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Design and optimization of new piperidines as renin inhibitors

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ABSTRACT

The discovery of a new series of piperidine-based renin inhibitors is described herein. SAR optimization upon the P3 renin sub-pocket is described, leading to the discovery of **9** and **41**, two bioavailable renin inhibitors orally active at low doses in a transgenic rat model of hypertension.

The renin–angiotensin–aldosterone system (RAAS) represents one of the major and most studied regulating systems of the arterial blood pressure in humans, and plays a primordial role in cardiovascular diseases, renal diseases, and other metabolic diseases.[1]

The development of a new class of piperidines as renin inhibitors is reported here. Piperidine-based renin inhibitors were first reported in the late nineties.[2] Unfortunately, and despite extensive optimization work, type **1** piperidine-based derivatives (Fig. 1) showed unsuitable pharmacokinetic properties.[3]

By de novo design and lead optimization, we have recently reported diazabicyclononene derivatives such as **2**[4] and tetrahydropyridine derivatives such as **3**[5] as renin inhibitors. Based on the knowledge accumulated during this work and from literature data,[2,6] we predicted that piperidines, comprising *trans*-arranged substituents in 3- and 4-positions such as, for example, in **4** would provide suitable exit vectors. Replacing the long methoxypropoxy linker present in piperidine derivatives of type **1** (Fig. 1) by the shorter 1,2-ethylenedioxy linker should improve the metabolic stability, as observed for diazabicyclononene based renin inhibitors.[4] Additionally, modification of the amide side chains should improve the potency of the corresponding inhibitors.[7]

The general synthetic pathway leading to 3,4-disubstituted piperidines of type **4** took advantage of the chemistry elaborated earlier for the tetrahydropyridine **3**.[5] The double bond of the known ester **5** was reduced using magnesium[8] to yield a mixture of the racemic *cis* and *trans* esters (Scheme 1). Isomerization to the thermodynamically more stable racemic, *trans* ester **6** was performed under basic conditions (MeONa/MeOH). Further ester hydrolysis and amide coupling using amine of type **7** where X = CH, $R^1 = R^2 =$ Me, and $R^3 =$ H afforded, after cleavage of the Boc-group, racemic piperidine **8** (Scheme 1 and Table 1, entry 1).

Piperidine *rac*-**8** was up to four times more potent toward renin than tetrahydropyridine **3**, measured either in buffer (IC_{50} = 0.31 nM vs 1.1 nM) or in plasma (IC_{50} = 12.8 nM vs 55.6 nM)[5] and as potent in plasma as **2** (IC_{50} = 19 nM),[4] establishing the proof of concept for the double bond reduction strategy. A slightly more basic secondary amine on the piperidine template compared to the tetrahydropyridine template (pK_a = 8.5 vs 8.0 on other, similar compounds), and slightly improved exit vectors at the 3- and 4-positions are possible explanations for this improved potency. Moreover, while the tetrahydropyridine series, for which compound **3**[5] is a good representative, significantly inhibited the CYP3A4 enzyme (IC_{50} = 0.8 μM), racemic piperidine **8** inhibited CYP3A4 only moderately (IC_{50} >7 μM).

Potency of this new derivative in 100% human plasma was not yet considered as sufficient. Earlier publications indicated that renin possesses a P3 sub-pocket ($P3^{sp}$).[3,6,7] In order to determine if it was possible to reach this $P3^{sp}$ with our 3,4-*trans*-substituted piperidines, and thereby improve potency in plasma, the known amine **7**[7] (X = CH, R^1 = Cl, R^2 = H, $R^3 = -(CH_2)_3OMe$, Scheme 1) was first chosen with the following intentions: the cyclopropyl ring should fill a renin sub-pocket identified earlier[4] while the *meta* aliphatic chain should better anchor the inhibitor by filling the $P3^{sp}$.

