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Brief Article

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Potent 11β-Hydroxylase Inhibitors with Inverse Metabolic Stability in Human Plasma and Hepatic S9 Fractions To Promote Wound Healing

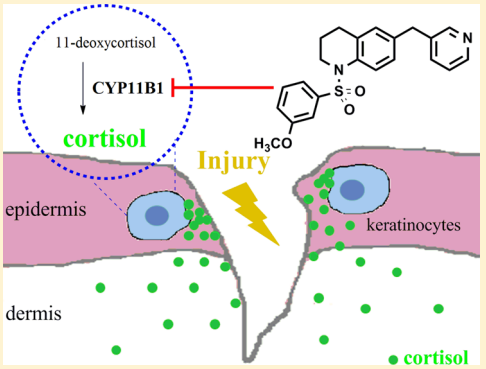
Weixing Zhu,[†,§] Qingzhong Hu,[†,§] Nina Hanke,[‡] Chris J. van Koppen,[‡] and Rolf W. Hartmann*[,†]

[†]Pharmaceutical and Medicinal Chemistry, Saarland University and Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus C2-3, D-66123 Saarbrücken, Germany

[‡]ElexoPharm GmbH, Im Stadtwald, D-66123 Saarbrücken, Germany

Supporting Information

ABSTRACT: Topical application of CYP11B1 inhibitors to reduce cutaneous cortisol is a novel strategy to promote healing of chronic wounds. Pyridyl substituted arylsulfonyltetrahydroquinolines were designed and synthesized resulting in a strong inhibitor **34** (IC_{50} = 5 nM). It showed no inhibition of CYP17 and CYP19 and no mutagenic effects. It exhibited inverse metabolic stability in plasma ($t_{1/2} \gg$ 150 min), which is similar to wound fluid in composition, and in liver S9 fractions ($t_{1/2}$ = 16 min).



INTRODUCTION

It has been reported that around 2% (6.5 million) of the general population in the U.S. are suffering from chronic wound healing,[1] which frequently appears as a comorbid condition with diabetes and/or obesity.[2] Actually, 15% of diabetic patients develop chronic nonhealing diabetic foot ulcers that significantly impair their lives and compose a major cause of hospitalization.[3] Despite the facts that a standard care guideline was recommended[4] and new treatments, such as skin substitutes and stem cell applications, were explored,[5] the outcomes of managing healing of chronic wounds are still not satisfying, whereas the costs (exceeding $50 billion per year) rapidly grow into a burden for society.[1] Therefore, novel therapeutic strategies are in urgent need. The wound healing process comprises four phases, namely hemostasis, inflammation, proliferation, and tissue remodeling,[6] which are strictly controlled regarding sequence, initiating and terminating timings, and intensity. Cortisol, as a major glucocorticoid, blocks inflammation, fibroblast proliferation, and collagen synthesis[2] and thus probably contributes to the formation of chronic wounds and ulcers when cutaneously overexpressed. This hypothesis was supported by numerous observations that elevated cortisol levels induced by stress or other reasons impair wound healing.[7] Recently, steroid 11β-hydroxylase (CYP11B1), which is the key enzyme in cortisol biosynthesis, was identified to be expressed in epidermis.[8] More important is that biosynthesis of this enzyme is up-regulated when tissues are injured and more cortisol impairing wound healing is produced in keratinocytes.[8] In vivo studies also revealed that topical application of metyrapone (Table 1, IC_{50} = 15 nM) to inhibit CYP11B1 accelerated wound closure.[8] These findings suggest that the inhibition of CYP11B1 could be a novel therapy to promote wound healing. Moreover, locally expressed estrogens have been shown to keep skin moisture, prevent skin aging, and in particular accelerate wound healing.[9] Since the cutaneous production of estrogens involve two important steroidogenic enzymes that are also expressed in epidermis, namely, 17α-hydroxylase-17,20-lyase (CYP17) and aromatase (CYP19),[10] inhibitors of CYP11B1 aiming at promoting wound healing should not inhibit these two enzymes. In contrast, to our best knowledge, aldosterone synthase (CYP11B2) is not detected in skin cells.[10] The selectivity over CYP11B2 is therefore not important for this indication. Furthermore, the applied compounds should be stable in wound fluid to maintain efficacious concentrations. Since the components of wound liquid including proteases are similar to that of plasma,[11] plasma stability is an important parameter to be considered for the development of compounds for cutaneous application. In fact, it is inevitable that minor amounts of topically administered CYP11B1 inhibitors diffuse into circulation. To avoid that such compounds interfere with adrenal steroid biosynthesis and thus lead to severe side effects, they should be fast metabolized by the liver. Similar strategies have been successfully applied for inhalant glucocorticoids in the treatment of asthma to achieve better safety profiles.[12] Accordingly, a suitable drug candidate to promote wound healing should show not only potent CYP11B1 inhibition and selectivity over CYP17 and CYP19 but also a high metabolic stability in plasma, whereas a short half-life ($t_{1/2}$) regarding hepatic S9 fraction, which contains

