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Thiophene-Based Vitronectin Receptor Antagonists

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Abstract—A series of $\alpha_v\beta_3$ antagonists based on a thiophene scaffold were synthesized via two routes and evaluated for in vitro biological activity. We have identified several structurally similar antagonists with different selectivities towards $\alpha_{IIb}\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$ at the cellular level. In addition, these antagonists exerted an antiangiogenic effect in the chick chorioallantoic membrane (CAM) assay.

The survival of vascular endothelial cells during angiogenesis is dependent on the interaction with extracellular matrices (ECM), which are mediated by the integrin family of cell adhesion receptors. Integrin $\alpha_v\beta_3$ is minimally expressed on resting blood vessels, but is significantly up-regulated on vascular cells within human tumors such as breast, and in response to growth factors in the chick chorioallantoic membrane (CAM).[1,2] Integrin $\alpha_v\beta_3$ shares a common β_3 subunit with the platelet fibrinogen receptor, $\alpha_{IIb}\beta_3$, which is a key player of platelet aggregation. Both these integrins bind to ECM proteins through the tripeptide sequence, arginine-glycine-aspartic acid (RGD). Disruption of $\alpha_v\beta_3$-ECM interactions with cyclic RGD peptides causes apoptosis of angiogenic endothelial cells, resulting in the disruption of neovascularization and inhibition of tumor growth.[3,4] Thus, the criteria for potential cancer therapeutics include potent $\alpha_v\beta_3$ compounds which are selective towards $\alpha_{IIb}\beta_3$, since antagonists of $\alpha_{IIb}\beta_3$ are associated with undesirable bleeding complications. Integrin $\alpha_v\beta_5$ is also an RGD dependent vitronectin adhesion receptor that plays a critical role in angiogenesis and is up-regulated in neuroblastoma.[5,6] The fact that the RGD tripeptide sequence serves as a recognition motif in multiple ligands for several different integrins (such as $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, $\alpha_v\beta_5$) has prompted considerable drug discovery efforts in designing peptidomimetics.[7] Most of the attention has been directed towards blockade of the $\alpha_v\beta_3$ but there is increasing evidence supporting the beneficial use of dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists for antiangiogenesis therapy.[8–10] It is clear, however, that $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are not the only integrins involved in angiogenesis since it has been recently shown that tumors in α_v-null mice displayed normal vascular development and even enhanced growth.[11] Recent experiments have highlighted the importance of integrins in the β1 family (such as $\alpha_5\beta_1$) in angiogenesis.[12]

Structural features important for both integrin affinity and specificity to $\alpha_v\beta_3$ have been derived from studies of cyclic RGD peptide antagonists[13] and other non-peptides.[14,15] Much of the patent literature describes $\alpha_v\beta_3$ antagonists that incorporate one or more rings as a central constraint,[7] including several disclosures covering the use of thiophenes.[16] The central constraint bears basic and acidic side chains, the 2,3-diaminopropanoic acid being the most extensively used,[17] which mimic the guanidine and carboxylate of the RGD sequence. The overall separation between the guanidine and carboxylic acid termini, and conformation of the antagonist are critical in achieving potency and specificity. With these considerations in mind, along with a survey of the literature for potent $\alpha_v\beta_3$ antagonists as our starting point, we focused our efforts on identifying potent and selective $\alpha_v\beta_3$ antagonists which could potentially prove useful in cancer therapy. Herein, we describe the synthesis and biological profile of a series of RGD peptidomimetic antagonists consisting of a guanidine linked via an aryl moiety to a central thiophene scaffold.[16c]

Our key strategy was to vary the positioning of the guanidinyl residue relative to the central thiophene

