Journal of Medicinal Chemistry

Article

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Design, Synthesis, and Evaluation of Conformationally Restricted Acetanilides as Potent and Selective β_3 Adrenergic Receptor Agonists for the Treatment of Overactive Bladder

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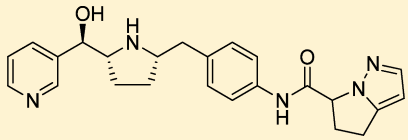
Supporting Information

ABSTRACT: A series of conformationally restricted acetanilides were synthesized and evaluated as β_3-adrenergic receptor agonists (β_3-AR) for the treatment of overactive bladder (OAB). Optimization studies identified a five-membered ring as the preferred conformational lock of the acetanilide. Further optimization of both the aromatic and thiazole regions led to compounds such as **19** and **29**, which have a good balance of potency and selectivity. These compounds have significantly reduced intrinsic clearance compared to our initial series of pyridylethanolamine β_3-AR agonists and thus have improved unbound drug exposures. Both analogues demonstrated dose dependent β_3-AR mediated responses in a rat bladder hyperactivity model.



INTRODUCTION

The discovery of the β_3-adrenergic receptor (β_3-AR) in the early 1980s[1] and the demonstration that activation of this receptor by small molecule agonists induced thermogenic[2] and antihyperglycaemic activity[3] in rodents led to a period of intense activity within the pharmaceutical industry aimed at novel treatments for type 2 diabetes and obesity.[4] A number of compounds entered clinical studies, however, ultimately they were unsuccessful, either because of a lack of efficacy, an unfavorable cardiovascular side effect profile, or poor pharmacokinetics, which led to a downturn in interest in this receptor as a target.

Overactive bladder (OAB) is a chronic and sometimes debilitating condition of the lower urinary tract that negatively impacts the quality of life of millions of people. A large multicountry survey recently estimated the prevalence of OAB at 11.8%, increasing with age.[5] Given that the elderly are the fastest-growing segment of the population, it is likely that this number will only increase. Muscarinic antagonists are the mainstay of pharmacotherapy for OAB,[6] and these drugs have been demonstrated to improve urgency, frequency of micturition, and urge incontinence, all of which are primary symptoms of OAB. However, through their anticholinergic mechanism, these agents have also demonstrated adverse effects, most notably dry mouth and constipation. While tolerability may be improved by the use of extended release formulations and agents with differing muscarinic receptor selectivities,[7] there remains a need for therapies with an improved side effect profile. Within the last 15 years, evidence for the presence of the β_3-AR on human and rat detrusor muscle has emerged.[8] In vitro studies have demonstrated that selective β_3-AR agonists cause relaxation of isolated rat and human detrusor muscle strips, and compelling in vivo evidence to support a role for β_3-AR in increasing bladder capacity in anesthetized rats has emerged.[9] This has led to renewed activity within the pharmaceutical industry in an attempt to discover potent and selective β_3-AR agonists as a potential new treatment for OAB, with the possibility for fewer side effect liabilities compared to antimuscarinics.[10] Several compounds such as **1** (YM-178, mirabegron)[11] and **2** (GW427353, solabegron),[12] as shown in Figure 1, have entered the clinic and, in the case of **1**, have been approved for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and urinary frequency.

Previous work in our laboratories has described the discovery of a series of pyridylethanolamine β_3-AR agonists with excellent potency and selectivity and possessing moderate oral bioavailability, culminating in the discovery of our first-generation clinical candidate **3**.[13] In an effort to address some of the liabilities

Received: November 8, 2013
Published: January 17, 2014

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

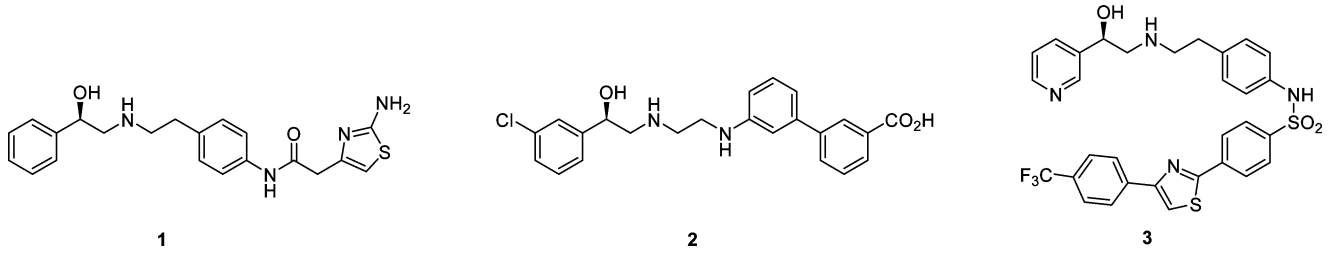


Figure 1. Structures of some of the β_3-AR agonists to have entered the clinic.

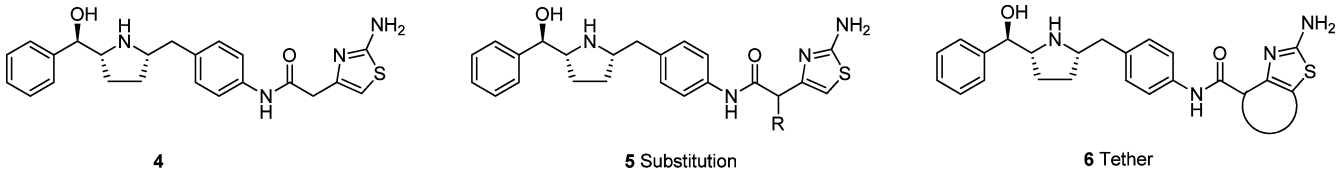
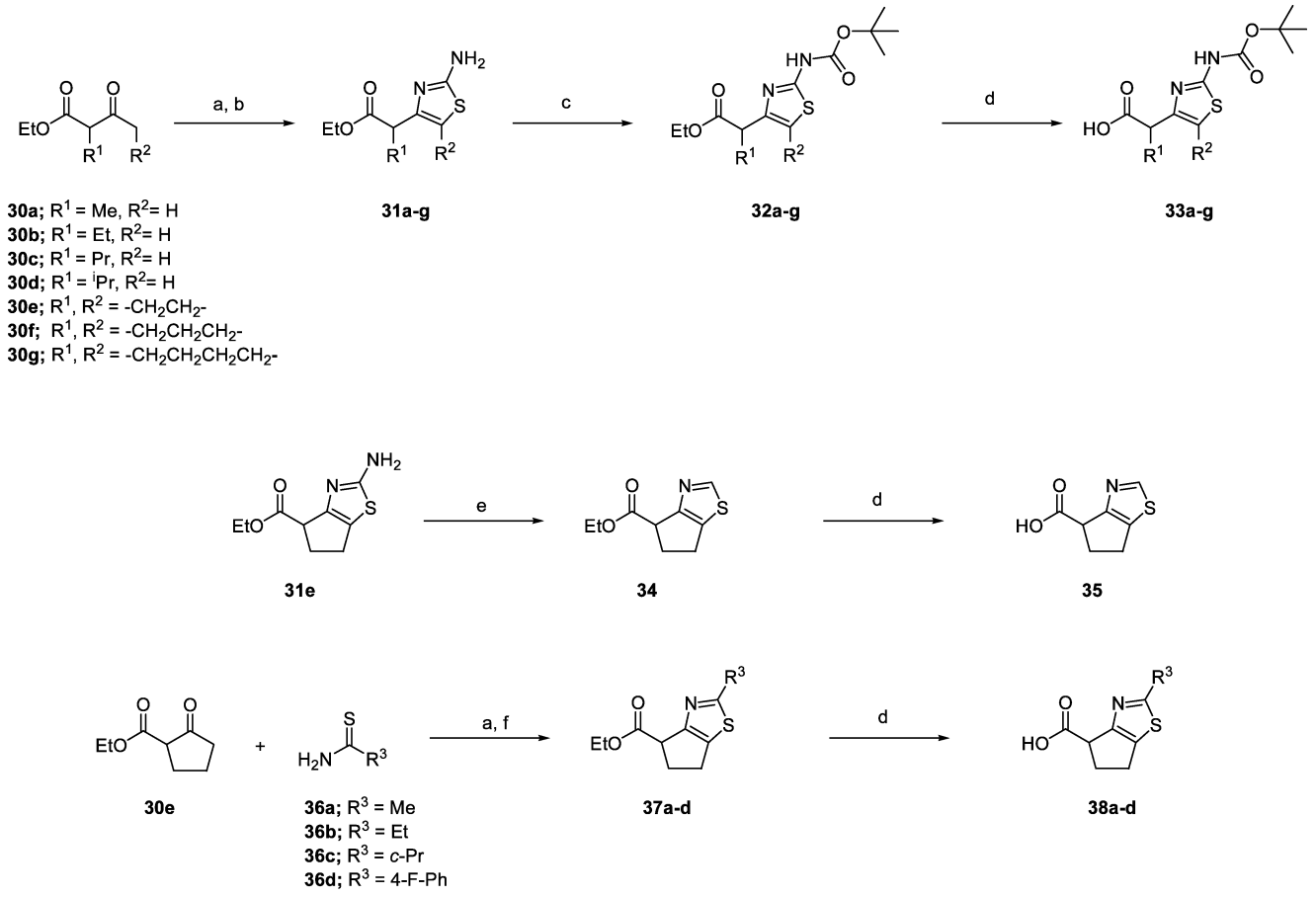


Figure 2. Structures of second-generation pyrrolidine based β_3-AR agonists.

Scheme 1. Preparation of RHS-Thiazole Intermediates[a]



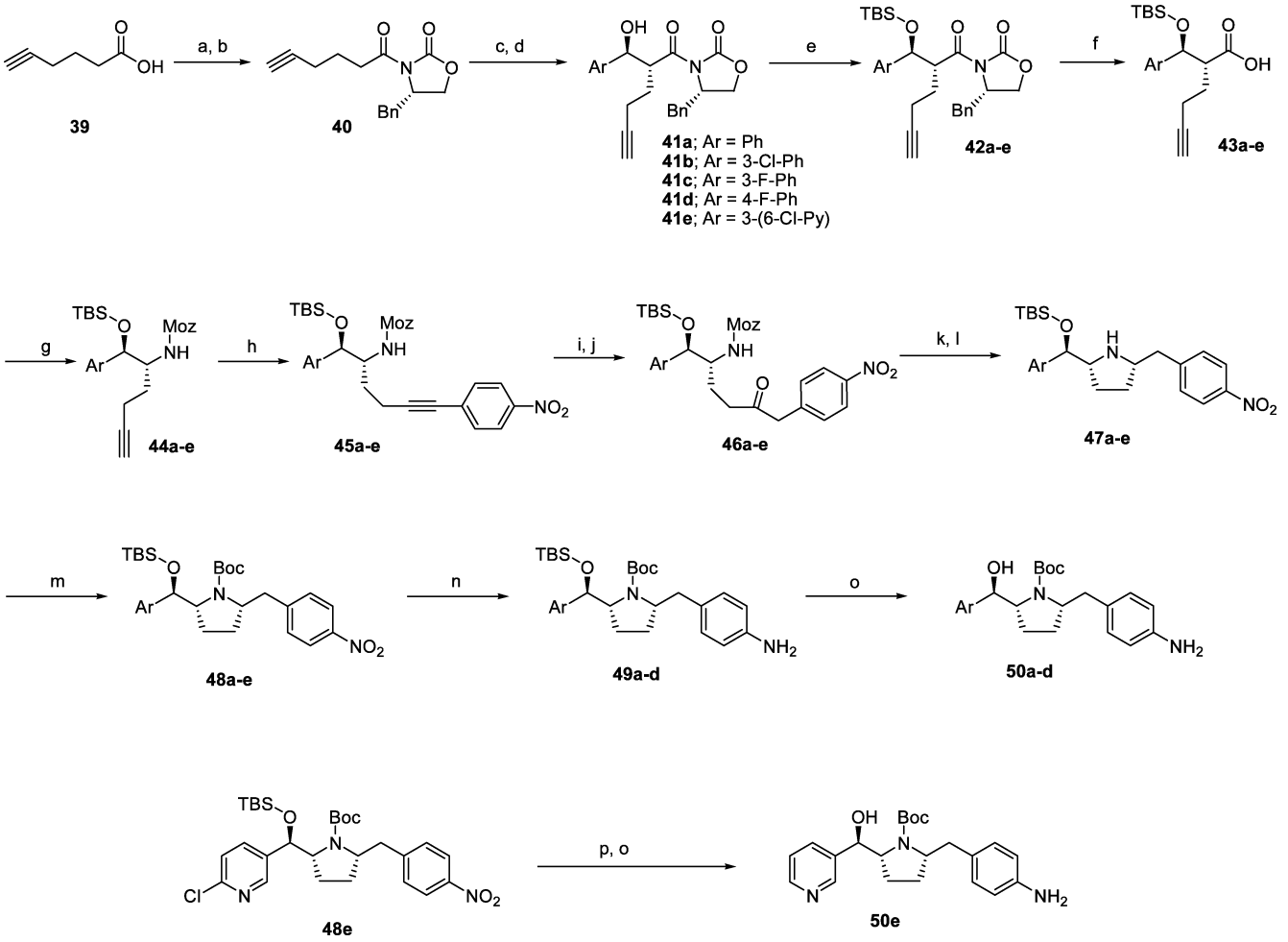
[a]Reagents: (a) Br_2, $CHCl_3$, 0 °C; (b) thiourea, EtOH, reflux; (c) $(Boc)_2O$, Et_3N, DMAP, CH_2Cl_2; (d) LiOH, THF, MeOH, H_2O; (e) *tert*-butylnitrite, DMF, 50 °C; (f) R^3-thioamide **36a−e**, EtOH, reflux.

associated with this series,[14] we proceeded to report the design and synthesis of second generation β_3-AR agonists containing a novel pyrrolidine scaffold, exemplified by acetanilide derivative **4**.[15] Compound **4** demonstrated potent human β_3-AR agonist activity and was also very selective over the β_1 and β_2 receptors. We have also disclosed the structure−activity relationships (SAR) surrounding some acetanilide derivatives in a linear (nonpyrrolidine) series and demonstrated the beneficial effects on potency and selectivity of substitution at the position α to the amide carbonyl group.[16] We now wish to report the incorporation and extension of this SAR into the novel pyrrolidine based series.

