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Synthesis of 2,5-thiazole butanoic acids as potent and selective $\alpha_v\beta_3$ integrin receptor antagonists with improved oral pharmacokinetic properties

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Abstract—We describe a series of 2,5 thiazole containing compounds, which are potent antagonists of the integrin $\alpha_v\beta_3$ and show selectivity relative to the other integrins, such as $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_6$. These analogs were demonstrated to have high bioavailability relative to other relative heterocyclic analogs.

The cell surface integrin superfamily that consists of non-covalently linked, heterodimeric, transmembrane receptors has attracted much scientific interest.[1] A main focus in our laboratories is the receptor, $\alpha_v\beta_3$. This receptor is found on activated endothelial cells, smooth muscle cells, osteoclasts, and many tumor cells. Its primary role in angiogenesis is described in many publications. The $\alpha_v\beta_3$ receptor is very promiscuous and recognizes a variety of extracellular matrix proteins, such as vitronectin, fibronectin, fibrinogen, thrombospondin, osteopontin, bone sialoprotein, and denatured collagen.[2] The primary receptor recognition site on these extracellular matrix proteins involves the arginine-glycine-aspartic acid (RGD) tripeptide sequence contained within these proteins. Attachment of these proteins to the $\alpha_v\beta_3$ receptor results in a chain of events, that initiates the process of new blood vessel growth from existing vasculature. Antagonists of $\alpha_v\beta_3$ have been demonstrated to inhibit angiogenesis in vivo and thereby have potential utility in inhibiting tumor growth.[3] The $\alpha_v\beta_3$ receptor is also the prevalent integrin found on the surface of osteoclasts, that is responsible for cellular attachment, and subsequent bone resorption.[4] Antagonists of $\alpha_v\beta_3$ inhibit bone loss in animal models of osteoporosis[5] and appear to play a significant role in several other pathophysiological conditions, including restenosis after angioplasty,[6] ocular neovascularization,[7] and rheumatoid arthritis.[8] Antagonists of this integrin thereby may be a beneficial therapy for the treatment of a wide variety of diverse disease states.

Previously, we identified several new chemical series of $\alpha_v\beta_3$ antagonists. The initial chemical scaffold was very similar to the RGD sequence in vitronectin. These compounds demonstrated potent anti-tumor efficacy in the mouse Leydig cell tumor model,[9] but contained several amide moieties that limited its PK exposure. Other series, such as the cinnamic acids, were revealed to be potent and selective toward the integrin $\alpha_v\beta_3$ but also

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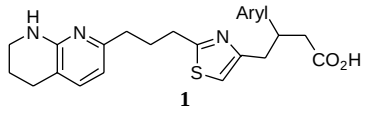
did not have optimal PK.[10] This paper follows the newly published discovery of a class of peptidomimetic β-substituted 1,2,4-oxadiazole butanoic acids, that were potent and selective antagonists toward the integrin $\alpha_v\beta_3$ and possessed promising rat PK.[11] The replacement of the hetrocyclic core was the focus of this SAR study targeting the optimization of affinity, selectivity, and pharmacokinetic properties. This investigation revealed the 2,5-thiazole analogs (**1**) yielded $\alpha_v\beta_3$ affinity, integrin selectivity, and enhanced pharmacokinetic properties, relative to the 1,2,4-oxadiazole series.



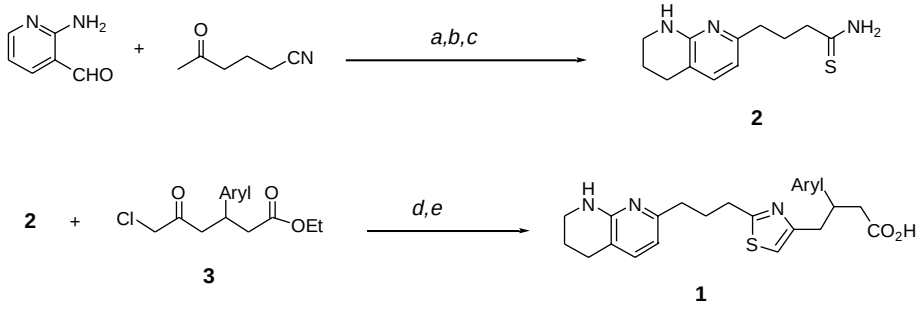
1

The synthesis of the 2,5 thiazole ring exploited a thioamide intermediate **2** (Scheme 1). The 1,8-tetrahydronaphthyridine moiety was utilized due to its effectiveness in generating potency in our 1,3,4-oxadiazole series.[11] A subsequent reaction of thioamide **2** with α-chloro-ketone **3** afforded 2,5-thiazole analogs in high yield. This overall route interfaced well with 1,2,4-oxadiazole analogs, such that, many common intermediates were utilized. This methodology increased output and simplified the overall SAR. The limiting factor was accessibility of the glutaric anhydrides. However, various established methods were used to generate these common intermediates (Scheme 2).

