Bioorganic & Medicinal Chemistry Letters 21 (2011) 2430–2436

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery and synthesis of potent zwitterionic inhibitors of renin

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ARTICLE INFO

Article history:
Received 11 January 2011
Accepted 15 February 2011
Available online 18 February 2011

Keywords:
Renin
Hypertension
Piperidine
Zwitterion
hERG
CYP3A4

ABSTRACT

The incorporation of a carboxylic acid within in a series of 3-amido-4-aryl substituted piperidines (represented by general structure **32**) led to the discovery of potent, zwitterionic, renin inhibitors with improved off-target profiles (CYP3A4 time-dependent inhibition and hERG affinity) relative to analogous non-zwitterionic inhibitors of the past (i.e., **3**). Strategies to address the oral absorption of these zwitterions are also discussed within.

The renin-angiotensin-aldosterone system (RAAS) is an important physiological regulator of blood pressure and fluid homeostasis. The inhibition of either the formation or the action of angiotensin (Ang) II, the main product of the RAAS, represents a major therapeutic approach in the treatment of hypertension and the prevention of associated comorbidities such as heart and kidney failure, myocardial infarction, and stroke. Today, important drugs modulating the RAAS include inhibitors of the angiotensin-converting enzyme (ACE) and antagonists of the Ang II type-1 receptor (AT1R).[1] It has long been hypothesized that inhibitors of renin, the rate-limiting enzyme in the RAAS cascade responsible for the cleavage of angiotensinogen to Ang I, may represent the most attractive therapeutic strategy to block the RAAS.[2]

Initial efforts directed toward inhibiting renin involved scaffolds that were either peptide based or peptidomimetics.[3] While potency could be attained in the nanomolar range, the development of these compounds were discontinued due to their metabolic instability, excessive production costs and poor overall pharmacokinetics (high clearance and low oral bioavailability).[4] Nonetheless, these prototypical candidates were important in that they were able to demonstrate a more effective blood pressure lowering than an ACE inhibitor when dosed intravenously.[5] By the late 1990s, two new classes of non-peptidic inhibitors emerged (Fig. 1), one of which culminated in the eventual disclosure of aliskiren **1**, the first and only direct renin inhibitor approved for the treatment of essential hypertension.[6]

Concomitantly, the discovery of piperidine-based renin inhibitors by Roche represents a more significant structural departure from the original peptide-like molecules.[7] Initially identified from a high throughput screen,[7b] a series of optimizations, guided by crystallography, led to the eventual identification of potent, orally active, direct inhibitors of renin as in **2** (Fig. 1).[7d] Since then, several potent piperidine-like analogues have been reported, including: diazabicyclo-nonenes;[8] achiral tetrahydropyridines;[9] morpholines;[10] ketopiperazines;[11] aminopropanamides;[12] and non-chiral indole-3-carboxamides.[13]

Recently, Actelion reported the discovery of a new series of potent piperidine-based renin inhibitors bearing a 3-amido-4-aryl substitution pattern with impressive anti-hypertensive effects after oral administration in a double transgenic hypertensive rat expressing human renin and angiotensinogen (e.g., **3**, Fig. 1).[14] Although many of these compounds were found to suffer from time-dependent CYP3A4 inhibition (TDI), attempts to eradicate these liabilities via the installation of polarity were met with only modest success. Moreover, it was recently found that compound **3** exhibited undesirable affinity toward the hERG channel with a K_i value of 243 nM (unpublished data). Herein, we wish to disclose the discovery of potent, zwitterionic inhihitors of renin based on the 3-amido-4-aryl substituted piperidine core, and our attempts at addressing both CYP3A4 and hERG inhibition.

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doi:10.1016/j.bmcl.2011.02.067

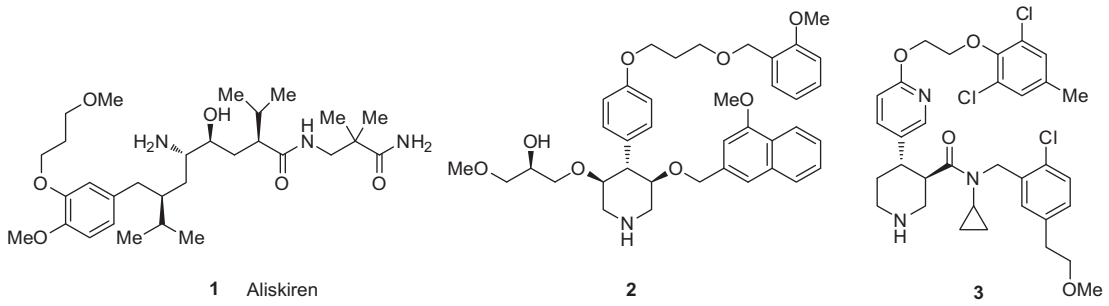


Figure 1. Non-peptidic inhibitors of renin.