We initially targeted simple substitution with a methyl group to establish if the previously observed SAR would translate to this series and then further elaborated with higher alkyl homologues (**5**) (Figure 2). We have previously demonstrated in the linear

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

Scheme 2. Preparation of LHS-Aromatic Intermediates[a]



[a](a) Trimethylacetyl chloride, Et_3N, THF; (b) (*S*)-4-benzyl-2-oxazolidinone, LiCl, THF; (c) ArCHO, $MgCl_2$, TMS-Cl, Et_3N, EtOAc; (d) TFA, MeOH; (e) TBS-triflate, 2,6-lutidine, CH_2Cl_2; (f) H_2O_2, LiOH, THF, H_2O; (g) DPPA, Et_3N, 4-methoxybenzyl alcohol, toluene, 80 °C; (h) 4-iodonitrobenzene, $PdCl_2$(dppf), CuI, Et_3N, DMF; (i) pyrrolidine, DMF, 85 °C; (j) AcOH, H_2O; (k) TFA, CH_2Cl_2; (l) $NaCNBH_3$, MeOH; (m) $(Boc)_2O$, i-Pr_2NEt, CH_2Cl_2; (n) H_2, 10% Pd/C, MeOH or Zinc, AcOH, EtOH 80 °C; (o) TBAF, THF; (p) H_2 50 psi, 10% Pd/C, KOAc MeOH.

series that substitution with a methyl group on the thiazole-5-position also improved potency. We therefore realized that with an open C−H bond at the thiazole-5-position, there was the opportunity to form a tether between the thiazole and the α position (**6**). In principle, the introduction of this conformational constraint could have a positive effect on potency through a reduction in the entropic penalty required to achieve the optimal compound−receptor interaction.

In this article, we will describe the synthesis and SAR of various ring size tethers as well as compounds with modifications to both the left-hand side (LHS) aromatic and to the thiazole-2-position. Finally, we explored an alternative to the thiazole by replacement with a pyrazole. These studies have led to the discovery of a novel series of conformationally restricted acetanilide derivatives. In this paper, we describe in vitro SAR, pharmacokinetic properties, and evaluation of certain compounds from this new series in an in vivo rat bladder hyperactivity model.

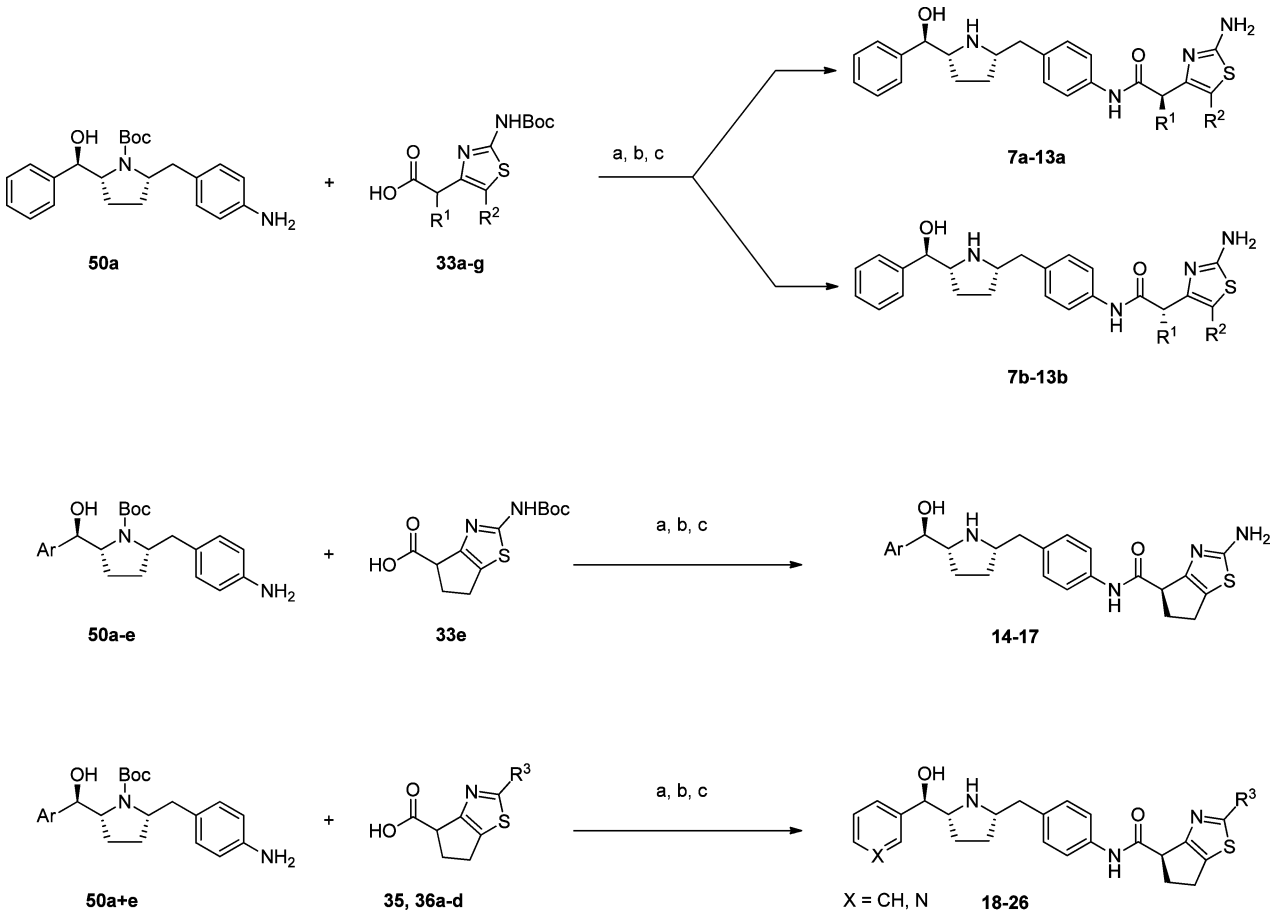
CHEMISTRY

Thiazole acid intermediates **33a−g**, **35**, and **38a−d** were synthesized following the routes illustrated in Scheme 1. Keto-esters (**30**) were brominated by the addition of bromine in chloroform at 0 °C. The initially formed α-brominated product is rearranged to the desired γ-brominated product by blowing wet air through the reaction mixture,[17] and the resultant crude material is treated with thiourea in refluxing ethanol to afford amino thiazoles **31a−g**. *N*-Boc protection under standard conditions was performed in order to facilitate purification of later intermediates. Saponification of the esters **32a−g** afforded the acids **33a−g**. A similar sequence was performed on ethyl 2-oxocyclopentane carboxylate (**30e**), replacing thiourea with thioamides **36a−d** to yield the desired substituted thiazoles **37a−d**. Hydrolysis of the ester under standard conditions afforded acids **38a−d**. The unsubstituted thiazole **35** was prepared by direct deamination of amino-thiazole **31e** by heating with *tert*-butyl nitrite in DMF to give ester **34**, followed by hydrolysis to the acid **35**.

Intermediates with modifications to the LHS aromatic ring were prepared according to the procedure shown in Scheme 2. 5-Hexynoic acid (**39**) was coupled with (*S*)-4-benzyl-2-oxazolidinone to afford the *N*-acyloxazolidinone **40**, which underwent magnesium chloride catalyzed anti-Aldol reaction[18] with benzaldehydes, followed by desilylation with TFA in methanol to give the adduct **41a−e** in high diastereoselectivity. Protection of the alcohol using TBS-triflate and 2,6-lutidine afforded products **42a−e**. Removal of the oxazolidinone auxiliary with

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

Scheme 3. General Synthesis Route to Targets 7−26[a]



[a](a) EDC, HOAt, i-Pr_2NEt, DMF; (b) chiral HPLC separation, (*R*,*R*)-Whelk-O column, eluent IPA/heptanes; (c) TFA, CH_2Cl_2.

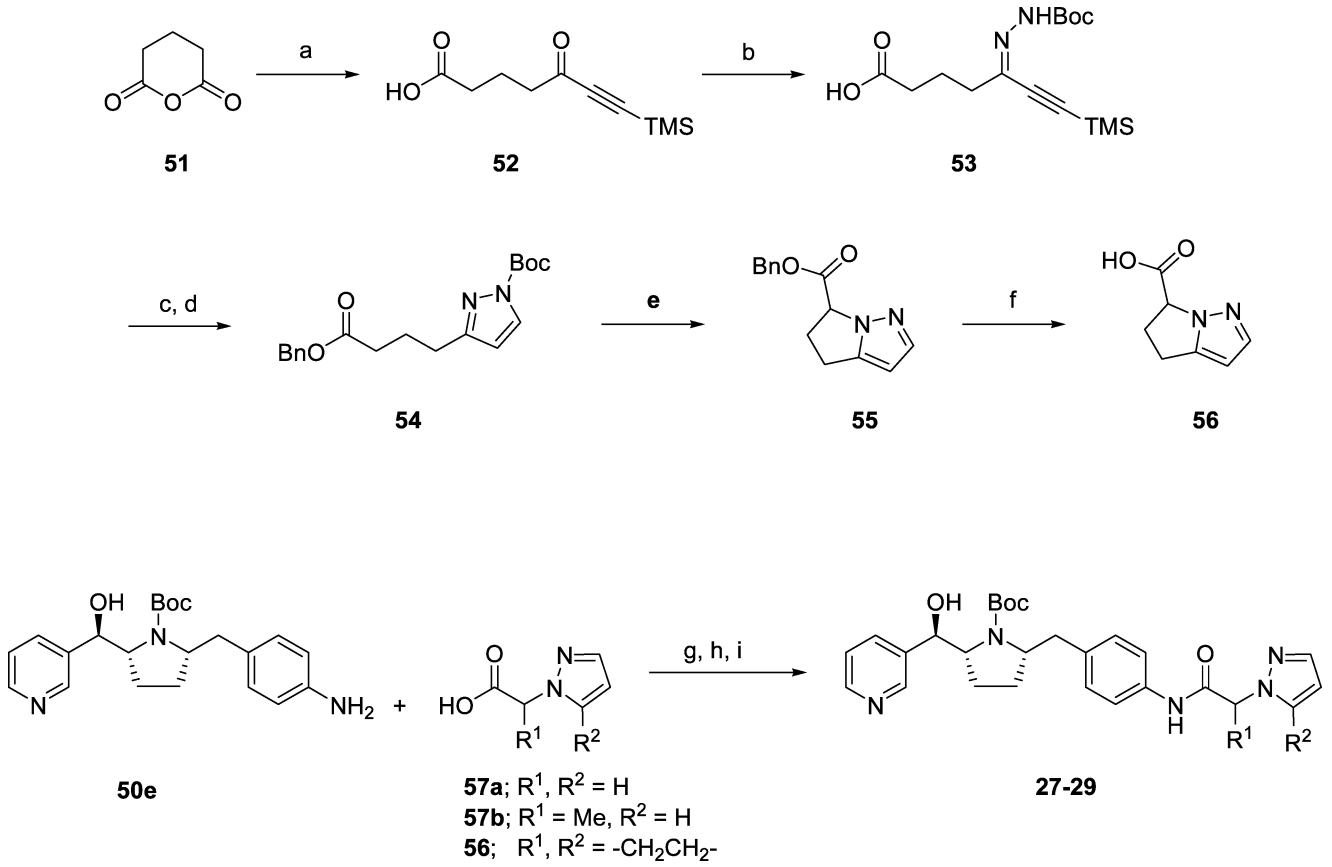
LiOH in the presence of 30% H_2O_2 provided acids **43a−e**, which underwent Curtius rearrangement in the presence of 4-methoxybenzyl alcohol to afford the Moz-protected amines **44a−e**. Sonogashira coupling of **44a−e** with 4-iodonitrobenzene provided the 4-nitrophenylacetylenes **45a−e**, which were converted to ketones **46a−e** using a procedure developed by Fukuyama.[19] Regioselective conjugate addition of pyrrolidine to the acetylene afforded an intermediate enamine, which was then hydrolyzed under acidic conditions to the ketone (**46a−e**). Removal of the Moz protecting group by treatment with TFA and concomitant cyclization afforded a 2,3-dihydropyrrole intermediate, which was reduced to a mixture of *cis* and *trans* pyrrolidine isomers using sodium cyanoborohydride. During the reductive amination step, selective formation of the *cis* over the *trans* pyrroline was always observed, with selectivities generally in the 80:20 range, although selectivity as low as 60:40 was observed. The desired *cis* pyrrolidine isomers **47a−e** could be readily separated from the *trans* isomers by silica gel chromatography. Boc protection under standard conditions gave the Boc-pyrrolidines **48a−e**. Anilines **50a−d** were prepared by reduction of nitro intermediates **48a−d** using either hydrogenolysis or zinc in acetic acid, followed by TBS deprotection. Hydrogenolysis of **48e** under pressure in the presence of potassium acetate reduced the nitro group and removed the pyridyl chlorine and following TBS deprotection afforded **50e**. Aniline **50a** was identical spectroscopically to the same intermediate prepared by a different route previously, and whose identity had been fully characterized by 2D NMR and X-ray analysis.[15]

Standard EDC coupling of the anilines **50a−e** with thiazole acetic acids **33a−g**, **35**, and **38a−d** (Scheme 3) afforded the desired coupled products as a mixture of two diastereoisomers. The diastereoisomers were readily separated utilizing chiral preperative HPLC methodology, all mixtures being separated using an (*R*,*R*)-Whelk-O column with mixtures of IPA and heptanes as eluent. The resultant pure diastereoisomers were Boc deprotected under standard conditions to give the target agonists **7−26**.

Assignment of an absolute configuration to the more active acid component in the final derivatives was determined by the following method. Separation of the enantiomers of acid **38a** was achieved utilizing chiral SFC HPLC. Coupling of the individual acids to aniline **50e** using standard EDC conditions (including Hunig's Base) resulted in complete racemization of the thiazole component, however, EDC coupling with the omission of Hunig's Base proceeded smoothly with no sign of racemization. Deprotection and biological evaluation of the individual diastereoisomers allowed us to determine which acid resulted in the more potent agonist. X-ray crystallographic analysis of this acid determined the absolute configuration to be *R*. By inference, given that the more potent agonist was always derived from the protected precursor which eluted from the (*R*,*R*)-Whelk-O column first, we have assigned this diastereoisomer as also having the *R* configuration.

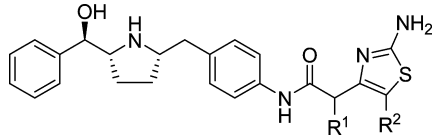
dx.doi.org/10.1021/jm4017224 | *J. Med. Chem.* 2014, 57, 1437−1453

Scheme 4. Synthesis Route to Pyrazole Derivatives[a]



[a](a) Bis(trimethylsilyl)acetylene, $AlCl_3$, CH_2Cl_2; (b) Boc-hydrazine, i-PrOH; (c) TBAF, THF; (d) benzyl bromide, K_2CO_3, DMF; (e) (i) LiHMDS, TMS-Cl, THF, (ii) NBS, (iii) TFA, CH_2Cl_2, (iv) K_2CO_3, NaI, acetone 58 °C; (f) H_2, Pd(C), EtOH; (g) EDC, HOAt, Hunig's base, DMF; (h) chiral HPLC separation, (*R*,*R*)-Whelk-O column, eluent IPA/heptanes; (i) TFA, DCM.