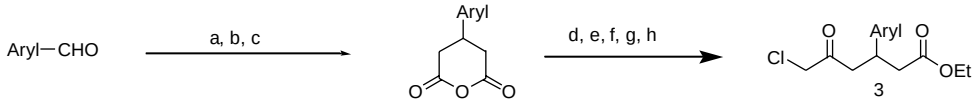
Aromatic aldehyde was condensed with ethyl acetoacetate in the presence of piperidine. The resulting carbocyclic intermediate was saponified to give 3-aryl glutaric diacids. These diacids were then readily converted to cyclic anhydrides by heating with excess acetic anhydride. These anhydride intermediates were then converted to both the racemic and *S*-isomer α-chloro-ketones (**3**) by the route described in Scheme 2.[12]

A series of β-substituted 2,5-thiazole-butanoic acids were prepared (Table 1) and tested in the $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, and $\alpha_v\beta_6$ solid-phase receptor binding assays (SPRA).[4] Most analogs demonstrated at least 100-fold selectivity over the related platelet integrin, $\alpha_{IIb}\beta_3$, and 1000-fold selectivity over the integrin $\alpha_v\beta_6$. We sought selectivity from the $\alpha_v\beta_6$ integrin due to the physiological deficits in the B_6 knockout mouse.[13] Variation of a β-substituent with substituted aromatic rings, and bicyclic aromatic and heterocyclic rings resulted in good affinity with dioxolane analog (**8**) affording the best affinity.

While the 1,8-tetrahydronaphthyridine moiety was an effective guanidine mimetic for these 2,5-thiazole containing $\alpha_v\beta_3$ antagonists, we sought to explore other guanidine surrogates such as to optimize $\alpha_v\beta_3$ antagonist affinity. A selection of guanidine mimetic variants is shown in Table 2. These mimetics were selected based on their calculated increased basicity relative to the 1,8-tetrahydronaphthyridine moiety. In addition, variation of the β-substituent, as either a 3,4-methylenedioxyphenyl (*S*-enantiomer) or a 4-methoxypyridyl group (racemic), was utilized to further explore the SAR. The 2-amino-4-cycloaminopyridinyl containing *S*-isomer analogs (**17**–**19**) revealed a common theme of possessing comparable affinity to the *rac*-1,8-tetrahydronaphthyridine analog (**8**). Further data analysis reveals that variation of the β-position from the *S*-isomeric 3,4-methylenedioxyphenyl group (**17**–**19**) to the *rac*-4-methoxypyridyl group (**21**–**23**) reveals comparable, but not improved, $\alpha_v\beta_3$ antagonist affinity. The final guanidine surrogate analyzed was the 1-methyl-1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazine (**20**, **24**). These 2,5-thia-

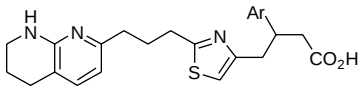


Scheme 1. Reagents and condition: (a) (i) L-Proline, EtOH; (ii) chromatographic isomer separation; (b) H_2/Pd; (c) H_2S, pyridine, triethylamine; (d) 1,4-dioxane, Δ; (e) LiOH, THF.

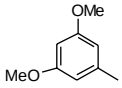
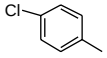
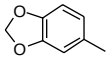
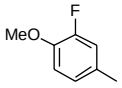
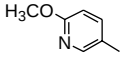
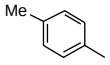
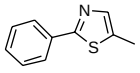
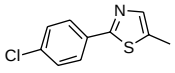
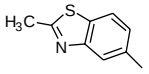
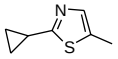
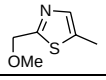


Scheme 2. Representative methods for forming α-chloroketo γ-esters. Racemic **3**. Reagents and conditions: (a) ethyl acetoacetate, piperidine; (b) (i) NaOH, H_2O, EtOH, Δ; (ii) HCl; (c) Ac_2O, Δ; (d) EtOH, reflux; (e) oxalyl chloride, CH_2Cl_2; (f) $TMSCHN_2$, THF; (g) HCl in Et_2O. *S*-isomer **3**. Reagents and conditions: (a) Ethyl acetoacetate, piperidine; (b) (i) NaOH, H_2O, EtOH, Δ; (ii) HCl; (c) Ac_2O, Δ; (d) EtOH, HCl, reflux; (e) Chirazyme L-2, pH 7.4; (f) oxalyl chloride, CH_2Cl_2; (g) $TMSCHN_2$, THF; (h) HCl in Et_2O.

