inhibition of this step would be a very effective antihypertensive strategy. Any appropriate medication affecting the RAS might also result in optimal end-organ protection, in particular for heart and kidney as shown in animal models.[2] This might also be accompanied by a diminished potential for cough side effects, affecting 5–35% of patients treated with ACE inhibitors.[5]

Accordingly, substantial efforts were reported over the last decades to discover renin inhibitors for clinical use, for example, those structures shown in Figure 1 and to overcome issues like low oral bioavailability.[6] Aliskiren (SPP100), an orally active renin inhibitor with four chiral centers (Fig. 1), is currently the only compound, which has reached the market.[2b,7] Consequently, several research groups have reported novel renin inhibitors on diverse scaffolds with different renin active-site binding topologies.[6]

In this publication, we describe the identification, structure–activity relationship and optimization of a novel series of indole-3-carboxamides as potent and non-chiral renin inhibitors.[8] The first active molecule **1**[9] was discovered from high-throughput screening (HTS) of our internal collection and rapidly optimized to result in phenoxy- and benzyl-derivatives **2a** and **2m** at the indole-2 position, displaying increased binding activity (Fig. 2). Consequently these derivatives were used as starting point for further inhibitor design.

Docking[10–12] these molecules into public renin X-ray structures (e.g., PDB 1rne)[13] in addition to our internal experience with other aspartyl-proteases led us to postulate that the piperazine moiety is situated close to both catalytic aspartate residues Asp^{32} and Asp^{215}, engaged in ionic hydrogen-bonding interactions. The internal

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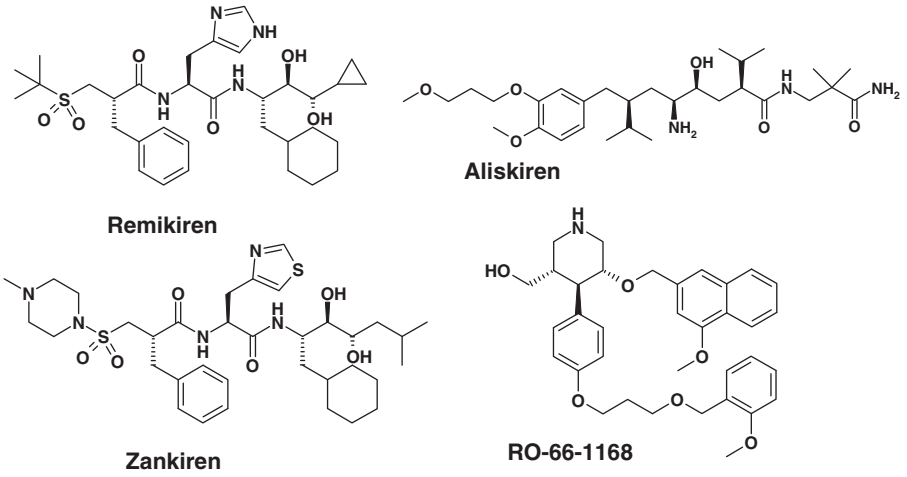
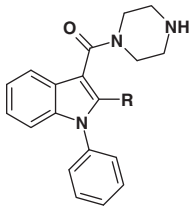


Figure 1. Chemical structures of potent renin inhibitors Remikiren, Aliskiren, Zankiren and RO-66-1168.



Cpd	R=	Renin IC_{50} [μM]
1	N-piperidinyl-	3.800
2a	Phenoxy-	0.420
2m	Benzyl-	0.091

Figure 2. Initial indole-3-carboxamides as basis for design. IC_{50} data was obtained as described in the text and Ref. 8.

X-ray structures of **2a** and **2m** at resolutions of 2.9 and 2.75 Å nicely confirmed these assumptions, thus supporting further lead optimization (Fig. 3).[14]

The protein-binding pocket adopts the 'closed-flap' conformation, as also observed in renin X-ray structures of peptidomimetics (1rne)[13] or Aliskiren (2v0z).[16] The protonated piperazine amine is in hydrogen-bonding interactions with the carboxylates of both Asp^{32} and Asp^{215}. A further hydrogen-bond interaction is present between Thr^{77}-Oγ**H** and the carboxamide oxygen in **2a**. This side-chain hydroxyl-functionality is also in a slightly longer hydrogen-bonding distance to the phenoxy-oxygen; although di-aromatic ether oxygens normally do not reveal any hydrogen-bond acceptor tendency. Interestingly, replacement of this oxygen in **2a** results in improved binding affinity,[17] which could be due to a more favorable conformational effect of the benzyl substituent in **2m**, as deduced from conformational analyzes. This preference might be attributed to the close 3.12 Å distance between carbonyl oxygen and ether oxygen atoms in **2a**, resulting in repulsive electrostatic interactions of their lone pair electrons, which is not possible in **2m**.