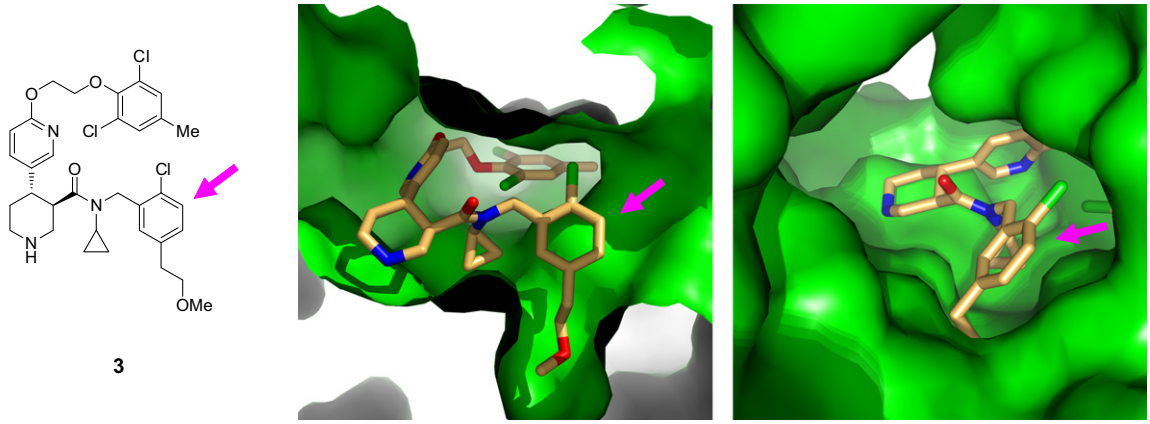


Figure 2. Modeling of compound **3** into active site of renin depicting site for water accessibility.

Given the promiscuous and hydrophobic nature of the CYP3A4 binding pocket[15] and the fact that the hERG-encoded channel is known to be blocked by lipophilic amines,[16] we postulated that the off-target activities observed with **3** were associated with its chemical structure (i.e., cationic piperidine flanked by two lipophilic tails). Consequently, we set out to design new renin inhibitors with added polarity. In order to ensure that these new alterations would not jeopardize the intrinsic potency already realized, we specifically opted to place a second polar appendage at the *meta*-position of the benzyl amide ring in the renin S3 pocket, as this was suggested by modeling to be an ideal place for attaching a solvent exposed hydrophilic cap (Fig. 2).[17]

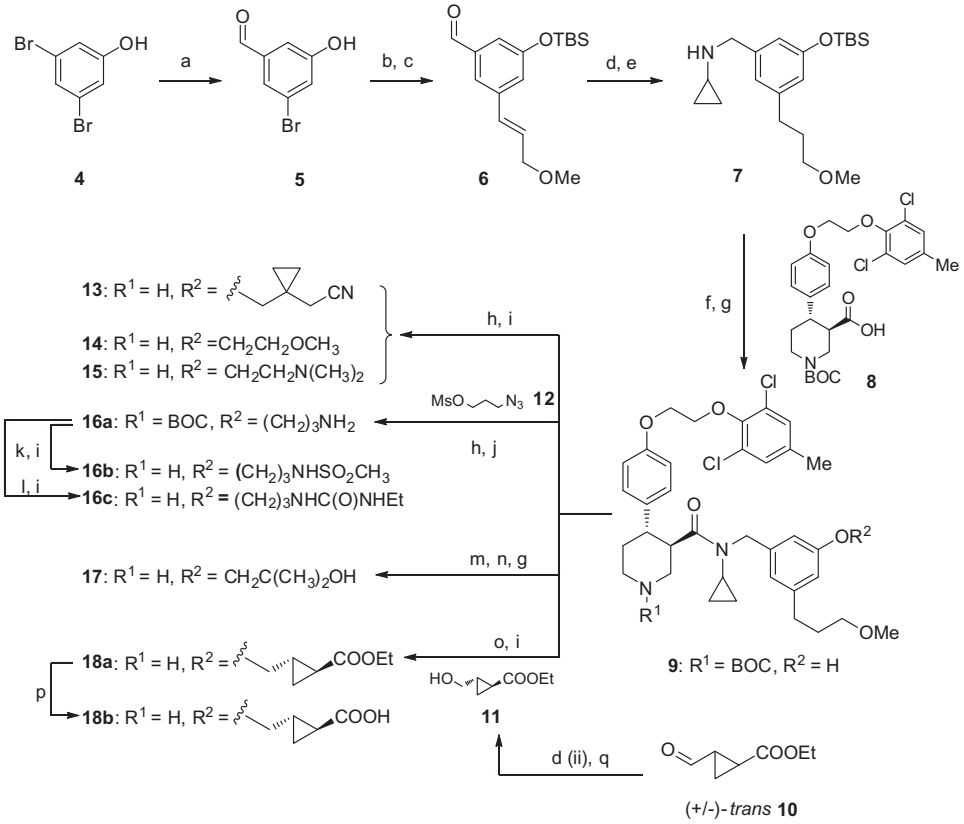
Guided by this hypothesis, we chose to work with the advanced phenol intermediate **9**, as this provided a suitable handle for rapidly introducing diverse hydrophilic attachments. The synthesis of **9**, which can be accessed in seven steps from commercial reagent **4**, was prepared as shown in Scheme 1. Treatment of 3,5-dibromophenol **4** with *n*-butyllithium and *n*-butylmagnesium chloride followed by quenching with DMF afforded benzaldehyde **5**. Subsequent coupling with commercially available 2-[(1E)-3-methoxyprop-1-en-1-yl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane using standard Suzuki conditions followed by protection with TBDMSCl afforded the aldehyde **6**. The aldehyde was then subjected to a stepwise reductive amination and the double bond reduced under standard balloon hydrogenation conditions to afford benzyl amine **7**. Amide coupling with acid **8**[18] proceeded smoothly without epimerization using HATU as the coupling agent, and subsequent deprotection with TBAF yielded the desired phenol intermediate **9**. At this stage, phenol **9** could be derivatized to the final analogues **13**–**18** via an alkylation, Mitsunobu, or an epoxide-ring opening reaction, followed by a BOC-deprotection. Unless otherwise shown, the hydrophilic reagents used to functionalize **9** were either accessible from commercial sources or synthesized from known literature procedures.

