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Supporting Information for:

Combinatorial and rational strategies to develop non-peptidic α4β7-integrin antagonists from cyclic peptides

Dirk Gottschling, Jürgen Boer, Anja Schuster, Bernhard Holzmann, Horst Kessler*

General. Reactions on solid support were performed in syringes with a fritted disc (2, 5 or 10 mL) from Vetter-Laborbedarf, Tübingen, Germany. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 as solvent and internal reference. Thin layer chromatography was performed on silica gel 60 F_{254} plates from Merck. Flash chromatography was performed on silica gel 60 (230-400 mesh ASTM) from Merck. Analytical HPLC analyses were performed on a YMC column, 4.6 mm × 25 mm, 5 µm C_{18}, 1 mL/min, 30 min linear gradient from H_2O (0.1% TFA) and CH_3CN (0.1% TFA), 220 nm. ESI-mass spectra were obtained on a LCQ Finnigan mass spectrometer.

General procedure 1 (synthesis of 3-amino-3-arylpropionic acid derivatives): An aromatic aldehyde (20 mmol), malonic acid (2.08 g, 20 mmol) and ammonium acetate (3.08, 40 mmol) were refluxed in ethanol (30 mL) for 6 h. The reaction mixture was kept overnight at room temperature and the precipitated amino acid was removed by filtration. The product was used without further purification.

General procedure 2 (introduction of the Fmoc-protecting group): A solution of Fmoc-chloride (5.5 mmol) in dioxane (7.5 mL) was slowly added to a ice-cooled solution of amino acid (5.0 mmol) in dioxane (5 mL) and aqueous sodium carbonate (10 %, 15 mL). After stirring 1 h at 0°C and 12 h at room temperature, the reaction mixture was poured into ice water (150 mL) and extracted with cold diethyl ether (2 x 150 mL). The pH of the aqueous solution was adjusted with 1N HCl to 2. The precipitate was removed by filtration and dried under reduced pressure. The product was used without further purification.

General procedure 3 (synthesis of 3-arylglutaric acids): Piperidine (0.405 mL, 4.1 mmol) was added slowly to an ice cooled solution of an aromatic aldehyde (30 mmol) in ethyl acetoacetate (7.65 mL, 60 mmol). The reaction mixture was left at room temperature for 3 days without stirring. The precipitate was dissolved in ethanol (16 mL) and refluxed for 1 h. After cooling to room temperature, the crystalline product was filtered off and dried under vacuo. The product was added stepwise to a solution of NaOH (4.1 g, 102.5 mmol) in water (3.5 mL) at 90°C and stirred for 2 h at 80°C. Ice (23

g) and ethyl acetate (5.9 mL) was added. The aqueous phase was separated and the pH was adjusted to 1 with concentrated, aqueous HCl. The precipitate was removed by filtration, washed with water and dried under vacuo.

Solid phase synthesis

General procedure 4 (peptide synthesis): Peptide synthesis was either performed manually or using an automatic robot Syro II (Multisynthec, Germany) on chlorotrityl-resin. The loaded resin (1.0 g) was washed with DMF (3 × 10 mL, 3 min) and treated with a 0.1-0.25 M solution of Fmoc-protected amino acid (3.0 equiv), HATU (3.0 equiv), HOAt (3.0 equiv) and collidine (30 equiv) in DMF. The suspension was shaken for 1-2.5 h and washed with DMF (5 × 10 mL).

General procedure 5 (Fmoc deprotection): The resin (1.0 g) was treated with a solution of 20% piperidine in DMF (2 × 10 mL) for 15 min and washed with DMF (5 × 10 mL).

General procedure 6 (cyclization of linear peptides): The fully protected linear amino acid sequences were cleaved from the resin using a 8:1:1 mixture of CH_2Cl_2, TFE and acetic acid. Cyclization of the linear sequences was performed in DMF at concentrations of 1.25×10^{-3} M using HATU (1.1 equiv), HOAt (1.1 equiv) and collidine (11 equiv).

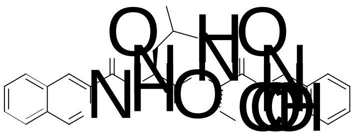
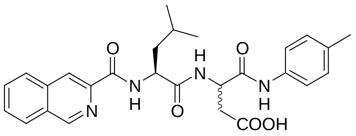
General procedure 7 (incorporation of Fmoc-azaXaa-Cl): The resin (1.0 g) was washed with anhydrous DMF (3 × 10 mL) and a 0.1 M solution of Fmoc-azaXaa-Cl (5 equiv) and DIEA (5.5 equiv) in anhydrous DMF was then added. The reaction mixture was shaken at room temperature for 15 h and washed with DMF (5 × 7 mL).

General procedure 8 (reductive amination): The resin (1.0 g) was washed with anhydrous DMF (3 × 10 mL) and TMOF (3 x 10 mL). A 0.17 M solution of a Fmoc protected aminoaldehyde (5 equiv) in TMOF was added and the suspension was shaken for 5 h. After washing with CH_2Cl_2 (3 × 10 mL) a 0.1 M suspension of sodium triacetoxyborhydride (5 equiv) in CH_2Cl_2 was added and shaken overnight at room temperature. The resin was washed with DMF (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL).