Based on previous work, we predicted that the active enantiomer should possess the (3*R*,4*S*) absolute configuration. To experimentally confirm this, the enantiomeric pair of *trans* racemic ester **6** was separated by preparative HPLC using a chiral stationary phase (Regis R,R Whelk 01 column) followed by ester hydrolysis, amide coupling, and deprotection. The active enantiomer **9**

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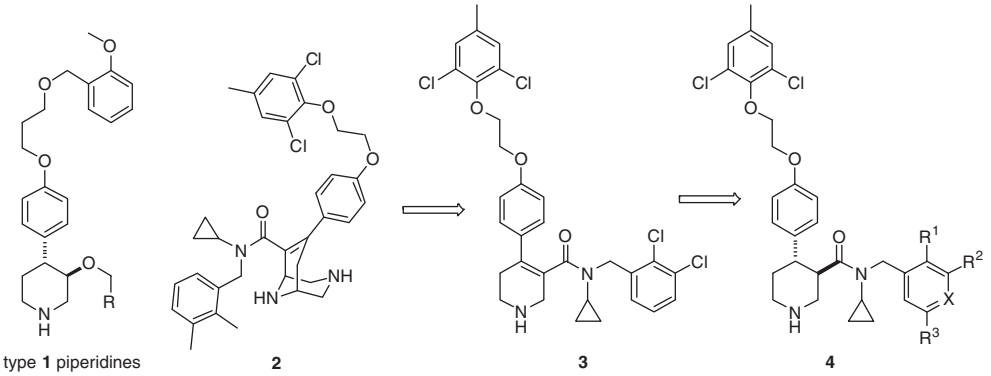
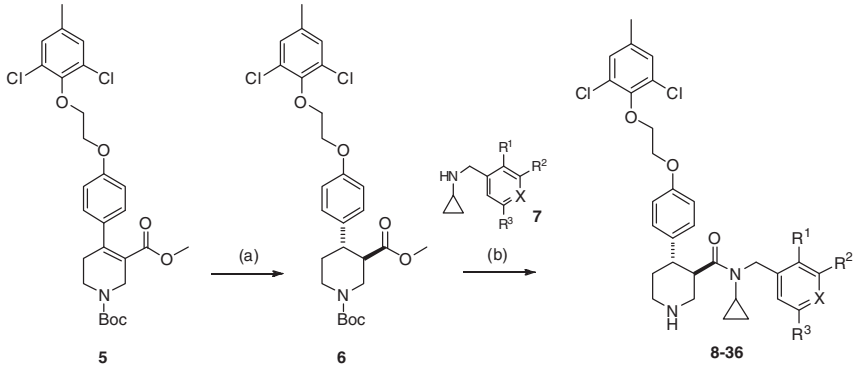


Figure 1. Design leading to 3,4-disubstituted piperidines of type **4** from type **1** based piperidines, diazabicyclonene **2** and tetrahydropyridine **3**.



Scheme 1. Synthetic pathway leading to 3,4-disubstituted piperidines of type **8**–**36** from known ester **5**. Reagents and conditions: (a) (i) 1.2 equiv Mg, MeOH, rt, 2 h, 89%; (ii) 2.0 equiv MeONa, MeOH, 70 °C, 16 h, 95%; (b) (i) 3.5 equiv NaOH, MeOH, rt, 48 h; (ii) 1.25 equiv HOBt, 4.0 equiv DIPEA, 0.25 equiv DMAP, 1.5 equiv amine **7**; (iii) 15 equiv 4 M HCl in dioxane, CH_2Cl_2, 0 °C, 1.5 h, 20–70%.