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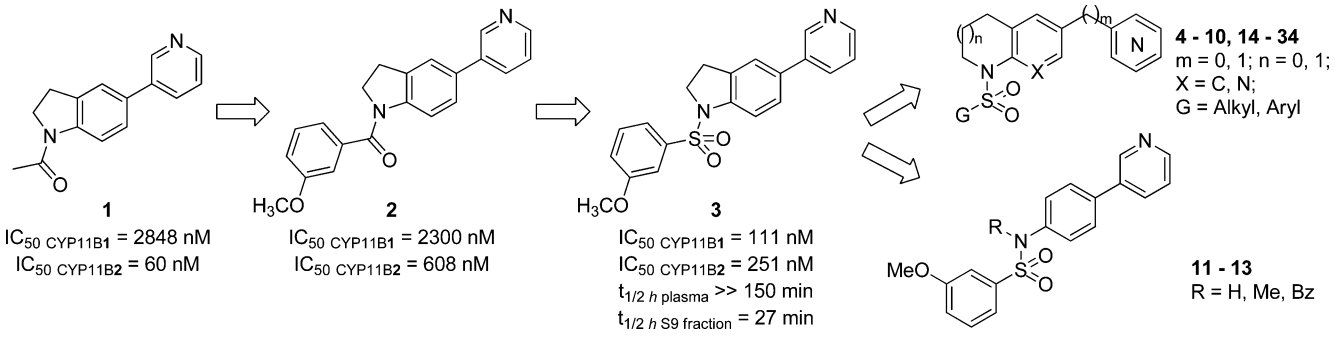
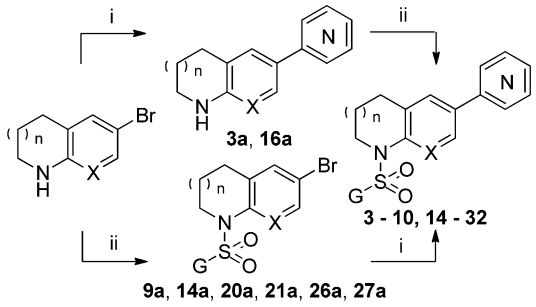
Chart 1. Design Concept and Title Compounds



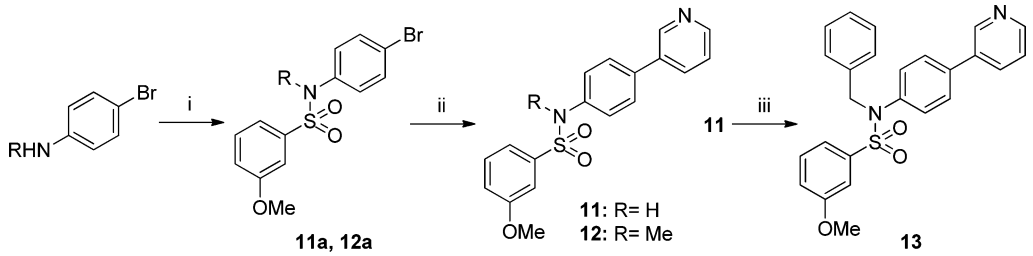
Scheme 1[a]



	X	n	Py	G		X	n	Py	G		X	n	Py	G
3	C	0	3-	3-OMe Ph	**15**	C	1	3-	3-OMe Ph	**24**	C	1	3-	3-Cl Ph
4	C	0	3-	3-F Ph	**16**	C	1	3-	Ph	**25**	C	1	3-	4-Cl Ph
5	C	0	3-	Me	**17**	C	1	3-	4-OMe Ph	**26**	C	1	3-	3-CF_3 Ph
6	C	0	3-	Et	**18**	C	1	3-	3-Me Ph	**27**	C	1	3-	4-CF_3 Ph
7	C	0	3-	*i*-Pr	**19**	C	1	3-	4-Me Ph	**28**	C	1	3-	3-CN Ph
8	C	0	3-	*i*-Bu	**20**	C	1	3-	3-OCF_3 Ph	**29**	C	1	3-	4-CN Ph
9	N	0	4-	3-OMe Ph	**21**	C	1	3-	4-OCF_3 Ph	**30**	C	1	3-	2-Thienyl
10	N	0	3-	3-OMe Ph	**22**	C	1	3-	3-F Ph	**31**	C	1	3-	2-Naphthyl
14	C	1	4-	3-OMe Ph	**23**	C	1	3-	4-F Ph	**32**	C	1	3-	Cyclohexyl

