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Design and synthesis of novel potent and selective integrin $\alpha_v\beta_3$ antagonists—Novel synthetic routes to isoquinolinone, benzoxazinone, and quinazolinone acetates

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Abstract—An unexpected ring contraction of benzazepinone based $\alpha_v\beta_3$ antagonists led to the design of quinolinone-type derivatives. Novel and efficient synthetic routes to isoquinolinone, benzoxazinone, and quinazolinone acetates were established. Nanomolar $\alpha_v\beta_3$ antagonists based on these new scaffolds were prepared. Moreover, benzoxazinones **15a** and **15b** exhibited high microsomal stability and good permeability.

The $\alpha_v\beta_3$ receptor belongs to the integrin class of cell adhesion receptors which are capable of mediating both cell–cell and cell-extracellular matrix (ECM) interactions.[1] The integrins were named in 1986 by Hynes to emphasize their role in integrating the intracellular cytoskeleton with the external milieu.[2] These cell surface receptors are composed of noncovalently associated α and β chains that combine to give a wide array of heterodimers with distinct cellular and adhesive specificities. At least 18 α subunits and 8 β subunits form a superfamily of 24 receptors. These multifunctional molecules have been shown to play a role in the regulation of cellular adhesion, migration, invasion, proliferation, apoptosis, and gene expression. Antagonists of integrins $\alpha_{IIb}\beta_3$ (also called GPIIb/IIIa) and $\alpha_v\beta_3$ have typically been designed after the bioactive arginine–glycine–aspartate (RGD) conformations of peptides derived from their primary ligands, fibrinogen and vitronectin, respectively.[3] Examples of the disease states that have a strong β_3 integrin-dependent component in their etiologies are thrombosis (integrin $\alpha_{IIb}\beta_3$), unstable angina (GPIIb/IIIa), and restenosis (integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$).[4] The selective inhibition of the $\alpha_v\beta_3$ integrin has been postulated to present a therapeutic approach for the treatment of a variety of diseases, including diabetic retinopathy and a variety of remodeling disorders such as osteoporosis, restenosis, and the angiogenesis component of cancer.[1,5]

In a previous report,[6] we showed that the oral bioavailability (in rat) of a tetrahydrobenzazepinone derivative could be substantially enhanced when administered as ethyl ester prodrug (compound **2b**, Scheme 1). Two ways were tried in parallel for its preparation[7]: transesterification of the *tert*-butyl ester **2a**, or esterification of the corresponding acid **1**. Surprisingly, under transesterification conditions,[7] the expected 1,3,4,5-tetrahydrobenzazepinone ethyl-acetate **2** was obtained only as side product.[8] The structure of the major product could be elucidated by means of 2D NMR experiments as the 3,4-dihydroquinolinone ethylpropanoate **3** (see Supporting Information). The following mechanism was postulated for the ring contraction[9]: cleavage of the amide bond of the 7-membered lactam under acidic attack and formation of the thermodynamically favored 6-membered ring (Scheme 1). The nanomolar activity of **4** (IC_{50} 27.3 nM, in a competitive ELISA using vitronectin as natural ligand) prompted us to look in more detail

