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Synthesis and biological evaluation of ureido derivatives as VLA-4 antagonists

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Bioisosteric replacement based design strategy was employed in order to improve pharmacokinetic properties of TBC-3486, a potent VLA-4 antagonist. However, the newly synthesized analogs did not show any significant activity.

Keywords*:* VLA-4 antagonists, bioisosteres, ureido derivatives

IPC Code: Int. Cl.[8] C07D

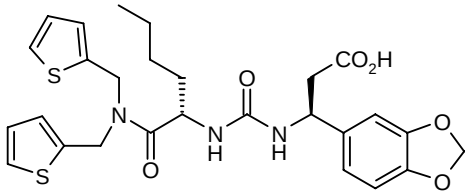
VLA-4 (also known as the very late antigen-4 or $\alpha_4\beta_1$) has been identified as a relatively new target for the development of "non-steroidal" alternatives for the treatment options for asthma and related inflammatory diseases. One of the cell adhesion processes, which involves the interaction of the integrin, VLA-4 with extracellular matrix proteins such as fibronectin and cell-surface ligands such as vascular cell adhesion molecule-1 (VCAM-1) is largely responsible for the pathogenesis of inflammatory and autoimmune diseases[1-4]. Monoclonal antibody such as Natalizumab[5] directed against the α_4 integrin has been approved for the treatment of mutiple sclerosis and Crohn's disease and several small molecule VLA-4 antagonists are under early clinical or preclinical development[6-9].

While early work on VLA-4 antagonists was based on peptides or variants of the LDV sequence, more recent efforts have been directed towards small molecules or peptidomimetics based on LDV mimics and acylphenylalanines. Based on LDV peptidomimetics, TBC-3486 (**1**) has been identified as a potent VLA-4 antagonist (IC_{50} = 0.4 nM) and was also found to be active *in vivo*[6,10,11]. However, the molecule has been associated with poor oral availability (<3%) and hence there has been no further development.

Design Strategy

It has been our objective to develop orally available and potent small molecule VLA-4 antagonists. Bioisosteric replacements of active moiety(ies) of a pharmacophore constitute a viable and effective approach to rational drug design[12,13]. This approach has also been effectively utilized for the optimization of pharmacokinetic (PK) properties. Thus, we set out to change the key structural elements in TBC-3486 (**1**) in order to develop novel VLA-4 antagonists with improved oral availability.

The lead molecule, TBC-3486 (**1**) consists of three main functionalities – a carboxyl, an amide and a urea. Structure-activity relationship (SAR) data on VLA-4 antagonists suggest that the presence of a negative ionizable group *viz.*, the acid moiety is critical for the desired biological activity. Our approach therefore, was to look into the replacements of amide and urea moieties by isosteric groups[14] in order to improve upon the pharmacokinetic properties of **1** and yet retain the desired biological activity. Interestingly, amide and urea are isosteric to each other and this prompted us to study the impact of interchanging these functionalities in **1** leading to new pharmacophore of general structure **2**. In order to understand the SAR of the isosteric replacements as in pharmacophore **2**, a single point variation was undertaken. Thus, R_3 and Ar were maintained as *iso-*



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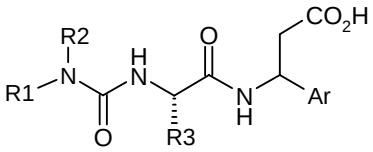

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butyl and 3,4-methylenedioxyphenyl groups, respectively, while the point of diversity was brought in by changing R_1 and R_2. As a starting point, based on the lead molecule, thienylmethyl groups were chosen for R_1 and R_2, leading to the target molecule **3**.

Besides bioisosteric replacements, we also considered other factors that could help achieve improved oral availability. One of the key factors that contributes to good oral availability is absorption and this is determined by highly interdependent influences of aqueous solubility, ionizability (*p*Ka) and lipophilicity (LogP)[15]. While retaining the ionizability of the molecules, we addressed modifications by introducing hydrophobic groups such as *o*-tolyl (compound **4)**, indolyl (compound **5**) or N-*t*-butylcarbamate (compound **6**) as R_1 and R_2 that would impact the LogP and the solubility of the molecules. Comparison of the theoretical LogP[16] values of the designed molecules with TBC-3486 (**1**) and its leucine analog **7** brought in the desired reduction in the calculated LogP values (by 0.66-1.1 units) (**Table I**) and interestingly compounds **4-6** in fact showed a dramatically improved predicted solubility (5-25 times higher). It was, therefore, expected that these newly designed compounds would exhibit better bioavailability.