Table 1. SPRA data for racemic β-substituted analogs



1

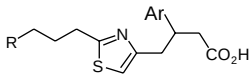
Compound	Ar	IC_{50} (nM)[a]		
		$\alpha_v\beta_3$ SPRA	$\alpha_{IIb}\beta_3$ SPRA	$\alpha_v\beta_6$ HT29
4		2.80	559	1308
5		3.49	484	2119
6		1.54	350	1743
7		3.81	636	2444
8		1.18	263	2390
9		2.17	405	3263
10		1.23	[b]	1323
11		11.06	2004	5057
12		1.77	252	2671
13		2.09	559	4301
14		2.82	95.1	>10,000
15		1.22	[b]	313
16		1.20	[b]	841

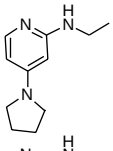
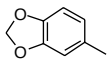
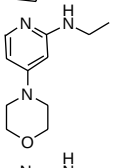
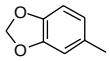
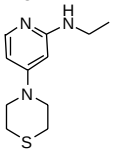
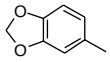
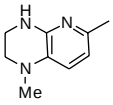
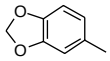
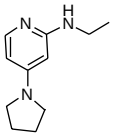
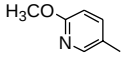
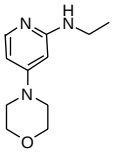
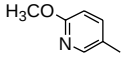
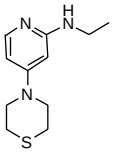
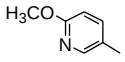
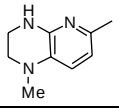
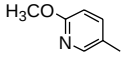
[a] Average of at least three determinations.
[b] Not tested.

zole analogs revealed the greatest affinity of the guanidine mimetic investigated but only comparable affinity to the 1,8-tetrahydronaphthyridine analogs (**8**, **10**).

Representative synthetic protocols for analogs **17**–**19** and **21**–**23** are shown in Scheme 3. For analogs **20** and **24** alternate thioamides were prepared (Scheme 4) and combined with their respective α-chloro-ketones as shown in Scheme 1.

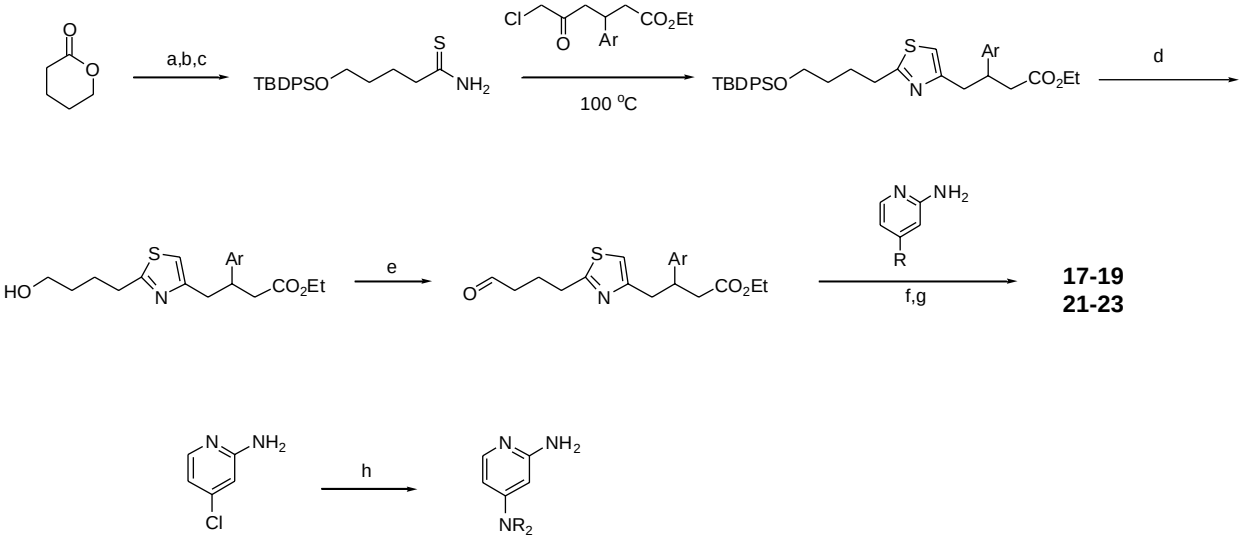
Table 2. SPRA data for alternate guanidine mimetic analogs