Table 1 highlights our initial modifications and their effect on renin potency, hERG affinity and their potential for CYP3A4 time-dependent inhibition (TDI). We were pleased to see that all of the analogues synthesized were highly potent on the renin enzyme, in agreement with our structural analysis of **3** in complex with renin. Moreover, these compounds exhibited low nanomolar activity in the presence of human plasma. Contrary to our initial hypothesis however, many of the polar appendages had little effect

Table 1
Renin potency, hERG affinity, and NADPH-dependent CYP3A4 inhibition of select renin compounds

Compd	Renin IC_{50}[a] (nM)		hERG K_i (μM)	NADPH-dependent CYP3A4 inhibition[b]		
	Buffer	Plasma		–NADPH IC_{50} (μM)	+NADPH IC_{50} (μM)	IC_{50} Shift
13	0.028	10.9	0.06	19.6	1.4	14.1
14	0.021	4.4	0.08	21.6	2.4	9.0
15	0.046	4.5	0.18	22.7	1.4	16.1
16b	0.028	2.9	0.08	27.6	1.6	16.8
16c	0.022	3.3	0.15	6.3	2.4	2.6
17	0.097	10.6	0.13	>50	5.6	>8.9
18a	0.064	11.0	0.08	19.3	1.4	13.98
18b	0.027	1.1	1.07	>50	>50	~1

[a] Average of at least two replicates. See Ref. 12 for assay protocols.
[b] NADPH dependent CYP3A4 inhibition: measures change in the IC_{50} for CYP3A4 during a 30 min preincubation in the absence and presence of NADPH. IC_{50} shift ⩾1.5-fold suggests that compound could be a potential TDI. Conversely, IC_{50} shift <1.5-fold signifies that the compound may not be a TDI. The higher the IC_{50} shift ratio, the greater the TDI potential. See Ref. 20 for complete protocol.



Scheme 1. Reagents and conditions: (a) (i) *n*-BuLi (2.5 equiv), *n*-BuMgCl (0.6 equiv), toluene, −10 °C, 30 min; (ii) DMF (20 equiv), −40 °C to rt, 1 h; (b) 2-[(1E)-3-methoxyprop-1-en-1-yl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1 equiv), Na_2CO_3 (2 M in water, 4 equiv), $Pd(OAc)_2$ (10 mol %), PPh_3 (20 mol %), DMF, 80 °C, 16 h; (c) TBDMSCl, imidazole, DMF, rt, 16 h; (d) (i) cyclopropyl amine (2 equiv), $MgSO_4$ (1.5 equiv), DCM, rt, 12 h; (ii) $NaBH_4$ (1.5 equiv), MeOH, 0 °C to rt, 1.5–3 h; (e) H_2 balloon, 10% Pd/C (10 mol %), EtOAc, rt, 1.5 h; (f) acid **8** (1 equiv), amine **9** (1 equiv), HATU (1.5 equiv), DIPEA (3 equiv), DCM, rt, 18 h; (g) TBAF (0.1 M, 1.3–2 equiv), THF, rt, 1 h; (h) RBr, RCl or ROMs (1.3–2.5 equiv), Cs_2CO_3 or K_2CO_3 (1.3–4.4 equiv) DMF, 80 °C; (i) HCl (4 M in dioxane, 10 equiv), DCM, rt; (j) PPh_3 (1.2 equiv), THF, H_2O, 50 °C, 8 h; (k) $MeSO_2Cl$ (1.2 equiv), Et_3N (1.5 equiv), DCM, 0 °C, 2 h; (l) EtNCO (1.5 equiv), THF, 0 °C, 2 h; (m) Cs_2CO_3 (2 equiv), 2,2-dimethyloxirane (25 equiv), DMF, 50–80 °C, 22.5 h; (n) TMSI (2 equiv), MeCN, 0 °C, 10 min; (o) 1,1′-(azodicarbonyl)dipiperidine (1.2 equiv), *n*-Bu_3P (1.2 equiv), 80 °C, 18 h; (p) NaOH (1 N aq, 1.3 equiv), EtOH, 75 °C, 18 h; (q) Chiral Pak AD preparative column (10% EtOH/hexanes), faster eluting enantiomer.

Table 2
Bioavailability and exposure of compound **18a** or **18b** after oral administration in Sprague Dawley rats or Beagle dogs

Entry	Formulation[a]	F[b] (%)	$AUC_{0-24\ h}$[b] (μM h)
Dosed as parent acid **18b**			
1	0.5% methocel	1	0.1
2	60% PEG 200	4	0.5
3	60% PEG 200 (pH 2.5)	1	0.1
4	Peanut oil	1	0.1
5	Lyposin II	0	0.03
6	Na salt in 0.5% methocel	1	0.1
7	Ca salt in 0.5% methocel	0	0.3
8	Intra-duodenum[c]	0	0
Dosed as ethyl ester prodrug **18a**			
9	0.5% Methocel, rats	52	5.7
10	0.5% Methocel, dogs[d]	6	0.01 (0.03)[e]