Results and Discussion

Synthesis of compounds **3-6** was therefore undertaken to test the above mentioned hypotheses. A general approach involving amide and urea bond formation starting from appropriately substituted β-amino acid ester was utilized for the synthesis of the newly designed compounds **3-6**. Thus, racemic β-amino acid ester **8** was prepared by modified Rodionow reaction using piperonal and monoethyl malonate in the presence of ammonium acetate in ethanol in 46% yield[17-20]. Aminoester **8** was coupled with N-Boc-l-leucine[21] **9** using well-established amide bond formation reaction conditions (EDC and HOBt in the presence of N-methylmorpholine in DMF) to afford the corresponding N-Boc amidoester **10** in 69% yield (**Scheme I**). The Boc group was removed (trifluoroacetic acid in dichloromethane) to obtain the aminoester intermediate **11** in quantitative yield. The aminoester **11** was then coupled with amine[10] **12** in the presence of carbonyldiimidazole to give the desired urea **13**; which finally, on hydrolysis with lithium hydroxide afforded the desired acid **3** in 78% yield in two-steps.

Similarly, target compounds **4** and **5** were prepared by coupling aminoester intermediate **11** with *o*-tolyl isocyanate and indoline, respectively, followed by hydrolysis (**Scheme II**). Hydrolysis of carbamate ester **10** using lithium hydroxide furnished the carbamate acid **6** in 96% yield.

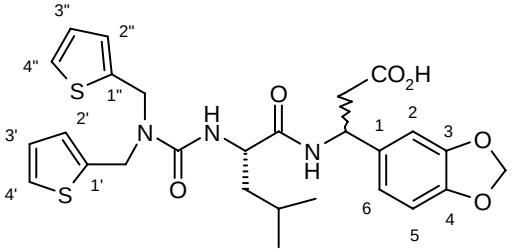
Structures of all the new compounds made were established on the basis of their spectral data.

Biological Evaluation

Compounds **3-6** were tested *in vitro* for their potential to inhibit VLA-4-VCAM-1 interactions. All compounds exhibited an IC_{50} of >10 μM in the cell adhesion assays. The results suggest that interactions of the amido and ureido functionalities (as present in **1** or **7**) with the target protein are critical for biological activity[10]. The results also suggest that bioisosteres may not always elicit similar biological response and that the exact nature of the interactions of the ligand with the biological system varies even with these bioisosteres.

Conclusions

The present work reports on a new investigation on bioisosteric analogs structurally related to a known potent VLA-4 antagonist, TBC-3486. The newly synthesized compounds were found to be inferior and the loss in the biological activity when compared to TBC-3486 could be attributed to lack of interactions of the newly introduced bioisosteric groups with the target protein.



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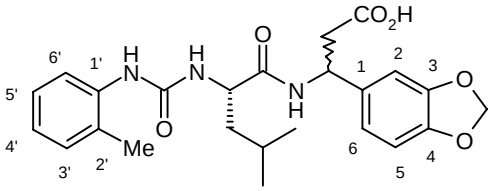

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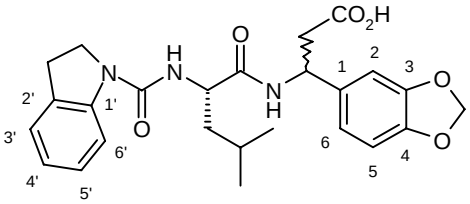

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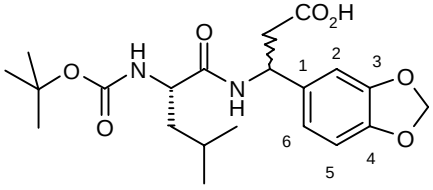

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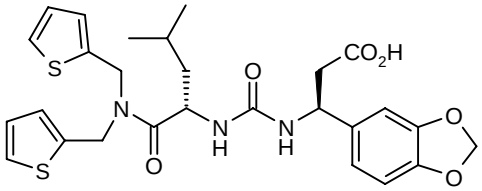