Keywords: Ring contraction; Benzazepinone; Isoquinolinone; Benzoxazinone; Quinazolinone; Integrin; $\alpha_v\beta_3$; 2D NMR; ELISA; Microsomal metabolic stability; Caco-2 permeability.
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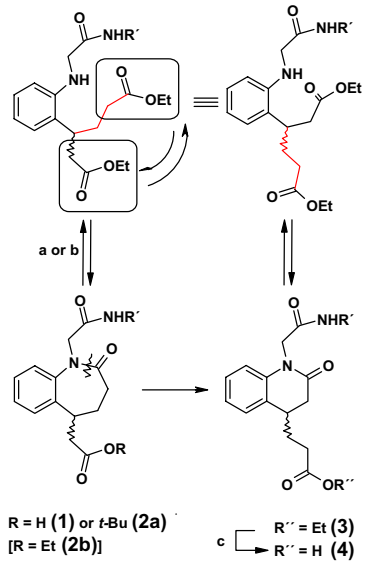
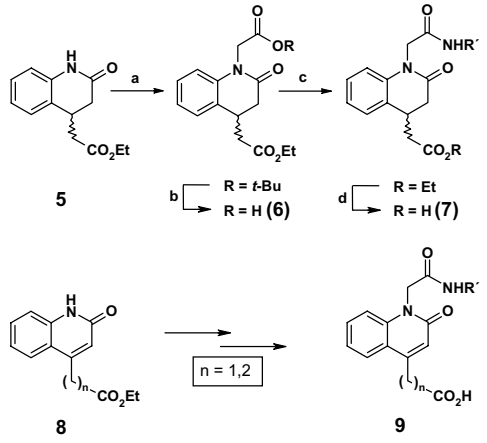


Scheme 1. Reagents and conditions: (a) from **1**: EtOH, HCl, 5 d, rt; (b) from **2a**: EtOH, HCl, 4 h, reflux, 83%; (c) NaOH, EtOH, H_2O, 1 h, reflux, 35%.

at quinolinone-type derivatives for their potential as $\alpha_v\beta_3$ antagonists.

Here we present the synthesis and SAR of novel $\alpha_v\beta_3$ antagonists based on the 3,4-dihydro-1*H*-quinolin-2-one, 1,4-dihydro-benzo[*d*][1,3]oxazin-2-one, 3,4-dihydro-1*H*-quinazolin-2-one, 1*H*-quinolin-2-one, and 3,4-dihydro-2*H*-isoquinolin-1-one cores.

3,4-Dihydro-1*H*-quinolin-2-one derivatives **7** were prepared starting from ethyl ester **5**[10] after N-alkylation, cleavage of the *tert*-butyl ester, condensation of acid **6** with the corresponding amine [(4-*trans*-aminomethyl-cyclohexyl)-(1*H*-benzoimidazol-2-yl)-amine or (4-aminomethyl-phenyl)-(1*H*-benzoimidazol-2-yl)-amine)] using TOTU[11] as coupling reagent, and subsequent saponification (Scheme 2). 1*H*-Quinolin-2-one derivatives **9** (Table 2) were prepared from **8**[12,13] following the same route.



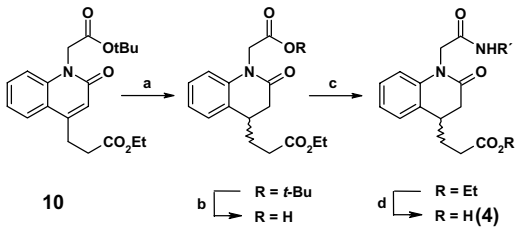
Scheme 2. Reagents and conditions: (a) *tert*-butyl bromo acetate, K_2CO_3, DMF, 12 h, rt, quant.; (b) TFA, CH_2Cl_2, 5 h, rt, 72%; (c) R′NH_2, TOTU, NMM, DMF, 12 h, 0 °C-rt, 84% (Q = 1,4-*trans*-cyclohexyl)/34% (Q = 1,4-phenyl); (d) NaOH (1 M), dioxane, H_2O, 3 h, rt, 90% (Q = 1,4-*trans*-cyclohexyl)/84% (Q = 1,4-phenyl).

For the analogous propionic acids (compounds **4**), an alternative synthetic route to the ring contraction of the corresponding benzazepinone derivative (Scheme 1) was established. Synthesis started with diester **10**,[13] hydrogenation was carried out smoothly on PtO_2, and final compounds **4** were obtained following a 3-step sequence similar to the one described above (Scheme 3).