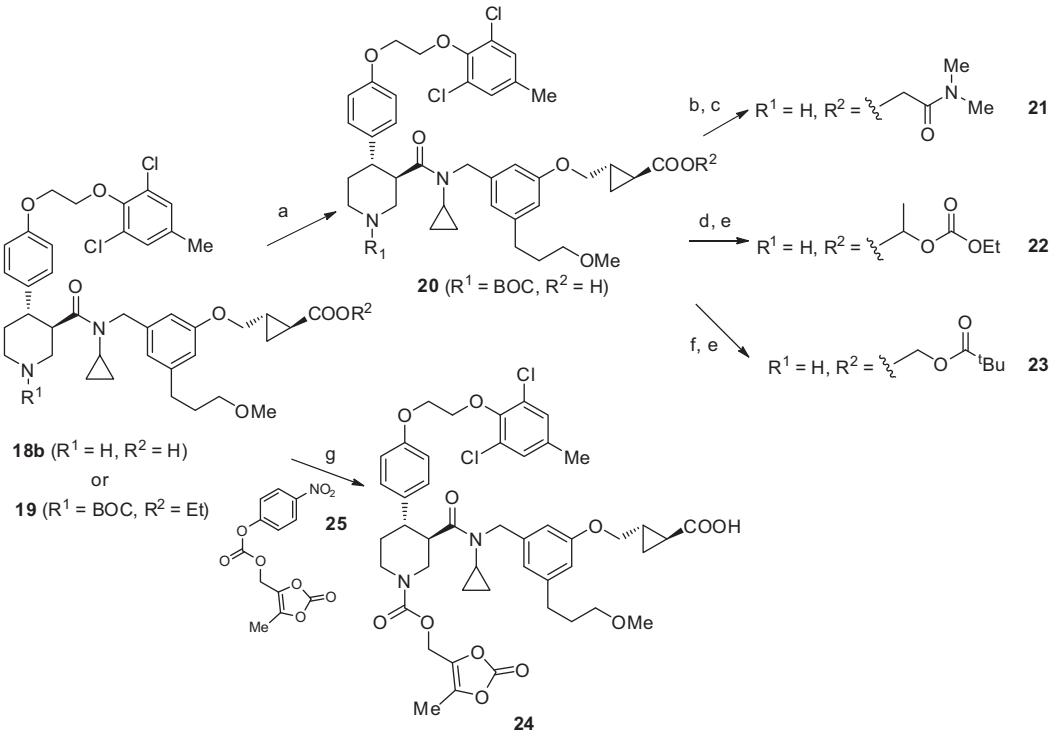
[a] Administered orally in Sprague Dawley rats ($n = 2$) at 30 mpk, 5 mL/kg.
[b] Unless otherwise noted, AUC and F are with respect to **18b**.
[c] Administered intra-duodenum at 20 mpk, 1 mL/kg in 5% dextrose.
[d] Administered orally to Beagle dogs ($n = 2$) at 3 mpk, 5 mL/kg.
[e] Value in parenthesis is exposure levels of prodrug **18a**.

Table 3
Assessing the hERG affinity, exposure, and extent of hydrolysis of the zwitterionic prodrugs **18a**, **21**–**24** in rat and human whole blood/liver

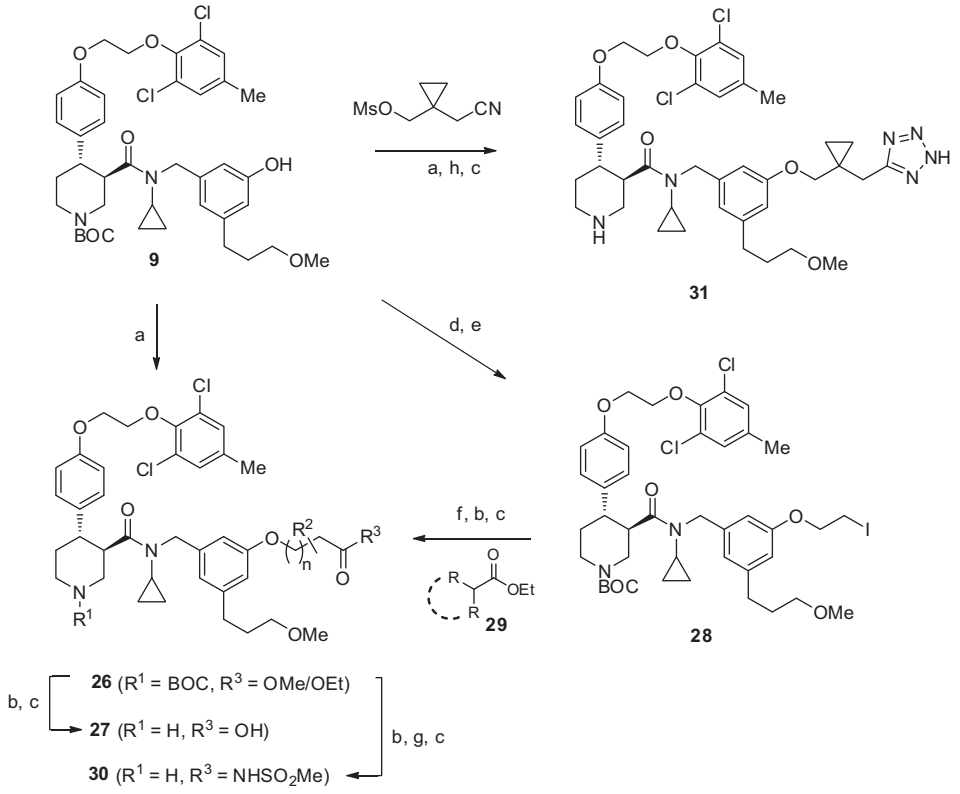
Compd	hERG K_i (μM)	% Conversion to **18b**[a]				$AUC_{0-24\ h}$[b] (μM h)
		Whole blood		Liver S9		
		Rat	Human	Rat	Human	
Acid-based prodrugs						
18a	0.08	99	3	60	56	5.7
21	0.2	100	1	66	––	3.5
22	––	100	38	81	90	0
23	0.04	100	56	83	95	0.2
Piperidine-based prodrugs						
24	3.7	93	11	98	99	0.2