[a]Reagents and conditions. (i) Method A: corresponding boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 95 °C. (ii) Method B: corresponding sulfonyl chloride, pyridine, DMAP, dry THF, rt to 60 °C. Or method C: corresponding sulfonyl chloride, NaH, dry THF, 60 °C.

Scheme 2[a]



[a]Reagents and conditions. (i) Method B: corresponding sulfonyl chloride, pyridine, DMAP, dry THF, rt to 60 °C. (ii) Method A: corresponding boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 95 °C. (iii) Benzyl bromide, NaH, dry DMF, 0−80 °C.

microsomes and cytosol and thus is a good indicator of phase I and II metabolism in liver, is also required.

Metyrapone, which is a clinically used CYP11B1 inhibitor to reduce circulating cortisol levels for patients with Cushing syndrome, meets the criteria of potency, selectivity, and plasma stability ($t_{1/2\ plasma}$ > 150 min). However, it exhibits a half-life of 49 min in human liver S9 fractions, which is most likely too long for this indication. Similarly, although some of our previously identified CYP11B1 inhibitors[13] fulfill most of the requirements, their hepatic metabolism is not rapid enough. In this study, we report a novel series of CYP11B1 inhibitors exhibiting the desired inverse metabolic stability profile. The design of novel CYP11B1 inhibitors was inspired by an observation in the pyridylindoline class[14] of CYP11B2 inhibitors (Chart 1). Compound **1**, bearing an acetyl group at the indoline core, is a potent CYP11B2 inhibitor ($IC_{50\ CYP11B2}$ = 60 nM) showing only weak inhibition of CYP11B1 with an IC_{50} value of 2848 nM. Replacing the small acetyl group by a bulky 3-methoxybenzoyl moiety (**2**)[14] reduced CYP11B2 inhibition by 10-fold to 608 nM, whereas inhibition of CYP11B1 was slightly increased (IC_{50} = 2300 nM). Surprisingly, in preliminary studies we found that further exchange of the amido group by the corresponding sulfonamide moiety (**3**) resulted in a significant elevation of CYP11B1 inhibition by 20-fold to 111 nM. More important is that **3** exhibits the desired inverse metabolic stability profile: the half-life in plasma was far longer than 150 min, whereas the one in hepatic S9 fractions was 27 min. The latter is about half of that of metyrapone, which makes **3** more attractive as a lead compound than metyrapone. This encouraged us to modify the structure to further improve CYP11B1 inhibition and the metabolic profile. Various groups (alkyl or aryl) were introduced at the sulfonamide moiety. The pyrrolidine ring of the indoline core was expanded or opened. A methylene bridge was inserted between the core and the pyridyl substituent. Furthermore the position of the pyridyl N (3- or 4-pyridyl) was also investigated (Chart 1). To improve selectivity, the addition of an N atom in the core was attempted as well. These efforts led to **4**−**34** which were subsequently biologically evaluated: the inhibition of CYP11B1 and CYP11B2 was determined and

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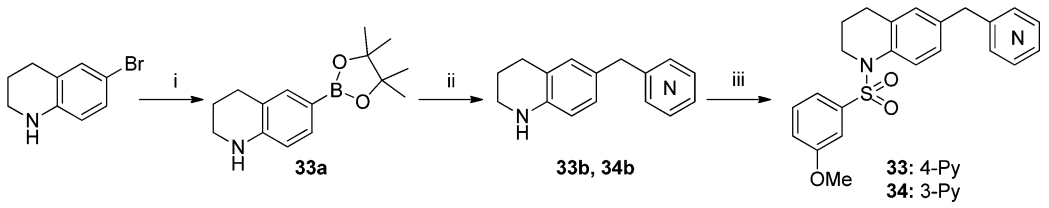
Scheme 3[a]



[a]Reagents and conditions. (i) Bis(pinacolato)diboron, $Pd(dppf)Cl_2$, CH_3COOK, dry 1,4-dioxane, 80 °C. (ii) Method A: corresponding bromomethylpyridine, $Pd(PPh_3)_4$, Na_2CO_3, DME/H_2O, 95 °C. (iii) Method B: corresponding sulfonyl chloride, pyridine, DMAP, dry THF, rt to 60 °C.

the most potent CYP11B1 inhibitors were further tested for inhibition of CYP17 and CYP19, metabolic stability in human plasma and hepatic S9 fractions, and mutagenicity.