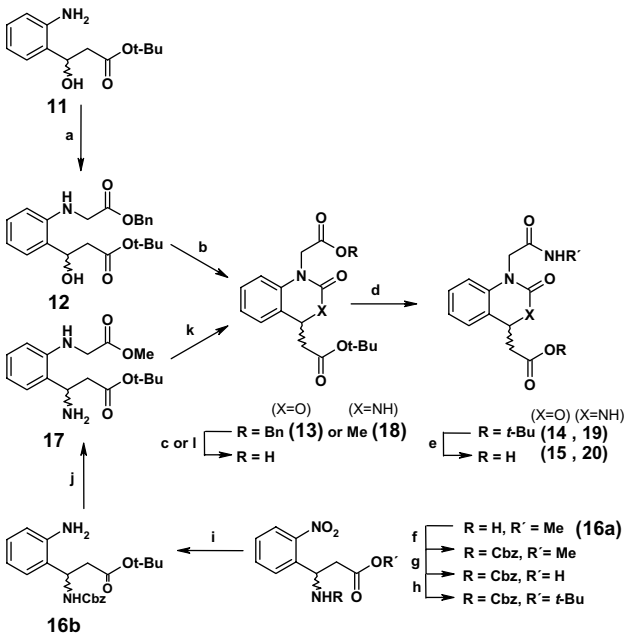
A novel synthetical path, which is common to both the 1,4-dihydro-benzo[*d*][1,3]oxazin-2-one (**15**, X = O) and the 3,4-dihydro-1*H*-quinazolin-2-one (**20**, X = NH) derivatives, was designed starting from the corresponding 3-hydroxy-3-(2-amino-phenyl)-propionic ester **11**[14] and the protected 3-amino-3-(2-amino-phenyl)-propionic ester **16b**,[15] respectively. Anilines **11** and **16b** were N-alkylated and the formed intermediates **12** and **17** cyclized using 'triphosgene' or CDI, respectively. Final compounds **15** and **20** were obtained after cleavage of esters **13** and **18**, condensation with the required amine, and final cleavage of the *tert*-butyl esters **14** and **19** (Scheme 4).

The commercially available 2-cyanomethyl-benzoic acid ethyl ester **21** was chosen as starting material for the synthesis of the 3,4-dihydro-2*H*-isoquinolin-1-one derivatives **26**. The benzylic position of **21** was deprotonated with LDA and quenched with *tert*-butyl bromo acetate. Nitrile **22** was hydrogenated to afford intermediate **23** which cyclized spontaneously (yield 64%). Finally after N-alkylation, saponification of the methyl ester, condensation, and *tert*-butyl ester cleavage, compounds **26** were isolated with 64–66% yield (Scheme 5).

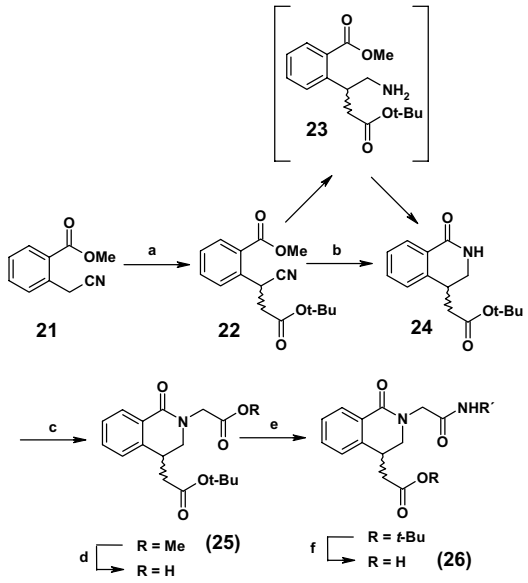
Compounds **4**, **7**, **9**, **15**, **20**, and **26** were evaluated for $\alpha_v\beta_3$ inhibition by means of competitive ELISA using vitronectin as natural ligand. Compounds displaying IC_{50} values >10 μM were considered as 'not active'. Specificity versus $\alpha_{IIb}\beta_3$ was examined routinely for compounds displaying an IC_{50} $\alpha_v\beta_3$ < 100 nM. All compounds discussed showed at least 1000-fold selectivity. If not indicated otherwise, compounds were screened as racemic mixtures.