[a] After 20 min preincubation ($n = 2$).
[b] Administered orally in Sprague Dawley rats ($n = 2$) at 30 mg/kg, 5 mL/kg in 0.5% methocel for **18a**, **21**–**23** and 20 mg/kg; 5 mL/kg in 0.5% methocel for **24**. AUC is with respect to **18b**.

on reducing the hERG binding and the CYP3A4 IC_{50} shift (compounds **13**–**18a**). The only exception was the zwitterionic inhibitor **18b** which demonstrated a favorable reduction in hERG affinity (K_i = 1.07 μM) with no observable CYP3A4 activity in the presence or absence of NADPH. Indeed, attempts to reduce either the hERG affinity or CYP3A4 inhibitory activity via the incorporation of a carboxylic acid has been previously documented in the literature.[19] Encouraged by these findings, **18b** was selected for further pharmacokinetic profiling in Sprague Dawley rats. Unfortunately, when the compound was administered orally at 30 mpk as a 0.5%



Scheme 2. Reagents and conditions: (a) **19** (1 equiv), NaOH (3 equiv), EtOH, MW 100 °C, 5 min; (b) Cs_2CO_3 (2 equiv), 2-chloro-*N*,*N*-dimethylacetamide (1.2 equiv), DMF, 80 °C; (c) 4 M HCl in dioxane (10 equiv), DCM, rt; (d) 1-chloroethyl ethyl carbonate (1.3 equiv), NaI (0.1 equiv), TEA (2 equiv), acetone, 75 °C, 3 h; (e) $ZnBr_2$ (10 equiv), DCM, rt, o/n; (f) chloromethyl pivalate (1.1 equiv), DBU (1.1 equiv), ACN:DCM (1:1), rt, 10 h; (g) **18b** (1 equiv), **25** (1.2 equiv), DMF, rt, 2 h.



Scheme 3. Reagents and conditions: (a) (i) RBr, RCl or ROMs (1.3–2.5 equiv), Cs_2CO_3 or K_2CO_3 (1.3–4.4 equiv), DMF, 80 °C; or (ii) 1,1′-(azodicarbonyl)dipiperidine (1.2 equiv), *n*-Bu_3P (1.2 equiv), 80 °C, o/n; (b) NaOH (1 N aq, 1.3 equiv), EtOH, 75 °C, o/n; (c) 4 M HCl in dioxane (10 equiv), DCM, rt; (d) 1,2-dibromoethane (20 equiv), 3 M NaOH (2–5 equiv), $Bu_4N^+HSO_4^-$, 70 °C, o/n; (e) NaI (1.5 equiv), acetone, reflux; (f) (i) LDA (1.1 equiv), **29** (1.05 equiv), THF, −78 °C to −20 °C; (ii) **28** (1 equiv), THF, −78 °C to rt; (g) $MeSO_2NH_2$ (3 equiv), EDCI (1.5 equiv), DMAP (1.7 equiv), DCM, rt, 3 days; (h) tributyltin azide (3 equiv), dioxane, 150 °C, o/n.

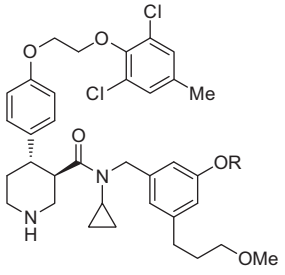
methocel suspension, it was found to have extremely low bioavailability (Table 2, entry 1).

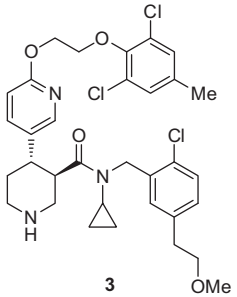
In an effort to improve the oral exposure, compound **18b** was dosed in various vehicles, as either the parent acid or its alkali salt, and at different pH. Representative examples of these studies are highlighted in Table 2. In summary, all of the formulations attempted offered negligible improvement in terms of oral bioavailability and exposure, including direct administration into the duodenum (entries 1–8). Speculating that poor cellular permeability was a key factor limiting compound **18b**'s bioavailability, we then turned to a prodrug approach as this method was found to be successful for numerous zwitterionic drugs lacking oral absorption.[21] Pleasingly, when **18b** was dosed in the rat as its ethyl ester prodrug **18a**, a dramatic increase in bioavailability and exposure was realized, with no measurable levels of ester prodrug **18a** observed (entry 9). Unfortunately, this finding did not translate into the dog where conversion to the corresponding acid was incomplete (entry 10).

To ascertain the relevance of these two dichotomous results to human, the ethyl ester prodrug **18a** was incubated in the whole blood of various species. Significantly different rates of ester hydrolysis between the species were observed, with the rat being the most efficient (98%) (rhesus monkey (15%) >dog (2%) ≈ human (3%)). These results, taken together with the fact that **18a** exhibits a strong affinity for the hERG channel (K_i = 0.08 μM), necessitated the design of a more labile prodrug. Specifically, well absorbed prodrugs which demonstrated rapid and quantitative conversion in human whole blood and/or liver were sought.