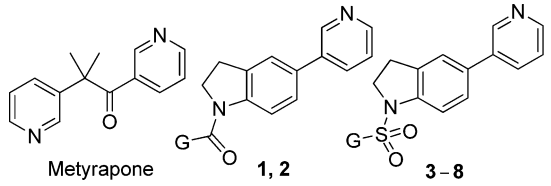
RESULTS AND DISCUSSION

Chemistry. The synthesis of the final compounds **3**–**12** and **14**–**32** (Schemes 1 and 2) was achieved in two steps. For **3**–**8**, **16**–**19**, **22**–**25**, and **28**–**32**, the route started from commercially available 5-bromoindoline or 6-bromo-1,2,3,4-tetrahydroquinoline using a Suzuki coupling reaction to introduce the pyridyl moiety. Subsequently, a sulfonylation reaction was employed to insert the corresponding sulfonamide group resulting in the desired products. For **9**–**12**, **14**, **15**, **20**, **21**, **26**, and **27**, the same reactions were applied in reversed order. In some sulfonylations NaH was employed as a stronger base instead of pyridine to ensure satisfying yields. Compound **11** was further substituted with a benzyl group by reacting with benzyl bromide leading to compound **13** (Scheme 2). To introduce the methylene bridge into **33** and **34**, 6-bromo-1,2,3,4-tetrahydroquinoline was first converted into the borate before coupling with the corresponding bromomethylpyridine under conventional Suzuki coupling conditions (Scheme 3). 3-Methoxyphenylsulfonyl was subsequently inserted to give the final compounds.

Inhibition of Human CYP11B1. To assess the inhibitory activities, the synthesized compounds were tested in an assay using V79 MZh cells expressing human CYP11B1 with 11-deoxycorticosterone (100 nM) as the substrate.[15] The results are presented in Tables 1–3 in comparison to metyrapone. As mentioned above, the replacement of the small acetyl group (**1**) by a bulky aromatic 3-methoxybenzoyl moiety (**2**) increased CYP11B1 inhibition by 20% (Table 1), while exchange of the carbonyl group by a sulfone moiety leading to the corresponding sulfonamide **3** boosted CYP11B1 inhibition by a factor of around 20, to 111 nM. This dramatic elevation led to the speculation that the sulfonamide moiety could form more favorable interactions like H-bonds than the carboxamide group. Another explanation was that the different geometry of the sulfonamide was more appropriate for stretching the aromatic ring into a nearby hydrophobic pocket. In any case pyridyl substituted sulfonylindolines could be considered as promising starting points for the design of potent CYP11B1 inhibitors. The influence of alkyl and phenyl substitution at the sulfonyl moiety was first explored. As expected, methylsulfonyl **5** (IC_{50} = 1674 nM) is more potent than the corresponding acetyl analogue **1** (IC_{50} = 2848 nM). With increasing bulkiness of the sulfonyl substituents, namely, Me (**5**) to Et (**6**) and *i*-Pr (**7**), the inhibition was promoted by around 2-fold. However, the *i*-Bu **8** only showed a weak inhibition of around 2 μM. This observation might be due to the length of the group, which might result in clashes to the pocket wall rather than bulkiness because the large 3-OMe Ph (**3**) and 3-F Ph (**4**) groups lead to potent CYP11B1 inhibitors with IC_{50} of 111 and 327 nM, respectively. Of course, the aromaticity of these phenylsulfonyl moieties providing the possibility of π–π interactions could be a significant contributor of affinity.