The phenoxy- or benzyl substituents are located in the hydrophobic renin S1 pocket, lined by the lipophilic side-chains of residues Phe^{117}, Trp^{39}, Val^{120}, Tyr^{75} and Val^{30}. This aromatic substituent is in optimal arrangement for favorable π–π interactions[18], in particular an edge-to-face interaction with Tyr^{75}. This pocket is filled with an isopropyl group by Aliskiren. The N^1-Phenyl-substituent occupies the slightly more polar S3 pocket, favorably interacting with Pro^{111} and Phe^{117} side chains. Aliskiren fills this region with a methoxy-phenyl group. The indole core is engaged in lipophilic interactions to Ser^{219}Cβ, Met^{289}Cε and Thr77Cγ moieties. These X-ray structures suggest that the indole-3-carboxamide scaffold could serve as template for exploration of both the S1 and S3 pockets.

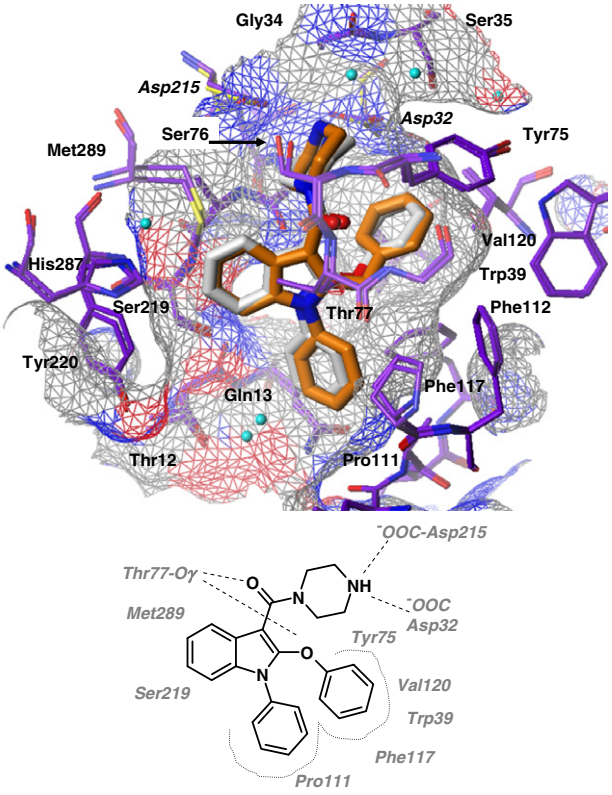
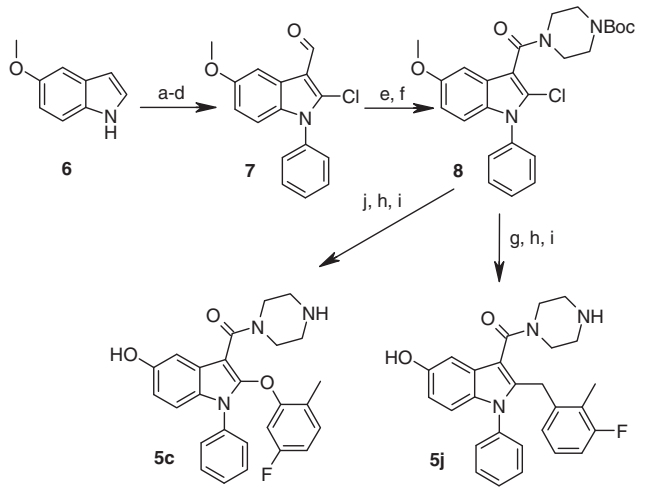


Figure 3. Upper: X-ray crystal structures of two indole-3-carboxamides 2a (gray carbons, 2.9 Å resolution) and **2m** (orange carbons, 2.75 Å resolution) in complex with human renin. The protein binding site is indicated using a MOLCAD[15] hydrogen bond surface (red = ligand acceptor expected, blue = donor expected). Crystallographic water is indicated by cyan spheres. Lower: Schematic view of interactions between **2a** and renin.