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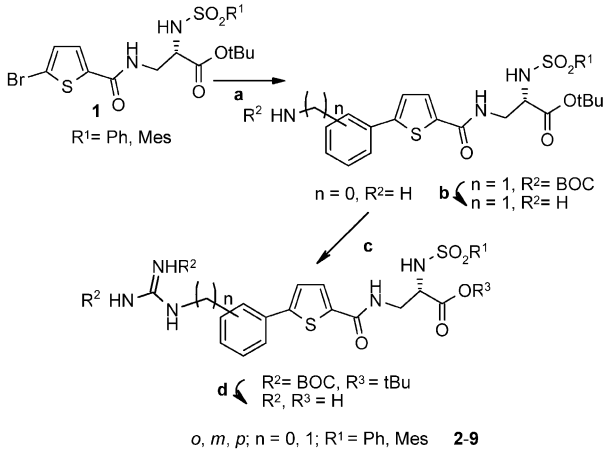
scaffold so as to mimic different RGD conformations, as well as find the optimal separation of the guanidinyl residue from the carboxylic acid terminus and determine their effects on integrin affinity and selectivity. Based on earlier work done in our laboratories, we decided to conformationally restrict the basic side chain by introduction of an aryl group in hopes of improving potency. Hence a series of *o*-, *m*-, and *p*-substituted phenyl or benzyl guanidines were prepared. The aryl-linked thiophenes were rapidly accessed using a palladium catalyzed cross-coupling with in situ generation of the stannanes, which has been cited in the literature for the preparation of various biaryls[18] (Scheme 1). The coupled products were obtained in modest yields along with homo-coupled side-products. The guanidines were prepared according to literature procedures[19] in varying yields, with the ortho giving the lowest yield, presumably due to steric crowding. Simultaneous cleavage of the *tert*-butyl ester and the bis-BOC guanidine afforded the final compounds, **2–9** (Table 1), in quantitative yields as the TFA salts.

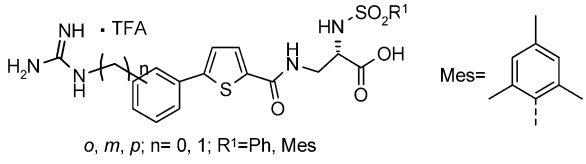


Scheme 1. (a) 3-Bromo-BOC benzyl amine or 3-bromo-BOC aniline, $(SnBu_3)_2$, $(Ph_3P)_2PdCl_2$, dioxane, 90 °C, (27–38%); (b) HCl (4 M in 1,4-dioxane), 3 min, rt (100%); (c) (*tert*-butoxycarbonylimino-pyrazol-1-yl-methyl)-carbamic acid *tert*-butyl ester, diisopropylethylamine, DMAP, DMF, 55 °C, (14–83%); (d) TFA/CH_2Cl_2 (1:1), rt (60–99%).

Antagonists (**3**, **5**, **7**, **9**), which were further profiled, were efficiently prepared on a multi-gram scale employing a Suzuki cross-coupling.[20] We found it convenient to use a reusable polymer supported $Pd(PPh_3)_4$ catalyst as described by Fenger and Le Drian to carry out the cross-coupling.[21] Thus, commercially available 3-aminophenyl boronic acid hemisulfate (**12**), thiophene **1**, polymer-supported $Pd(PPh_3)_4$ catalyst and aqueous sodium carbonate in DME, was degassed and heated at 80 °C affording the coupled aniline-thiophenes quantitatively (Scheme 2). Guanidinylation and deprotection was effected as before giving the final compounds **7** and **9** in 52 and 46% overall yields, respectively. In the case of the benzylamine-thiophene antagonists, intermediate **11** was prepared in two steps by guanidinylation followed by conversion to the boronic acid (Scheme 2). Thus, to **10** was added one equivalent of methyl lithium to abstract the NH protons followed by one equivalent of *t*-butyl lithium to effect lithium-halogen exchange.[22] Quenching with triisopropyl borate and subsequent hydrolysis afforded the boronic acids in good yields. The cross-coupling proceeded in acceptable yields giving a mixture of bis-BOC and mono-BOC compounds. After final deprotection, **3** and **5** were obtained in 55 and 65% overall yields, respectively.

