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DISSERTATION

In vitro and *in vivo* characterization of small molecular inhibitors of the (pro)renin receptor ((P)RR) signal transduction pathway

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"So eine Arbeit wird eigentlich nie fertig,

man muss sie für fertig erklären,

wenn man nach Zeit und Umständen

das Möglichste getan hat"

Wolfgang von Goethe

"Italienische Reise" Zweiter Teil (16. März 1787)

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Summary

English abstract

The (pro)renin receptor ((P)RR) plays a crucial role in the development of cardial and renal end-organ damage. Within an academic setting our group aimed to develop small molecular inhibitors of the (P)RR and therefore named renin/ prorenin receptor blockers (RERBs). In this thesis, the effects of two inhibitors of the (P)RR pathway was investigated: the V-ATPase (vacuolar adenosine triphosphatase) inhibitor bafilomycin and genistein which blocks the translocation of promyelocytic leukemia zinc finger (PLZF) to the nucleus. Bafilomycin reduces cell number in a dose dependent manner, genistein in contrast reduces cell number only in high concentrations. In the context of Wnt signalling bafilomycin represses Wnt3a-induced stimulation of axin2. After our group has performed a high-throughput screen for the identification of novel RERB compounds the microsomal stabilities of 121 compounds and the *in vivo* half-lives of 22 compounds have been determined. *In vivo* half-lives and predicted *in vivo* hepatic clearance were correlated showing r^2 values of 0,31 for the rat and 0,27 for the human species.

Our data support that the (P)RR is a promising target for oncological indications. Pharmacokinetic data helped to identify drug candidates for future *in vivo* proof-of-concept animal experiments. The weak coefficients of determination of *in vitro* and *in vivo* pharmacokinetic data question the benefit of using microsomal stability as a tool to select compounds for further testing.

German abstract

Der Renin-/ Prorenin-Rezeptor (RER oder (P)RR) spielt eine Schlüsselrolle bei der Entstehung kardialer und renaler Endorganschäden. Es war das Ziel unserer Arbeitsgruppe innerhalb eines akademischen Umfeldes niedermolekulare Inhibitoren des (P)RR zu identifizieren, die Renin-/ Prorenin-Rezeptor-Blocker (RERBs) genannt werden. Innerhalb dieser Arbeit wurden zwei Inhibitoren des (P)RR Signalweges untersucht: der V-ATPase (vakuolare Adenosintriphosphatase) Inhibitor Bafilomycin und Genistein, welches die Translokation von PLZF (*promyelocytic leukemia zink finger*) in den Kern inhibiert. Bafilomycin reduziert die Zellzahl dosisabhängig, Genistein hingegen reduziert die Zellzahl nur in sehr hohen Dosen. Im Zusammenhang mit dem Wnt Signalweg unterdrückt Bafilomycin die Wnt3a-induzierte Stimulation von axin2. Nach einem *high-throughput screening,* das zur Identifizierung von neuen RERB Substanzen durchgeführt wurde, haben wir die mikrosomale Stabilität von 121 Substanzen und die *in vivo* Halbwertszeit von 22 Substanzen bestimmt. Die *in vivo* Halbwertszeiten und die vorhergesagten *in vivo* Clearances wurden korreliert. Es zeigten sich r^2-Werte von 0,31 für die Korrelation mit der Nagerspezies und r^2-Werte von 0,27 für die Korrelation mit der humanen Spezies.

Unsere Daten untermauern, dass der (P)RR ein vielversprechender onkologischer Angriffspunkt ist. Die schwache Korrelation pharmakokinetischer Daten *in vivo* und *in vitro* stellt den Nutzen von mikrosomaler Stabilitätstestung in frühen Phasen der Medikamentenentwicklung in Frage.