The synthetic pathway for preparation of compounds **2–5** is outlined in Scheme 1, here exemplified for compounds **5c** and **5j**. Indole **6** was first arylated under Copper catalysis, followed by chlorination with NCS and subsequent hydrolysation under acidic conditions.[19] Vilsmeyer–Haack formylation with concomitant chlorination led to aldehyde **7**. Oxidation to the carboxylic acid and coupling with *N*-Boc-piperazine provided key intermediate **8**. To gain compound **5j** 3-fluoro-2-methylbenzyl bromide was transformed into the benzyl zinc compound, transmetallated to the



Scheme 1. Synthesis of compounds **5c** and **5j**. Reagents and conditions: (a) CuI, proline, Phenyliodide, K_2CO_3, DMSO, 125 °C, microwave, 63%; (b) NCS, DCM, rt; (c) HOAc, H_3PO_4, 70 °C→130 °C, 65% over both steps; (d) $POCl_3$, DMF, $CHCl_3$, pyridine, 0 °C→rt, 68%; (e) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 0 °C→80 °C; (f) *N*-Boc-piperazine, EDC, HOAt, NMM, DMF, rt, quant. over both steps; (g) (i) 3-fluoro-2-methylbenzyl bromide, Zn, THF, 0 °C, (ii) 9-MeO-9-BBN, −78 °C→rt, (iii) $Pd(OAc)_2$, *S*-Phos, DMF, 100 °C, 53%; (h) TFA, DCM, rt, quant.; (i) BBr_3, DCM, −78 °C→rt, 47–58%; (j) 5-fluoro-2-methylphenol, NaH, NMP, rt→150 °C, microwave, 50%.

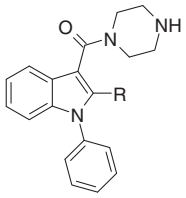
corresponding organoboron species and finally coupled with **8** in a Palladium-mediated reaction. Deprotection of the piperidine and hydroxyl group let to **5j**. Alternatively **8** submitted to nucleophilic substitution, followed by deprotection to provide the arylether **5c**.

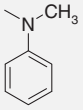
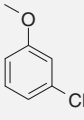
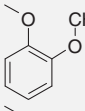
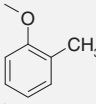
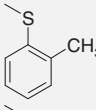
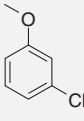
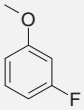
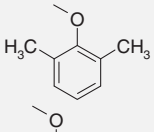
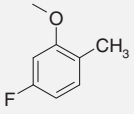
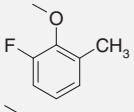
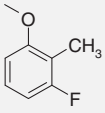
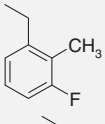
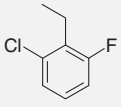
Initial variations of the phenoxy-group in **2a** group led to a significant improvement of renin binding affinity by optimally filling the hydrophobic S1 subsite. A representative collection of synthesized molecules and experimentally determined renin inhibition IC_{50} values [μM] is given in Table 1. To calculate this IC_{50} value, the activity of an inhibitor was evaluated in a 10 point concentration ranging, in duplicate (intraplate), and two to four independent experiments were performed. The IC_{50} is then expressed as geometric mean ± standard deviation (SD),[8] which in general was low for this assay.

In particular the favorable effect of *ortho*-Methyl-substitution on affinity directed towards a small subpocket near Val^{30} and Gly^{217} was remarkable (**2e**: 0.021 μM). Adding another fluorine-substituent further enhances binding (**2l** with *meta*-F: 0.011 μM; **2j** with *meta′*-F: 0.005 μM) for both the phenoxy- and benzyl-indole scaffolds (e.g., **2n** with benzyl and *meta*-F: 0.009 μM). Further molecular modeling suggested that the favorable effect of the *meta′*-F could be attributed to a close contact to the aromatic plane of the Tyr^{75} ring system.[20] The *meta*-F substituent on the other hand interacts with another subpocket, in particular with $Val^{30}C\gamma$ (3.4 Å) and the side-chain carbon atoms of Asp^{32} (3.7 Å). Representative distances are taken from the X-ray structure of **5k** in Figure 4 (see below).

Initial modifications at the piperazine ring did not result in increased binding affinity, as shown in Table 2. Hence we then investigated the S3 pocket by variation of the indole N^1-substituent. Representative variations of synthesized molecules are shown in Table 3. While 2-pyridyl and 4-fluorophenyl groups attached to the N^1 position were less efficient for binding, the incorporation of a methoxy–propoxy substituent increased affinity, in particular if added to the *ortho*-position at the N^1-phenyl ring pointing towards the renin $S3_{sp}$ subpocket, which itself is accessible from the S3 pocket.[13] This is obvious from comparing **2a** (0.420 μM) with **4c** (0.175 μM) in Table 3. A similar substitution is present in

Table 1
In vitro renin activity (IC_{50}) for compounds **2a**–**2o**[a]



Compds	R	Renin IC_{50}[a] (μM)
2a	Phenoxy	0.420
2b		3.160
2c		9.000
2d		0.680
2e		0.021
2f		0.071
2g		0.773
2h		0.063
2i		0.064
2j		0.005
2k		0.016
2l		0.011
2m	Benzyl	0.091
2n		0.009
2o		0.018

[a] IC_{50} data were obtained as described in the text and in Ref. 8.