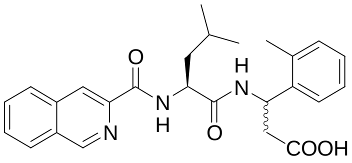
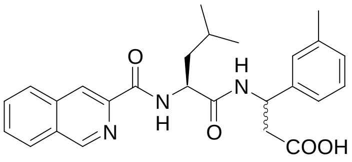
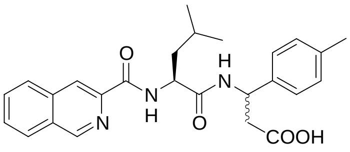
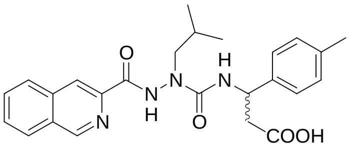
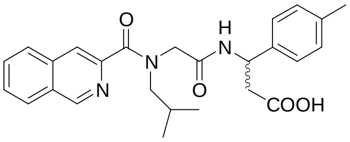
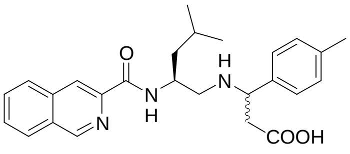
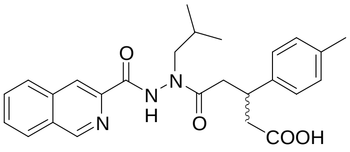
General procedure 9 (*tert*-butyl deprotection and cleavage from the resin): The resin (1.0 g) was washed with CH_2Cl_2 (2 × 10 mL) and treated with a 95:2.5:2.5-mixture of TFA, TIPS and water (10 mL) for 1.5 h at room temperature. The resin was washed with CH_2Cl_2 (3 × 10 mL). The solutions were mixed, evaporated under reduced pressure and purified by HPLC.

Characterization

Characterization of the synthesized peptidomimetics:

sequence	no.	Σ	MG	ESI [M+H$^+$]	R_t	%
c(F-L-D-F-D-p)	**1**	$C_{37}H_{46}N_6O_{10}$	734.81	735.3	19.61	48%
c(F-L-D-*Phg*-D-p)	**2a**	$C_{36}H_{44}N_6O_{10}$	720.78	721.3	18.38	nt
	2b	$C_{36}H_{44}N_6O_{10}$	720.78	721.3	18.44	nt
	**4**	$C_{27}H_{30}N_4O_5$	490.55	491.1	24.37	9%
	**5a**	$C_{27}H_{30}N_4O_5$	490.55	491.1	25.67	12%
	5b	$C_{27}H_{30}N_4O_5$	490.55	491.1	25.82	7%

Characterization of the compounds containing dipeptide mimetics:

sequence	no.	Σ	MG	ESI [M+H$^+$]	R$_t$	%
	**6a**	$C_{26}H_{29}N_3O_4$	447.53	448.1	25.21	44%
	6b	$C_{26}H_{29}N_3O_4$	447.53	448.0	25.86	49%
	**7a**	$C_{26}H_{29}N_3O_4$	447.53	448.0	25.35	31%
	7b	$C_{26}H_{29}N_3O_4$	447.53	448.0	25.91	31%
	**8a**	$C_{26}H_{29}N_3O_4$	447.53	448.1	24.38	nt
	8b	$C_{26}H_{29}N_3O_4$	447.53	448.1	25.03	nt
	**9**	$C_{25}H_{28}N_4O_4$	448.51	449.0	23.93	39%
	**10**	$C_{26}H_{29}N_3O_4$	447.53	448.0	21.83	60%
	**11a**	$C_{26}H_{31}N_3O_3$	433.54	434.3	22.69	33%
	11b	$C_{26}H_{31}N_3O_3$	433.54	434.1	22.84	29%
	**12**	$C_{26}H_{29}N_3O_4$	447.53	448.0	25.71	21%

Biological Evaluation

Adhesion buffer: Click´s RPMI, 1 % BSA, 1.0 mM $CaCl_2$, 1.0 mM $MgCl_2$

Cell adhesion assay: 96-well plates were coated with donkey anti-human IgG (Jackson ImmunoResearch Laboratories Inc.) (0.5 µg in 100 µL PBS/well) overnight at 4°C. The plates were washed with adhesion buffer and incubated with MAdCAM-Ig (100 ng/well) for 30 min at room temperature. Alternatively, the plates were coated overnight at 4°C with VCAM-1 (400 ng/well) in PBS. After washing with adhesion buffer, the plates were blocked with adhesion buffer for 1 h at 37°C. 38C13-ß7 lymphoma cells ($\alpha 4\beta 1^-$, $\alpha 4\beta 7^+$) or Jurkat lymphoma cells ($\alpha 4\beta 1^+$, $\alpha 4\beta 7^-$) were labelled for 30 min at 37°C with H33342 dye (12 µg/mL) (Calbiochem, LA Jolla, CA) in adhesion buffer and washed with PBS containing 1 mM EDTA and with PBS. The cells (38C13-ß7 lymphoma cells: 8 x 10^5 cells/mL, Jurkat lymphoma cells: 6.5 x 10^5 cells/mL) were resuspended in adhesion buffer. The compounds were preincubated with the cells at the final concentration of 1 mg/mL for 10 min at 37°C. The cell/compound suspension (100 µl/well) was put onto 96-microtiter plates and centrifuged 10 min at 15 g. Subsequently, cells were allowed to adhere for 30 min at 37°C and non-adherent cells were removed by inverse centrifugation for 10 min at 50 g. Adhesion assays were quantified by fluorimetry using a Cytofluor 2300 (Millipore, Bedford, MA).