After the observation that the aromatic sulfonyl moiety was important for CYP11B1 inhibition, the 3-OMe phenyl substituent was kept constant and modifications on the indoline core were performed (Table 2). Also the influence of a 3- or 4-pyridyl group was investigated. Since an N atom inside the central ring was demonstrated to improve the

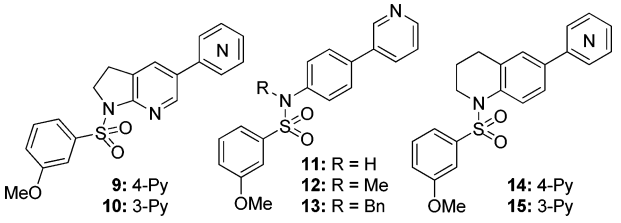
Table 1. Inhibition of CYP11B1 by Compounds 3–8



compd	G	IC_{50} (nM)[a]	compd	G	IC_{50} (nM)[a]
1	Me	2848	**5**	Me	1674
2	3-OMePh	2300	**6**	Et	690
3	3-OMePh	111	**7**	*i*-Pr	499
4	3-FPh	327	**8**	*i*-Bu	2003
metyrapone		15			

[a]Mean value of at least three independent tests, standard deviation less than 25%. Hamster fibroblasts expressing human CYP11B1; substrate 11-deoxycorticosterone, 100 nM.

Table 2. Inhibition of CYP11B1 by Compounds 9–15



compd	IC_{50} (nM)[a]	compd	IC_{50} (nM)[a]
metyrapone	15	**12**	68
3	111	**13**	2001
9	1750	**14**	>5000
10	587	**15**	28
11	188		

[a]Mean value of at least three independent tests, standard deviation less than 25%. Hamster fibroblasts expressing human CYP11B1; substrate 11-deoxycorticosterone, 100 nM.

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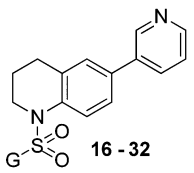
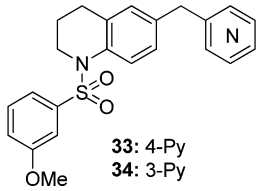
Table 3. Inhibition of CYP11B1 by Compounds 16−34



16 - 32



33: 4-Py
34: 3-Py

compd	G	IC_{50} (nM)[a]	compd	G	IC_{50} (nM)[a]	compd	G	IC_{50} (nM)[a]
metyrapone		15	**21**	*p*-OCF_3Ph	929	**28**	*m*-CNPh	65
16	Ph	65	**22**	*m*-FPh	71	**29**	*p*-CNPh	114
15	*m*-OMePh	28	**23**	*p*-FPh	49	**30**	2-thienyl	60
17	*p*-OMePh	334	**24**	*m*-ClPh	62	**31**	2-naphthyl	663
18	*m*-MePh	26	**25**	*p*-ClPh	137	**32**	*c*-hexyl	1167
19	*p*-MePh	278	**26**	*m*-CF_3Ph	309	**33**		6
20	*m*-OCF_3Ph	382	**27**	*p*-CF_3Ph	1224	**34**		5

[a]Mean value of at least three independent tests, standard deviation less than 25%. Hamster fibroblasts expressing human CYP11B1; substrate 11-deoxycorticosterone, 100 nM.

selectivity over CYP17 and CYP19,[13] it was inserted into the benzene nucleus of the indoline core. However, in this series of compounds, this structural modification reduced the potency by a factor of 5 (**10**). The exchange of the 3-pyridyl substituent by a 4-pyridyl ring further decreased inhibition to 1750 nM (**9**). Upon opening of the pyrrolidine nucleus of the indoline core to provide more flexibility to the arylsulfonyl moiety, a 2-fold improvement of CYP11B1 inhibition was observed for the *N*-Me analogue **12** (IC_{50} = 68 nM) compared to the parent **3** (IC_{50} = 111 nM). In contrast, the *N*-H **11** was less potent (IC_{50} = 188 nM) compared to the *N*-Me **12**, indicating that the hydrogen is unfavorable. Substitution on the N with a benzyl moiety reduced CYP11B1 inhibition to 2 μM probably because of a steric clash. The pyrrolidine nucleus was also expanded leading to 1,2,3,4-tetrahydroquinoline as a new core. In contrast to the total loss of inhibitory potency induced by 4-pyridyl (**14**), the 3-pyridyl substituted 1,2,3,4-tetrahydroquinoline analogue **15** exhibited a 4-fold elevation of CYP11B1 inhibition compared to lead **3**, almost reaching the potency of metyrapone (IC_{50} of 28 and 15 nM, respectively).