Table 1
SAR of selected renin compounds: amide side chain modification

Entry	Compds	X	R^1	R^2	R^3	Renin IC_{50} (nM)		Buffer to plasma ratio	CYP3A4 IC_{50} (μM)		CYP3A4 TDI_{30}
						Buffer	Plasma		Mid	Test	
1	*rac*-**8**	CH	Me	Me	H	0.31	12.8	41	7.4	11	7
2	**9**	CH	Cl	H	$-(CH_2)_3OMe$	0.22	12.3	56	4.8	8.1	18
3	**10**	CH	Cl	H	$-(CH_2)_2OMe$	0.18	8.1	45	3.2		
4	**11**	CH	Cl	H	$-(CH_2)_2NHMe$	3.90	91.3	23	14	16	1
5	**12**	CH	Cl	H	$-CH_2NHMe$	1.39	35.6	26	15	17	2.1
6	**13**	CH	Cl	H	$-CH_2NHEt$	2.17	16.1	7	14	17	
7	**14**	CH	Cl	H	$-(CH_2)_2NHEt$	0.40	19.1	48	15	18	1.7
8	**15**	CH	Cl	H	$-(CH_2)_2NH$cylopropyl	0.56	8.2	15			
9	**16**	CH	Cl	H	$-(CH_2)_2NHCH_2CHF_2$	0.05	3.1	62			
10	**17**	CH	Cl	H	$-CH_2NH$cylopropyl	0.31	7.5	24			
11	**18**	CH	Cl	H	$-CH_2NHCH_2CH_2F$	0.15	7.4	49	7.3	9.9	8.4
12	**19**	CH	Cl	H	$-CH_2NHCH_2CHF_2$	0.28	14.5	41	3.1	4.8	18
13	**20**	CH	Cl	H	$-(CH_2)_2NHAc$	0.18	1.4	8	2.4	6	8.7
14	**21**	CH	Cl	H	$-(CH_2)_2C(O)NHMe$	0.11	1.9	17	2.9	8.9	21
15	**22**	CH	Cl	H	$-(CH_2)_2NHC(O)CH_2CF_3$	0.41	9.5	23	2.8	4.5	6.9
16	**23**	CH	Cl	H	$-(CH_2)_2NHC(O)CH_2O$*t*Bu	0.26	3.7	14			
17	**24**	CH	Cl	H	$-CH_2C(O)NHEt$	0.11	2.5	23	2.1	7.1	8.7
18	**25**	CH	Cl	H	$-CH_2NHAc$	0.16	4.1	26	5.1	8.4	4.1
19	**26**	CH	Cl	H	$-CH_2C(O)NHMe$	0.11	3.4	31			
20	**27**	CH	Cl	H	$-CH_2NHC(O)Et$	0.13	4.6	35			
21	**28**	CH	Cl	H	$-CH_2C(O)NH$cyclopropyl	0.24	3.4	14			
22	**29**	CH	Cl	H	$-CH_2NHC(O)$cyclopropyl	0.31	2.8	9			
23	**30**	CH	Cl	H	$-CH_2NHC(O)CH_2CF_3$	0.38	6.9	18	4	7.9	4.5
24	**31**	CH	Cl	H	$-CH_2OC(O)NHMe$	0.62	4.4	7			
25	**32**	CH	Cl	H	$-(CH_2)_2NHC(O)OMe$	0.57	10.5	14			
26	**33**	CH	Cl	H	$-(CH_2)_2OC(O)NHMe$	0.27	7.3	27			
27	**34**	CH	Cl	H	$-CH_2NHC(O)OMe$	0.09	2.0	22	3.5	6.5	12
28	**35**	N	Cl	H	$-(CH_2)_3OMe$	0.28	3.5	13	5.9		13
29	*rac*-**36**	NO	Cl	H	$-(CH_2)_3OMe$	0.04	0.5	13			

Mid: midazolam as a marker. Test: testosterone as a marker. The CYP3A4 TDI_{30} value represents the ratio of the CYP3A4 IC_{50} at t_0 to the CYP3A4 IC_{50} after 30 min of pre-incubation in the presence of liver microsomes.

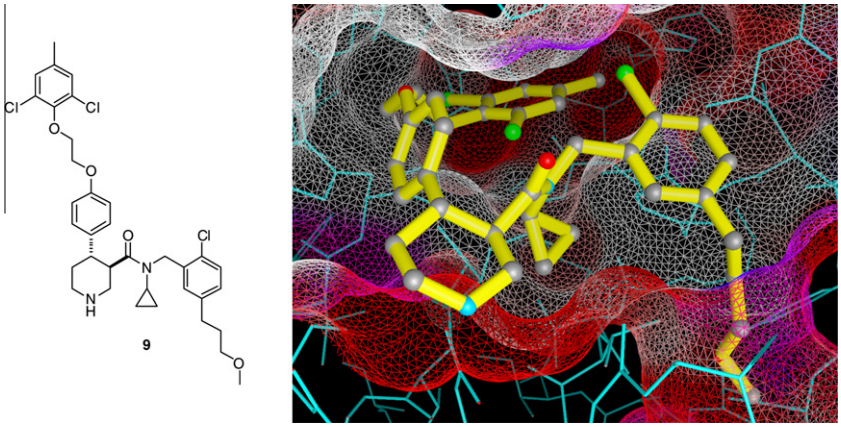


Figure 2. **9** soaked into renin crystals. Resolution of 2.4 Å. PDB code 3O9L.