Scheme 3. Reagents and conditions: (a) H_2, PtO_2, 9 h, rt, 67%; (b) TFA, CH_2Cl_2, 12 h, rt, 59%; (c) R′NH_2, TOTU, NMM, DMF, 14 h, 0 °C-rt, 25% (Q = 1,4-*trans*-cyclohexyl)/29% (Q = 1,4-phenyl); (d) NaOH (1 M), dioxane, H_2O, 4 h, rt, 79% (Q = 1,4-*trans*-cyclohexyl)/76% (Q = 1,4-phenyl).



Scheme 4. Reagents and conditions: (a) benzyl bromo acetate, DIPEA, DMF, 3 d, rt, 77%; (b) 1—bis(trichloromethyl)carbonate ('triphosgene'), DIPEA, THF, 1.5 h, rt; 2—CH_3CN, DMAP, 1 h, rt; (c) if X = O: H_2, Pd/C, EtOAc, 40 min, rt, 75%; (d) R′NH_2, TOTU, NMM, DMF, 1 h, 0 °C, X = O 69% (Q = 1,4-*trans*-cyclohexyl)/75% (Q = 1,4-phenyl); X = NH 52% (Q = 1,4-*trans*-cyclohexyl)/84% (Q = 1,4-phenyl); (e) HCl(4 N)/AcOH (1:1, vol/vol), dioxane, 3–12 h, rt, X = O 35% (Q = 1,4-*trans*-cyclohexyl)/12% (Q = 1,4-phenyl); X = NH 67% (Q = 1,4-*trans*-cyclohexyl)/51% (Q = 1,4-phenyl); (f) CbzCl, DIPEA, CH_2Cl_2, 12 h, rt, 95%; (g) NaOH (1 M), dioxane, H_2O, 12 h, rt, quant.; (h) Perchloric acid, *tert*-butyl acetate, 6 h, rt, 82%; (i) H_2, RaNi, MeOH, 1 h, rt, 97%; (j) 1—Methyl bromo acetate, DIPEA, DMF, 50 h, rt, 84%; 2. H_2, Pd/C (10%), MeOH, 40 min, rt, 98%; (k) CDI, dioxane, 12 h, rt, 72%; (l) if X = NH: NaOH (1 M), dioxane, H_2O, 30 min, 10 °C, 81%.

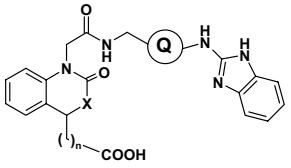


Scheme 5. Reagents and conditions: (a) LDA, *tert*-butyl bromo acetate, THF, 1 h, −78 °C, 57%; (b) H_2, PtO_2, EtOH, AcOH, 2.5 h, rt, 64%; (c) methyl bromo acetate, K_2CO_3, DMF, 2 d, rt; (d) NaOH (1 M), dioxane, H_2O, 4 h, rt, 46%; (e) R′NH_2, TOTU, NMM, DMF, 2 d, 0 °C-rt, 69% (Q = 1,4-*trans*-cyclohexyl)/48% (Q = 1,4-phenyl); (f) HCl(4 N)/AcOH (1:1, vol/vol), dioxane, 12 h, rt, 66% (Q = 1,4-*trans*-cyclohexyl)/64% (Q = 1,4-phenyl).