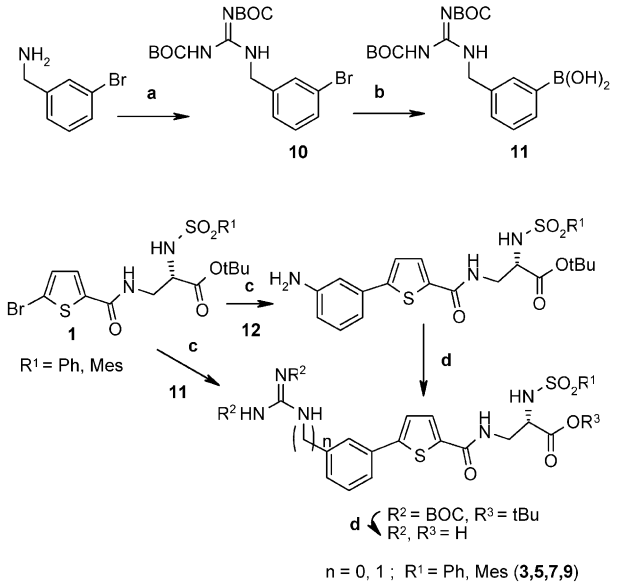
Compounds **2–9** were tested for their ability to inhibit biotinylated-fibrinogen from binding to $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ purified proteins in a solid-phase receptor binding assay. The IC_{50} values are summarized in Table 1. Once the IC_{50} values fell in the nM range, this assay did not prove useful in differentiating compounds. Therefore, a cell adhesion assay was chosen to assess the potency of the $\alpha_v\beta_3$ antagonists as this was observed to be a more stringent assay for differentiating between them. The IC_{50} values for the compounds to inhibit the binding of K562 cells engineered to overexpress the $\alpha_v\beta_3$ receptor from attaching to immobilized fibrinogen are summarized in

Table 1. Thiophene-based antagonists **2–9**



Compd	*n*		R_1	$\alpha_v\beta_3$ Fg IC_{50} (nM ± SD)[a]	K562/$\alpha_v\beta_3$-Fg IC_{50} (nM ± SD)[a]	$\alpha_{IIb}\beta_3$ Fg IC_{50} (nM ± SD)[a]	$\alpha_{IIb}\beta_3/\alpha_v\beta_3$
2	1	*o*	Ph	0.04 ± 0.001	21 ± 9.9	0.0058 ± 0.0036	<0.1
3	1	*m*	Ph	2.2 ± 1.7	0.74 ± 0.45[b]	0.34 ± 0.13[b]	<0.5
4	1	*p*	Ph	2.1 ± 0.07	730 ± 480	6.1 ± 5.0	<0.1
5	1	*m*	Mes	3.4 ± 4.1	11 ± 7.4	0.16 ± 0.07[b]	<0.1
6	0	*o*	Ph	5500	5330 ± 4490	0.25 ± 0.18	<0.1
7	0	*m*	Ph	1.0 ± 0.9	0.19 ± 0.13	0.59 ± 0.28[b]	3
8	0	*p*	Ph	71.5 ± 40.3	3790 ± 2920	0.0013 ± 0.00014	<0.1
9	0	*m*	Mes	1.4 ± 1.3	0.00092 ± 0.00083[b]	0.31 ± 0.16[b]	300

[a]The IC_{50} values denote the concentration required to reduce the binding of biotinylated fibrinogen (Fg) to purified $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ and K562 cells expressing $\alpha_v\beta_3$ (K562/$\alpha_v\beta_3$) to immobilized fibrinogen by 50%. Results are shown as a means ± sd based on a minimum of at least two independent experiments performed in triplicate. See ref 24 for full experimental details.
[b]Standard average error of mean (SEM) was determined.



Scheme 2. (a) As in (d), (55%); (b) MeLi (1 equiv), *t*-BuLi (1 equiv), $B(iOPr)_3$, ether, −78 °C to rt, o/n (65%); (c) polymer supported $Pd(PPh_3)_4$, $NaCO_3$ (aq, 2 M), DME, 80 °C (55–99%); (d) (*tert*-butoxycarbonylimino-pyrazol-1-yl-methyl)-carbamic acid *tert*-butyl ester, DIPEA, DMAP, DMF, 55 °C, (51–64%); (e) TFA/CH_2Cl_2 (1:1), rt (91–99%).

Table 2. Thiophene-based antagonists **3**, **5**, **7** and **9**

Compd	HT29/VN $\alpha_v\beta_5$ IC_{50} (nM ± SEM)[a]	$\alpha_v\beta_5$/ $\alpha_v\beta_3$	K562/FN $\alpha_5\beta_1$ IC_{50} (nM ± SEM)[a]	$\alpha_5\beta_1$/ $\alpha_v\beta_3$
3	72 ± 36	97	640 ± 261	864
5	11 ± 5.7	1	71 ± 36	6
7	6.4 ± 3.7	33	95 ± 47	500
9	2.5 ± 1.0	2717	26 ± 13	28,260

[a]The IC_{50} values denote the concentration required to reduce the binding of HT29 cells expressing $\alpha_v\beta_5$ to vitronectin (VN) and K562 cells expressing $\alpha_5\beta_1$, to fibronectin (FN) by 50%. Results are shown as a means ± SEM based on a minimum of at least three independent experiments performed in triplicate. See ref 24 for full experimental details.