1.1 Introduction

1.1.1 The (pro)renin receptor and its pathophysiological significance

In the last years increasing scientific interest focuses on the (pro)renin receptor ((P)RR). The (P)RR, which has been cloned in 2002, is a component of the renin angiotensin system, binds renin and prorenin and is described to consist of 350 amino acids with a single transmembrane domain[1]. The initial reports that the (P)RR has no homology with any known membrane protein[1] turned out to be wrong. The coding sequence of ATP6AP2, an accessory protein of a vacuolar proton-transporting ATPases (adenosine triphosphatase) and the coding sequence of the C-terminal part of the (P)RR are identical[2]. It was shown that the binding of renin to the (P)RR results neither in a change of intracellular cyclic adenosine monophosphate (cAMP) levels nor in a change of intracellular Ca^{2+} concentration, but it increases the activity of the MAP (mitogen-activated protein kinases) ERK1/2 (extracellular signal-regulated kinase 1/2)[1]. To analyze the function of the (P)RR as a V-ATPase (vacuolar ATPase) Advani *et al.*[3] used the pleomacrolide antibiotic bafilomycin, which acts as a selective inhibitor of V-ATPases, to show that bafilomycin inhibits renin-induced, (P)RR-mediated ERK 1/2 activation. These results are similar to results seen by knocking down the (P)RR by small inhibitory RNA (siRNA)[3].

A soluble form of the (P)RR (s(P)RR) is generated by cleavage of the (P)RR by furin and/or ADAM19 (a disintegrin and metalloproteinase 19) into the s(P)RR and the V-ATPase-associated (P)RR isoform named M8-9[4-6].

A new signal transduction pathway of the (P)RR involving physical interaction of the transcription factor promyelocytic leukemia zink finger (PLZF) was disclosed by our group[7]. PLZF translocates into the nucleus after stimulation of the (P)RR by renin or prorenin and suppresses the RER promoter[7,8]. The translocation of PLZF to the nucleus was shown to be inhibited by the small molecule genistein[9]. The (P)RR seems to play an important role in cell proliferation and apoptosis. It was shown that stimulation of the (P)RR in epithelial cells induces pro-proliferative effects[8]. This corresponds to the finding that prorenin increases the proliferation and migration of endothelial cells and the finding that melanoma-xenografts stably transfected with prorenin have an increased growth rate[10]. The direct interaction of the (P)RR and PRL-1 (phosphatase of regenerating liver–1) an ubiquitously expressed phosphatase involved in cellular proliferation[5,11,12], implicates the involvement of the (P)RR

in an oncological context. There is also an involvement of the (P)RR in the Wnt pathway. The pioneering work of Cruciat *et al.* reveals an important role of the (P)RR in the Wnt signalling pathways[13]. They demonstrated the interaction of the (P)RR with the Wnt (co)receptors frizzled 8 and LRP 6 (low-density lipoprotein receptor-related protein 6)[13] e.g. by using siRNA against the (P)RR. Furthermore, they showed that the (P)RR is necessary for induction of Wnt3a downstream genes. It is known that the unspecific V-ATPase inhibitor bafilomycin can inhibit the (P)RR pathway[3], but also the Wnt pathway[13].

The importance of the (P)RR concerning cardiorenal end-organ damage is discussed below.

1.1.2 Development of prorenin/ renin receptor blockers

Hypertension causes 60-80% of all heart failures[14,15], around 70% of all dialysis-dependent kidney disease[16,17] and up to 30% of all blindness in industrial nations[18-20]. It represents one of the currently most severe medical problems. Until today there is no sufficient therapy for end-organ damage caused by hypertension and diabetes. All gold standard therapies such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin AT1 receptor blockers (ARBs), β blockers or oral antidiabetic drugs are only able to decelerate the development of end-organ damage, but are not able to prevent them[21,22]. The development of renin/ prorenin receptor blockers (RERBs) opens a new possible strategy in fighting against these global problems[5,23]. The interest regarding (P)RR blockage and thereby preventing end-organ damage started when Ichichara et al. found that a decoy peptide which inhibits the binding of prorenin to the (P)RR can ameliorate or even completely block the tissue damage induced by diabetes or hypertension in mice and rats[24-26]. There are numerous publications about the beneficial effect of these so-called handle region peptides (HRPs)[24-28]. The HRPs showed their effect in a model of streptozotocin (STZ) induced diabetes in rats[24] and diabetic angiotensin type 1_a receptor ($AT1_aR$) deficient mice[26], prevented the development of glomerulosclerosis in transgenic rats overexpressing the human (P)RR and showed a beneficial effect in creatinine clearance and left ventricular function in spontaneously hypertensive rats on a high salt diet[28]. These findings correspond to those of Satofuka et al. describing a beneficial effect of HRPs in an STZ induced diabetic mouse model of retinal inflammation[29]. In all these studies the HRPs did not affect blood pressure. Although these results do underline the importance of the (P)RR there are shortcomings of using these HRPs as a medication in humans. These peptides must be administered parenterally due to poor