Based on the SAR gained with previous series,[6,16,17] two basic moieties were selected to test the potential of the new scaffolds as cores for $\alpha_v\beta_3$ antagonists. These two aminobenzimidazole residues, whose spacer to the arginine mimetic differs in terms of length and flexibility (in one case a cyclohexyl, in the other a phenyl group), have been shown to contribute to high affinity to the $\alpha_v\beta_3$ receptor when combined with various other cores.[6,16,17]

Compared to the data obtained within the corresponding benzazepinone series **1**,[6] the fused 6-membered ring (X = CH_2) is better tolerated when Q is cyclohexyl and $n = 1$ (compound **7a**, Table 1). The diminished distance between the Asp and Arg mimetics—induced by the smaller ring size—cannot be compensated by a longer spacer to the acidic function ($n = 2$, compounds **4a** and **4b**), as one might have thought. As already reported by us,[6] replacement of phenyl by cyclohexyl favors nanomolar and in one case sub-nanomolar activity (compound **15a**, X = O). The variation of X within the cyclohexyl series only plays a 'fine tuning' role (IC_{50} of ca. 10 nM and below), whereas the activity of the phenyl derivatives highly depends on the central bicycle and varies between 4.2 and 50 nM (compounds **15b** and **7b**, resp.). However, the geometry of the benzoxazinone

Table 1. Dihydroquinolinones, dihydrobenzoxazinones, and dihydroquinazolinones



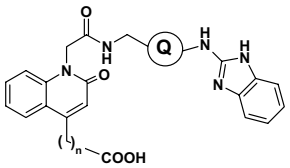
Compound	X	*n*	Q	$\alpha_v\beta_3$[a] IC_{50} (nM)
1a[6]	$(CH_2)_2$	1	Cyclohexyl	1.5
1b[6]	$(CH_2)_2$	1	Ph	4.4
7a	CH_2	1	Cyclohexyl	7.4
7b	CH_2	1	Ph	50
4a	CH_2	2	Cyclohexyl	27
4b	CH_2	2	Ph	5′000
15a	O	1	Cyclohexyl	0.7
15b	O	1	Ph	4.2
20a	NH	1	Cyclohexyl	13
20b	NH	1	Ph	37

[a] Values are means of three experiments; intra-assay variation <10%, inter-assay variation < factor 2; cyclohexyl: 1,4-*trans*-cyclohexyl; Ph: 1,4-phenyl.

scaffold (X = O) seems to be preferred affording the most active compounds in the two series (**15a** and **15b**). The large difference in activity between derivatives **4a** and **4b** in the elongated series ($n = 2$) was unexpected: the more flexible cyclohexyl group seems to better accommodate the elongated propionic acid chain.

The former observations were confirmed in the 1*H*-quinolin-2-one series **9** (Table 2):

Table 2. Quinolinones



Compound	*n*	Q	$\alpha_v\beta_3$[a] IC_{50} (nM)
9a	1	Cyclohexyl	10
9b	1	Ph	50
9c	2	Cyclohexyl	1000
9d	2	Ph	500

[a] Values are means of three experiments; intra-assay variation <10%, inter-assay variation < factor 2; cyclohexyl: 1,4-*trans*-cyclohexyl; Ph: 1,4-phenyl.

- elongation of the spacer ($n = 2$) disfavors the $\alpha_v\beta_3$ activity (compounds **9c** and **9d**);
- when *n* is 1, only the cyclohexyl spacer allows low nM activity (compound **9a**).

The retro-orientation of the lactam in the 3,4-dihydro-2*H*-isoquinolin-1-one series is detrimental for the $\alpha_v\beta_3$ activity (compounds **26a** and **26b**, Table 3). Compared to the benzazepinone series (compounds **27a** and **27b**),[6] this effect is even more pronounced, again confirming that the distance between the acidic and the basic group has a major influence on the activity of $\alpha_v\beta_3$ antagonists.[1] However, the spatial arrangement of the acidic and basic groups is also a determining parameter: comparison of benzazepinones **27** with the more constrained isoquinolinones **26** suggests that the more flexible 7-membered ring might compensate more efficiently the shorter distance between the 2 groups.