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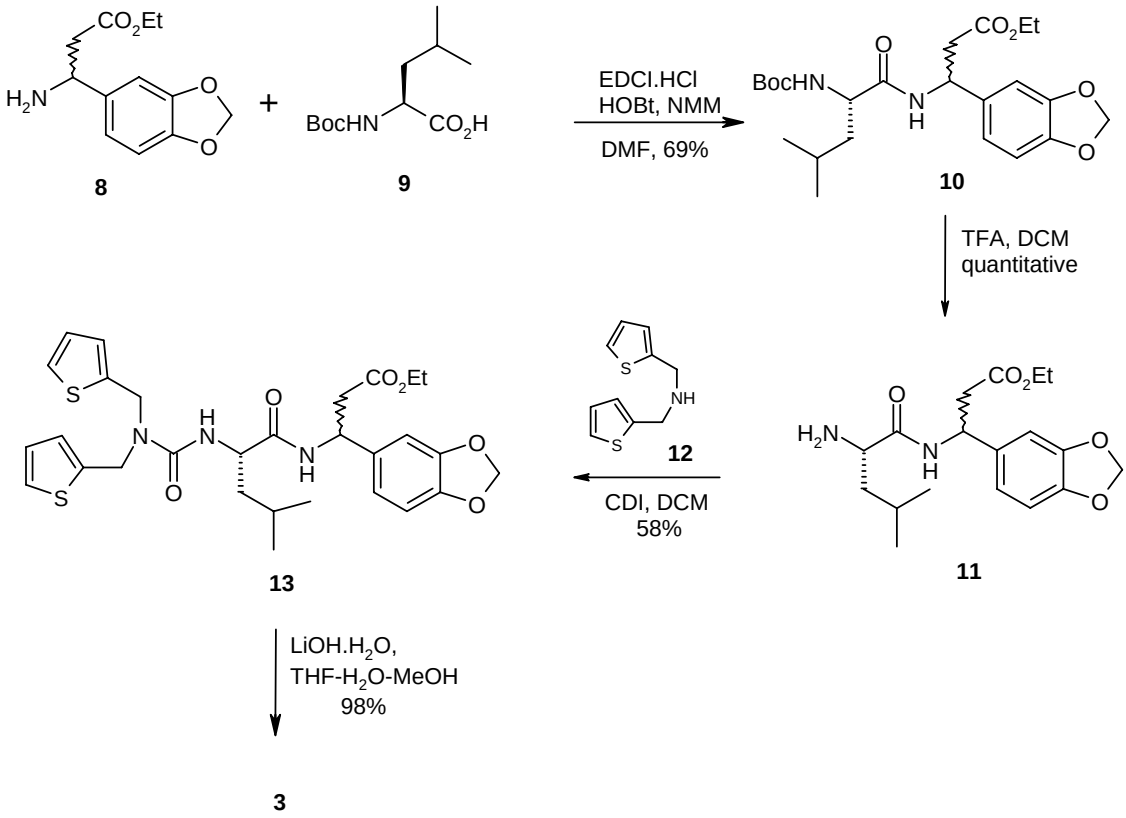
Table I — Predicted values for LogP and aqueous solubility

Compd	Log P	Solubility (g/Lt)	
		*p*H 2.5	*p*H 7.4
1	4.62 ± 0.63	4.8e-4	0.53
7	4.43 ± 0.63	6.3e-4	0.71
3	4.43 ± 0.63	6.3e-4	0.71
4	3.77 ± 0.59	5.1e-3	7.04
5	3.47 ± 0.59	3.6e-3	4.86
6	3.32 ± 0.55	0.017	24.06

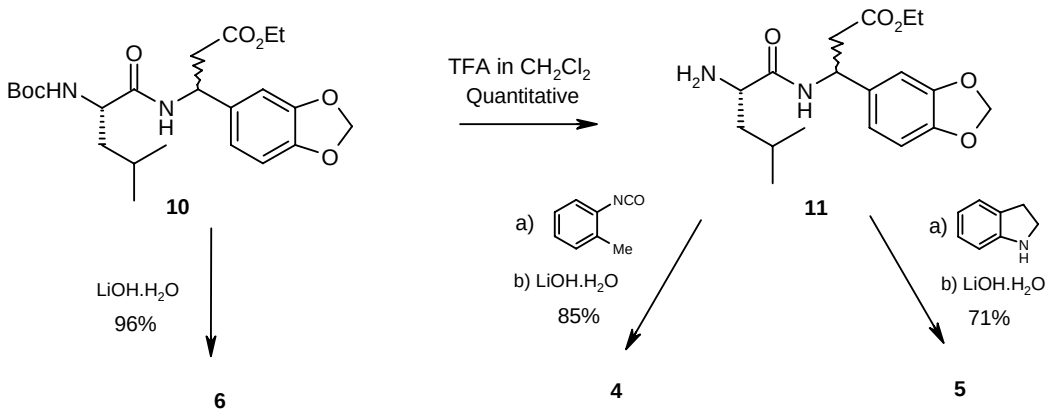
Experimental Section

TLC was carried out on precoated silica gel $60F_{254}$ plates using either UV absorption or iodine staining for visualization. Column chromatography was carried out using 100-200 mesh silica gel. HPLC analysis were carried out using Waters 2695 Separations Module HPLC system with Waters 2996 photodiode array (PDA) detector using a YMC PRO C-18 (250 × 4.6 mm) column; mobile phase: sodium acetate buffer (*p*H 5.5)/acetonitrile (80-20:20-80); flow rate 1 mL/min, run time 60 mins. Melting points were determined on a Buchi instrument and are uncorrected. IR spectra were recorded on a Paragon 1000pc FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise noted, at 400 MHz and 100 MHz with a Bruker DRX and Ultra Shield spectrometers, respectively using TMS as internal standard (chemical shifts in δ, ppm). LCMS spectra were obtained from a PE-SCIEX AP_1 3000 LC/MS/MS system using electron spray ionization at 80-250ºC using 6 mM ammonium acetate buffer of *p*H 6.7, using a positive ion mode.