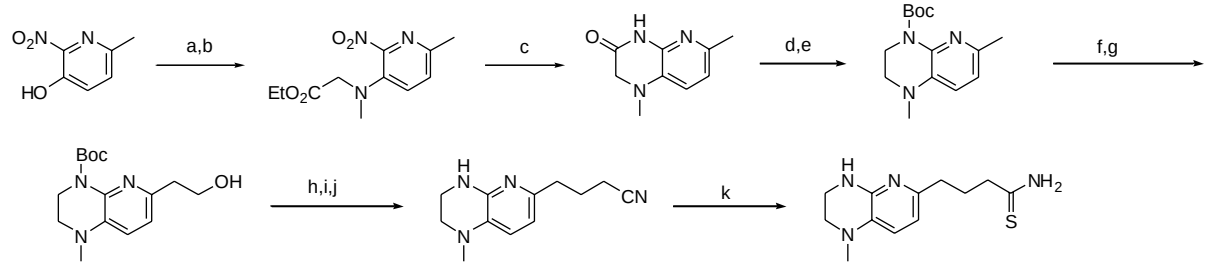
Compound	R	Ar	IC_{50} (nM)[a] $\alpha_v\beta_3$ SPRA
17[c]			1.26
18[c]			1.56
19[c]			1.70
20			0.61
21			3.00
22			3.54
23			3.73
24			1.24[b]

[a] Average of at least three determinations.
[b] Average of two determinations.
[c] *S*-isomer.

Rat PK was determined on selected analogs illustrated in Table 3. It was apparent that the 2,5-thiazole class of antagonists possessed a different pharmacokinetic profile than the 1,2,4-oxadiazole series (**8** vs **25**[15]). The significant improvement of % *F*, $t_{1/2}$, C_{max}, and AUCs of **8** versus **25** may be due to the lower clearance of **8**. While we did see enterohepatic recirculation in an earlier series, it was not determined as to what role enterohepatic recirculation played in these higher %F values. The *S*-isomer of **8** also possessed a similar PK profile in rat and exhibited good bioavailability in other species such as dog ($t_{1/2}$ = 13 h, % *F* = 60) and cynomonkey ($t_{1/2}$ = 11.2 h,



Scheme 3. Synthesis of compounds **17**–**19** and **21**–**23**. Reagents and conditions: (a) NH_3, MeOH; (b) *t*-butyldimethylsilyl chloride, imidazole; (c) Lawesson's reagent, benzene, 60–70 °C; (d) TBAF, THF; (e) TPAP, NMO, sieves; (f) $NaBH(OAc)_3$, R = pyrrolidine, azepine, morpholine, thiomorpholine; (g) LiOH; (h) NR_2 = pyrrolidine, azepine, morpholine, thiomorpholine; dimethylacetamide, 200 °C, 5 min, CEM microwave.



Scheme 4. Synthesis of alternate thioamides for the preparation of compounds **20** and **24**. (a) Reagents and conditions: $(CF_3SO_2)_2O$, Et_3N; (b) $MeNHCH_2CO_2Et$; (c) H_2, Pd/C; (d) $LiAlH_4$; (e) Boc_2O, Et_3N; (f) LDA, diethylcarbonate; (g) $LiBH_4$; (h) Ph_3P, imidazole, I_2; (i) ethylcyanoacetate, NaH, DMF; (j) KOH, ethylene glycol, Δ; (k)H_2S, Pyridine, Et_3N.

Table 3. Rat pharmacokinetic data

Compound	% *F*	$t_{1/2}$ (h)	AUC-PO/dose (μg h/ml/mg/kg)	AUC-IV/dose (μg h/ml/mg/kg)	C_{max} (μg/mL)	CL (mL/min/kg)	V_z (mL/kg)
6	98	6.1	2.2	2.2	6.1	7.3	2040
9	95	4.3	1.8	1.9	9.1	9.0	1290
15	>100	3.4	2.5	2.4	22.0	6.8	1020
10	>100	6.3	1.6	1.4	6.6	12.3	2950
16	90	7.1	1.7	1.9	3.0	8.7	2680
8	>100	7.0	6.8	5.2	19.6	3.3	897
25	95	1.4	1.5	1.6	12	10.9	733

% $F = 64$). Although not shown, most β-substituted 2,5-thiazoles listed in this table also revealed greater rat pharmacokinetic properties when compared directly to those of other β-substituted heterocyclic analogs, such as 1,3,4-thiadiazoles and 1,3,4-oxadiazoles.[14]

In summary, synthetic routes of a series of readily accessible 2,5-thiazole butanoic acids possessing potent $\alpha_v\beta_3$ antagonist activity was developed. The overall SAR of this series illustrates the modification of the β-aryl ring and incorporation of an appropriate guanidine mimetic yields novel analogs with low to sub-nanomolar $\alpha_v\beta_3$ affinity possessing a 100-fold selectivity over $\alpha_{IIb}\beta_3$ and a 100-fold selectivity over $\alpha_v\beta_6$. In addition, these 2,5-thiazole derivatives possessed an increased oral rat pharmacokinetic profile relative to similar 1,2,4-oxadiazole analogs. Future communications from our laboratories will be reported in time on the variation of the heterocyclic core as well as the in vivo pharmacology of this class of molecules.

References and notes

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