Table 1. SAR Results for Alkylated and Ring Constrained Amino-Thiazole Analogues



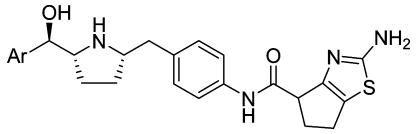
			human EC_{50} (nM)[a] (%Act)[b]	human IC_{50} (nM)[c]	
compd	R^1	R^2	β_3	β_1	β_2
4	H	H	0.98 ± 0.59 (92)	>20000	>20000
7a isomer 1	Me	H	0.18 ± 0.04 (84)	>20000	>20000
7b isomer 2	Me	H	0.67 ± 0.02 (85)	>20000	>20000
8a isomer 1[d]	Et	H	0.83 ± 0.13 (85)	>20000	>20000
9a isomer 1[d]	*n*-Pr	H	0.88 ± 0.01 (66)	>20000	>20000
10a isomer 1[d]	*iso*-Pr	H	1.60 ± 0.01 (85)	>20000	>20000
11a isomer 1	$-CH_2CH_2-$		0.15 ± 0.03 (91)	>20000	>20000
11b isomer 2	$-CH_2CH_2-$		44.2 ± 6.00 (91)	>20000	>20000
12a isomer 1[d]	$-CH_2CH_2CH_2-$		0.21 ± 0.03 (103)	>20000	>20000
13a isomer 1[d]	$-CH_2CH_2CH_2CH_2-$		1.86 ± 0.16 (70)	>20000	>20000

[a]Unless otherwise noted, the results are shown as the mean ± SD ($n \geq 3$) or presented as the average of two experiments. [b]% Activation defined as the fitted maximal value of the test compound concentration response expressed as a percent of the maximal response to *R*-(−)-isoproterenol. [c]$n = 1$. [d]Single diastereoisomer with only more potent isomer indicated.

Preparation of the pyrazole derivatives **27**−**29** was achieved using similar methodology utilizing commercially available acids **57a**−**b** and the cyclic acid **56**, which was prepared as outlined in Scheme 4. Glutaric anhydride (**51**) and bis(trimethylsilyl)-acetylene was treated with anhydrous $AlCl_3$ to afford keto-acid **52**. Condensation of **52** with Boc-hydrazine gave hydrazone **53**, which following TMS deprotection and benzyl ester formation afforded pyrazole **54**. Formation of a trimethylsilylketene acetal followed by bromination and acidic removal of the Boc group, and then base induced cyclization generated the cyclic pyrazole

dx.doi.org/10.1021/jm4017224 | *J. Med. Chem.* 2014, 57, 1437−1453

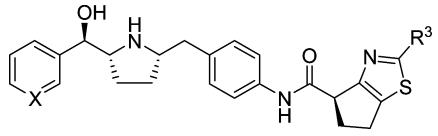
Table 2. SAR Results of LHS Aromatic Variation



compd[d]	Ar	human EC_{50} (nM)[a] (%Act)[b] β_3	human IC_{50} (nM)[c] β_1	human IC_{50} (nM)[c] β_2	hERG IC_{50} (μM)
11a	Ph	0.15 ± 0.03 (103)	>20000	>20000	12.7
14	3-ClPh	0.15 ± 0.06 (93)	>20000	8451 ± 2368[e]	1.5
15	3-FPh	0.04 ± 0.002 (94)	>20000	>20000	4.0
16	4-FPh	0.13 ± 0.02 (72)	>20000	>20000	5.6
17	3-Py	0.37 ± 0.02 (101)	>20000	>20000	23.3

[a]Unless otherwise noted, the results are shown as the mean ± SD ($n \geq 3$) or presented as the average of two experiments. [b]% Activation defined as the fitted maximal value of the test compound concentration response expressed as a percent of the maximal response to *R*-(−)-isoproterenol. [c]$n = 1$. [d]Single diastereoisomer with only more potent isomer indicated. [e]$n = 2$.

Table 3. SAR Results for Substitution at the Thiazole-2-Position



compd[d]	X	R^3	human EC_{50} (nM)[a] (%Act)[b] β_3	human IC_{50} (nM)[c] β_1	human IC_{50} (nM)[c] β_2
18	CH	H	0.64 ± 0.53 (101)	>20000	>20000
19	N	H	1.36 ± 0.69 (103)	>20000	>20000
20	CH	Me	0.48 ± 0.13 (96)	>20000	>20000
21	N	Me	1.15 ± 0.57 (89)	>20000	>20000
22	CH	Et	1.95 ± 0.45 (37)	>20000	>20000
23	N	Et	13.6 ± 2.36 (49)	>20000	>20000
24	N	*c*-Pr	12.4 ± 4.74 (43)	>20000	>20000
25	CH	4-F-Ph	186 ± 114.6 (76)	4329 ± 140[e]	2513 ± 539[e]
26	N	4-F-Ph	8.78 ± 1.32 (15)	13340 ± 1183[e]	2471 ± 585[e]

[a]Unless otherwise noted, the results are shown as the mean ± SD ($n \geq 3$) or presented as the average of two experiments. [b]% Activation defined as the fitted maximal value of the test compound concentration response expressed as a percent of the maximal response to *R*-(−)-isoproterenol. [c]$n = 1$. [d]Single diastereoisomer with only more potent isomer indicated. [e]$n = 2$.

55. Cleavage of the ester under hydrogenolyis conditions afforded acid **56**.

RESULTS AND DISCUSSION

All final compounds were evaluated as human β_3 adrenergic receptor agonists by measuring their ability to stimulate increases in intracellular cAMP in Chinese hamster ovary (CHO) cell lines stably expressing the human β_3 receptor. Selectivity over human β_1 and β_2-AR was assessed by measuring the binding affinity of compounds for these receptors using membranes prepared from recombinant cells expressing either β_1 or β_2-AR.[20]

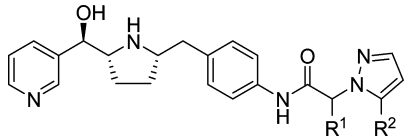
We initially investigated the effect of substitution with alkyl groups at the position α to the carbonyl in compound **4**. The results of this study are summarized in Table 1. In line with previous observations in the nonpyrrolidine containing series of acetanilides, introduction of a methyl group α to the carbonyl improved potency.[15] For example, diasteroisomer **7a** is 5-fold more potent than the unsubstituted analogue **4**. Increasing the size of the alkyl group gives compounds with decreasing activity over the methyl analogue; for example, the ethyl **8a** and *n*-propyl **9a** analogues are equipotent to the unsubstituted analogue **4**, and the iso-propyl analogue **10a** shows a loss in activity compared to **4**. Of note is that in all cases the receptor demonstrated a distinct stereochemical preference for one diastereomer over the other.

We next focused our attention on analogues in which a ring of various sizes has been formed between the thiazole-5-position and the α position. Constraining the side chain through formation of a five-membered (**11a**) or six-membered ring (**12a**) also improved β_3-AR activity (4−5-fold) over the unsubstituted compound while also maintaining the excellent selectivity profile over the β_1 and β_2-AR. Increasing the ring size to a seven-membered ring (**13a**) caused a 2-fold decrease in β_3-AR activity over the unsubstituted compound. While compounds **11a** and **12a** were comparable to **4** in terms of potency, pharmacokinetic evaluation in the rat revealed that both compounds displayed an inferior pharmacokinetic (PK) profile compared to **4** (Table 5), with both demonstrating increased clearance (Cl_p = 50 mL/min/kg for **11a** and 185 mL/min/kg for **12a**), decreased bioavailability (F = 6% for **11a** and 0% for **12a**), and decreased unbound drug exposures. The five-membered analogue **11a** appeared slightly superior to the six-membered analogue, and thus all further studies focused on fused five-membered ring analogues.

We next explored replacement of the LHS phenyl group in **11a** with several substituted phenyl groups (Table 2). Addition of a

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

Table 4. SAR Results for Pyrazole Derivatives



			human EC_{50} (nM)[a] (%Act)[b]	human IC_{50} (nM)[c]	
compd[d]	R^1	R^2	β_3	β_1	β_2
27	H	H	76.7 ± 13.68 (72)	>20000	>20000
28	Me	H	16.7 ± 10.33 (102)	>20000	>20000
29	$-CH_2CH_2-$		3.7 ± 2.86 (84)	>20000	>20000

[a]Unless otherwise noted, the results are shown as the mean ± SD ($n \geq 3$) or presented as the average of two experiments. [b]% Activation defined as the fitted maximal value of the test compound concentration response expressed as a percent of the maximal response to *R*-(−)-isoproterenol. [c]$n = 1$. [d]Single diastereoisomer with only more potent isomer indicated.

Table 5. Mean Pharmacokinetic Parameters of Selected β_3 Adrenergic Agonists in Non Clinical Species[a]

compd	species	Cl_p mL/min/kg	$T_{1/2}$ (h)	PO AUC $_{total}$ (nM·h/mpk)[b]	PO AUC $_{unbound}$ (nM·h/mpk)[b]	*F* (%)	% unbound	rat LM Cl_{int} mL/min/kg	P_{app} ($\times 10^{-6}$ cm/s)
3[d]	rat	62	8	70	1	17	2	ND	ND
	dog	20	14	290	6	27	2		
	monkey	19	9	50	1	4	2		
4	rat	11	8	440	26	12	6	38	5
11a	rat	50	4	40	7	6	18	59	5
12a	rat	185	3	<LQ[c]	<LQ[c]	0	14	63	8
17[c]	rat	32	4	<LQ[c]	<LQ[c]	0	unstable	57	<1
18	rat	80	1	60	6	12	10	68	24
19	rat	33	2	480	91	41	19	<15	13[e]
	dog	27	6	980	686	67	70		
	monkey	36	2	90	90	8	100		
20	rat	87	1	30	2	6	5	135	26
21	rat	25	1	700	77	46	11	62	17
28	rat	34	2	420	ND	29	ND	ND	ND
29	rat	24	3	590	212	35	36	55	6[e]
	dog	34	8	1680	1176	100	70		
	monkey	36	4	120	120	11	100		

[a]Dose: iv, 1 mpk; po, 2 mpk. [b]Cassette dosing: iv, 0.13 mpk; po, 0.25 mpk. [c]<LQ = below limits of quantification. [d]Data taken from ref 24. [e]Transport was observed in control cells, hence permeability is likely overestimated. ND: not determined.

chlorine atom to the three position (such as that found in salabegron) resulted in a compound (**14**) equipotent to the phenyl analogue, however, selectivity over β_2-AR and the human Ether-à-go-go Related Gene (hERG) potassium channel is reduced. Replacement of the chlorine with a fluorine (**15**) resulted in a 4-fold increase in β_3-AR activity and also improved selectivity over hERG. Shift of the fluoro group to the 4-position (**16**) maintained β_3-AR activity but also resulted in reduced selectivity over hERG. Finally, replacement of the phenyl moiety with a 3-pyridyl (**17**) group led to a slight reduction in potency (EC_{50} = 0.37nM) with similar selectivity over hERG, however, this compound demonstrated no bioavailability (*F* = 0%).

We sought to improve the bioavailability in both the phenyl and pyridyl series by further examination of the thiazole RHS. Of particular interest was the replacement of the amino-group from the 2-position of the thiazole based upon the metabolism-induced toxicity potential of this class of heterocycles.[21] Transformation of the amino group to either a hydrogen (**18** and **19**) or a methyl group (**20** and **21**) resulted in a 3−4-fold loss in activity in both the phenyl and pyridyl cases, however, this was counterbalanced by much improved rat bioavailability in the pyridyl LHS analogues (*F* = 41% for **19** and 46% for **21**). Further homologation to an ethyl (**22** and **23**) or a cyclopropyl group (**24**) decreased potency further but more importantly rendered these analogues partial agonists. Replacement with a 4-fluorophenyl group either decreased potency still further (**25**) or led to a dramatic loss in receptor activation (**26**) and in both cases showed decreased selectivity over $\beta_{1/2}$-AR.

Finally, we examined replacement of the thiazole with alternative heterocycles (Table 4). In our previous work, we demonstrated that a pyrazole was an effective replacement for the thiazole.[15] Given the improved rat bioavailability observed with the pyridyl LHS in the thiazole series, we chose to explore thiazole replacement in this series. The parent pyrazole acetamide (**27**) showed significantly reduced β_3-AR potency (EC_{50} = 77nM) compared to **4**, however, good selectivity over β_1-AR and β_2-AR was maintained. As expected, the introduction of a methyl group at the α position furnished a compound (**28**) with improved β_3-AR activity (EC_{50} = 16.7nM) and which also had good rat bioavailability (*F* = 29%). Fusion of the α position to the pyrazole 5-position through a five-membered ring resulted in **29**, which had a further gain in β_3-AR activity (EC_{50} = 3.7nM) and also maintained good rat bioavailability (*F* = 35%). To try and understand what was driving the differences observed in bioavailability with these analogues, we examined intrinsic clearance (Cl_{int}) in liver microsomal (LM) incubations and

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

Table 6. Off-Target Activities of Selected β_3 Adrenergic Agonists

	P450 inhibition IC_{50}s (μM)[a]			ion channel IC_{50}s (μM)			
compd	2C9	2D6	3A4	hERG[b]	$hCa_v1.2$[c]	$hNa_v1.5$[d]	SERT[d] IC_{50} (nM)
4	>100	2.2	>100	11.2	8.2	>30	1613
11a	>100	18.3	86	12.7	>30	5.6	1218
19	>100	78.8	>100	6.1	>30	>30	422
21	67.2	>100	41.8	12	>30	>30	89
29	>100	>100	>100	>30	>30	>30	898

[a]Human liver microsome P450 marker enzyme activities with diclofenac α'-hydroxylation for 2C9, dextromethorphan *O*-demethylation for 2D6, and testosterone 6β-hydroxylation for 3A4. [b]IKr binding assay, ref 23. [c]Binding assay with [^{3}H]-diltiazem, ref 23. [d]Reference 23.

passive permeability (P_{app}) in MDCKII cells (Table 5). Examination of the in vitro intrinsic clearance alone did not appear to explain the differences in oral bioavailability in rats. Low passive permeability likely contributed to the low oral bioavailability of several compounds, however, compounds **19** and **29** had improved oral bioavailability despite low passive permeability, indicating the potential role of uptake transporters in absorption. In support of this hypothesis, a recent paper suggested mirabegron (**1**) also has low-to-moderate membrane permeability and P-gp is likely to be involved in its efflux and that it could be transported by intestinal influx transporters.[22]

On the basis of the β_3-AR activity and rat pharmacokinetic profiles, compounds **19**, **21**, and **29** were selected for further evaluation. Further examination of these analogues against ancillary targets (Table 6) demonstrated a clean P450 inhibition profile for all three analogues, with compound **21** being the least selective, showing an IC_{50} of 42 μM against CYP3A4. Binding at the hERG, human cardiac calcium channel, and human cardiac sodium channels was measured to examine possible cardiac liabilities of these compounds. All three compounds were clean (IC_{50} > 30 μM) at the calcium and sodium channels, however, **19** and **21** do show some inhibition of the hERG potassium channel (IC_{50} = 6 and 12 μM, respectively). Additional in vitro profiling in an extensive panel of receptor and ion channel binding and enzyme inhibition assays showed these compounds possessed activity at the serotonin transporter (SERT), the target of the selective serotonin reuptake inhibitor (SSRI) class of antidepressants. Compound **21** proved to be the least selective (77-fold), with compounds **19** and **29** being somewhat more selective (310- and 243-fold respectively).