After optimization of the core resulting in 1,2,3,4-tetrahydroquinoline, the substituents on the phenylsulfonyl moiety were further scrutinized (Table 3). Various groups with different bulkiness, electrostatic properties, and potential of forming hydrogen or halogen bonds were introduced into the *m*- or *p*-position of the phenylsulfonyl moiety. With the exception of the F substituted compounds **22** and **23**, all *m*-substituted analogues were 2- to 10-fold more potent than the corresponding *p*-substituted ones (see compound pairs **15**, **17**−**21** and **24**−**29**). This finding together with the observation that the long *i*-Bu sulfonyl substituent strongly decreased potency might indicate that the hydrophobic pocket occupied by these moieties is rather shallow. The nonsubstituted phenylsulfonyl **16** showed a strong inhibition of CYP11B1 with an IC_{50} of 65 nM. *m*-Substitution with electron donating groups OMe (**15**) and Me (**18**) increased the potency by 2-fold to less than 30 nM. In contrast, electron withdrawing groups F (**22**), Cl (**24**), and CN (**28**) did not show much impact on CYP11B1 inhibition compared to the nonsubstituted **16**. Interestingly, although exhibiting different electrostatic potentials, the OCF_3 (**20**) and CF_3 (**26**) groups reduced inhibitory potencies by a factor of about 5, which might indicate multi F substitution is not tolerated. The replacement of the phenyl moiety by other rings was also attempted. In contrast to the similar potency rendered by a 2-thienyl group (**30**, IC_{50} = 60 nM), substitution with the more bulky naphthyl ring (**31**) led to a 10-fold decrease of CYP11B1 inhibition compared to the phenylsulfonyl **16**. In accordance with the observation that alkyl compounds are less potent than aryl analogues (Table 1), substitution with a *c*-hexyl ring (**32**) reduced potency by a factor of nearly 20 compared to the phenyl **16**. As a result of this investigation, *m*-OMe- and *m*-Me-phenylsulfonyl substitution was identified as most suitable for CYP11B1 inhibition.

Introduction of a methylene bridge[16] disrupts the conjugation between core and heme-binding heterocycle, provides the molecule more flexibility, and changes orientation of the compound with regard to the coordination of its sp^2 hybrid N with the heme iron. These changes in molecular shape and conformational flexibility consequently lead to alterations in binding affinity and inhibitory profiles toward the CYP enzyme targets.[17] To exploit these opportunities, a methylene bridge was inserted accompanied by a 3- or 4-pyridyl substituent (**34** and **33**, respectively). Surprisingly, both compounds showed a 10-fold improvement of CYP11B1 inhibition compared to the parent **16**, resulting in IC_{50} of around 5 nM. Thus, the compounds were more potent than metyrapone (IC_{50} = 15 nM).

Metabolic Stabilities in Human Plasma and Hepatic S9 Fractions. As discussed above, a CYP11B1 inhibitor for the promotion of wound healing should be stable in plasma but metabolically labile in liver to achieve both efficacy and safety. Compound **3** as lead had exhibited the desired inverse metabolic stabilities. As a result from optimization, **34** showed not only a very long half-live in human plasma ($t_{1/2\ plasma} \gg 150$ min) but also instability in human liver S9 fraction ($t_{1/2\ S9}$ = 16 min), being much better than metyrapone ($t_{1/2\ S9}$ = 49 min).

Inhibition of Human CYP17 and CYP19. Because of their crucial roles in the biosynthesis of sex hormones, inhibition of CYP17[18] and CYP19[19] has been exploited in the treatment of hormone sensitive prostate and breast cancers, respectively. These two enzymes are also expressed in the skin and are involved in the cutaneous production of estrogens, which are known to accelerate wound healing. The selectivity over CYP17 and CYP19 was therefore considered as safety criteria, and four of the most potent CYP11B1 inhibitors, namely, **15**, **18**, **33**, and **34**, were tested for inhibition of these two enzymes. Since

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these compounds had shown IC_{50} below 30 nM toward human CYP11B1, a more than 60-fold higher concentration (2 μM) was selected for these selectivity assays. All tested compounds exhibited less than 15% inhibition of CYP17 (Table 4). As for CYP19, only the 4-pyridyl analogue **33** showed strong inhibition (88%), whereas the other compounds exhibited inhibition values of around 30%. Accordingly, these compounds were considered to be selective enough for a safe application.