bioavailability. Therefore, there is a putative medical need to develop a small molecule having the same target and end-organ protective properties, but which is suitable for a use in a human context.

1.1.3 Aim of the dissertation

It was the aim of this study to further clarify the effect of bafilomycin and genistein in relation to (P)RR signalling and to investigate their effect on cell number as well as the effect of bafilomycin on the interaction of the (P)RR with the Wnt pathway.

Previous work of our group identified several chemical series of small molecules interfering with the (P)RR signalling pathway by performing a high-throughput screen in a luciferase-based assay with the library of Evotec[30] and by performing subsequent secondary assays such as dose-response analysis, toxicity and proliferation assays. This study aimed to characterize the pharmacokinetic (PK) properties of these compounds as a prerequisite for further *in vivo* proof-of-concept experiments. Moreover, we analyzed whether *in vitro* PK testing can substitute for *in vivo* PK experiments.

1.2 Methods

All materials and methods used are listed and described in the publications. In this section, only the main methods are briefly described.

1.2.1 Cell culture experiments

Cells were maintained in sterile plastic flasks for culture. HepG2 (human hepatoma) , KELLY (human neuroblastoma) and B-16V cells (mouse melanoma) were grown in RPMI medium supplemented with 10% FCS (fetal calf serum) and 5% penicillin/ streptomycin. Hek293T cells were cultured in High Glucose DMEM supplemented with 10% FCS and 5% penicillin/ streptomycin. Cells were grown in a humidified incubator at 37 °C and 5% CO_2. Medium was changed twice weekly. Cells were incubated with vehicle, bafilomycin or genistein for 48 hours using the indicated concentrations. All substances were diluted in 100% dimethyl sulfoxide (DMSO). Final DMSO concentration in the well was 1%. In order to measure proliferation, we determined the number of viable cells by Cell proliferation assay XTT (AppliChem, Darmstadt, Germany). XTT tetrazolium is reduced to XTT formazan, an orange colored water soluble dye, by mitochondrial enzymes of living cells. For assessment of cytotoxicity, we measured lactate dehydrogenase (LDH) activity in supernatant of cells cultured as described above. Upon addition of pyruvate, LDH generates lactate and reduces $NADH^+$ to NAD (nicotinamide adenine dinucleotide). The decline of $NADH^+$ is measured photometrically and is proportional to the amount of LDH in the sample. Cell titer glo Luminiscent Cell Viability assay (Promega, Mannheim, Germany) was used to determine the number of viable cells in culture based on quantification of the ATP present. The amount of total protein correlates with the number of cells in cell culture[31]. This was assessed by the Bradford assay, a colorimetric protein assay, which is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250. IC50 was calculated by the program GraphPad PRISM, (GraphPad Software Inc., La Jolla, CA, USA) after log transformation of concentration and normalization of response.

The function of the (P)RR within the Wnt pathway was analyzed by using Wnt3a-conditioned medium. Cells were incubated with bafilomycin for 24 hours in Wnt3a-conditioned or control medium. RNA was isolated using Nucleo-Spin RNA II Kit (Macherey-Nagel, Düren, Germany). RNA concentration was measured spectrophotometrically. Reverse transcription was performed by using M-MLV reverse transcriptase. PCR was performed with Power SYBR

Green PCR Master Mix (Applied Bioscience, Darmstadt, Germany) and primers for ubiquitin c (UBC) and axin2. PCR reactions were performed on the Stratagene Mx300P (Stratagene, La Jolla, CA, USA) or the 7300 Real-time PCR System (Applied Biosystems) in a 2-step protocol.