The dog pharmacokinetic profiles of **19** and **29** revealed good oral bioavailabilities (F_{dog} = 67−100%). However, both compounds exhibit poor biovailability in rhesus monkeys (8−11%). Examination of the stability of these compounds in liver microsomes across a range of species (Table 7) indicates that **19** and **29** both have a much higher in vitro intrinsic clearance in monkey liver microsomes than in rat, dog, and human liver microsomes, which may lead to greater hepatic extraction in this species.

Table 7. In Vitro Intrinisc Clearance of Compounds 19 and 29 in Liver Microsomes

	intrinsic clearance (mL/min/kg body weight)			
compd	human	rat	dog	monkey
19	<10	<15	<10	205
29	19	55	31	261

A metabolism and excretion study was conducted in bile duct cannulated rats after oral dosing with ^{3}H-labeled **29** at 10 mg/kg. Urine and bile were collected for up to 48 h and analyzed by LC/MS/MS. **29** was well absorbed, with 73% of the administered dose recovered in urine and bile. **29** was eliminated renally (~50% of recovered dose) and through metabolism (~50% of recovered dose). This differs significantly from **3**, which was poorly absorbed and metabolized extensively, and hepatic extraction was the major route for clearance of this compound in rats.[14,24]

In comparison to our first-generation pyridylethanolamine β_3-AR agonist, **3**, both **19** and **29** demonstrated significantly different properties such as lower Log *D* (4.6 for **3**, compared to 0.9−1.2 for **19** and **29** at pH 7.3) reduced plasma protein binding (~2% unbound for **3**, compared to 20−100% unbound for **19** and **29**) and reduced in vivo intrinsic clearance (in rats estimated as ~4000 mL/min/kg for **3** compared to less than 200 mL/min/kg for **19** and **29**). The lower intrinsic clearance results in significantly improved unbound drug exposures in vivo for **19** and **29** compared to **3** (Table 5).

Compounds **19** and **29** were evaluated in an acetic acid induced bladder hyperactivity model in anesthetized rats (Figure 1).[25,26] Before conducting in vivo experiments, we confirmed the in vitro potency of these compounds at β_3-AR across a number of species as shown in Table 8. We observed significant differences between human and rat β_3-AR activity with **19** and **29**, which is expected based on previous observations.[26] The EC_{50} values for the rat β_3-AR of compounds **19** and **29** showed significantly reduced potency (**19**, 20-fold; **29**, 100-fold) relative to human β_3-AR activity. After intravenous dosing, both **19** and **29** caused a reduction in micturition pressure with a trend for dose dependence (Figure 3). Reductions in micturition pressure are an indirect measure of detrusor muscle relaxation. As anticipated by its greater potency at the rat β_3-AR, the thiazole analogue **19** showed a reduction in micturation pressure (59%) at a lower unbound plasma level (1.2 μM) than the pyrazole analogue **29**. Compound **29** was able to elicit a greater reduction in micturation pressure overall (69%) but at an unbound plasma level of 27 μM.

CONCLUSIONS

In summary, we have investigated changes to both the LHS aromatic and RHS heterocycle of a series of pyrrolidine containing β_3 adrenergic receptor agonists with the aim of addressing issues related to pharmacokinetics (e.g., low rat oral bioavailability) and potential metabolic/toxicological issues (e.g., toxicity potential of amino-thiazole, ion channel inhibition). To accomplish these goals, we developed a novel route to the pyrrolidine core to allow modification of the LHS aromatic ring. We also expanded the SAR in the pyrrolidine series by preparing conformationally restricted acetanilide RHS heterocycles. Excellent on-target potency was maintained with a number of examples; compounds **19** and **29** no longer contain an amino-thiazole group and demonstrate marked improvements in rat in

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

Table 8. β_3-AR Activity of Selected in Compounds in Different Species

	EC_{50} (nM)[a] (%Act)[b]			
compd	human	rat	dog	Rhesus
11a	0.15 ± 0.03 (103)	1.3 ± 0.39 (100)	0.19 (102)[c]	0.06 (78)[c]
19	1.4 ± 0.69 (103)	27.6 ± 6.9 (89)	5.0 (82)[c]	2.0 (98)[c]
29	3.7 ± 2.86 (84)	434 ± 171 (98)	22 (86) ± 12.41	4.7 (93) ± 3.19

[a]Unless otherwise noted, the results are shown as the mean ± SD ($n \geq 3$) or presented as the average of two experiments. [b]% Activation defined as the fitted maximal value of the test compound concentration response expressed as a percent of the maximal response to *R*-(−)-isoproterenol. [c]$n = 1$.

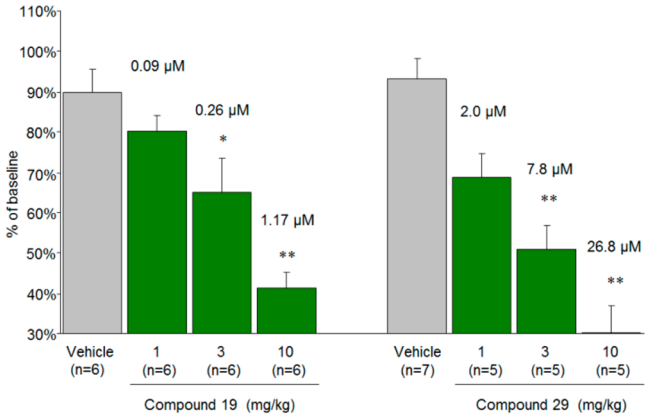


Figure 3. Reduction in micturation pressure produced by increasing doses of **19** and **29** in acetic acid induced bladder hyperactivity model with unbound plasma levels. *, $P < 0.05$, and **, $P < 0.01$, versus vehicle group. Each bar represents mean ± SEM.

vivo intrinsic clearance and unbound drug exposures compared to our original clinical candidate, **3**. Both compounds also demonstrated a good selectivity profile in vitro against a panel of receptors, ion channels and enzymes, which made them suitable candidates for additional in vivo preclinical studies. For the first time in this series, we were able to demonstrate an in vivo pharmocodynamic effect. In rats, compounds **19** and **29** were able to dose-dependently reduce bladder pressure following intravenous dosing, albeit at unbound plasma concentrations above their intrinsic EC_{50s}. This model is able to afford a rank order of potency which agrees with differences in in vitro activity. Additional SAR analysis, as well as in vivo efficacy of related compounds, will be disclosed in future articles.

EXPERIMENTAL SECTION

General. Unless otherwise stated, all reactions were carried out under an atmosphere of dry argon or nitrogen in dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 °C. All solvents and reagents were obtained from commercial sources and were used as received. Analytical thin layer chromatography (TLC) was performed with Whatman TLC plates (Partisil K6F, 60 Å, 250 μm). Visualization was accomplished by irradiation under a 254 nm UV lamp. Strong cation exchange was performed using Varian Bond Elut SCX columns. Chromatography on silica gel was carried out using prepacked silica gel cartridges using the indicated solvent system on either a Biotage Horizon flash chromatography system or a Teledyne ISCO Combiflash Companion flash chromatography system. If needed, products were purified by reverse phase chromatography, which was performed using a Gilson purification system employing a YMC Pro-Pac C18 column (5 μm, 20 mm × 100 mm). The mobile phase was a mixture of acetonitrile and H_2O, each containing 0.1% trifluoroacetic acid. If needed, products were purified by preparative TLC on silica gel gf precoated 20 cm × 20 cm 1000 μm from Analech. ^{1}H NMR spectra were recorded on a Varian Unity Inova AS500 500 MHz spectrometer. The reported compounds are of ≥95 HPLC area % purity, unless otherwise noted. Purity analysis was carried out by LC/MS analysis using an Agilent 1100 system fitted with a Waters Micromass ZQ2000 quadrupole spectrometer.[28]

(*R*)-2-(2-Aminothiazol-4-yl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)propanamide (7a) and (*S*)-2-(2-Aminothiazol-4-yl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-propanamide (7b). To a solution of (2*S*,5*R*)-*tert*-butyl 2-(4-aminobenzyl)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidine-1-carboxylate (**50a**) (100 mg, 0.26 mmol) and 2-(2-((*tert*-butoxycarbonyl)-amino)thiazol-4-yl)propanoic acid (**33a**) (71 mg, 0.26 mmol) in DMF (3 mL) was added EDC (100 mg, 0.52 mmol), HOBt (60 mg, 0.39 mmol), and triethylamine (0.146 mL, 1.05 mmol), and the resulting mixture stirred at room temperature for 4 h. The mixture was poured into water (15 mL) and extracted with ethyl acetate (3 × 5 mL). The combined extracts were washed with brine (15 mL), dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (Biotage Horizon: SNAP 10g: elution with gradient 0−50% ethyl acetate in hexanes) to give (2*S*,5*R*)-*tert*-butyl 2-(4-(2-(2-((*tert*-butoxycarbonyl)amino)thiazol-4-yl)propanamido)benzyl)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidine-1-carboxylate. The diastereoisomers were separated by preparative HPLC (Pirkle (R,R)-Whelk-O column: eluent 25% isopropyl alcohol in heptanes) to give first eluting isomer (2*S*,5*R*)-*tert*-butyl 2-(4-((*R*)-2-(2-((*tert*-butoxycarbonyl)-amino)thiazol-4-yl)propanamido)benzyl)-5-((*R*)-hydroxy(phenyl)-methyl)pyrrolidine-1-carboxylate (34 mg, 20%) and second eluting isomer (2*S*,5*R*)-*tert*-butyl 2-(4-((*S*)-2-(2-((*tert*-butoxycarbonyl)-amino)thiazol-4-yl)propanamido)benzyl)-5-((*R*)-hydroxy(phenyl)-methyl)pyrrolidine-1-carboxylate (26 mg, 16%). A mixture of (2*S*,5*R*)-*tert*-butyl 2-(4-((*R*)-2-(2-((*tert*-butoxycarbonyl)amino)thiazol-4-yl)-propanamido)benzyl)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidine-1-carboxylate (34 mg, 0.053 mmol) was dissolved in dichloromethane (0.5 mL) and trifluoroacetic acid (0.1 mL, 1.3 mmol) and the resulting mixture stirred at room temperature for 2 h. The mixture was evaporated and the free base formed by eluting through an SCX cartridge with 2 M ammonia in methanol and concentrating the filtrate. The residue was purified by preparative thin layer chromatography (elution with 0.5% ammonium hydroxide and 10% methanol in dichloromethane) and afforded the title compound **7a** (9.3 mg, 40%). LC/MS *m*/*e* 437.1 $(M + H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.49 (d, $J = 7.1$ Hz, 3H), 1.49−1.60 (m, 3H), 1.78−1.85 (m, 1H), 2.78 (dd, $J = 13.0$ and 6.8 Hz, 1H), 2.85 (dd, $J = 13.0$ and 7.3 Hz, 1H), 3.30−3.40 (m, 2H), 3.70 (q, $J = 7.1$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 6.35 (s, 1H), 7.19 (d, $J = 8.5$ Hz, 2H), 7.27 (t, $J = 6.9$ Hz, 1H), 7.31−7.37 (m, 4H), 7.50 (d, $J = 8.5$ Hz, 2H). In a similar manner, (2*S*,5*R*)-*tert*-butyl 2-(4-((*S*)-2-(2-((*tert*-butoxycarbonyl)amino)thiazol-4-yl)propanamido)benzyl)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidine-1-carboxylate (26 mg, 0.04 mmol) was deprotected to afford **8a** (6 mg, 34%). LC/MS *m*/*e* 437.1 $(M + H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.49 (d, $J = 7.1$ Hz, 3H), 1.49−1.60 (m, 3H), 1.78−1.85 (m, 1H), 2.78 (dd, $J = 13.0$ and 6.8 Hz, 1H), 2.85 (dd, $J = 13.0$ and 7.3 Hz, 1H), 3.30−3.40 (m, 2H), 3.70 (q, $J = 7.1$ Hz, 1H), 4.47 (d, $J = 8.0$ Hz, 1H), 6.35 (s, 1H), 7.19 (d, $J = 8.5$ Hz, 2H), 7.27 (t, $J = 6.9$ Hz, 1H), 7.31−7.37 (m, 4H), 7.50 (d, $J = 8.5$ Hz, 2H).

(*R*)-2-(2-Aminothiazol-4-yl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy-(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)butanamide (8a). Starting from aniline **50a** and intermediate **33b**, the procedures summarized above provided **8a**. LC/MS *m*/*e* 451.2 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.87 (t, $J = 7.5$ Hz, 3H), 1.27−1.34 (m, 2H), 1.38−1.44 (m, 1H), 1.57−1.64 (m, 1H), 1.74−1.82 (m, 1H), 1.84−1.93

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

(m, 1H), 2.58 (dd, J = 13.0 and 7.0 Hz, 1H), 2.67 (dd, J = 13.0 and 6.5 Hz, 1H), 3.12 (q, J = 7.5 Hz, 1H), 3.22 (quint, J = 7.0 Hz, 1H), 3.45 (dd, J = 9.0 and 6.5 Hz, 1H), 4.26 (d, J = 7.5 Hz, 1H), 6.26 (s, 1H), 6.88 (br s, 2H), 7.13 (d, J = 8.5 Hz, 2H), 7.22 (t, J = 7.0 Hz, 1H), 7.27−7.33 (m, 4H), 7.52 (d, J = 8.5 Hz, 2H), 9.94 (s, 1H).

(*R*)-2-(2-Aminothiazol-4-yl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)pentanamide (9a). Starting from aniline **50a** and intermediate **33c**, the procedures summarized above provided **9a**. LC/MS m/e 465.2 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 (t, J = 7.0 Hz, 3H), 1.22−1.36 (m, 4H), 1.38−1.46 (m, 1H), 1.56−1.65 (m, 1H), 1.68−1.76 (m, 1H), 1.82−1.90 (m, 1H), 2.58 (dd, J = 13.0 and 6.5 Hz, 1H), 2.68 (dd, J = 13.0 and 7.0 Hz, 1H), 3.14 (q, J = 7.0 Hz, 1H), 3.22 (quint, J = 7.0 Hz, 1H), 3.56 (t, J = 8.5 and 6.0 Hz, 1H), 4.27 (d, J = 7.5 Hz, 1H), 6.25 (s, 1H), 6.88 (br s, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.22 (t, J = 7.0 Hz, 1H), 7.27−7.34 (m, 4H), 7.51 (d, J = 8.5 Hz, 2H), 9.95 (s, 1H).