Ethyl 3-amino-3-(3,4-methylenedioxy-phenyl)-propionate 8. Piperonal (3,4-methyl-enedioxybenzaldehyde, 37 g, 0.246 mole) and ammonium acetate (37.98, 0.49 mole) were added to ethanolic solution (500 mL) of ethyl hydrogen malonate (32.47 g, 0.246 mole). The mixture was heated at 80ºC for 15 hr and concentrated. The oily residue was diluted with water (250 mL), basified (*p*H ~9) with solid sodium bicarbonate, and extracted with ethyl acetate (2×200 mL). The combined organic phase was then extracted with 10% aqueous HCl. (2×200 mL). Aqueous extract was washed with ethyl acetate (2×100 mL), basified with sodium bicarbonate and extracted with ethyl acetate (2×250 mL). Organic



Scheme I



Scheme II

layer was washed with water and brine (2×200 mL), dried over anhyd. sodium sulphate and concentrated to obtain the title compound **8** as oil, yield 26.8g (46%). IR (DCM): 3372, 1729, 1608, 1488, 1442, 1372, 1342, 1305, 1241, 1098, 1037, 933, 863, 813, 728 cm^{-1}; ^1H NMR: δ 1.21 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 2.66-2.69 (2H, d, *J* = 6.9 Hz, CH_2CO), 4.13 (2H, q, *J* = 7.2 Hz, CH_2CH_3), 4.38 (1H, t, *J* = 6.8 Hz, $CHNH_2$), 5.96 (2H, s, OCH_2O), 6.74-6.90 (m, 3H, Ar-H); LCMS(m/z): 238.2 (M^++1) ($C_{12}H_{15}NO_4$).

3-Benzo[1, 3]dioxol-5yl-3-[(S)-2-*tert*-butoxycarbonylamino-4-methylpentanoylamin-o] - propionic acid ethyl ester 10. N-Boc-l-leucine[21] **9** (1.94 g, 8.4 mmoles), N-hydroxy-benzotriazole (HOBt) (1.29 g, 8.4 mmoles) and N-methylmorpholine (1 g, 8.4 mmoles) were added to a solution of amine **8** (2 g, 8.4

mmoles) in DMF (10 mL) at 0ºC. Reaction mixture was stirred for 30 min at 0ºC and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC) (1.61 g, 8.4 mmoles) was added. Reaction mixture was allowed to warm to room temperature and stirred overnight, diluted with water (25 mL) and extracted with ethyl acetate (2×25 mL). The combined organic phase was washed with water and brine (2×20 mL each) and dried (anhyd. Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (eluent:ethyl acetate-hexane; 2:8) afforded the title compound **10** as oil, yield 2.6 g (69%). ^{1}H NMR (~1:1 mixture of diastereomers): δ 0.93 (6H, d, *J* = 6 Hz, CH(C*H*$_3$)$_2$), 1.17-1.21 (3H, t, *J* = 6 Hz, CH_2CH_3), 1.42 (9H, s, C(CH$_3$)$_3$), 1.52-1.72 (3H, m, CH_2CH), 2.73-2.88 (2H, m, $COCH_2$), 4.04-4.11 (2H, q, *J* = 6 Hz, CH_3CH_2), 4.73-4.76 (1H, m, NHC*H*CO), 5.27-5.30 (1H, m, ArC*H*NH)), 5.93 (2H, d, *J* = 2.4 Hz, OCH_2O), 6.74–6.77 (3H, m, Ar-H), 7.13 (1H, m, NH); LCMS (m/z): 451.4 (M^++1) ($C_{23}H_{34}N_2O_7$).

3-Benzo[1, 3]dioxo-5yl-3-[(S)-2-(3, 3-bis-thiophen-2-ylmethyl-ureido)-4-methylpentano-ylamino] propionic acid 3.