1.2.2 *In vitro* pharmacokinetics

For the determination of *in vitro* half-lives, the compounds were incubated with either rat liver microsomes or human liver microsomes as given in Schrezenmeier et al.[32]. At six time points, including a basal time point, aliquots of the mixture were taken. Samples were analyzed using liquid chromatography–mass spectrometry (LC-MS/MS).

1.2.3 *In vivo* pharmacokinetics

In vivo pharmacokinetic studies were performed in male Spraque-Dawley rats. They were weighted on the day of the PK study. Each animal received an intravenous bolus of 1.5 ml of solved compounds via the lateral tail vein. Compounds were solved in wellsolve (Celeste Co., Tokyo, Japan) and saline. The exact compound concentration in the solution was determined later via LC-MS/MS. Blood samples were taken when the animals were narcotized with isofluran. Blood samples for determination of compound plasma concentration were taken at a pre-application time point, 15 min, 30 min, 1 h, 2 h, 4 h and 8 h after dose administration from the contralateral tail vein. Plasma was gained by centrifugation and was stored at -80 °C until shipping. The maintenance and all study protocols were approved by the Landesamt für Gesundheit und Soziales (LaGeSo) Berlin (G 0229/09).

1.2.4 Bioanalysis

For LC-MS/MS analysis API 4000 Triple Quad with TurboSpray ion source (AB Sciex, Framingham, MA, USA) was used as mass spectrometer. Calibration curves were conducted using serial dilution of compound prepared at 1 mg/ml.

1.2.5 Data analysis

Half-lives from the microsomal stability assay were calculated using the formula $t_{1/2} = 0.693 / \text{slope}$. *In vitro* clearances were also calculated in this study. Intrinsic (*in vitro*) clearance (Cl_{int}) was calculated using the formula $t_{1/2} = 0.693 \cdot V / Cl_{int}$ with volume of incubation (V) of 2 ml/mg and Cl_{int} given in ml/min/mg microsomal protein. The *in vitro* Cl_{int} is then scaled up following equations based on the well-stirred model[33-35]:

(1) Scaled Cl_{int} [ml/min/kg] = Cl_{int} [ml/min/mg protein] · microsomal protein content per liver weight [mg/g] · liver weight per body weight [g/kg],

(2) predicted *in vivo* hepatic clearance (Cl_{pred}) [ml/min/kg] = (scaled Cl_{int} [ml/min/kg] · hepatic blood flow [ml/min/kg]) / (scaled Cl_{int} [ml/min/kg] + hepatic blood flow [ml/min/kg]).

Regarding the rat species, the *in vivo* microsomal protein content per liver weight is 45 mg/g, the liver weight per body weight is 40 g/kg and the liver blood flow is 70 ml/min/kg body weight; regarding the human species, the *in vivo* microsomal protein content per liver weight is 49 mg/g, the liver weight per body weight is 26 g/kg and the liver blood flow is 20 ml/min/kg body weight. Cl_{pred} refers to a blood clearance. It is linked to plasma clearance in a linear fashion via the hematocrit.

1.3 Results

1.3.1 The effect of genistein and bafilomycin on proliferation

Figure 7D (publication 2) shows results of the XTT, Cell titer glo, Brandford and LDH assay in KELLY cells, HepG2 cells and B-16V cells, which were incubated with increasing doses of bafilomycin and genistein. Bafilomycin reduced cell number in a dose-dependent manner. Genistein inhibited cell number increase only at a high dose (100 µM). The dose causing 50% inhibition (IC50) in KELLY cells was 3.7 nM for bafilomycin and 16 µM for genistein in the XTT assay. Bradford total protein measurement was performed as an independent method to assess cell number. Also in this assay, bafilomycin inhibited cell number increase in a dose dependent manner while genistein is only effective at a dose of 100 µM. The maximum reduction is about 55% of control with bafilomycin in HepG2 cells in the Bradford assay. Further testing of number of viable cells was performed by measuring ATP content in the three cell lines after stimulation with bafilomycin or genistein with the luciferase-based Cell titer glo assay. The effect of bafilomycin and genistein on cellular ATP content is comparable in all three tested cell lines. The IC50 of genistein is also comparable in all cell proliferation assays. Additionally, we measured the release of the cytoplasmic enzyme LDH into the supernatant as a parameter for cytotoxicity. As a control, a full kill with 1% Triton X 100 was performed showing values around 700% of vehicle. LDH activity was measured in the supernatant after an incubation time of 48 h with bafilomycin and genistein. Bafilomycin increases LDH activity slightly with increasing concentrations. Genistein, up to a concentration of 10 µM, does not affect LDH activity in the supernatant.