Benzoxazinones **15** with nanomolar and sub-nanomolar $\alpha_v\beta_3$ activity were further characterized and exhibited (Table 4):

- high selectivity *versus* $\alpha_{IIb}\beta_3$ and $\alpha_5\beta_1$;
- microsomal metabolic stability[18] in rat, dog, and human;
- good permeability in the Caco-2 model.[19]

In summary, novel routes for the synthesis of 3,4-dihydro-1*H*-quinolin-2-ones **7**, 1,4-dihydro-benzo[*d*][1,3]-oxazin-2-ones **13**, 3,4-dihydro-1*H*-quinazolin-2-ones **18**, and 3,4-dihydro-2*H*-isoquinolin-1-ones **24** were presented. These new scaffolds could serve as central core for the design of potent and selective integrin $\alpha_v\beta_3$ antagonists (e.g., benzoxazinones **15** with nanomolar and sub-nanomolar $\alpha_v\beta_3$ activity). Based on their favorable in vitro ADME profile, PK studies as well as in vivo screening in different disease models are planned for **15a** and **15b**.

Acknowledgments

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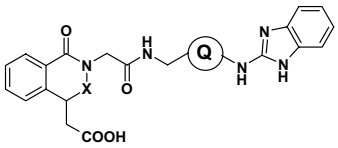
Table 3. Dihydro-isoquinolinones



Compound	X	Q	$\alpha_v\beta_3$[a] IC_{50} (nM)
27a[6]	$(CH_2)_2$	Cyclohexyl	11
27b[6]	$(CH_2)_2$	Ph	122
26a	CH_2	Cyclohexyl	10,000
26b	CH_2	Ph	10,000

[a] Values are means of three experiments; intra-assay variation <10%, inter-assay variation < factor 2; cyclohexyl: 1,4-*trans*-cyclohexyl; Ph: 1,4-phenyl.

Table 4. Potency, selectivity, and ADME parameters of selected compounds

Compound	$\alpha_V\beta_3$/VN ELISA[a] IC_{50} (nM)	$\alpha_{IIb}\beta_3$/Fg ELISA[a] IC_{50} (nM)	$\alpha_5\beta_1$/FN Adhesion[b] Inhibition at 10^{-5} M	Caco-2 Permeation assay P_{app}[c] ($cm/s \times 10^{-6}$)
15a	0.7	>10,000	25%	3.23
15b	4.2	>10,000	8%	3.48

[a] Values are means of three experiments; intra-assay variation <10%, inter-assay variation < factor 2.

[b] Cell adhesion and migration: values are means of 4 experiments; intra-assay variation <20%, inter-assay variation < factor 2.

[c] P_{app} = Apparent Permeability coefficient; $n = 2$, intra-assay variation <20% (P_{app} values >2e−7 cm/s are considered as medium, >2e−6 cm/s as high transport rate). *Abbreviations:* VN, vitronectin; Fg, fibrinogen; FN, fibronectin; r-d-h, rat, dog, human.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.089.

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7. Transesterification has been run under acidic conditions in ethanol for 2 h at reflux; esterification of acid **1** in ethanol catalyzed by HCl for 5 d at rt.

8. HPLC analysis after transesterification and esterification[7] revealed 2 peaks depicting the same molecular mass (LC–MS: 532) but with opposite (HPLC-) ratios: ca. 30:70 after transesterification and ca. 80:20 after esterification. When reflux was continued (further 2 h) under transesterification conditions, a single product was obtained (corresponding to the major component of the former mixture). Structure assignment of 3,4-dihydroquinolinone ethylpropanoate **3** was achieved by means of 2D NMR experiments (HSQC, HMBC, see Supporting Information). The corresponding 1,3,4,5-tetrahydrobenzazepinone ethyl-acetate **2** could be finally prepared by activating the carboxyl group with the BOP reagent in smooth conditions, according to: Diago-Meseguer, J.; Palomo-Coll, A. L.; Fernandez-Lizarbe, J. R.; Zugaza-Bilbao, A. *Synthesis* **1980**, 547 and could be attributed to the second peak of the HPLC-chromatogram (minor peak after transesterification, major one after esterification).
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