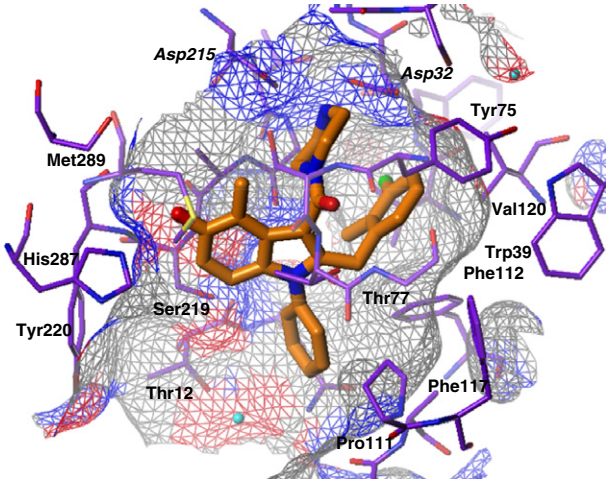
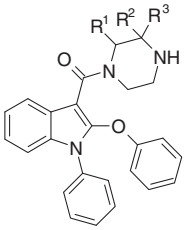


Figure 4. X-ray crystal structure of indole-3-carboxamide **5k** (orange carbons, 2.5 Å resolution) in complex with human renin resulting from rational compound optimization. The protein binding site is indicated using a MOLCAD[15] hydrogen-bonding surface. Crystallographic water is indicated by cyan spheres.

Table 2
In vitro activity for compounds **3a**–**3e**[a]



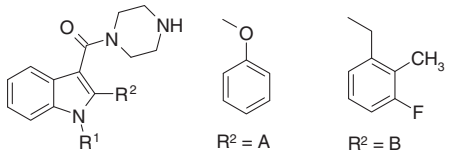
Compds	R^1	R^2	R^3	Renin IC_{50}[a] (μM)
2a	H	H	H	0.420
3a	H	*rac*-CH_3	H	6.460
3b	H	CH_3	CH_3	7.300
3c	*rac*-Benzyl	H	H	4.500
3d	H	*rac*-CH_2OH	H	1.600
3e	Homopiperazine			3.410

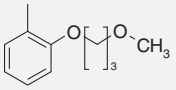
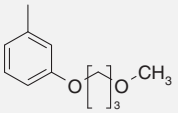
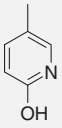
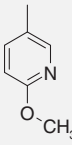
[a] IC_{50} data were obtained as described in the text and in Ref. 8.

Aliskiren in this area.[7] However, molecular modeling suggests that the corresponding vector for the indole scaffold is slightly deviating from the narrow entrance into this subpocket, which might explain the less pronounced effect of this substitution compared to other series like Aliskiren analogs. Furthermore, the replacement of phenyl by aliphatic rings did not result in a dramatic loss of affinity, underscoring the hydrophobic nature of the favorable interactions in this S3 site (**4f**: 0.020 μM vs **2n** with 0.009 μM).

Finally we focused on variations at the indole core to explore polar and hydrophobic interactions with the binding site to increase affinity. The resulting molecules are summarized in Table 4. Several polar substitutions display a slightly favorable effect on affinity, especially the 5-OH substitution in **5c** (0.002 μM), when compared to its parent **2j** (0.005 μM). Small, polar groups like hydroxyl in this area in the protein pocket are acceptable, while a 5-methoxy group resulted in a reduction of binding affinity (**5b**: 1.350 μM). As docking and the subsequent X-ray structure of the close analog **5k** suggests (see below), this 5-hydroxyl-group favorably interacts with the imidazole ring of His^{287}, while there is not enough space in the binding site to accommodate an additional methyl group in **5b**.

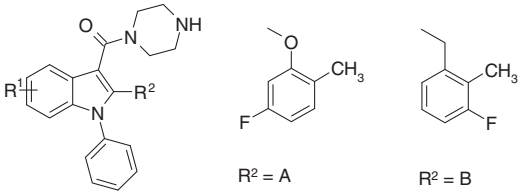
Table 3
In vitro activity for compounds **4a**–**4i**[a]



Compds	R^1	R^2	Renin IC_{50}[a] (μM)
2a	Phenyl	A	0.420
4a	2-Pyridyl	A	4.970
4b	4-Fluorphenyl	A	0.707
4c		A	0.175
4d		A	0.763
2n	Phenyl	B	0.009
4e	Cyclohexyl	B	0.034
4f	Cyclopentyl	B	0.020
4g	4-Pyranyl	B	0.752
4h		B	9.350
4i		B	0.360

[a] IC_{50} data were obtained as described in the text and in Ref. 8.