1.3.2 The effect of genistein and bafilomycin on Wnt signalling

The effect of bafilomycin on Wnt signalling was investigated using Wnt3a conditioned medium followed by real-time PCR analysis of axin2. Data were standardized to UBC. The V-ATPase inhibitor bafilomycin inhibits the Wnt3a induced activation of axin2 on messenger RNA (mRNA) level (publication 3, fig. 4B).

1.3.3 Pharmacokinetic *in vitro* testing

121 compounds, which were structurally diverse and compliant with Lipinski´s rule of five[36], were investigated for their microsomal stability in human and rat liver microsomes in duplicate measurements using six time points (publication 1, fig. 1). The coefficient of determination (r^2) was 0.3998 between human and rat liver microsomes. To account for

microsomal protein concentration, intrinsic clearance (Cl_{int}) was calculated; to account for the *in vivo* situation, we calculated the scaled intrinsic clearance which considers protein content per liver weight and liver weight per body weight. Predicted *in vivo* clearance was calculated by using the well-stirred model. All these calculations are based on the same dataset. The coefficients of determination between the rat and the human human species are identical or similar to 0.3998 because only linear (from Cl_{int} to scaled Cl_{int}) or nonlinear transformation has been performed (publication 1, fig. 1).

1.3.4 *In vivo* half-lives in the rat species

It was our goal to select compounds with long *in vivo* half-lives. We used previously described thresholds to distinguish long from short *in vitro* half-lives (15 min regarding rat liver microsomes[37] and 30 min regarding human liver microsomes[38]). We prioritized 15 compounds that were fulfilling at least one of these criteria and we selected 7 compounds not fulfilling these criteria as controls. A representative plasma concentration time curve is shown in figure 2 of publication 1.

1.3.5 Correlations of *in vitro* half-lives and *in vivo* half-lives

For these 22 compounds, we examined the correlation between compound half-lives, which were determined either in rat or in human liver microsomes, and compound half-lives, which were determined *in vivo*. We observed an r^2 of 0.09 for rat microsomes and 0.07 for human microsomes demonstrating a weak correlation (publication 1, fig.3). Subset analyses regarding compounds which were below or above the thresholds were performed, all showing weak correlations.

1.3.6 Correlations of *in vitro* clearances and *in vivo* clearances

In a last step we analyzed whether there is a correlation between *in vivo* clearance determined in rats and predicted *in vivo* hepatic clearance. Predicted *in vivo* hepatic clearance is calculated based on data determined *in vitro*, since *in vitro* half-life depends on microsomal protein concentration available. Unlike the weak in *vivo-in vitro* half-life correlations, the clearance correlations are higher being in the intermediate range (publication 1, fig. 4).

1.4 Discussion

Within publication 2 the effect of the small molecules bafilomycin and genistein were addressed simultaneously for the first time. Our data show that genistein does not significantly inhibit cell number increase, but that bafilomycin reduces cell number in a dose dependent manner in several cells of different origin, such as HepG2, KELLY and B-16V cells. Furthermore, we used an LDH assay to exclude a direct cytotoxic effect of bafilomycin and genistein. Results are comparable in different assays measuring mitochondrial enzyme activity, ATP concentration or total protein concentration, validating or results. Our data are consistent with the results of Rusin et al. who have determined two digit micromolar IC50 values for the antiproliferative effect of genistein in several cancer cell lines[39]. Also in accordance with our data, bafilomycin was found to decrease the growth of different tumor cell lines *in vitro*[40] as well as xenograft growth *in vivo*[40,41]. Experiments given in publication 2 indicate that the cell number effects observed using XTT, Cell titer glo and Bradford assay are caused by decreased proliferation as demonstrated by BrdU assay.