(*R*)-2-(2-Aminothiazol-4-yl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-3-methylbutanamide (10a). Starting from aniline **50a** and intermediate **33d**, the procedures summarized above provided **10a**. LC/MS m/e 465.3 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.80 (d, J = 4.5 Hz, 3H), 0.93 (d, J = 4.5 Hz, 3H), 1.32−1.41 (m, 2H), 1.41−1.49 (m, 1H), 1.60−1.68 (m, 1H), 2.23−2.32 (m, 1H), 2.63 (dd, J = 13.0 and 7.0 Hz, 1H), 2.76 (dd, J = 13.0 and 6.0 Hz, 1H), 3.18−3.25 (m, 2H), 3.28 (t, J = 6.5 Hz, 1H), 4.36 (d, J = 7.0 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 6.90 (br s, 2H), 7.12 (d, J = 8.0 Hz, 2H), 7.24 (t, J = 6.5 Hz, 1H), 7.27−7.35 (m, 4H), 7.51 (d, J = 8.0 Hz, 2H), 9.94 (s, 1H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (11a). Starting from aniline **50a** and intermediate **33e**, the procedures summarized above provided **11a**. LC/MS m/e 449.1 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25−1.33 (m, 2H), 1.36−1.44 (m, 1H), 1.55−1.63 (m, 1H), 2.45−2.53 (m, 1H), 2.53−2.60 (m, 2H), 2.62−2.70 (m, 2H), 2.74−2.81 (m, 1H), 3.10 (q, J = 7.0 Hz, 1H), 3.21 (quint, J = 7.0 Hz, 1H), 3.78 (dd, J = 8.0 and 6.0 Hz, 1H), 4.23 (d, J = 7.5 Hz, 1H), 6.86 (br s, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.21 (t, J = 7.0 Hz, 1H), 7.27−7.32 (m, 4H), 7.51 (d, J = 8.5 Hz, 2H), 10.00 (s, 1H).

(*S*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (11b). Starting from aniline **50a** and intermediate **33e**, the procedures summarized above provided **11b**. LC/MS m/e 449.1 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.20−1.35 (m, 2H), 1.35−1.45 (m, 1H), 1.51−1.64 (m, 1H), 2.43−2.60 (m, 3H), 2.60−2.72 (m, 2H), 2.74−2.85 (m, 1H), 3.08 (q, J = 7.0 Hz, 1H), 3.19 (quint, J = 7.0 Hz, 1H), 3.78 (m, 1H), 4.21 (d, J = 7.5 Hz, 1H), 6.86 (br s, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.21 (t, J = 7.0 Hz, 1H), 7.27−7.32 (m, 4H), 7.51 (d, J = 8.5 Hz, 2H), 10.00 (s, 1H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-4,5,6,7-tetrahydrobenzo[*d*]thiazole-4-carboxamide (12a). Starting from aniline **50a** and intermediate **33f**, the procedures summarized above provided **12a**. LC/MS m/e 463.0 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.27−1.37 (m, 2H), 1.39−1.48 (m, 1H), 1.57−1.69 (m, 2H), 1.87−1.98 (m, 3H), 2.44−2.54 (m, 2H), 2.59 (dd, J = 13.0 and 7.5 Hz, 1H), 2.71 (dd, J = 13.0 and 6.5 Hz, 1H), 3.17 (t, J = 7.5 Hz, 1H), 3.25 (quint, J = 7.0 Hz, 1H), 3.55 (m, 1H), 4.29 (d, J = 7.0 Hz, 1H), 6.74 (br s, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.22 (t, J = 6.5 Hz, 1H), 7.28−7.32 (m, 4H), 7.52 (d, J = 8.5 Hz, 2H), 10.03 (s, 1H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*d*]thiazole-4-carboxamide (13a). Starting from aniline **50a** and intermediate **33g**, the procedures summarized above provided **13a**. LC/MS m/e 477.0 $(M + H)^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.46−1.60 (m, 3H), 1.60−1.69 (m, 1H), 1.77−1.95 (m, 5H), 2.29−2.37 (m, 1H), 2.61 (dd, J = 13.5 and 6.0 Hz, 1H), 2.73−2.81 (m, 2H), 2.85 (dd, J = 13.5 and 7.0 Hz, 1H), 3.32−3.40 (m, 2H), 3.85 (dd, J = 6.5 and 2.5 Hz, 1H), 4.47 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 7.26 (t, J = 7.0 Hz, 1H), 7.31−7.36 (m, 4H), 7.48 (d, J = 8.5 Hz, 2H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-(3-chlorophenyl)(hydroxy)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (14). Starting from aniline **50b** and intermediate **33e**, the procedures summarized above provided **14**. LC/MS m/e 483.3/485.3 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19−1.28 (m, 1H), 1.30−1.38 (m, 1H), 1.38−1.46 (m, 1H), 1.54−1.61 (m, 1H), 2.44−2.58 (m, 3H), 2.61−2.69 (m, 2H), 2.73−2.80 (m, 1H), 3.11 (dd, J = 7.0 Hz, 1H), 3.19 (quint, J = 6.5 Hz, 1H), 3.77 (t, J = 6.0 Hz, 1H), 4.29 (d, J = 6.5 Hz, 1H), 6.85 (br s, 2H), 7.10 (d, J = 8.5 Hz, 2H), 7.25−7.33 (m, 3H), 7.38 (s, 1H), 7.50 (d, J = 8.5 Hz, 2H), 9.99 (s, 1H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-(3-fluorophenyl)(hydroxy)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (15). Starting from aniline **50c** and intermediate **33e**, the procedures summarized above provided **15**. LC/MS m/e 467.3 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25−1.38 (m, 2H), 1.41−1.48 (m, 1H), 1.56−1.63 (m, 1H), 2.44−2.60 (m, 3H), 2.62−2.70 (m, 2H), 2.73−2.80 (m, 1H), 3.12 (q, J = 7.0 Hz, 1H), 3.21 (quint, J = 7.5 Hz, 1H), 3.77 (m, 1H), 4.31 (d, J = 6.5 Hz, 1H), 6.85 (br s, 2H), 7.03 (td, J = 8.5 and 2.0 Hz, 1H), 7.11 (d, J = 8.5 Hz, 2H), 7.12−7.18 (m, 2H), 7.30−7.35 (m, 1H), 7.51 (d, J = 8.5 Hz, 2H), 10.01 (s, 1H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-(4-fluorophenyl)(hydroxy)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (16). Starting from aniline **50d** and intermediate **33e**, the procedures summarized above provided **16**. LC/MS m/e 467.3 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.21−1.33 (m, 2H), 1.34−1.42 (m, 1H), 1.55−1.62 (m, 1H), 2.44−2.59 (m, 3H), 2.62−2.69 (m, 2H), 2.73−2.80 (m, 1H), 3.10 (q, J = 7.0 Hz, 1H), 3.21 (quint, J = 7.0 Hz, 1H), 3.77 (m, 1H), 4.27 (d, J = 7.0 Hz, 1H), 6.85 (br s, 2H), 7.07−7.13 (m, 4H), 7.34 (dd, J = 8.5 and 6.0 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H), 10.00 (s, 1H).

(*R*)-2-Amino-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(pyridin-3-yl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (17). Starting from aniline **50e** and intermediate **33e**, the procedures summarized above provided **15**. LC/MS m/e 450.1 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19−1.26 (m, 1H), 1.30−1.44 (m, 2H), 1.56−1.62 (m, 1H), 2.45−2.58 (m, 3H), 2.62−2.70 (m, 2H), 2.74−2.81 (m, 1H), 3.15−3.25 (m, 2H), 3.77 (m, 1H), 4.34 (d, J = 7.0 Hz, 1H), 6.86 (br s, 2H), 7.11 (d, J = 8.5 Hz, 2H), 7.32 (dd, J = 8.0 and 5.0 Hz, 1H), 7.51 (d, J = 8.5 Hz, 2H), 7.72 (dt, J = 8.0 and 2.0 Hz, 1H), 8.43 (dd, J = 5.0 and 1.5 Hz, 1H), 8.53 (d, J = 2.0 Hz, 1H), 10.00 (s, 1H).

(*R*)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (18). Starting from aniline **50a** and intermediate **35**, the procedures summarized above provided **18**. LC/MS m/e 434.1 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.26−1.34 (m, 2H), 1.38−1.45 (m, 1H), 1.57−1.64 (m, 1H), 2.58 (dd, J = 13.5 and 7.0 Hz, 1H), 2.66−2.76 (m, 3H), 2.85−2.92 (m, 1H), 2.97−3.04 (m, 1H), 3.12 (q, J = 7.0 Hz, 1H), 3.23 (quin, J = 7.0 Hz, 1H), 4.06 (t, J = 7.5 Hz, 1H), 4.26 (d, J = 7.0 Hz, 1H), 7.14 (d, J = 8.5 Hz, 2H), 7.21 (t, J = 6.5 Hz, 1H), 7.27−7.33 (m, 4H), 7.53 (d, J = 8.5 Hz, 2H), 8.92 (s, 1H), 10.20 (s, 1H).

(*R*)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(pyridin-3-yl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (19). Starting from aniline **50e** and intermediate **35**, the procedures summarized above provided **19**. LC/MS m/e 435.2 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.35−1.54 (m, 3H), 1.64−1.71 (m, 1H), 2.66 (dd, J = 13.0 and 7.5 Hz, 1H), 2.73 (q, J = 7.0 Hz, 2H), 2.81 (dd, J = 13.0 and 5.5 Hz, 1H), 2.86−2.92 (m, 1H), 2.97−3.04 (m, 1H), 4.06 (t, J = 7.0 Hz, 1H), 4.51 (d, J = 7.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 2H), 7.35 (dd, J = 7.5 and 5.0 Hz, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 7.5 Hz, 1H), 8.46 (d, J = 5.0 Hz, 1H), 8.56 (s, 1H), 8.92 (s, 1H), 10.24 (s, 1H).

(*R*)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(phenyl)methyl)pyrrolidin-2-yl)methyl)phenyl)-2-methyl-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (20). Starting from aniline **50a** and intermediate **38a**, the procedures summarized above provided **20**. LC/MS m/e 448.1 $(M + H)^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25−1.33 (m, 2H), 1.36−1.44 (m, 1H), 1.56−1.63 (m, 1H), 2.57 (dd, J = 13.5 and 6.5 Hz, 1H), 2.60 (s, 3H), 2.63−2.69 (m, 3H), 2.80−2.87 (m, 1H), 2.91−2.99 (m, 1H), 3.10 (q, J = 7.0 Hz, 1H), 3.21 (quint, J = 7.0 Hz, 1H), 3.98 (t, J = 7.5 Hz, 1H), 4.23 (d, J = 7.5 Hz, 1H), 7.13 (d, J = 8.5 Hz,

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

2H), 7.21 (t, J = 7.0 Hz, 1H), 7.27−7.32 (m, 4H), 7.53 (d, J = 8.5 Hz, 2H), 10.16 (s, 1H).

(*R*)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(pyridin-3-yl)methyl)-pyrrolidin-2-yl)methyl)phenyl)-2-methyl-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (21). Starting from aniline **50e** and intermediate **38a**, the procedures summarized above provided **21**. LC/MS m/e 449.1 (M + H)$^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.17−1.16 (m, 1H), 1.29−1.36 (m, 1H), 1.36−1.44 (m, 1H), 1.55−1.62 (m, 1H), 2.55 (dd, J = 13.0 and 7.0 Hz, 1H), 2.60 (s, 3H), 2.61−2.69 (m, 3H), 2.80−2.87 (m, 1H), 2.91−2.99 (m, 1H), 3.14−3.24 (m, 2H), 3.98 (t, J = 7.5 Hz, 1H), 4.34 (d, J = 7.5 Hz, 1H), 7.13 (d, J = 8.5 Hz, 2H), 7.32 (dd, J = 7.5 and 5.0 Hz, 1H), 7.53 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 7.5 Hz, 1H), 8.43 (dd, J = 5.0 and 1.5 Hz, 1H), 8.53 (d, J = 1.5 Hz, 1H), 10.16 (s, 1H).

(*R*)-2-Ethyl-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)methyl)-pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]-thiazole-4-carboxamide (22). Starting from aniline **50a** and intermediate **38b**, the procedures summarized above provided **22**. LC/MS m/e 462.0 (M + H)$^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.33 (t, J = 7.5 Hz, 3H), 1.48−1.61 (m, 3H), 1.79−1.87 (m, 1H), 2.70−2.95 (m, 5H), 2.98 (q, J = 7.5 Hz, 2H), 3.01−3.09 (m, 1H), 3.34−3.43 (m, 2H), 3.98 (m, 1H), 4.49 (d, J = 7.5 Hz, 1H), 7.21 (d, J = 8.5 Hz, 2H), 7.26 (m, 1H), 7.31−7.38 (m, 4H), 7.54 (d, J = 8.5 Hz, 2H).

(*R*)-2-Ethyl-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(pyridin-3-yl)-methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (23). Starting from aniline **50e** and intermediate **38b**, the procedures summarized above provided **23**. LC/MS m/e 463.3 (M + H)$^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19−1.27 (m, 1H), 1.23 (t, J = 7.5 Hz, 3H), 1.30−1.44 (m, 2H), 1.55−1.62 (m, 1H), 2.55 (dd, J = 13.0 and 7.0 Hz, 1H), 2.62−2.68 (m, 3H), 2.79−2.86 (m, 1H), 2.91 (q, J = 7.5 Hz, 2H), 2.92−2.99 (m, 1H), 3.16−3.25 (m, 2H), 3.98 (t, J = 7.0 Hz, 1H), 4.35 (d, J = 7.0 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 7.31 (dd, J = 7.5 and 5.0 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.71 (dd, J = 7.5 and 2.0 Hz, 1H), 8.43 (dd, J = 5.0 and 1.5 Hz, 1H), 8.52 (d, J = 2.0 Hz, 1H), 10.16 (s, 1H).

(*R*)-2-Cyclopropyl-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(pyridin-3-yl)-methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (24). Starting from aniline **50e** and intermediate **38c**, the procedures summarized above provided **24**. LC/MS m/e 475.3 (M + H)$^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.81−0.88 (m, 2H), 1.00−1.06 (m, 2H), 1.18−1.30 (m, 2H), 1.31−1.46 (m, 2H), 1.56−1.64 (m, 1H), 2.25−2.32 (m, 1H), 2.52−2.71 (m, 3H), 2.76−2.83 (m, 1H), 2.87−2.96 (m, 1H), 3.18−3.27 (m, 2H), 3.93 (dd, J = 7.5 and 6.0 Hz, 1H), 4.38 (d, J = 7.0 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 7.32 (dd, J = 8.0 and 5.0 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.72 (dt, J = 8.0 and 2.0 Hz, 1H), 8.43 (dd, J = 5.0 and 1.5 Hz, 1H), 8.53 (d, J = 2.0 Hz, 1H), 10.13 (s, 1H).