(absolute configuration established as 3*R*,4*S* based on X-ray obtained via soaking into renin crystals, see Fig. 2) was about 500 times more potent than its enantiomer (0.22 nM vs 92 nM in buffer and 12.3 nM vs 2615 nM in plasma). While the introduction of the methoxypropyl side chain provided an acceptable pharmacokinetic profile in rat with a bioavailability of 20%, compound **9** inhibited CYP3A4 more strongly than *rac*-**8**. Furthermore, inhibitor **9** showed a more important time-dependant inhibition of CYP3A4 after 30 min of pre-incubation in the presence of liver microsomes (TDI_{30},Table 1, entry 2 vs entry 1).

From the work toward the design and optimization of a substituted amino propanamide series as renin inhibitor,[7] it was known that a high degree of freedom for structural variations of the *meta* position on the phenyl ring of the amide side chain was tolerated. Accordingly, we considered this as an opportunity to improve the compound's overall profile (potency in plasma and CYPs inhibition) by further modifying this side chain starting from enantiomerically pure ester **6**.

Compound **10** (Table 1, entry 3) displayed no substantial differences when compared with *rac*-**8** or **9** (entries 1 and 2) with respect to the inhibitory potency against purified recombinant human renin in buffer. However, introduction of a small, secondary amine led to a reduced potency (Table 1, entries 4–6). This is probably due to the polar functionality which needs to be desolvated in order to fit into the small $P3^{sp}$. The buried NH functionality does not seem to find a suitable H-bonding partner which explains the overall unfavorable energy balance. However, the buffer to plasma shift is reduced, and both the competitive and the time-dependant inhibition constants (TDI) of CYP3A4 are improved, showing that introducing hydrophilic, potentially protonated residues allow to tackle CYP3A4 related issues.

Extending the side chain length and introducing two terminal fluorines greatly improved potency (entry 9 vs entry 4), but failed to reduce the buffer to plasma ratio. Slight increase of steric bulk (entries 7 and 8) also allowed regaining potency. Shorter secondary amines yielded similar trends but less pronounced. Modulating the steric hindrance and/or nitrogen basicity led to increased potency (entries 10–12 vs entry 5) but at the same time CYP3A4-linked issues reappeared.

Amides or inverse amides (entries 13–23) were suitable regarding potency, demonstrating the high tolerance of the $P3^{sp}$ for different functional groups and substituents. Carbamates (entries 24–27) also displayed a nice activity profile. Finally, as already seen in the amino propanamide series,[7] pyridine and *N*-oxide pyridine derivatives were well tolerated as phenyl replacements (entries 28 and 29). While these compounds could improve the buffer to plasma shift, amides or carbamates failed to improve the CYP3A4 profile. Compounds **20** and **30** (entries 13 and 23) led to a moderate blood pressure decrease in our double transgenic rat model (−9 mmHg for **20** and −19 mm Hg for **30**, at 3 mg/kg, po), presumably due to a modest bioavailability. The potency of the *N*-oxide *rac*-**36** in plasma is remarkable, with a value in the subnanomolar range, at least 25 times more potent than diazabicyclonene **2**.[4]

Interestingly, the X-ray structure analysis of compound **15a**, analogous to compound **15** (Table 1 entry 6), in renin delivered unexpected results since the *meta* substituent intended to fill the $P3^{sp}$ showed an unexpected, previously unreported interaction through formation of an hydrogen bond with the OH group of Ser230 (Fig. 3). This interaction offers new opportunities in the design of renin inhibitors.

The removal of the double bond from the tetrahydropyridine scaffold gave us the opportunity to introduce small polar groups at the 4-position of the piperidine (Scheme 2) to modulate the CYP3A4 activity. Starting from the known β-keto ester **37**,[5] a convergent synthesis was designed to take advantage of the already known 4-substituent side chain chemistry.[4] The β-keto ester **37** was condensed in refluxing toluene with the desired amine followed by addition of the lithiated derivative of aryl **39** leading to the desired *rac*-derivative in a 4:1 *cis*–*trans* ratio. Removal of the Boc-group followed by HPLC separation using a chiral stationary phase (Regis R,R Whelk 01 column) afforded the desired pure, *trans*-[3*S*,4*R*] stereoisomer.