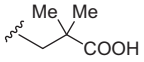
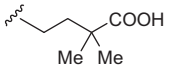
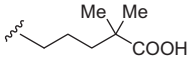
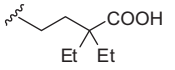
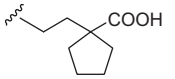
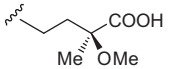
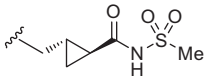
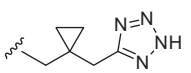
Table 3 highlights some of the many acid and piperidine-based prodrugs that were assessed for their ability to hydrolyze in both human and rat whole blood and liver. Access to the acid-based prodrugs **21**–**23** could be achieved from the corresponding

Table 4
Renin buffer and plasma potency, hERG binding, and rat pharmacokinetics of select zwitterionic compounds





3

Compd	R	Renin IC_{50}[a] (nM)		hERG K_i (μM)	NADPH-dependent CYP3A4 inhibiton[b]			Pharmacokinetics in rats[c]		
		Buffer	Plasma		−NADPH IC_{50} (μM)	+NADPH IC_{50} (μM)	IC_{50} shift	F%	po $AUC_{0-24\ h}$ (μM h)	Cl (mL/min/kg)
3		0.05	3.3	0.2	>50	1.6	>31.8	72	8.6	62
18b		0.03	1.1	1.1	>50	>50	~1	1	0.1	60
27a		0.04	5.6	1.0	>50	>50	~1	2	0.2	49
27b		0.06	29.6	0.6	>50	>50	~1	9	1.1	44
27c		0.02	9.0	0.4	>50	>50	~1	13	2.1	43
27d		0.01	6.1	0.2	>50	>50	~1	22	16	9.7
27e		0.02	23.4	0.4	>50	>50	~1	26	5.8	29
27f		0.01	12.4	0.5	>50	>50	~1	15	3.3	27
27g		0.06	2.1	2.3	>50	>50	~1	3	1.2	23
30		0.01	2.1	0.5	>50	>50	~1	0	0	26
31		0.03	5.3	0.5	>50	>50	~1	0	0	46

[a] Average of at least two replicates. See Ref. 12 for assay protocols.
[b] NADPH dependent CYP3A4 inhibition: measures change in the IC_{50} for CYP3A4 during a 30 min preincubation in the absence and presence of NADPH. IC_{50} shift ⩾1.5-fold suggests that compound could be a potential TDI. Conversely, IC_{50} shift <1.5-fold signifies that the compound may not be a TDI. The higher the IC_{50} shift ratio, the greater the TDI potential. See Ref. 20 for complete protocol.
[c] Administered in Sprague Dawley rats (n = 2): PO = 30 mpk, 5 mL/kg in 0.5% methocel; iv = 3–5 mpk; 1 mL/kg in 60% PEG 200.

intermediate acid **20** via alkylation with a commercially available chloromethyl alkylating reagent. Conversely, the carbamylation of **18b** with the known (oxodioxolenyl)-methyl carbonate **25**[22] afforded the piperidine-based prodrug **24** (Scheme 2). Not surprisingly, all of the prodrugs investigated exhibited undesirable affinities toward the hERG channel with the exception of the piperidine-based prodrug **24** (K_i = 3.7 μM). More importantly, prodrugs **22**–**24** demonstrated significant conversion to **18b** after either whole blood or liver incubations in both rat and human. Unfortunately, prodrugs **22**–**24** also failed to afford comparable oral exposures realized with **18a** in the rat. As a result, efforts in the prodrug series were discontinued and new zwitterionic inhibitors with inherently improved pharmacokinetic properties were sought.

Focusing our efforts around the acid moiety, several lipophilic acid analogues of varying length, substitution and functionality were synthesized in hope that the added lipophilicity would translate into compounds with enhanced oral absorption. Once again, intermediate **9** served as a useful starting point for accessing the intended zwitterions (Scheme 3). Thus, an alkylation or Mitsunobu reaction between **9** and an appropriately substituted alkyl halide, mesylate or alcohol provided access to the ester intermediate **26**. Subsequent ester hydrolysis followed by BOC deprotection provided access to the final zwitterions **27a**–**g** (Table 4). Alternatively, a more efficient access to butyric acid analogues could be achieved from the deprotonation of a commercially available ester **29** and its subsequent reaction with an alkylating agent as in **30**. The acylsulfonomide **30** could be easily obtained in three steps from **26** via hydrolysis of the ester, coupling with methanesulfonamide and BOC deprotection. Finally, tetrazole **31** could be prepared directly from the corresponding nitrile by treating it with tributyltin azide in refluxing dioxane.

In general, all of the acid analogues were found to be highly potent inhibitors of renin, again confirming the accommodating nature of the channel situated off the renin S3 pocket (Table 4). Moreover, none of the acids demonstrated a potential for time dependent inhibition against CYP3A4, unlike the prototypical compound **3**. In terms of chain length, butanoic and pentanoic acid derivatives seemed to offer the best compromise between hERG and renin plasma potency and exposure in the rat (e.g., **27b** vs **27c** vs **27d**). More importantly, zwitterions with proximal, nonpolar groups gave the best results in terms of absorption, exposure and clearance. In fact, we were pleased to find that the zwitterions **27d–f** could provide similar or better exposures and clearances to either the ethyl ester prodrug **18a** (Table 2, entry 9) or the historical compound **3** (Table 4) when administered orally in the rat. Unfortunately, the gain in bioavailability, exposure and clearance observed with such analogues was met with a concomitant loss in renin plasma potency. Moreover, the added lipophilicity resulted in compounds with increased off-target binding with respect to hERG. Consequently, efforts to remediate this loss, either through the introduction of additional polarity within the alkyl appendage (e.g., **27g**) or through replacement of the carboxylic acid by an even more polar isostere (**30** and **31**), could be partially realized but at the expense of bioavailability and exposure. As a result, an alternative strategy aimed at decreasing the inherent lipophilicity and hence off-target activities associated with **3** was sought. Indeed, a more promising approach involving the truncation of the northern lipophilic appendage within **3** can offer potent renin inhibitors with dramatically improved hERG and CYP3A4 profiles. The results of this novel strategy will be disclosed in an upcoming publication.[23]