(*R*)-2-(4-Fluorophenyl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(phenyl)-methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (25). Starting from aniline **50a** and intermediate **38d**, the procedures summarized above provided **25**. LC/MS m/e 528.1 (M + H)$^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.22−1.31 (m, 2H), 1.35−1.42 (m, 1H), 1.54−1.62 (m, 1H), 2.56 (dd, J = 13.0 and 6.5 Hz, 1H), 2.63 (dd, J = 13.0 and 6.5 Hz, 1H), 2.67−2.77 (m, 2H), 2.90−2.99 (m, 1H), 3.03−3.10 (m, 1H), 3.19 (quint, J = 6.5 Hz, 1H), 4.09 (t, J = 7.5 Hz, 1H), 4.21 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 8.5 Hz, 2H), 7.21 (t, J = 7.0 Hz, 1H), 7.26−7.32 (m, 6H), 7.55 (d, J = 8.5 Hz, 2H), 7.90 (dd, J = 8.5 and 5.5 Hz, 2H), 10.24 (s, 1H).

(*R*)-2-(4-Fluorophenyl)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-hydroxy(pyridin-3-yl)methyl)pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxamide (26). Starting from aniline **50e** and intermediate **38d**, the procedures summarized above provided **25**. LC/MS m/e 529.0 (M + H)$^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.45−1.62 (m, 3H), 1.77−1.85 (m, 1H), 2.75 (dd, J = 13.0 and 7.0 Hz, 1H), 2.79−2.92 (m, 3H), 2.96−3.04 (m, 1H), 3.10−3.18 (m, 1H), 3.30−3.38 (m, 2H), 4.12 (t, J = 7.0 Hz, 1H), 4.56 (d, J = 7.5 Hz, 1H), 7.18 (t, J = 9.0 and 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.42 (dd, J = 7.5 and 5.0 Hz, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.86 (d, J = 8.0 Hz, 1H), 7.92 (dd, J = 8.5 and 5.0 Hz, 2H), 8.44 (d, J = 5.0 Hz, 1H), 8.55 (s, 1H).

(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(pyridin-3-yl)methyl)pyrrolidin-2-yl)methyl)phenyl)-2-(1*H*-pyrazol-1-yl)acetamide, TFA Salt (27). Starting from aniline **50e** and 1*H*-pyrazol-1-ylacetic acid **57a**, the procedures summarized above provided **27**. LC/MS m/e 392.1 (M + H)$^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.81−1.90 (m, 1H), 1.90−2.00 (m, 2H), 2.05−2.14 (m, 1H), 2.96 (dd, J = 13.7 and 8.7 Hz, 1H), 3.13 (dd, J = 13.7 and 6.5 Hz, 1H), 3.73−3.81 (m, 1H), 3.84−3.91 (m 1H), 5.00 (m, 3H), 6.55 (t, J = 2.2 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 7.55 (m, 3H), 7.71 (d, J = 2.2, 1H), 7.86 (dd, J = 8.0 and 5.4 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.72 (dd, J = 5.4 and 1.0 Hz, 1H), 8.85 (d, J = 1.9 Hz, 1H).

(*S*)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(pyridin-3-yl)methyl)-pyrrolidin-2-yl)methyl)phenyl)-2-(1*H*-pyrazol-1-yl)-propanamide, TFA Salt (28). Starting from aniline **50e** and 2-(1*H*-pyrazol-1-yl)propanoic acid **57b**, the procedures summarized above provided **27**. LC/MS m/e 406.2 (M + H)$^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.62−1.78 (m, 3H), 1.67 (d, J = 7.0 Hz, 3H), 1.82−1.90 (m, 1H), 2.84 (dd, J = 13.5 and 9.4 Hz, 1H), 3.07 (dd, J = 13.5 and 5.5 Hz, 1H), 3.56−3.66 (m, 1H), 3.68−3.78 (m 1H), 4.83 (d, J = 8.2 Hz, 1H), 5.19 (q, J = 7.0 Hz, 1H), 6.28 (t, J = 2.1 Hz, 1H), 7.18 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 1.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.57 (dd, J = 8.0 and 5.1 Hz, 1H), 7.85 (d, J = 2.1 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 8.50 (q, J = 8.2 Hz, 1H), 8.62 (q, J = 5.1 and 1.4 Hz, 1H) 8.70 (d, J = 1.8 Hz, 1H), 9.47 (t, J = 4.8 Hz, 1H), 10.33 (s, 1H).

(*S*)-*N*-(4-(((2*S*,5*R*)-5-((*R*)-Hydroxy(pyridin-3-yl)methyl)-pyrrolidin-2-yl)methyl)phenyl)-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]-pyrazole-6-carboxamide, TFA Salt (29). Starting from aniline **50e** and intermediate **56**, the procedures summarized above provided **27**. LC/MS m/e 418.4 (M + H)$^+$. ^{1}H NMR (500 MHz, CD_3OD) δ 1.81−1.95 (m, 3H), 2.06−2.13 (m, 1H), 2.70−2.77 (m, 1H), 2.89−3.08 (m, 4H), 3.14 (dd, J = 13.5 and 6.1 Hz, 1H), 3.78 (quint, J = 7.1 Hz, 1H), 3.85 (q, J = 8.2 Hz, 1H), 4.94 (d, J = 8.0 Hz, 1H), 5.01 (dd, J = 8.2 and 4.4 Hz, 1H), 6.06 (d, J = 1.6 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 7.53 (d, 1.6 Hz, 1H), 7.58 (d, J = 8.5 Hz, 2H), 7.71 (dd, J = 8.0 and 5.3 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.65 (dd, J = 5.3 and 1.4 Hz, 1H), 8.76 (d, J = 1.9 Hz, 1H).

General Procedure for the Synthesis of Thiazole Acids 33a−g. *Step A: Synthesis of **31a−g**.* Bromine was added in a dropwise manner to a solution of keto-esters (**30a−g**) in chloroform at 0 °C and the resulting mixture stirred at 0 °C for 30 min. Air was blown through the mixture for 1 h, and the mixture was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in ethanol and thiourea added and the resulting mixture heated at reflux overnight. The mixture was cooled and concentrated under reduced pressure and the residue partitioned between CH_2Cl_2 and satd $NaHCO_3$, and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 100% EtOAc gradient) to afford thiazoles **31a−g**.

*Step B: Synthesis of Boc-amino-thiazoles (**32a−g**).* To a solution of aminothiazoles **31a−g** in CH_2Cl_2 was added Boc-anhydride, Et_3N, and DMAP and the resulting mixture stirred at room temperature overnight. The reaction mixture was washed with 1N HCl and satd NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified by flash chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 40% EtOAc gradient) to afford the products **32a−g**.

*Step C: Synthesis of Thiazole Acids (**33a−g**).* To a solution of ethyl esters **32a−g** in a mixture of THF and methanol was added lithium hydroxide solution and the resulting mixture stirred at room temperature overnight. The organics solvents were removed by evaporation and the aqueous residue diluted with more water and extracted with EtOAc. The aqueous layer acidified by the addition of conc HCl, extracted with EtOAc, and the organic layer dried over $MgSO_4$ and concentrated under reduced pressure. The residue was triturated with acetonitrile, filtered, and dried to give the products **33a−g**.

*2-(2-((tert-Butoxycarbonyl)amino)thiazol-4-yl)propanoic Acid (**33a**).* Starting from ethyl 2-methyl-3-oxo-butanoate **30a** and following the procedures above provided compound **33a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56 (s, 9H), 1.57 (d, J = 7.4 Hz, 3H), 3.84 (q, J = 7.4 Hz, 1H), 6.69 (s, 1H), 11.05 (br s, 1H).

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437−1453

*2-(2-((tert-Butoxycarbonyl)amino)thiazol-4-yl)butanoic Acid (**33b**).* Starting from ethyl 2-ethyl-3-oxobutyrate **30b** and following the procedures above provided compound **33b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, J = 7.5 Hz, 3H), 1.54 (s, 9H), 1.92 (m, 1H), 2.13 (m, 1H), 3.67 (t, J = 7.5 Hz, 1H), 6.74 (s, 1H), 11.58 (br s, 1H).

*2-(2-((tert-Butoxycarbonyl)amino)thiazol-4-yl)pentanoic Acid (**33c**).* Starting from ethyl 2-propyl-3-oxobutanoate **30c** and following the procedures above provided compound **33c**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (t, J = 7.5 Hz, 3H), 1.36 (m, 2H), 1.55 (s, 9H), 1.86 (m, 1H), 2.06 (m, 1H), 3.73 (t, J = 7.5 Hz, 1H), 6.72 (s, 1H), 11.70 (br s, 1H).

*2-(2-((tert-Butoxycarbonyl)amino)thiazol-4-yl)-3-methylbutanoic Acid (**33d**).* Starting from ethyl 2-isopropyl-3-oxobutanoate **30d** and following the procedures above provided compound **33d**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (d, J = 6.5 Hz, 3H), 1.10 (d, J = 6.5 Hz, 3H), 1.54 (s, 9H), 2.41 (m, 1H), 3.48 (d, J = 9.5 Hz, 1H), 6.81 (s, 1H), 10.70 (br s, 1H).

*2-((tert-Butoxycarbonyl)amino)-5,6-dihydro-4H-cyclopenta[d]-thiazole-4-carboxylic Acid (**33e**).* Starting from ethyl 2-oxo-cyclopentanecarboxylate **30e** and following the procedures above provided compound **33e**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.55 (s, 9H), 2.71 (m, 1H), 2.88 (m, 2H), 3.05 (m, 1H), 3.97 (m, 1H), 10.98 (br s, 1H).

*2-((tert-Butoxycarbonyl)amino)-4,5,6,7-tetrahydrobenzo[d]-thiazole-4-carboxylic Acid (**33f**).* Starting from ethyl 2-oxo-cyclohexanecarboxylate **30f** and following the procedures above provided compound **33f**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.55 (s, 9H), 1.87 (m, 1H), 2.00 (m, 1H), 2.06 (m, 1H), 2.25 (m, 1H), 2.65 (m, 1H), 2.72 (m, 1H), 3.70 (t, J = 5.5 Hz, 1H), 11.53 (br s, 1H).

*2-((tert-Butoxycarbonyl)amino)-5,6,7,8-tetrahydro-4H-cyclohepta[d]thiazole-4-carboxylic Acid (**33g**).* Starting from ethyl 2-oxo-cycloheptanecarboxylate **30g** and following the procedures above provided compound **33g**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (s, 9H), 1.57 (m, 1H), 1.85 (m, 1H), 2.00 (m, 3H), 2.31 (m, 1H), 2.74 (m, 1H), 2.86 (m, 1H), 4.00 (t, J = 4.6 Hz, 1H), 11.23 (br s, 1H).

Ethyl 5,6-Dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxylate (34). To a solution of amino-thiazole **31e** (9g, 42.4 mmol) in anhydrous DMF (100 mL) warmed at 50 °C was added dropwise a solution of *tert*-butyl nitrite (5.5 mL, 46.6 mmol). After addition was complete, the mixture was heated at 50 °C and continued for 30 min. The mixture was cooled and partitioned between EtOAc (300 mL) and water (500 mL); the aqueous layer was separated and washed with water (2 × 400 mL) and satd NaCl (100 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification flash chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 50% EtOAc in hexanes gradient) gave the desired product (1.8 g, 21% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (t, J = 7.0 Hz, 3H), 2.87 (m, 2H), 2.94 (m, 1H), 3.10 (m, 1H), 4.05 (t, J = 7.0 Hz, 1H), 4.23 (q, J = 7.0 Hz, 2H), 8.69 (s, 1H).

5,6-Dihydro-4*H*-cyclopenta[*d*]thiazole-4-carboxylic Acid (35). To a solution of thiazole ester **34** (1.8g, 9.13 mmol) in a mixture of THF (30 mL) and methanol (10 mL) was added lithium hydroxide (10 mL of a 1 M aqueous solution, 10 mmol) and the resulting mixture stirred at room temperature for 2 h. The organics were removed by evaporation in vacuo and the aqueous residue diluted with water (25 mL). The mixture was extracted with EtOAc (×2) and the organic layes discarded. The remaining aqueous was acidified to pH ~ 4 with 1N HCl and extracted with EtOAc (×3). The combined EtOAc layers were washed with satd NaCl, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was triturated with acetonitrile, filtered, and dried to give 900 mg (58%) as an off white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.64 (m, 1H), 2.74 (m, 1H), 2.86 (m, 1H), 2.96 (m, 1H), 3.86 (t, J = 7.0 Hz, 1H), 8.92 (s, 1H), 12.44 (s, 1H).

General Procedure for the Synthesis of Thiazole Acids 38a–d. *Step A: Synthesis of **37a–d**.* To a solution of ethyl 2-oxocyclopentanecarboxylate in anhydrous chloroform cooled at 0 °C was added bromine dropwise over 15 min. After complete addition the mixture was allowed to warm to room temperature and stirred overnight. Air was bubbled through mixture for 1 h and then washed with satd $NaHCO_3$ and satd NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was taken up in ethanol (100 mL) and thioacetamide added and the resulting mixture stirred at room temperature for 1 h then heated at reflux overnight. The mixture was cooled evaporated in vacuo and the residue partitioned between satd $NaHCO_3$ and CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 20% EtOAc in hexanes gradient) afforded thiazoles **37a–d**.

Step B. To a solution of esters **37a–d** in a mixture of THF and methanol was added lithium hydroxide solution and the resulting mixture stirred at room temperature for 5 h. Organic solvents removed by evaporation and the remaining aqueous extracted with diethyl ether (100 mL). The aqueous layer was acidified by the addition of 1N HCl and extracted with CH_2Cl_2 (3×); combined CH_2Cl_2 extracts dried over $MgSO_4$ and concentrated in vacuo. The residue was triturated with acetonitrile, filtered, and dried to give thiazole acids **38a–d**.

*2-Methyl-5,6-dihydro-4H-cyclopenta[d]thiazole-4-carboxylic Acid (**38a**).* Starting from thioacetamide (**36a**) following the procedures above provided compound **38a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.73 (s, 3H), 2.82 (m, 3H), 3.04 (m, 1H), 4.03 (m, 1H), 8.53 (br s, 1H).

*2-Ethyl-5,6-dihydro-4H-cyclopenta[d]thiazole-4-carboxylic Acid (**38b**).* Starting from thiopropionamide (**36b**) following the procedures above provided compound **38b**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (t, J = 7.5 Hz, 3H), 2.84 (m, 3H), 2.82 (m, 3H), 3.06 (m, 3H), 4.04 (dd, J = 8.5 and 7.0 Hz, 1H), 10.14 (br s, 1H).