Step 1 — 3-Benzo[1,3]dioxo-5yl-3-[(S)-2-(3,3-bis-thiophen-2ylmethylureido) - 4-methy-lpentanoyl-amino]propionic acid ethyl ester 13: Amine **10** (1 g, 8.8 mmoles) was added to a mixture of TFA and dichloromethane (20% v/v, 20 mL). The mixture was stirred at room temperature for 2 hr and concentrated under reduced pressure to afford **11** as oil, yield 1.1 g. This was used as such for the next step without any purification and characterization. Triethylamine (253 mg, 2.5 mmoles) was added to a solution of compound **11** (500 mg, 1.14 mmole) in dichloromethane (5 mL) at 0ºC and the reaction mixture was stirred for 15 min. Carbonyldiimidazole (272 mg, 1.68 mmole) was then added and stirring was continued for 30 mins at 0ºC. Bis-thiophen-2-ylmethylamine hydrochloride[10] **12** (280 mg, 1.14 mmoles) was added at 0°C and the reaction mixture was brought to room temperature and stirred overnight. Reaction mixture was diluted with ethyl acetate (20 mL), washed with water and brine (20 mL each) and dried (anhyd. Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (eluent: ethyl acetate-hexane; 4:6) afforded the title compound **13** as solid, yield 386 mg (58%). m.p. 95-98ºC. IR (DCM): 3287, 3072, 2955, 1736, 1663, 1621, 1490, 1488, 1443, 1370, 1241, 1166 cm^{-1}; ^{1}H NMR (~1:1 mixture of diastereomers): δ 0.83 (d, *J* = 7.8 Hz) and 0.85 (d, *J* = 7.6 Hz) [6H, CH(C*H*$_3$)$_2$], 1.17 (t, *J* = 4.8 Hz) and 1.18 (t, *J* = 4.8 Hz) [3H, CH_2CH_3], 1.40-1.62 (3H, m, CH_2CH), 2.74 (dd, *J* = 11.8 and 6.5 Hz) and 2.89 (dd, *J* = 14.1 and 6.5 Hz) [2H, $COCH_2$], 4.06 (q, *J* = 4.8 Hz) and 4.07 (q, *J* = 4.8 Hz) [2H, CH_3CH_2], 4.40 (1H, bs, NHC*H*CO), 4.61-4.76 (4H, m, 2 × HetC*H*$_2$N), 5.25 (1H, m, ArC*H*NH), 5.92 (s) and 5.93 (s) [2H, OCH_2O], 6.72-6.79 (m, 3H), 6.92-6.96 (m, 2H) and 7.22–7.26 (m, 4H) [Ar-H]; LCMS (m/z): 586.2 (M^++1) ($C_{29}H_{35}N_3O_6S_2$).

Step 2 — 3-Benzo[1,3]dioxo-5yl-3-[(S)-2-(3,3-bis-thiophen-2ylmethylureido) 4-methyl-pentanoyl-amino]propionic acid 3: Lithium hydroxide monohydrate (54 mg, 1.28 mmoles) was added to a solution of propionic acid ethyl ester **13** (375 mg, 0.64 mmole) in tetrahydrofuran (3 mL), methanol (1 mL) and water (1 mL). Reaction mixture was stirred at room temperature for 4 hr and concentrated under reduced pressure. The oily residue was diluted with water (20 mL) and extracted with ethyl acetate (2 × 15 mL). The aqueous layer was acidified with aqueous potassium hydrogen sulphate to pH 3 and then extracted with ethyl acetate (2 × 15 mL). The combined organic phase was washed with water and brine (15 mL each) and dried (anhyd. Na_2SO_4) and concentrated *in vacuo* to afford title compound **3** as white solid, yield 350 mg (98%), (mixture of diastereomers; 45:53 by HPLC, t_R = 25.03 and 25.15 mins; PDA 235 nm); m.p. 78-81ºC. IR (DCM): 3316, 2956, 1711, 1622, 1538, 1442, 1368, 1240, 1039 cm^{-1}; ^{1}H NMR (~1:1 mixture of diastereomers): δ 0.74 (d, *J* = 6.3 Hz) and 0.80 (d, *J* = 6.3 Hz) [6H, CH(C*H*$_3$)$_2$], 1.25-1.41 (3H, m, CH_2CH), 2.80-2.91 (2H, m, $COCH_2$), 4.53-4.67 (5H, m, 2 × HetC*H*$_2$, NHC*H*CO), 5.26-5.29 (1H, m, ArC*H*NH), 5.91 (s) and 5.93 (s) [2H, OCH_2O], 6.69-6.80 (3H, m), 6.91-6.95 (3H, m) and 7.20–7.26 (3H, m) [Ar-H]; ^{13}C NMR (~1:1 mixture of diastereomers): δ 20.08 (CH_3), 20.92 (CH_3), 22.66 and 22.83 (*C*HCH$_3$), 37.52 (*C*H$_2$CH), 39.51 and 40.14 (*C*H$_2$CO$_2$H), 43.45 and 43.49 (N*C*HAr), 46.92 (NCH$_2$ × 2), 50.88 and 51.19 (NCHCO), 99.11 (OCH_2O), 105.24 (C-2), 106.30 and 106.37 (C-5), 117.62 and 117.71 (C-6), 123.85 and 123.90 (C-4' and C-4"), 124.46 and 124.54 (C-3' and C-3"), 124.99 (C-2' and C-2"), 132.64 and 132.96 (C-1), 138.06 and 138.13 (C-1' and C-1"), 144.77 (C-4), 145.86 (C-3), 155.54 (NCONH), 170.86 and 170.95