Piperidine *rac*-**40** (Scheme 2 and Table 2) revealed a good tolerance for the newly introduced alcohol in terms of potency. Compound **41**, whose stereochemistry was determined to be (3*S*,4*R*) by analogy to compound **41a**, soaked into a renin crystal (Fig. 4), was slightly more potent than piperidine **10**, while its (3*R*,4*S*) enantiomer **42** was about 150-fold less active in buffer. Unfortunately, the introduction of the hydroxyl moiety at 4-position did not improve the CYP3A4 profile as exemplified by compound **41** (CYP3A4 IC_{50mid} = 1.4 μM, CYP3A4 IC_{50test} = 4.6 μM).

To determine the effect of these modifications on the in vivo efficacy, compounds **9** (Table 1 entry 2) and **41** (Table 2) were selected for further profiling. 4-Hydroxypiperidine **41** displayed the same potency in buffer (0.24 nM) as the tetrahydropyridine analogue **43**[9] (Fig. 5, 0.28 nM) but with an improved potency in human plasma (5.6 nM vs 26.6 nM). When administered at a dose of 3 mg/kg, po to double transgenic rats harboring both the human angiotensinogen and the human renin gene,[10] 4-hydroxypiperidine **41** was superior to its closest analogue tetrahydropyridine **43** with a twofold improvement in the maximal mean arterial pressure (MAP) reduction (−36 vs −18 mmHg) and a 3–4-fold increase for the area between the curves (ABC) (−1411 vs −382 mmHg h). Compound **9**

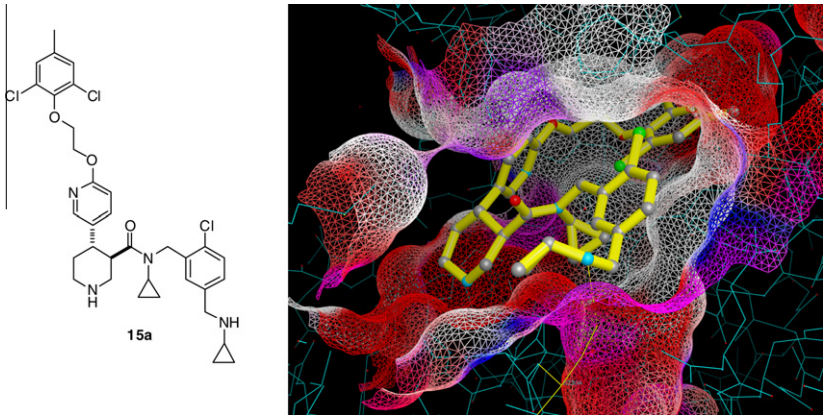
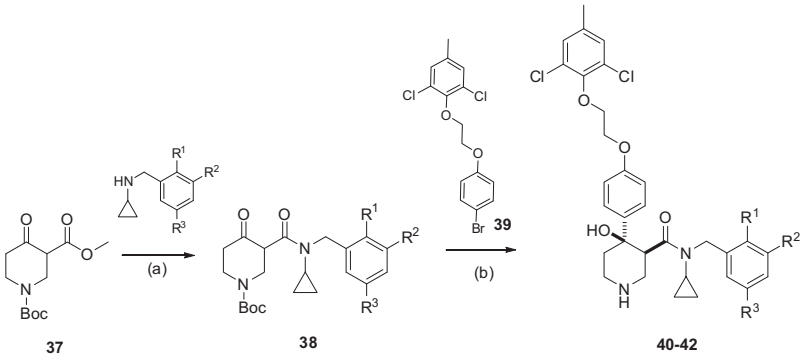


Figure 3. Compound **15a** soaked into renin crystals. PDB code 3OAG.



Scheme 2. Synthetic pathway leading to 4-hydroxy piperidines **40**–**42**. Reagents and conditions: (a) 1.1 equiv of amine, 0.13 equiv *p*-TsOH, toluene, 110 °C, 16 h, 75–85%; (b) (i) 2.5 equiv **39**, THF, 2.75 equiv *n*-BuLi, −78 °C, 30 min; (ii) 5.5 equiv DMPU, amide **38**, THF, −78 °C, 10 min, 10–15% of *cis*-rac and 42–50% of *trans*-rac product; (iii) 15 equiv HCl 4 M in dioxane, CH_2Cl_2, 0 °C, 1 h, 60–75%; (iv) chiral HPLC (Regis R,R Whelk 01 column), 20–45%.