Table 3. Dose-dependent inhibition of VEGF-mediated angiogenesis in the chick CAM assay by **3**, **5**, and **7**

[Inhibitor] (μg/mesh)	% Inhibition of capillary formation			
	3	**5**	**7**	**9**
3	29	65	29	
17	42	91	66	nd[a]
33	57	95	98	

[a]Not determined.

Table 1. For both series, ($n = 0,1$), it was found that when the guanidinylaryl moiety is positioned meta to the thiophene, the compounds are exceptionally potent against $\alpha_v\beta_3$ (**3**, **7** compared to **2**, **4**, **6**, **8**). In the phenyl sulfonyl series the *ortho* and *para* compounds were very active against $\alpha_{IIb}\beta_3$ hence the complete lack of selectivity for $\alpha_v\beta_3$, whereas the *meta* compounds were slightly selective. Interestingly, in the mesityl series, by shortening the space between the guanidinyl and phenyl moieties by one carbon (comparing **5** and **9**), there is a dramatic enhancement in potency and selectivity where compound **9** displays an activity against $\alpha_v\beta_3$ in the picomolar range and is 300-fold selective. We attribute the high potency to the combined effects of the mesityl group and the shorter overall separation between the guanidine and carboxylic acid, observations which have been documented in the literature as giving improved potency in compounds derived from 2,3-diaminopropanoic acid.[14,15,23]

Compounds **3**, **5**, **7** and **9** may additionally represent an interesting class of dual antagonists since they display good activities against $\alpha_v\beta_5$ directed cell adhesion to vitronectin (Table 2). Interestingly, compounds **5**, **7** and **9** were also found to antagonize $\alpha_5\beta_1$ mediated cell adhesion to fibronectin whereas **3** did not. There is an enhancement in $\alpha_5\beta_1$ potency when changing the phenyl for a mesityl group on the sulfonyl (comparing **3** to **5** and **7** to **9**), an observation which, on a different scaffold, has been noted in the literature.[16]

To assess whether selected compounds were able to exert an anti-angiogenic activity, they were tested in the chick mesh CAM assay using VEGF as an angiogenic stimulus (Table 3). All compounds inhibited VEGF-induced angiogenesis in a dose-dependent manner, with **5** being particularly potent at low concentrations compared to **3** and **7**.

We have identified potent $\alpha_v\beta_3$ antagonists by systematic positioning of the guanidinyl moiety around the aryl-thiophene, giving compounds with varying integrin affinities as determined by cell adhesion studies. Compound **9** is a highly potent $\alpha_v\beta_3$ antagonist which exhibited the best selectivity against $\alpha_{IIb}\beta_3$ of the series. Moreover, **9** showed activity against $\alpha_v\beta_5$ and $\alpha_5\beta_1$, yet still exhibited preferential selectivity for $\alpha_v\beta_3$. In contrast, while compounds **3**, **5** and **7** showed activity against $\alpha_v\beta_5$ and $\alpha_5\beta_1$, **5** was the most non-selective for all integrins tested, and **3** and **7** were somewhat selective for $\alpha_v\beta_3$. Antagonists **3**, **5** and **7** demonstrated an anti-angiogenic effect in the CAM assay. We suggest that the ability for compound **5** to non-selectively inhibit $\alpha_v\beta_3$/ $\alpha_v\beta_5$/$\alpha_5\beta_1$ compared to the more selective inhibition of compounds **3** and **7** towards $\alpha_v\beta_3$, contributes to its increased potency in the CAM assay. These inhibitors have been further characterized in endothelial cell in vitro assays to determine the integrin selectivity required for anti-angiogenic activity and the results will be presented in due course.[24] Having identified potent vitronectin receptor antagonists, it still remains a significant challenge to identify those exhibiting good pharmacokinetics. This series of compounds are not expected to exhibit a good pharmacokinetics profile due to the presence of the guanidino function. However, there have been significant advances reported by numerous groups who have identified effective guanidine equivalents, which in combination with simple ester prodrugs, provide compounds with acceptable oral bioavailability.[25] Further work is required to address these pharmacokinetic issues.

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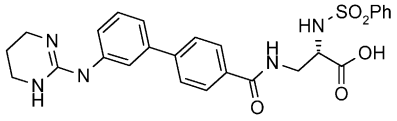

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