Table 4. Inhibition of CYP17 and CYP19 by Selected Compounds

	% inhibition[a]	
compd	CYP17[b]	CYP19[c]
metyrapone	3	0
15	11	32
18	9	29
33	14	88
34	9	26

[a]Mean value of at least three independent tests, standard deviation less than 25%. [b]*E. coli* expressing human CYP17; substrate progesterone, 25 μM; inhibitor concentration, 2.0 μM. [c]Human placental CYP19; substrate androstenedione, 500 nM; inhibitor concentration, 2.0 μM.

Inhibition of Human CYP11B2. CYP11B2 is the pivotal enzyme responsible for the biosynthesis of aldosterone, and its inhibition was therefore proposed as a novel therapy for related cardiovascular and renal diseases.[20] Despite the challenges in achieving selectivity between CYP11B1 and CYP11B2, which is due to the high homology between these two enzymes (>93%), selective inhibitors of CYP11B1[13] and CYP11B2[21] have been developed. Although the selectivity over CYP11B2 is not that important for cutaneous application required in wound healing, this series of CYP11B1 inhibitors was still tested for inhibition of CYP11B2 because the compounds originated from pyridyl substituted indoline type of CYP11B2 inhibitors and the information obtained on inhibitory profiles could facilitate future design of selective inhibitors of both enzymes. Despite the fact that all compounds exhibited preference for CYP11B1 inhibition, the selectivity over CYP11B2 was not strong (Table S1 in Supporting Information). The tested compounds showed potent to modest inhibition of CYP11B2 with IC_{50} ranging from 5 to around 2000 nM. However, the compounds are expected to be rapidly metabolized and excreted after diffusion into circulation and thus have no chance to interfere with CYP11B1 or CYP11B2 in the adrenals.

Mutagenicity Evaluation. The Ames II mutagenicity assay was employed to assess the mutagenic potential of **34** using the genotypes of the TA98 and TAMix *Salmonella typhimurium* strains. No positive signal was observed in both strains with or without S9 mix at a compound concentration up to 100 μM, indicating that **34** was nonmutagenic.

CONCLUSION

Topical application of CYP11B1 inhibitors to reduce the cutaneous production of cortisol is a novel strategy to promote healing of chronic wounds. A suitable drug candidate for this indication should be selective over CYP17 and CYP19 that are also expressed in epidermis. Stability in wound fluid, which shows a very similar composition as plasma, is a must to maintain therapeutic concentrations. On the other hand the inhibitor is expected to be rapidly metabolized in the liver, as it cannot be excluded that minor amounts of the compound diffuse into circulation and interfere with adrenal steroid biosynthesis. In this study, pyridyl substituted arylsulfonyl-tetrahydroquinolines were designed and synthesized for this aim. As the best compound from this series, **34** is a highly potent CYP11B1 inhibitor (IC_{50} = 5 nM), being stronger than the lead **3** and metyrapone (the latter is being used clinically for the treatment of Cushing syndrome). It showed no mutagenic effects and no significant inhibition of CYP17 and CYP19 providing a wide enough therapeutic window at the treatment doses. In contrast to its long half-life ($t_{1/2\ plasma} \gg 150$ min) in human plasma, **34** is fast metabolized in human liver S9 fractions ($t_{1/2\ S9}$ = 16 min, being clearly superior to metyrapone). Therefore, it is considered for further in vivo evaluation.

EXPERIMENTAL SECTION

Biology. See Supporting Information for details.

Chemistry. The purities of the final compounds were higher than 95% as determined by HPLC.

Method A: Suzuki Coupling. To a solution of the corresponding bromide (0.5 mmol, 1.0 equiv) in 1,2-dimethoxyethane (12 mL) and water (4 mL) were added 3- or 4-pyridylboronic acid (1.0−2.0 equiv) and Na_2CO_3 (3.0−5.0 equiv). The mixture was degassed under reduced pressure and flushed with N_2 three times before $Pd(PPh_3)_4$ (5−10 mol %) was added. The resulting suspension was then heated to 95 °C for 4−8 h. After cooling to room temperature, water (20 mL) and EtOAc (40 mL) were added. The phases were separated, and the aqueous phase was extracted twice with EtOAc (40 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the crude product, which was purified with flash chromatography on silica gel.

Method B: Sulfonylation Reaction. To a solution of the corresponding amine (1 mmol, 1.0 equiv), 4-dimethylaminopyridine (DMAP, 0.2 equiv), and pyridine (3.0−5.0 equiv) in dry THF (8 mL) was added dropwise the corresponding sulfonyl chloride (1.0−2.0 equiv) in an ice bath. Subsequently, the resulting mixture was stirred at room temperature or heated to 60 °C for 1−6 h and the reaction was monitored with TLC until the starting materials were consumed. The mixture was then cooled to ambient temperature and quenched by the addition of saturated Na_2CO_3 aqueous solution (20 mL). It was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the crude product, which was further purified with flash chromatography on silica gel.