In summary, we have identified potent zwitterionic-based inhibitors of renin which offer improved off-target profiles (e.g., CYP3A4 and hERG inhibition) relative to analogous non-zwitterionic inhibitors of the past. The zwitterion 18b was found to possess the most interesting in vitro profile, with a plasma renin IC_{50} value of 1.1 nM, hERG K_i of 1.07 μM and no observed CYP3A4 TDI. While efforts to improve the oral bioavailability of **18b** could be realized with analogues such as **27d–f**, where the carboxylic acid is flanked by larger alkyl groups, this was unfortunately met with an increase in hERG activity and a reduction in renin plasma potency. As a result, work on the zwitterionic series was discontinued.

References and notes

1. Zaman, M. A.; Oparil, S.; Calhoun, D. A. *Nat. Rev. Drug Disc.* **2002**, *1*, 621.
2. (a) Skeggs, L. T.; Lentz, K. E.; Kahn, J. R.; Shumway, N. P.; Woods, K. R. *J. Exp. Med.* **1956**, *104*, 193; (b) Skeggs, L. T.; Kahn, J. R.; Lentz, K. E.; Shumway, N. P. *J. Exp. Med.* **1957**, *106*, 439.
3. Tice, C. M. *Ann. Reports Med. Chem.* **2006**, 155.
4. Fisher, N. D. L.; Hollenburg, N. K. *Expert Opin. Invest. Drugs* **2001**, *10*, 417.
5. Zimmerman, B. G. *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 370.
6. (a) Jensen, C.; Herold, P.; Brunner, H. R. *Nat. Rev. Drug Disc.* **2008**, *7*, 399; (b) Maibaum, J.; Stutz, S.; Göschke, R.; Rigollier, P.; Yamaguchi, Y.; Cumin, F.; Rahuel, J.; Baum, H. P.; Cohen, N. C.; Schnell, C. R.; Fuhrer, W.; Gruetter, M. G.; Schilling, W.; Wood, J. M. *J. Med. Chem.* **2007**, *50*, 4832.
7. (a) Oefner, C.; Binggeli, A.; Breu, V.; Bur, D.; Clozel, J. P.; D'Arcy, A.; Dorn, A.; Fischli, W.; Grüninger, F.; Güller, R.; Hirth, G.; Märki, H.; Mathews, S.; Müller, M.; Ridley, R. G.; Stadler, H.; Vieira, E.; Wilhelm, M.; Winkler, F.; Wostl, W. *Chem. Biol.* **1999**, *6*, 127; (b) Vieira, E.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Güller, R.; Hirth, G.; Märki, H. P.; Müller, M.; Oefner, C.; Scalone, M.; Stadler, H.; Wilhelm, M.; Wostl, W. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1397; (c) Güller, R.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Hirth, G.; Jenny, C.; Kansy, M.; Montavon, F.; Müller, M.; Oefner, C.; Stadler, H.; Vieira, E.; Wilhelm, M.; Wostl, W.; Märki, H. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1403; (d) Märki, H. P.; Binggeli, A.; Bittner, B.; Bohner-Lang, V.; Breu, V.; Bur, D.; Coassolo, P. H.; Clozel, J. P.; D'Arcy, A.; Doebeli, H.; Fischli, W.; Funk, C. H.; Foricher, J.; Giller, T.; Grüninger, F.; Guenzi, A.; Güller, R.; Hartung, T.; Hirth, G.; Jenny, C. H.; Kansy, M.; Klinkhammer, U.; Lave, T.; Lohri, B.; Luft, F. C.; Mervaala, E. M.; Müller, D. N.; Müller, M.; Montavon, F.; Oefner, C. H.; Qiu, C.; Reichel, A.; Sanwald-Ducray, P.; Scalone, M.; Schleimer, M.; Schmid, R.; Stadler, H.; Treiber, A.; Valdenaire, O.; Vieira, E.; Waldmeier, P.; Wiegand-Chou, R.; Wilhelm, M.; Wostl, W.; Zell, M.; Zell, R. *Farmaco.* **2001**, *56*, 21.
8. Bezençon, O.; Bur, D.; Weller, T.; Richard-Bildstein, S.; Remeň, L.; Sifferlen, T.; Corminboeuf, O.; Grisostomi, C.; Boss, C.; Prade, L.; Delahaye, S.; Treiber, A.; Strickner, P.; Binkert, C.; Hess, P.; Steiner, B.; Fischli, W. *J. Med. Chem.* **2009**, *52*, 3689.
9. Remeň, L.; Bezençon, O.; Richard-Bildstein, S.; Bur, D.; Prade, L.; Corminboeuf, O.; Boss, C.; Grisostomi, C.; Sifferlen, T.; Strickner, P.; Hess, P.; Delahaye, S.; Treiber, A.; Weller, T.; Binkert, C.; Steiner, B.; Fischli, W. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6762.
10. Akatsuka, H.; Sugama, H.; Awai, N.; Kawaguchi, T.; Takahashi, Y.; Iijima, T.; Shen, J.; Xia, G.; Xie, J. WO Patent 2008/153182 A1, **2008**.
11. (a) Holsworth, D. D.; Powell, N. A.; Downing, D. M.; Cai, C.; Cody, W. L.; Ryan, J. M.; Ostroski, R.; Jalaie, M.; Bryant, J. W.; Edmunds, J. J. *Bioorg. Med. Chem.* **2005**, *13*, 2657; (b) Powell, N. A.; Clay, E. H.; Holsworth, D. D.; Bryant, J. W.; Ryan, M. J.; Jalaie, M.; Zhang, E.; Edmunds, J. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2371; (c) Powell, N. A.; Clay, E. H.; Holsworth, D. D.; Bryant, J. W.; Ryan, M. J.; Jalaie, M.; Edmunds, J. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4713; (d) Holsworth, D. D.; Cai, C.; Cheng, X. M.; Cody, W. L.; Downing, D. M.; Erasga, N.; Lee, C.; Powell, N. A.; Edmunds, J. J.; Stier, M.; Jalaie, M.; Zhang, E.; McConnell, P.; Ryan, M. J.; Bryant, J.; Li, T.; Kasani, A.; Hall, E.; Subedi, R.; Rahim, M.; Maiti, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2500.
12. Chen, A.; Bayly, C.; Bezençon, O.; Richard-Bildstein, S.; Dubé, D.; Dubé, L.; Gagné, S.; Gallant, M.; Gaudreault, M.; Grimm, E.; Houle, R.; Lacombe, P.; Laliberté, S.; Lévesque, J. F.; Liu, S.; MacDonald, D.; Mackay, B.; Martin, D.; McKay, D.; Powell, D.; Remeň, L.; Soisson, S.; Toulmond, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2204.
13. Scheiper, B.; Matter, H.; Steinhagen, H.; Stilz, U.; Böcskei, Z.; Fleury, V.; McCort, G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6268.
14. (a) Corminboeuf, O.; Bezençon, O.; Grisostomi, C.; Remeň, L.; Richard-Bildstein, S.; Bur, D.; Prade, L.; Hess, P.; Strickner, P.; Fischli, W.; Steiner, B.; Treiber, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6286; (b) Corminboeuf, O.; Bezençon, O.; Remeň, L.; Grisostomi, C.; Richard-Bildstein, S.; Bur, D.; Prade, L.; Strickner, P.; Hess, P.; Fischli, W.; Steiner, B.; Treiber, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6291.
15. Ekroos, M.; Sjögren, T. *PNAS* **2006**, *103*, 13682.
16. (a) Stansfeld, P. J.; Sutcliffe, M. J.; Mitcheson, J. S. *Expert Opin. Drug Metab. Toxicol.* **2006**, *2*, 81; (b) Aronov, A. M. *Drug Disc. Today* **2005**, *10*, 149; (c) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5031; (d) Recanatini, M.; Poluzzi, E.; Masetti, M.; Cavalli, A.; De Ponti, F. *Med. Res. Rev.* **2005**, *25*, 133.
17. Chen, A.; Dubé, D.; Dubé, L.; Gagné, S.; Gallant, M.; Gaudreault, M.; Grimm, E.; Houle, R.; Lacombe, P.; Laliberté, S.; Liu, S.; Macdonald, D.; Mackay, B.; Martin, D.; McKay, D.; Powell, D.; Lévesque, J. F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5074.
18. Dubé, D.; Gallant, M.; Grimm, E.; Juteau, H.; Laliberté, S.; Wu, T. WO Patent 2008/058387.