(COOH), 171.80 and 171.89 (CONH); LCMS (m/z): 558.2 (M^++1) ($C_{27}H_{31}N_3O_6S_2$).

3-Benzo[1, 3]dioxol-5yl-3-[(S)-2-tert-butoxycarbonylamino-4-methylpentanoylamino] propionic acid 6. Hydrolysis of the propionic acid ethyl ester **10** (400 mg, 0.88 mmole) using lithium hydroxide monohydrate (75 mg, 0.17 mmole) in tetrahydrofuran (3 mL) methanol (1 mL) and water (1 mL), following the general procedure as described for the preparation of **3**, afforded the title compound **6** as white solid, yield 340 mg (96%), (mixture of diastereomers; 46:52 by HPLC, t_R = 36.01 and 36.44 mins; PDA 250 nm). m.p. 108-111ºC. IR (DCM): 3329, 2961, 1715, 1506, 1444, 1369, 1265, 1165, 1042 cm^{-1}; ^1H NMR (~1:1 mixture of diastereomers): δ 0.90 (6H, d, *J* = 5.7 Hz, $CH(CH_3)_2$), 1.41 (9H, bs, $C(CH_3)_3$), 1.46-1.62 (3H, m, CH_2CH), 2.78-2.96 (2H, m, $COCH_2$), 4.31-4.36 (1H, m, NHC*H*CO), 5.30-5.32 (1H, m, ArC*H*NH), 5.92 (s) and 5.94 (s) [2H, OCH_2O], 6.69-6.91 (3H, m, Ar-H), 7.60-7.63 (1H, m, NH); ^{13}C NMR (DMSO-d_6, ~1:1 mixture of diastereomers): δ 22.18 (CH_3), 22.73 (CH_3), 24.72 (*C*HCH_3), 28.25 (CH_3 × 3), 39.64 and 39.78 (*C*H_2CH), 41.02 and 41.58 (*C*H_2CO_2H), 49.09 (NCHAr), 52.73 and 52.93 (N*C*HCO), 80.68 (O*C*$(CH_3)_3$), 101.05 (OCH_2O), 106.92 and 107.02 (C-2), 108.24 (C-5), 119.42 and 119.55 (C-6), 134.35 and 134.54 (C-1), 146.82 (C-4), 147.82 (C-3), 156.26 (OCONH), 172.45 and 172.33 (COOH), 174.09 (CONH); LCMS (m/z): 423.4 (M^++1) ($C_{21}H_{30}N_2O_7$).

3-Benzo[1,3]dioxol-5yl-3-[(S)-4-methyl-2-(3-*o*-tolylureido)pen-tanoylamino]propionic acid 4.

Step 1— 3-Benzo[1,3]dioxol-5yl-3-[(S)-4-methyl-2-(3-*o*-tolylureido)pentanoylamino]-propionic acid ethyl ester. Triethylamine (55 mg, 0.55 mmole) was added to a solution of amine **11** (200 mg, 0.45 mmole) in dry dichloromethane (2 mL) at 0ºC and the mixture was stirred for 5 min. *o*-Tolyl isocyanate (61 mg, 0.45 mmole) was then added and reaction mixture was stirred at 0ºC for 30 min and subsequently at room temperature for 2 hr. Reaction mixture was diluted with dichloromethane (10 mL) and washed with water and brine (2 × 10 mL each). Organic phase was dried (anhyd. Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography (eluent:ethyl acetate-hexane; 4:6) afforded the title compound, yield 170 mg (77%). m.p. 168-172ºC. IR (DCM): 3355, 2957, 1735, 1638, 1558, 1489, 1446, 1242, 1167, 1041 cm^{-1}; ^1H NMR (~1:1 mixture of diastereomers): δ 0.89 (d, *J* = 5.4 Hz) and 0.94 (d, *J* = 5.4 Hz) [6H, $CH(CH_3)_2$], 1.16 (t, *J* = 7.2 Hz) and 1.18 (t, *J* = 7.2 Hz) [3H, CH_2CH_3], 1.40-1.60 (3H, m, CH_2CH), 2.16 (s) and 2.22 (s) [2H, $ArCH_3$], 2.76-2.77 (2H, m, $COCH_2$), 4.05 (q, *J* = 7.2 Hz) and 4.07 (q, *J* = 7.2 Hz) [2H, CH_3CH_2], 4.38-4.42 (1H, m, NHC*H*CO), 5.25 (1H, bs, ArC*H*NH), 5.88 (s) and 5.93 (s) [2H, OCH_2O], 6.67-6.78 (m, 3H) and 7.08-7.53 (m, 4H) [Ar-H]; LCMS (m/z): 484.2 (M^++1) ($C_{26}H_{33}N_3O_6$).