Table 2
SAR of selected renin inhibitors: modifications at the 4-position

Entry	R^1	R^2	R^3	Renin IC_{50} (nM)	
				Buffer	Plasma
rac-**40**	Me	Me	H	0.18	45
41 [3*S*,4*R*]	Cl	H	$-(CH_2)_2OMe$	0.24	5.6
42 [3*R*,4*S*]	Cl	H	$-(CH_2)_2OMe$	35.3	—

which displayed a similar in vitro profile as compound **43**, led to an even better in vivo efficacy (Fig. 5), presumably due to a more favorable pharmacokinetic profile.

To conclude, a novel series of piperidine-based renin inhibitors with an improved efficacy in a double transgenic rat model has been discovered. Piperidine **9** showed high efficacy at low dose (3 mg/kg), but, despite intense efforts, the unfavorable CYP interactions could not be eradicated. To tackle this issue, further modified

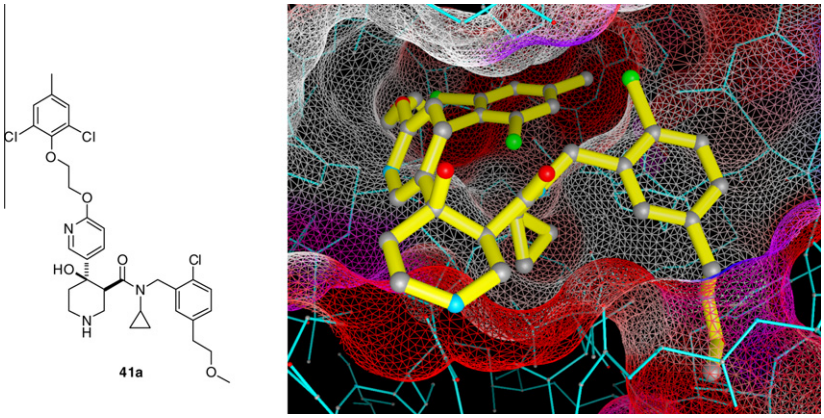
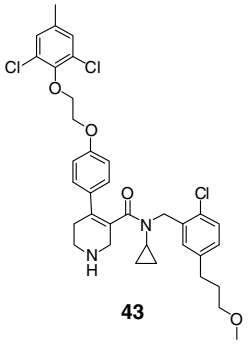


Figure 4. Compound **41a** soaked into renin crystals. PDB code 3OAD.



	43	9
IC_{50} buffer (nM)	0.28	0.22
IC_{50} plasma (nM)	26.6	12.3
ABC (mmHg·h)	-382	-1959
Max* (mmHg)	-18	-43

*maximal blood pressure reduction

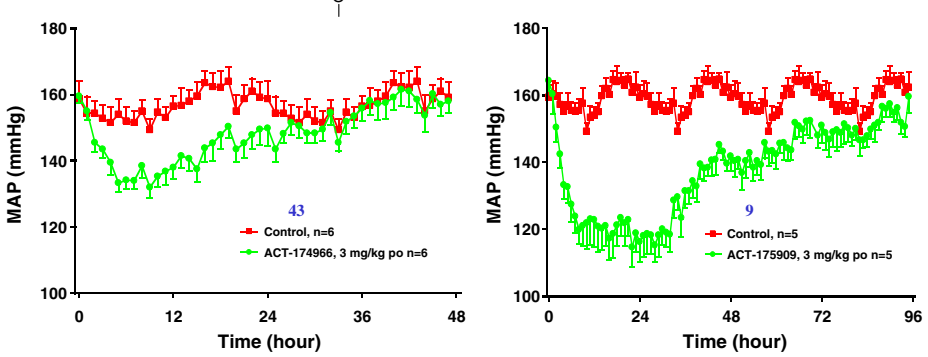


Figure 5. Pharmacodynamic behavior of compounds **43** and **9** when administered po at a dose of 3 mg/kg.

piperidines comprising hydrophilic groups at the 4-position are reported in the following Letter.

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