Bafilomycin and siRNA against the (P)RR similarly reduce cell number (comparison of own data and other data in publication 2). Additionally, other groups found that wild-type podocytes treated with bafilomycin have similar morphologic characteristics as podocytes with (P)RR deletion[42]. These observations indicate that bafilomycin can mimic the effects of a (P)RR knockdown.

In the context of our data, it is important to note that genistein inhibits the nuclear translocation of PLZF[9]. We conclude that genistein is a small molecule mimetic of siRNA against PLZF because both do increase (P)RR promotor acitivity and neither siRNA against PLZF nor genistein significantly decreases cell number in our phenotypical assays.

In the publication of Bernhard et al. our group was able to show for the first time that the (P)RR exerts a basal repression of the Wnt pathway and that endogenous axin2 is significantly upregulated after (P)RR knockdown[43]. In contrast to the full-length (P)RR, the V-ATPase domain seems to stimulate Wnt because the incubation with the V-ATPase inhibitor bafilomycin completely inhibits axin2 mRNA induction by Wnt3a.

Bafilomycin is known to inhibit the ligand-dependent[3] and Wnt-associated signal transduction of the (P)RR[13], but it is severely toxic what makes it unusable in clinical trials[44].

Concerning clinical trials of genistein, there was a recently performed phase II trial which showed no increased survival of patients with pancreatic cancer[45] consistent with or *in vitro*

data. The beneficial effect of genistein on proteinuria, albuminuria and glomerular deposits in streptozotocin-induced diabetic mice was demonstrated by Elmarakby et al.[46]. Genistein can also protect pancreatic β cells from high glucose-induced apoptosis[47]

Besides the interaction with the (P)RR signalling, genistein has a number of other effects. It can for example bind to estrogen receptors (ER) which leads to an activation of ER responsive genes[48]. Genistein can also inhibit tyrosines kinases[49] and aspects of Wnt signalling[50]. Furthermore, it was described that genistein can influence histone modifications and DNA methylation[51], NFkappaB[52] and Smad[53] signal transductions.

Bafilomycin and genistein interfere with (P)RR signalling, but the off-target effects discussed above, their lack of effect in clinical trials or their toxicity creates the need for other small molecules interfering with (P)RR signalling which are more specific and less toxic. Our group previously identified several chemical series of small molecules interfering with the (P)RR signalling pathway. A primary part of this thesis aimed to characterize the pharmacokinetic properties of these compounds as a prerequisite for further *in vivo* proof-of-concept experiments. Sufficient *in vivo* half-lives decides whether a compound can be used in such animal experiments.

The compounds analyzed in this study have been directly derived from a high-throughput screening without prior knowledge of any PK parameter. Besides the approach driven primarily by *in vivo* PK, we analyzed whether *in vitro* PK testing is useful to categorize compounds in early drug discovery. In our study, all coefficients of determination between PK parameters derived from *in vitro* experiments and those between PK parameters derived from *in vivo* single-dose applications have been in the intermediate to low range. Other publications have addressed the putative correlation of microsomal stability and murine *in vivo* PK. Even when working with known model compounds, a correlation is not necessarily observed[54,55] confirming our data in publication 1. The lack of predictability has a major impact on decision making in early academic drug discovery. We suggest to skip *in vitro* PK studies and to directly proceed to *in vivo* PK in an early drug discovery context in order to select hits/ (pre)leads for pharmacodynamic testing *in vivo*. This is further supported by the fact that experiments using microsomes, especially human ones, are not significantly cheaper compared to *in vivo* animal PK studies. This does not imply that cost should be a factor when considering animal replacement methods. Omitting microsomal stability testing also eliminates false-negative *in vitro* results which would exclude putative

pharmacologically promising compounds from further *in vivo* testing.