*2-Cyclopropyl-5,6-dihydro-4H-cyclopenta[d]thiazole-4-carboxylic Acid (**38c**).* Starting from cyclopropanethiocarboxamide (**36c**) following the procedures above provided compound **38c**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04 (m, 2H), 1.14 (m, 2H), 2.32 (m, 1H), 2.80 (m, 2H), 2.88 (m, 1H), 3.02 (m, 1H), 4.01 (t, J = 8.0 and 6.0 Hz, 1H), 7.83 (br s, 1H).

*2-(4-Fluorophenyl)-5,6-dihydro-4H-cyclopenta[d]thiazole-4-carboxylic Acid (**38d**).* Starting from 4-fluorothiobenzamide (**36d**) following the procedures above provided compound **38d**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.62 (m, 1H), 2.68 (m, 1H), 2.87 (m, 1H), 2.98 (m, 1H), 4.04 (dd, J = 8.5 and 5.5 Hz, 1H), 7.29 (t, J = 9.0 and 8.5 Hz, 2H), 7.90 (dd, J = 9.0 and 5.5 Hz, 2H).

(4*S*)-3-Hex-5-ynoyl-4-benzyl-1,3-oxazolidin-2-one (40). To a stirred solution of 5-hexynoic acid (69 g, 0.62 mol) and triethylamine (214 mL, 1.54 mol) in THF (1.0 L) at −25 °C was added neat 2,2-dimethylpropanoyl chloride (83 mL, 0.68 mol) dropwise over 20 min. The resulting mixture was stirred at −25 °C for 2 h, then solid lithium chloride (28.7 g, 0.677 mol) was added followed by (*S*)-4-benzyl-1,3-oxazolidin-2-one (101 g, 0.615 mol). The reaction mixture then allowed to warm to room temperature, stirred for an additional 12 h, then evaporated in vacuo to remove all volatiles. The off-white residue was diluted with water (400 mL) and extracted with ethyl acetate (3 × 300 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo to afford an off-white solid. The solid was recrystallized from an ethyl acetate and hexanes mixture to afford the desired compound as a white solid (135 g, 85%). LC/MS (EI) m/e: 272.3 $(M + H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.89–1.95 (m, 2H), 1.99 (t, J = 2.8 Hz, 1H), 2.32 (td, J = 7.1 and 2.8 Hz, 2H), 2.77 (dd, J = 13.5 and 9.6 Hz, 1H), 3.01–3.13 (m, 2H), 3.28 (dd, J = 13.2 and 3.2 Hz, 1H), 4.16–4.22 (m, 2H), 4.65–4.69 (m, 1H), 7.20 (d, J = 7.1 Hz, 2H), 7.27 (m, 1H), 7.33 (t, J = 7.6 and 6.8 Hz, 2H).

General Procedure for the Synthesis of Anilines 50a–d. *Step A: Synthesis of **41a–e**.* To a stirred solution of (4*S*)-3-hex-5-ynoyl-4-benzyl-1,3-oxazolidin-2-one **40** in ethyl acetate at room temperature was added sequentially triethylamine, solid magnesium(II) chloride, benzaldehyde, and chlorotrimethylsilane. After complete addition, the resulting mixture was stirred for 3 days. The reaction mixture was filtered through silica gel, which was subsequently washed with ethyl acetate. The filtrate was evaporated in vacuo, and the residue was suspended in a a mixture of ethyl acetate and methanol and stirred for 5 h, during which time all solids dissolved. The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography on a Biotage Horizon system (silica gel, 10% EtOAc in hexanes to 25% EtOAc in hexanes gradient) to afford the pure addition products **41a–e** as yellow/orange oils.

Step B: General Procedure for tert-Butyl-dimethylsilyl (TBS) Protection. To a solution of alcohols **41a–e** in CH_2Cl_2 cooled at 0 °C was added 2,6-lutidine followed by *tert*-butyldimethylsilyl trifluor-

dx.doi.org/10.1021/jm4017224 | J. Med. Chem. 2014, 57, 1437–1453

omethanesulfonate and the resulting mixture allowed to warm to room temperature and stirred overnight, diluted with satd NH_4Cl, and washed with 1N HCl, water and satd NaCl, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by column chromatography on a Biotage Horizon system (silica gel, 5% EtOAc in hexanes to 10% EtOAc in hexanes gradient) to give protected alcohols **42a−e** as oils.

Step C: General Procedure for Oxazolidinone Cleavage. To a solution of oxazolidinones **42a−e** in a mixture of THF and water cooled at 0 °C was added slowly 35% H_2O_2, keeping the internal temperature below 5 °C, followed by slow addition of an aqueous solution of LiOH. The resulting mixture was stirred overnight warming up to 15 °C in the process. The reaction was quenched by the addition of Na_2SO_3. The THF was removed by evaporation and the remaining aqueous cooled (a precipitate formed on cooling) and acidified to pH = 5 with 1N HCl, extracted with EtOAc (3×), combined EtOAc layers washed with satd NaCl, dried ($MgSO_4$), and evaporated in vacuo. A solid formed in the residue on standing which was diluted with hexanes and filtered. The filtrate evaporated and residue purified by column chromatography on a Biotage Horizon system (silica gel, 10% EtOAc in hexanes + 3% AcOH) to afford acids **43a−e** as a colorless oils.

Step D: General Procedure for Curtius Rearrangement. To a solution of acid **43a−e** in toluene was added triethylamine then diphenyl azidophosphate, and the resulting solution was stirred at ambient temperature for 5 h. Neat (4-methoxyphenyl)methanol was then added, and the reaction mixture was slowly heated to 80 °C for 1 h, during which time a vigorous evolution of nitrogen gas was observed. The reaction was then heated to 110 °C for 12 h, cooled to room temperature, and diluted with a saturated aqueous sodium bicarbonate solution. The phases were separated, and the aqueous layer was extracted with ethyl acetate (2 × 250 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo. The crude residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 25% EtOAc in hexanes gradient) to afford the desired compounds **44a−e** as colorless oils.

Step E: General Procedure for Sonagashira Coupling. To a solution of acetylenes **44a−e** and 1-bromo-4-nitrobenzene in DMF was added triethylamine, tetrakis(triphenylphosphine)palladium(0), and copper iodide. The mixture was degassed, flushed with nitrogen three times, then heated to 80 °C for 2 h. The reaction mixture was cooled, diluted with water, and extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 25% EtOAc in hexanes, gradient) to afford the desired coupled products **45a−e** as a orange oils.

Step F: General Procedure for Ketone Formation. To a solution of acetylenes **45a−e** in DMF was added pyrrolidine, and the resulting mixture was heated to 80 °C for 2 h. The reaction mixture was cooled to room temperature, diluted with a 10% aqueous acetic acid solution, and stirred for 3 h. The reaction mixture was then added to water and extracted with ethyl acetate (3×). The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 25% EtOAc in hexanes, gradient) to afford the desired ketones **46a−e** as yellow oils.

Step G: General Procedure for Pyrrolidine Formation. To a solution of ketones **46a−e** in dichloromethane was added trifluoroacetic acid and the resulting mixture stirred at room temperature for 1.5 h. The resulting mixture was evaporated in vacuo and the residue partitioned between dichloromethane and a saturated aqueous sodium bicarbonate solution. The phases were separated and the aqueous phase extracted with dichloromethane (2×). The combined organic phases were dried over $MgSO_4$ and evaporated in vacuo. The residue was then dissolved in dichloromethane cooled to 0 °C, and solid sodium triacetoxyborohydride was then added. The resulting mixture was allowed to warm to room temperature with stirring for 12 h. The reaction mixture was then carefully quenched with a saturated aqueous sodium bicarbonate solution, and the biphasic solution was stirred for 6 h. The phases were separated, and the aqueous phase extracted with dichloromethane (2×). The combined organic phases were dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100 hexanes to 20% EtOAc in hexanes gradient) to afford the desired pyrrolidines **47a−e** as clear oils.

Step H: General Procedure for tert-Butyloxycarbonyl (Boc)-Protection. To a solution of pyrrolidines **47a−e** in dichloromethane was added di-*tert*-butyl dicarbonate and *N*,*N*-diisopropylethylamine and the resulting mixture stirred at room temperature overnight. The reaction was then washed sequentially with a 1.0 M aqueous citric acid solution, water, a saturated aqueous sodium bicarbonate solution, and brine. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 15% EtOAc in hexanes gradient) to afford the desired protected pyrrolidines **48a−e** as white foams.

Step I: General Procedure for Nitro Group Reduction. To a nitrogen flushed solution of nitro compounds **44a**,**c−d** in methanol was added 10% palladium on carbon and the resulting mixture stirred under a balloon of hydrogen gas for 12 h. The reaction mixture was filtered through a plug of Celite, and the plug was washed with methanol. The combined filtrates were concentrated in vacuo to afford the desired anilines **49a**,**c−d** as a white foam.

Step J: General Procedure for tert-Butyl(dimethyl)silyl (TBS) Deprotection. To a solution of *tert*-butyl(dimethyl)silyl protected alcohols **49a−d** in tetrahydrofuran was added a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran and the resulting mixture heated at 55 °C for 2 h. After cooling to room temperature, the mixture was evaporated in vacuo and partitioned between ethyl acetate and water. The aqueous phase was extracted with ethyl acetate (2×), and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 75% EtOAc in hexanes gradient) to afford the desired alcohols **50a−d** as white foams.

*(2S,5R)-tert-Butyl 2-(4-Aminobenzyl)-5-((R)-hydroxy(phenyl)-methyl)pyrrolidine-1-carboxylate (**50a**).* Starting from benzaldehyde, the procedures summarized above provided compound **50a**. LC/MS (EI) *m*/*e*: 383.2 $(M + H)^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 1.49−1.48 (m, 2H), 1.55 (s, 9H), 1.70−1.63 (m, 2H), 2.51 (dd, *J* = 13.3 and 8.9 Hz, 1H), 3.01 (br d, *J* = 2.1 Hz, 1H), 3.62 (br s, 2H), 4.13−4.06 (m, 2H), 4.43 (br s, 1H), 6.19 (br s, 1 H), 6.67 (d, *J* = 8.2 Hz, 2H), 7.00 (d, *J* = 8.2 Hz, 2H), 7.36−7.29 (m, 5H).

*(2S,5R)-tert-Butyl 2-(4-Aminobenzyl)-5-((R)-(3-chlorophenyl)-(hydroxy)methyl)pyrrolidine-1-carboxylate (**50b**).* Starting from 3-chlorobenzaldehyde, the procedures summarized above provided compound **50b**. ^1H NMR (500 MHz, $CDCl_3$) δ 1.46 (m, 2H), 1.55 (s, 9H), 1.67 (m, 2H), 2.51 (dd, *J* = 13.5 and 9.0 Hz, 1H), 2.96 (br d, *J* = 10.0 Hz, 1H), 3.64 (br s, 2H), 4.06 (m, 2H), 4.39 (m, 1H), 6.33 (br s, 1 H), 6.67 (d, *J* = 8.0 Hz, 2H), 6.99 (d, *J* = 8.0 Hz, 2H), 7.22−7.30 (m, 3H), 7.37 (s, 1H).

*(2S,5R)-tert-Butyl 2-(4-Aminobenzyl)-5-((R)-(3-fluorophenyl)-(hydroxy)methyl)pyrrolidine-1-carboxylate (**50c**).* Starting from 3-fluorobenzaldehyde, the procedures summarized above provided compound **50c**. LC/MS (EI) *m*/*e*: 401 $(M + H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 1.24−1.54 (m, 11H), 1.60−1.81 (m, 2H), 2.62 (m, 1H), 3.86 (s, 1H), 4.03 (d, *J* = 6.0 Hz, 2H), 4.84 (m, 1H), 6.57 (d, *J* = 7.8 Hz, 2H), 6.74 (d, *J* = 7.3 Hz, 2H), 6.96 (t, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 10.0 Hz, 1H) 7.10 (d, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.8 Hz, 1H).

*(2S,5R)-tert-Butyl 2-(4-Aminobenzyl)-5-((R)-(4-fluorophenyl)-(hydroxy)methyl)pyrrolidine-1-carboxylate (**50d**).* Starting from 4-fluorobenzaldehyde, the procedures summarized above provided compound **50d**. LC/MS (EI) *m*/*e*: 401 $(M + H)^+$. ^1H NMR (500 MHz, CD_3OD) δ 1.49−1.55 (m, 11H), 1.57 (m, 2H), 1.98 (m, 1H), 2.70 (s, 1H), 3.93 (s, 1H), 4.07 (s, 1H), 4.80 (m, 1H), 6.63 (d, *J* = 7.0 Hz, 2H), 6.81 (d, *J* = 7.0 Hz, 2H), 7.05 (t, *J* = 8.6 Hz, 2H), 7.35 (t, *J* = 8.6 Hz, 2H).

*(2S,5R)-tert-Butyl 2-(4-Aminobenzyl)-5-((R)-hydroxy(pyridin-3-yl)-methyl)pyrrolidine-1-carboxylate (**50e**).* To a 500 mL Parr bottle was added 10% palladium on carbon (1.8g, 1.7 mmol) followed by a solution of *tert*-butyl (2*R*,5*S*)-2-[(*R*)-{[*tert*-butyl(dimethyl)silyl]oxy}(6-chloro-

dx.doi.org/10.1021/jm4017224 | *J. Med. Chem.* 2014, 57, 1437−1453

pyridin-3-yl)methyl]-5-(4-nitrobenzyl)pyrrolidine-1-carboxylate **48e** (19.2g, 34.2 mmol) and potassium acetate (5.03 g, 51.3 mmol) in ethanol (150 mL). The resulting mixture was agitated under a 50 psi atmosphere of hydrogen gas for 12 h. The reaction mixture was then filtered through a plug of Celite, the plug was washed with ethanol (200 mL), and the combined filtrates concentrated in vacuo. The residue was dissolved in tetrahydrofuran (100 mL), and a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (67 mL, 67 mmol) was then added. The resulting mixture was heated at 55 °C for 2 h. After cooling to room temperature, the mixture was evaporated in vacuo and partitioned between ethyl acetate and water. The aqueous phase was extracted with ethyl acetate (2 × 150 mL), and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 100% EtOAc in hexanes gradient) to afford the desired compound as white foam (11 g, 84%). LC/MS (EI) *m/e*: 384.2 $(M + H)^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 1.40−1.47 (m, 2H), 1.55 (s, 9H), 1.66−1.72 (m, 2H), 2.55 (dd, J = 13.5, 8.7, 1H), 2.95 (m, 1H), 2.95 (m, 1H), 3.64 (br s, 2H), 4.06−4.13 (m, 2H), 4.43 (br s, 1H), 6.45 (br s, 1H), 6.68 (d, J = 8.3 Hz, 2H), 7.00 (d, J = 8.3 Hz, 2H), 7.30 (m, 2H), 8.53 (br d, J = 3.7 Hz, 1H), 8.54 (m, 2H).