Step 2 —3-Benzo[1,3]dioxol-5yl-3-[(S)-4-methyl-2-(3-o-tolylureido)pentanoylamino]-propionic acid 4. Hydrolysis of the propionic acid ethyl ester (160 mg, 0.3 mmole), obtained from Step 1 above, using lithium hydroxide monohydrate (28 mg, 0.66 mmole) in tetrahydrofuran (3 mL), methanol (1 mL) and water (1 mL) following the general protocol as described for the preparation of **3**, furnished the title compound **4** as white solid, yield 140 mg (93%), (mixture of diastereomers; 46:52 by HPLC, t_R = 25.26 and 25.44 mins; PDA 235 nm). m.p. 108-111ºC. ^1H NMR (~1:1 mixture of diastereomers): δ 0.87 (d, *J* = 6 Hz) and 0.89 (d, *J* = 6 Hz) [6H, $CH(CH_3)_2$], 1.43-1.58 (3H, m, CH_2CH), 2.01 (s) and 2.05 (s) [3H, $ArCH_3$], 2.75-2.86 (2H, m, C*H*$_2$COOH), 4.47-4.49 (1H, m, NHC*H*CO), 5.28-5.29 (1H, m, ArC*H*NH), 5.93 (s) and 5.83 (s) [2H, OCH_2O], 6.73-6.78 (3H, m), 7.09-7.13 (3H, m) and 7.26-7.25 (1H, m) [Ar-H]; ^{13}C NMR (DMSO-d_6, ~1:1 mixture of diastereomers): δ 17.70 ($ArCH_3$), 22.21 (CH_3), 22.91 (CH_3), 24.15 and 24.20 (*C*HCH_3), 40.11 and 40.95 (*C*H_2CH), 42.46 (*C*H_2CO_2H), 49.20 (NCHAr), 51.26 and 51.35 (N*C*HCO), 100.81 (OCH_2O), 106.87 and 107.06 (C-2), 107.84 (C-5), 119.54 and 119.70 (C-6), 120.12 (C-6'), 121.70 (C-4'), 125.99 (C-5'), 126.32 and 126.38 (C-3'), 130.01 (C-2'), 136.30 and 136.45 (C-1), 138.13 (C-1'), 147.09 (C-4), 147.15 (C-3), 154.73 (NHCONH), 171.64 (CONH and COOH); LCMS (m/z): 456.2 (M^++1) ($C_{24}H_{29}N_3O_6$).

3-Benzo[1,3]dioxol-5yl-3-{(S)-2-[(2,3-dihydroindol-1-carbonyl)amino] - 4-methylpentanoylamino}-propionic acid 5.

Step 1 — 3-Benzo[1,3]dioxol-5yl-3-{(S)-2-[(2,3-dihydroindol-1-carbonyl)amino]-4-methylpentanoylamino}propionic acid ethyl ester. Reaction of the amine **11** (250 mg, 0.57 mmole) using triethylamine (174 mg, 1.72 mmole) in dichloromethane at 0ºC (5 mL) followed by carbonyldiimidazole (112 mg, 0.68 mmole) and indoline (68 mg, 0.57 mmole), following the general protocol as described for the preparation