To conclude, we substantiated the role of the (P)RR as an oncological drug target by *in vitro* experiments. Furthermore, we identified several small molecular compounds by *in vitro* PK testing suitable to be tested in future oncological *in vivo* proof-of-concept experiments as a further step for the drug discovery/ development of first-in-class renin/ prorenin receptor blockers.

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Declaration of any eventual publications

Eva Vanessa Schrezenmeier had the following share in the following publications

Publication 1: Schrezenmeier E, Zollmann FS, Seidel K, Böhm C, Schmerbach K, Kroh M, Kirsch S, Klare S, Bernhard S, Kappert K, Goldin-Lang P, Skuballa W, Unger T, Funke-Kaiser H., Moderate Correlations of in vitro versus in vivo Pharmacokinetics Questioning the Need of Early Microsomal Stability Testing, Pharmacology, 2012

Contribution in detail: Eva Vanessa Schrezenmeier performed the animal experiments, acquired and analyzed the data and drafted the figures of the manuscript. The manuscript was written in collaboration with the last author.

Publication 2: Kirsch S, Schrezenmeier E, Klare S, Zaade D, Seidel K, Schmitz J, Bernhard S, Lauer D, Slack M, Goldin-Lang P, Unger T, Zollmann FS, Funke-Kaiser H., The (pro)renin receptor mediates constitutive PLZF-independent pro-proliferative effects which are inhibited by bafilomycin but not genistein, International Journal of Molecular Medicine, 2014

Contribution in detail: Eva Vanessa Schrezenmeier performed the cell culture experiments, analyzed the data and drafted the figures of figure 7D. She contributed to the generation of the manuscript.

Publication 3: Bernhard SM, Seidel K, Schmitz J, Klare S, Kirsch S, Schrezenmeier E, Zaade D, Meyborg H, Goldin-Lang P, Stawowy P, Zollmann FS, Unger T, Funke-Kaiser H., The (pro)renin receptor ((P)RR) can act as a repressor of Wnt signalling, Biochemical Pharmacology, 2012

Contribution in detail: Eva Vanessa Schrezenmeier performed the cell culture experiments, analyzed the data and drafted the figure of figure 4B which was created during revision. She contributed to the generation of the manuscript.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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Publication 1

Moderate Correlations of in vitro versus in vivo Pharmacokinetics Questioning the Need of Early Microsomal Stability Testing, Pharmacology, 2012

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Publication 3

The (pro)renin receptor ((P)RR) can act as a repressor of Wnt signalling, Biochemical Pharmacology, 2012

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Affidavit (Eidesstattliche Versicherung)

„Ich, Eva Vanessa Schrezenmeier, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema:

In vitro and *in vivo* characterization of small molecular inhibitors of the (pro)renin receptor ((P)RR) signal transduction pathway

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Datum Unterschrift

Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of publications

Schrezenmeier E, Zollmann FS, Seidel K, Böhm C, Schmerbach K, Kroh M, Kirsch S, Klare S, Bernhard S, Kappert K, Goldin-Lang P, Skuballa W, Unger T, Funke-Kaiser H., Moderate Correlations of in vitro versus in vivo Pharmacokinetics Questioning the Need of Early Microsomal Stability Testing. Pharmacology, 2012;90:307–315

Bernhard SM, Seidel K, Schmitz J, Klare S, Kirsch S, Schrezenmeier E, Zaade D, Meyborg H, Goldin-Lang P, Stawowy P, Zollmann FS, Unger T, Funke-Kaiser H. The (pro)renin receptor ((P)RR) can act as a repressor of Wnt signalling. Biochemical Pharmacology, 2012;85:1643–1650

Kirsch S, Schrezenmeier E, Klare S, Zaade D, Seidel K, Schmitz J, Bernhard S, Lauer D, Slack M, Goldin-Lang P, Unger T, Zollmann FS, Funke-Kaiser H., The (pro)renin receptor ((P)RR) mediates constitutive, PLZF-independent pro-proliferative effects which are inhibited by bafilomycin but not genistein. International Journal of Molecular Medicine, 2014;33(4):795-808

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