7-(Trimethylsilyl)-5-oxohept-6-ynoic Acid (52). To a stirred solution of glutamic anhydride (32g, 280 mmol) in CH_2Cl_2 (1500 mL) at 0 °C was added bis(trimethylsilyl)acetylene (65.8 mL, 293 mmol) followed by slow addition of solid aluminum chloride (39.3g, 294 mmol) over 25 min. The resulting suspension was stirred for 3 h at 0 °C and then allowed to gradually warm to room temperature overnight. After cooling to 0 °C, the reaction was quenched slowly with 1 N aq HCl (500 mL) so as to keep the internal temperature below 10 °C. The layers were then separated, and the aqueous phase was extracted with DCM (2×). The combined organics were washed with 1 N HCl, water, and then brine and dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, eluent 50% EtOAc in hexanes) to afford the title compound (43.4g, 72.9% yield) as a yellow/brown oil. ^1H NMR (500 MHz, $CDCl_3$) δ 0.24 (s, 9H), 1.97 (m, 2H), 2.42 (t, J = 7.3 Hz, 2H), 2.66 (t, J = 7.3 Hz, 2H).

(5*Z*)-5-[(*tert*-Butoxycarbonyl)hydrazono]-7-(trimethylsilyl)-hept-6-ynoic Acid (53). To a stirred solution of 7-(trimethylsilyl)-5-oxohept-6-ynoic acid **52** (54.0g, 0.254 mol) in isopropyl alcohol (750 mL) was added *tert*-butyl carbazate (33.6g, 0.254 mol). The reaction mixture was stirred for 4 h at ambient temperature then evaporated in vacuo to remove all volatiles. This afforded the title compound as a yellow gum which was used without further purification (77g, 93%). LC/MS (EI) *m/e*: 327.2 $(M + H)^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 0.27 (s, 9H), 1.51 (s, 9H), 1.95 (m, 2H), 2.40 (t, J = 7.5 Hz, 2H), 2.46 (t, J = 7.5 Hz, 2H), 8.42 (br s, 1H).

***tert*-Butyl 3-[4-(Benzyloxy)-4-oxobutyl]-1*H*-pyrazole-1-carboxylate (54).** To a stirred solution of **53** (77.0g, 0.236Mol) in THF (500 mL) was added a 1.0 M solution of tetrabutylammonium fluoride in THF (350 mL, 0.35 mol) over 30 min, and the resulting mixture was stirred at ambient temperature for 48 h. The reaction mixture was evaporated to dryness in vacuo, and the residue was diluted with a 5% aqueous acetic acid solution (1 L). The aqueous phase was extracted with ethyl acetate (3 × 350 mL), and the combined organic layers were washed with water (2 × 100 mL) and brine (150 mL). The organic layers were dried over $MgSO_4$ and evaporated to dryness in vacuo. The crude residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, eluent 35% EtOAc in hexanes containing 3% acetic acid) to afford 4-[1-(*tert*-butoxycarbonyl)-1*H*-pyrazol-3-yl]-butanoic acid as (60 g, quantitative yield) as a yellow oil.

To a solution of the above acid (60.0 g, 0.236 mol) in DMF (200 mL) was added potassium carbonate (48.9g, 0.354 mol) followed by dropwise addition of benzyl bromide (40 mL, 0.30 mol) over 30 min. The resulting mixture was stirred for 24 h, quenched with water (1 L), and extracted with ethyl acetate (3 × 300 mL). The combined organic layers were washed with brine (150 mL), dried over $MgSO_4$, and evaporated to dryness in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 100% hexanes to 4% EtOAc in hexanes gradient) to afford the desired compound as a yellow oil (56 g, 68%). ^1H NMR (500 MHz, $CDCl_3$) δ 1.63 (s, 9H), 2.02 (quintet, J = 7.5 Hz, 2H), 2.42 (t, J = 7.5 Hz, 2H), 2.73 (t, J = 7.5 Hz, 2H), 5.11 (s, 2H), 6.19 (d, J = 2.5 Hz, 1H), 7.34 (m, 5H), 7.95 (d, J = 2.5 Hz, 1H).

Benzyl 5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-6-carboxylate (55). To a stirred solution of **54** (27.5g, 80.0 mmol) in THF (250 mL) cooled to −78 °C was added a 1.0 M solution of sodium bis(trimethylsilyl)amide in THF (88 mL, 88 mmol). The resulting dark-yellow solution was stirred for 1 h at −78 °C and then chlorotrimethylsilane (12 mL, 96 mmol) was added dropwise over 10 min, and the resulting mixture was stirred for 30 min. Solid *N*-bromosuccinimide (16g, 88 mmol) was then added in one portion, and the resulting mixture was stirred for 3 h at −78 °C followed by gradual warming to 0 °C over 1 h. The reaction was quenched with a saturated aqueous ammonium chloride solution and the aqueous phase extracted with ethyl acetate (3 × 200 mL). The combined organics were washed with brine, dried over $MgSO_4$, and evaporated to dryness in vacuo. To a stirred solution of the crude residue of in dichloromethane (50 mL) was added trifluoroacetic acid (50 mL), and the resulting mixture was stirred for 2 h at ambient temperature. All volatiles were then evaporated in vacuo, and the residue was diluted with toluene (50 mL) and evaporated again in vacuo to remove all residual trifluoroacetic acid. The crude material was then dissolved in acetone (125 mL), and solid potassium carbonate (14.0 g, 100 mmol) was slowly added over 15 min, followed by sodium iodide (1.2 g, 8.0 mmol), and the resulting mixture was heated at reflux for 16 h. The mixture was cooled to room temperature and evaporated to dryness in vacuo. The residue was diluted with a saturated aqueous ammonium chloride solution (100 mL) and then extracted with EtOAc (3 × 100 mL). The combined organics layers were washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by flash column chromatography on a Biotage Horizon system (silica gel, 10% EtOAc in hexanes to 80% EtOAc in hexanes gradient) to afford the desired compound as clear gum (6.2 g, 64%). ^1H NMR (500 MHz, $CDCl_3$) δ 2.63−2.70 (m, 1H), 2.85−3.01 (m, 3H), 4.99 (d, J = 9.4 Hz, 1H), 5.21 (s, 2H), 5.99 (d, J = 1.6 Hz, 1H), 7.31−7.38 (m, 5H), 7.58 (d, J = 1.6 Hz, 1H).

5,6-Dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole-6-carboxylic Acid (56). A 100 mL round-bottom flask under an atmosphere of nitrogen and charged with 10 wt % palladium on activated carbon (6 mg, 0.560 mmol), which was subsequently wet with ethanol (10 mL). A solution of **55** (6.0 g, 0.025 mol) in ethanol (40 mL) was then added and the mixture placed under a balloon of hydrogen for 4 h. The reaction was filtered through a pad of Celite, the pad was washed with ethanol (20 mL), and the filtrate evaporated in vacuo to afford the title compound as a colorless solid (3.7 g, 100%). LC-MS: *m/z* (ES) 153 $(MH)^+$. ^1H NMR (500 MHz, DMSO-d_6): δ 2.55 (m, 1H), 2.88 (m, 3H), 4.87 (dd, J = 8.9, 3.7 Hz, 1H), 5.98 (s, 1H), 7.44 (s, 1H), 11.90 (br s, 1H).

β_3 cAMP Assay. CHO cells, stably transfected with the cloned β_3-adrenergic receptor were harvested after 3 days of subculturing. Harvesting of cells is done with Enzyme-Free Dissociation Media (Specialty Media). Cells are then counted and resuspended in assay buffer (Hank's Balanced Salt Solution supplemented with 5 mM HEPES, 0.1%BSA) containing a phosphodiesterase inhibitor (IBMX, 0.6 mM). The reaction was initiated by mixing 6000 cells in with 6 μL of Alexa Fluor labeled cAMP antibody (LANCE kit), which was then added to an assay well containing 12 μL of compound (diluted in assay buffer to 2× final concentration). The reaction proceeded for 30 min at room temperature and was terminated by the addition of 24 μL of detection buffer (LANCE kit). The assay plate was then incubated for 1 h at room temperature and time-resolved fluorescence measured on a Perkin-Elmer Envision reader. The unknown cAMP level was determined by comparing fluorescence levels to a cAMP standard curve. The nonselective, full agonist β-adrenergic ligand isoproterenol was used to determine maximal stimulation. The human β_3-adrenergic receptor selective ligand (*S*)-*N*-[4-[2[[2-hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethyl]-phenyl]-4-iodobenzenesulfonamide was used as a control. Isoproterenol was titrated at a final concentration in the assay of 10^{-10} to 10^{-5} M, and the selective ligand (*S*)-*N*-[4-[2[[2-hydroxy-3-(4-hydroxyphenoxy)propyl]amino]ethyl]-

dx.doi.org/10.1021/jm4017224 | *J. Med. Chem.* 2014, 57, 1437−1453

phenyl]-4-iodobenzenesulfonamide was titrated at the β_3 receptor at concentration of 10^{-10} to 10^{-5} M. Unknown ligands were titrated at the β_3-adrenergic receptor at a final concentration in the assay of 10^{-10} to 10^{-5} M to determine the EC_{50}. The EC_{50} is defined as the concentration of compound that gives 50% activation of its own maximum. Data were analyzed using Microsoft Excel and Graphpad Prism, v4.

β_1 and β_2 Binding Assays. CHO cells recombinantly expressing β_1 and β_2 receptors were grown for 3−4 days post splitting; the attached cells were washed with PBS and then lysed in 1 mM Tris, pH 7.2 for 10 min on ice. The flasks are scraped to remove the cells then homogenized using a Teflon/glass homogenizer. Membranes were collected by centrifuging at 38000*g* for 15 min at 4 °C. The pelleted membranes were resuspended in TME buffer (50 mM Tris, pH 7.4, 5 mM $MgCl_2$, 2 mM EDTA) at a concentration of 1 mg protein/mL. The binding assay was performed by incubating together membranes (2−5 μg of protein), the radiolabeled tracer ^{125}I-cyanopindolol (^{125}I-CYP, 45 pM), 200 μg of WGA-PVT SPA beads (GE Healthcare), and the test compounds at final concentrations from 10^{-10} to 10^{-5} M in a final volume of 200 μL of TME buffer containing 0.1% BSA. The assay plate is incubated for 1 h with shaking at room temperature and then placed in a Perkin-Elmer Trilux scintillation counter. The plates were allowed to rest in the Trilux counter for approximately 10 h in the dark prior to counting. Data were analyzed using a standard four-parameter nonlinear regression analysis using Graphpad Prism software, v4. The IC_{50} is defined as the concentration of the title compound capable of inhibiting 50% of the binding of the radiolabeled tracer (^{125}I-CYP).

Serotonin Transporter Assay. HEK293 cells stably expressing the human serotonin transporter (SERT) were plated at a density of 20000 cells/well in a 384-well plate (BD Biocoat no. 35663) and incubated overnight at 37 °C. The following day, growth media was removed and replaced with assay buffer (HBSS containing 20 mM HEPES and 0.1% BSA; Sigma). Compounds (serially diluted in DMSO at 100× final concentration) were added to appropriate assay wells and the plate incubated for 30 min at 37 °C. Fluorescent transporter substrate was prepared following the manufacturer's instructions (Neurotransmitter Uptake Kit, no. R8173, Molecular Probes) and added to the assay plate. The plate was then incubated for an additional 30 min at 37 °C. Uptake of the fluorescent dye was then determined by measuring fluorescence in a bottom read mode fluorescence plate reader (440 nm excitation, 520 nm emission wavelengths). Each plate included 0% inhibition (DMSO only) and 100% inhibition (10 μM fluoxetine) control wells which were used to normalize the fluorescence data. Normalized data was then fit to a standard four-parameter curve and IC_{50} determined.

Rat Bladder Hyperactivity Model. Adult female Sprague−Dawley rats weighing 170−250 g (Charles River, Wilmington, MA) were used. All procedures were approved by the Institutional Animal Care and Use Committee at Merck and Co., Inc., Rahway, N.J. Cystometry was performed as described previously.[26,27] In brief, animals were anesthetized with urethane (1.1 g/kg ip). A lower abdominal midline incision was made to expose the bladder, and two polyethylene tubes (PE-50) were inserted into the bladder dome, secured with a 4−0 silk suture and connected to both a syringe pump (555920; Harvard Apparatus, Holliston, MA) and a pressure transducer via a three way stopcock. Physiological saline solution was intravesically infused at a rate of 0.05 mL/min. The intravesical pressure signal was recorded using a Powerlab unit (16/30; AD Instruments) at a sampling rate of 10 Hz. An intravenous catheter (PE-100) was implanted into a femoral vein, and the drug solutions were infused at a rate of 0.01 mL/min using an infusion pump (BS-9000-8, Braintree Scientific, Braintree, MA). After a stable micturition cycle was observed, a 0.5% acetic acid was intravesically infused for 2 h. The drug solution was continuously infused from 1 to 2 h after beginning the intravesical acetic acid infusion. Peak micturition pressures were measured pre- and postdose. Data are presented as the mean ± SEM. The mean values were compared with one-way ANOVA with Bonferoni posthoc test. A probability value less than or equal to 0.05 was considered significant.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Conditions for the separation of the isomers of **38a** and X-ray data collection on the isomer leading to more potent B_3-AR agonists, and procedures and characterization for compounds **31a−g**, **32a−g**, **33a**, **37a−d**, **38a**, **41a−e**, **42a−e**, **43a−e**, **44a−e**, **45a−e**, **46a−e**, **47a−e**, **48a−e**, **49a−d**, **50a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Judy Morris, Regina Black, and Lisa Frey for assistance with compound purification, Arlene McKeown and Richard Ball for collecting diffraction data, and Donna Hrenuik for in vitro assay support.

■ ABBREVIATIONS USED

AcOH, acetic acid; β-AR, β-adrenergic receptor; AUC, area under the pharmacokinetic exposure curve; b, broad; Boc, *t*-butyl carbamate; CHO cells, Chinese hamster ovary; CYP, cytochrome P450; d, doublet; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcabodiimide hydrochloride; EtOH, ethanol; hERG, human Ether-à-go-go Related Gene potassium channel; HOAt, 1-hydroxy-7-azabenzotriazole; HPLC, high performance liquid chromatography; IPA, propan-2-ol; LC/MS, liquid chromatography−mass spectroscopy; LHS, left-hand side; m, multiplet; MeOH, methanol; Moz, *p*-methoxybenzyl carbamate; NMR, nuclear magnetic resonance; OAB, overactive bladder; PK, pharmacokinetic; q, quartet; quin, quintet; RHS, right-hand side; s, singlet; SAR, structure−activity relationship; SFC, supercritical fluid chromatography; SSRI, selective serotonin receptor uptake; t, triplet; TBS, *t*-butyldimethylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, trimethylsilyl

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