19. (a) Chen, C.; Chen, Y.; Pontillo, J.; Guo, Z.; Huang, C. Q.; Wu, D.; Madan, A.; Chen, T.; Wen, J.; Xie, Q.; Tucci, F. C.; Rowbottom, M.; Zhu, Y.-F.; Wade, W.; Saunders, J.; Bozigianc, H.; Struthers, R. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3301; (b) Zhu, B. Y.; Jia, Z. J.; Zhang, P.; Su, T.; Huang, W.; Goldman, E.; Tumas, D.; Kadambi, V.; Eddy, P.; Sinha, U.; Scarborough, R. M.; Song, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5507; (c) Edmondson, S. D.; Mastracchio, A.; Beconi, M.; Colwell, L. F., Jr.; Habulihaz, B.; He, H.; Kumar, S.; Leiting, B.; Lyons, K. A.; Mao, A.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Zhu, L.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5151; (d) Edmondson, S. D.; Mastracchio, A.; Duffy, J. L.; Eiermann, G. J.; He, H.; Ita, I.; Leiting, B.; Leone, J. F.; Lyons, K. A.; Makarewicz, A. M.; Patel, R. A.; Petrov, A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3048.
20. *NADPH-dependent CYP3A4 inhibition assay:* 1 mg/mL of human liver microsomes and 0.05–50 μM of test compound were mixed with or without NADP-regenerating system and pre-incubated for 30 min at 37 °C. The preincubation medium was then diluted 10-fold with buffer containing 50 μM testosterone, and incubated 10 min at 37 °C. Samples were then quenched with 0.5% formic acid in acetonitrile, centrifuged and formation of 6β-hydroxy-testosterone monitored by HT-MS (RapidFire Technology).
21. Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Järvinen, T.; Savolainen, J. *Nat. Rev. Drug Disc.* **2008**, *7*, 255.
22. Alexander, J.; Bindra, D. S.; Glass, J. D.; Holahan, M. A.; Renyer, M. L., et al *J. Med. Chem.* **1996**, *39*, 480.
23. Chen, A. et al., manuscript in preparation.