6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline (33a). To the solution of 6-bromo-1,2,3,4-tetrahydroquinoline (530 mg, 2.5 mmol) and bis(pinacolato)diboron (1.27 g, 5 mmol) in dry 1,4-dioxane (12 mL) was added potassium acetate (1.22 g, 12.5 mmol). The mixture was degassed under reduced pressure and flushed with N_2 three times before $Pd(dppf)Cl_2$ (92 mg, 5 mol %) was added. The resulting suspension was then heated to 80 °C for 2 h. After cooling down, water (20 mL) and EtOAc (40 mL) were added. The phases were separated, and the aqueous phase was extracted twice with EtOAc (40 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give the crude product, which was purified with flash chromatography on silica gel (*n*-hexane/EtOAc, 20:1 to 5:1) to yield a white solid (440 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (m, 2H), 6.43 (d, J = 8.2 Hz, 1H), 4.06 (s, br, 1H), 3.30 (m, 2H), 2.75 (t, J = 6.3 Hz, 2H), 1.92 (m, 2H), 1.31 (s, 12H).

6-(Pyridin-3-ylmethyl)-1,2,3,4-tetrahydroquinoline (34b). The title compound was synthesized according to method A using **33a** (194 mg, 0.75 mmol), 3-(bromomethyl)pyridine hydrobromide (190 mg, 0.75 mmol), Na_2CO_3 (380 mg, 3.6 mmol), $Pd(PPh_3)_4$ (65 mg, 7.5 mol %), and 1,2-dimethoxyethane (18 mL)/water (6 mL) to yield the crude product, which was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 500:1 to 62.5:1) to yield a reddish solid (86 mg, 51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48 (d, J = 2.0 Hz,

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1H), 8.42 (dd, J = 4.8, 1.5 Hz, 1H), 7.47 (m, 1H), 7.18 (m, 1H), 6.75 (m, 2H), 6.41 (d, J = 8.3 Hz, 1H), 3.82 (s, 2H), 3.27 (m, 2H), 2.71 (t, J = 6.3 Hz, 2H), 1.91 (m, 2H).

1-((3-Methoxyphenyl)sulfonyl)-6-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydroquinoline (34). The title compound was synthesized according to method B using **34b** (56 mg, 0.25 mmol), 3-methoxybenzene-1-sulfonyl chloride (103 mg, 0.5 mmol), DMAP (6 mg, 0.05 mmol), pyridine (100 mg, 1.2 mmol), and dry THF (4 mL) to yield the crude product, which was purified by flash chromatography on silica gel (*n*-hexane/EtOAc, 20:1 to 4:1) to yield a light brown oil (70 mg, 72%). HPLC: 97% pure. MS (ESI) m/z = 395 [M^+ + H]. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.46 (m, 2H), 7.72 (d, J = 8.2 Hz, 1H), 7.45(m, 1H), 7.30 (dd, J = 8.4, 7.7 Hz, 1H), 7.20 (m, 2H), 7.02 (m, 2H), 6.97 (m, 1H), 6.80 (m, 1H), 3.90 (s, 2H), 3.77 (m, 2H), 3.61 (s, 3H), 2.37 (t, J = 6.6 Hz, 2H), 1.60 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.6, 150.1, 147.7, 140.6, 136.7, 136.3, 136.2, 135.3, 131.2, 130.0, 129.3, 127.0, 125.3, 123.4, 119.4, 119.2, 111.4, 55.4, 46.6, 38.4, 26.6, 21.4.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization of the not mentioned intermediates and final compounds, HPLC purities and retention times of all final compounds, inhibition of CYP11B2 by **3**−**34**, and descriptions of biological tests. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +(49) 681 302 70300. Fax: +(49) 681 302 70308. E-mail: rolf.hartmann@helmholtz-hzi.de. Home page: http://www.helmholtz-hzi.de/?id=3897.

Author Contributions

§W.Z. and Q.H. contributed equally to this paper.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, 17α-hydroxylase-17,20-lyase; CYP19, aromatase; $t_{1/2}$, biological half-life; equiv, equivalent; DMAP, 4-dimethylaminopyridine

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