of **13**, furnished (purification by column chromatography; eluent: ethyl acetate-hexane; 1:1) the title ester as solid, yield 153 mg (54%). m.p. 118-120ºC. IR (DCM): 3447, 2957, 1737, 1638, 1596, 1539, 1485, 1443, 1398, 1354, 1285, 1241, 1163, 1040 cm^{-1}; ^1H NMR (~1:1 mixture of diastereomers): δ 0.89 (d, *J* = 5.7 Hz) and 0.96 (d, *J* = 5.7 Hz) (6H, $CH(CH_3)_2$), 1.15 (t, *J* = 6.6 Hz) and 1.18 (t, *J* = 6.6 Hz) [3H, CH_2CH_3], 1.66-1.70 (3H, m, CH_2CH), 2.78-2.83 (2H, m, CH_2COOEt), 3.17-3.20 (2H, m, $ArCH_2$), 3.93-4.09 (4H, m, OCH_2 and NCH_2], 4.44-4.47 (1H, bs, NHC*H*O), 5.26-5.28 (1H, m, NHC*H*Ar), 5.87 (s) and 5.94 (s) [2H, OCH_2O], 6.65-6.67 (1H, m), 6.76-6.86 (2H, m), 6.91-6.96 (1H, m), 7.14-7.16 (2H, m) and 7.83-7.89 (1H, m) [Ar-H]; LCMS (m/z): 496.2 (M^++1) ($C_{27}H_{33}N_3O_6$).

Step 2—3-Benzo[1,3]dioxol-5yl-3-{(S)-2-[(2,3-dihydroindol-1-carbonyl)amino]-4-methylpentano-ylamino}propionic acid 5. Hydrolysis of the propionic acid ethyl ester (145 mg, 0.29 mmole) obtained in Step 1 above, using lithium hydroxide monohydrate (25 mg, 0.58 mmole) in tetrahydrofuran (1.5 mL), methanol (0.5 mL), and water (0.5 mL), following the general protocol as described for compound **3**, furnished title compound **5** as white solid, yield 120 mg (88%), (mixture of diastereomers; 45:54 by HPLC, t_R = 25.94 and 26.07 min; PDA 250 nm). m.p. 105-107ºC. IR (DCM): 3426, 2958, 1714, 1634, 1536, 1485, 1442, 1399, 1355, 1241, 1102, 1040 cm^{-1}; ^1H NMR (~1:1 mixture of diastereomers): δ 0.83 (d, *J* = 6 Hz) and 0.90 (d, *J* = 6 Hz) [6H, $CH(CH_3)_2$], 1.55-1.65 (3H, m, CH_2CH), 2.85-3.10 (2H, m, CH_2COOH), 3.10-3.50 (2H, m, $ArCH_2CH_2N$), 3.86-3.92 (2H, m, NCH_2), 4.73-4.76 (1H, m, NHC*H*CO), 5.33 (2H, bs, ArC*H*N*H*), 5.87 (s) and 5.94 (s) [2H, OCH_2O], 6.64-6.93 (4H, m), 7.10-7.15 (2H, m), 7.76 (1H, d, *J* = 9 Hz) [Ar-H], 8.23 (1H, m, NH); ^{13}C NMR (~1:1 mixture of diastereomers): δ 22.30 (CH_3), 22.77 (CH_3), 24.73 and 24.92 (*C*HCH_3), 27.69 (CH_2Ar), 39.40 and 39.52 (*C*H_2CH), 42.03 and 42.44 (*C*H_2CO_2H), 46.96 (NCH_2), 48.83 and 49.03 (NCHAr), 52.09 and 52.53 (N*C*HCO), 101.03 (OCH_2O), 107.031 (C-2), 108.20 (C-5), 115.03 (C-6'), 119.35 and 119.54 (C-6), 122.32 (C-4'), 124.59 (C-3'), 127.54 (C-5'), 130.51 (C-2'), 134.26 and 134.89 (C-1), 143.06 (C-1'), 146.64 and 146.71 (C-4), 147.77 (C-3), 154.96 and 155.03 (NHCONH), 173.05 and 173.17 (COOH), 173.83 (CONH); LCMS (m/z): 468.4 (M^++1) ($C_{25}H_{29}N_3O_6$).

Biology

Compounds **3-6** were tested for VLA-4 ($\alpha_4\beta_1$) antagonistic activity in Jurkat cell adhesion assays[22]. VCAM-1 (100 ng/well) was coated in Maxisorp microtitre module at 4°C overnight. Non-specific blocking was carried out with 3% BSA for 2 hr and the wells washed with TBS (50 mM Tris, 0.15 *M* NaCl *p*H 7.4, 0.1 m*M* $CaCl_2$, 0.1 m*M* $MgCl_2$, 0.1 m*M* $MnCl_2$). U937 cells were suspended in fresh medium and incubated at 37°C for 2 hr before the assay. Cells were then washed in TBS solution and 180 μL of cell suspension (1×10^6 cells/mL in TBS buffer) added per well in VCAM-1 coated wells. 20 μL of test compound solution in 50% DMSO and 50% TBS was then added and the cells incubated at 37°C for 1 hr. 3 to 5 dilutions of each test compound were tested in duplicate. After incubation, the non-adherent cells were removed by washing with TBS and the number of adhered cells quantified by LDH activity estimation. The percent adhesion was calculated as compared to control.

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