Table 4
In vitro activity for compounds **5a**–**5o**[a]



Compds	R^1	R^2	Renin IC_{50}[a] (μM)
5a	6-OCH_3	A	0.024
5b	5-OCH_3	A	1.350
5c	5-OH	A	0.002
5d	5-$CONH_2$	A	0.006
5e	6-CO_2H	A	0.122
5f	6-$OCH_2CH_2N(CH_3)_2$	A	0.029
5g	6-OCH_2CO_2H	A	0.130
5h	6-OCH_3	B	0.012
5i	6-OH	B	0.004
5j	5-OH	B	0.004
5k	4-CH_3, 5-OH	B	0.002
5l	7-F	B	0.084
5m	6-OCH_2CH_2OPh	B	0.024
5n	6-OCH_2CH_2OH	B	0.031
5o	6-SO_2CH_3	B	0.044

[a] IC_{50} data were obtained as described in the text and in Ref. 8.

Other tolerated substitutions are the 5-carboxamide (**5d**: 0.006 μM) and 6-OH group (**5i**: 0.004 μM). Interestingly, the 6-methoxy substitution in **5h** (0.012 μM) is significantly more active compared to the 5-methoxy-substitution in **5b**. This was explained

by docking and the subsequent X-ray structure of **5k** (see below), revealing that the His^{287} side chain limits the cavity size for 5-OMe substitution in this region. In contrast, substituents at the indole-6 position point to a more open area limited by Ser^{219} and Tyr^{220}, where slightly larger and lipophilic substitutions might be accommodated. The further inspection of available X-ray structures also suggests that more lipophilic substituents might be tolerated in position 6 in addition to the small polar substitution in position 5. This resulted in synthesis of **5k** with an IC_{50} of 0.002 μM.

We then solved the X-ray structure of compound **5k** in complex with human renin by X-ray structure analysis at 2.5 Å (Fig. 4), thus validating our hypothesis about favorable protein–ligand interactions for biological affinity.[14] In addition to the interaction of the piperazine with the catalytic aspartates, the Thr^{77}-OγH hydrogen bond and the lipophilic interactions of both phenyl rings in S1 and S3, several other interactions further contribute to the observed high binding affinity of **5k**.

The 2-methyl-3-fluoro substituted phenol optimally fills the S1 pocket with fluorine directed towards a small lipophilic subpocket formed by side-chain carbon atoms of Val^{30}, Val^{120} and Asp^{32} with distances of 3.4 and 3.7 Å to Val^{30}Cγ and Asp^{32}Cγ═O), respectively. The close distance and geometric arrangement between fluorine and Asp^{32} carboxylate also suggests an involvement of orthogonal multipolar interactions to complex stabilization.[21,22] The *ortho*-methyl group is pointing towards Gly^{217}Cα, Val^{30}Cγ and the aromatic ring in Phe^{117}, thus also contributing to binding.

The substitutions at the indole core also favorably contribute to binding affinity. The 5-OH substituent is engaged in an additional hydrogen-bonding interaction to the imidazole-nitrogen in His^{287}, which also lower activity, when adding a methyl group to the phenolic OH (**5b**: 1.350 μM). The 4-methyl group is involved in lipophilic contacts to the side-chain atoms of Thr^{77}Cγ, and somewhat more distant Ser^{76}Cβ and Ala^{218}Cα. This combination of favorable interactions thus accounts for the improved binding affinity of **5k** in comparison to the template **2m**.

In summary, we have described the discovery and structure–activity relationship of a novel series of potent and non-chiral renin inhibitors with various modifications around an indole scaffold. The incorporation of substituted phenoxy- or benzyl substituents in S1 combined with an adequate substitution at the indole core led to a significant improvement of biological activity. The best compounds (e.g., **5k**) display IC_{50} values of 2 nM against renin without significant inhibition of related proteases. Thanks to the availability of experimental information from several X-ray structures of renin-inhibitor complexes during our optimization, we were able to understand the influence of key structural elements to affinity, which might allow for further design around this versatile scaffold. Hence, this series constitutes a promising area for lead optimization supported by structure-based design. Further details on these studies will be reported in due time.

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