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Selective cell adhesion inhibitors: Barbituric acid based α4β7—MAdCAM inhibitors

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Abstract—A novel series of barbituric acid derivatives were identified as selective inhibitors of α4β7 MAdCAM (mucosal addressin cell adhesion molecule-1) interactions via a high throughput screening exercise. These inhibitors were optimized to submicromolar potencies in whole cell adhesion assays, retaining their selectivity over α4β1 VCAM.

The circulation and homing of leukocyte subsets is characterized by an intricate series of cell–cell interactions that collectively contribute to a tissue specific homing event.[1,2] This multistep process that leads to the infiltration of leukocytes into target tissues involves multiple interactions, which include the association of carbohydrates with selectins, chemokine receptors with chemokines, and integrins with immunoglobulin-like (Ig-like) cell adhesion molecules. Lymphocytes bearing the integrin α4β7, a heterodimeric protein expressed on T-lymphocytes, home specifically to tissues expressing the cell adhesion molecule mucosal addressin cell adhesion molecule (MAdCAM).[3–6] This cell adhesion molecule is specifically expressed on specialized vascular endothelial cells within gut associated lymphoid tissues and within lamina propria as well. Under inflammatory activation conditions the expression of MAdCAM is highly upregulated and results in increased recruitment of α4β7+ lymphocytes. This phenomenon is associated with tissue damage in both murine and non-human primate models of inflammatory bowel disease (IBD) where anti-α4β7 mAbs have shown efficacy.[7,8]

MAdCAM is known to interact with its ligand α4β7 using a very select group of residues in the c–d loop of its first immunoglobulin domain where the recognition binding sequence is leucine–aspartic acid–threonine (**1**, LDT).[9,10] The literature contains several examples of modified tripeptides,[11–13] tripeptide mimics,[14,15] and modified amino acids (phenylalanine derivatives)[16–21] as integrin antagonists. Close mimics of the recognition binding motif including modified LDT analogs[22] and mannose scaffolded LDT mimics[23] have proven to provide highly selective molecules. Most members of the phenylalanine class of α4β7-MAdCAM inhibitors are non-selective and inhibit both α4β7-MAdCAM and α4β1-VCAM mediated cell adhesion. Recent reports in the literature regarding potential undesirable immunomodulatory effects via the inhibition of α4β1-VCAM illustrate the importance of selective inhibitors for use in the treatment of diseases driven by α4β7/MAdCAM lymphocyte trafficking.[24] In our efforts to develop small molecule antagonists of leukocyte trafficking, we identified **2** as an inhibitor of cell adhesion mediated by the leukocyte cell surface integrin α4β7 and the cell adhesion molecule MAdCAM via a high throughput screen of our small molecule collection.

We herein report on the chemistry involved in our optimization efforts in this series of novel and selective barbituric acid based integrin antagonists. The starting molecule **2**, proved to be a selective inhibitor of

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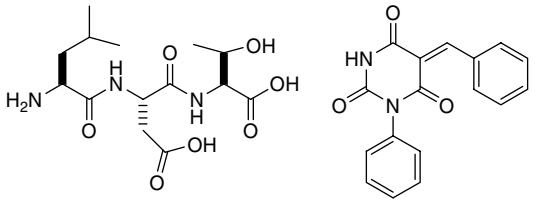
α4β7 binding to MAdCAM, had an $IC_{50} = 2.2\ \mu M$ (Fig. 1).

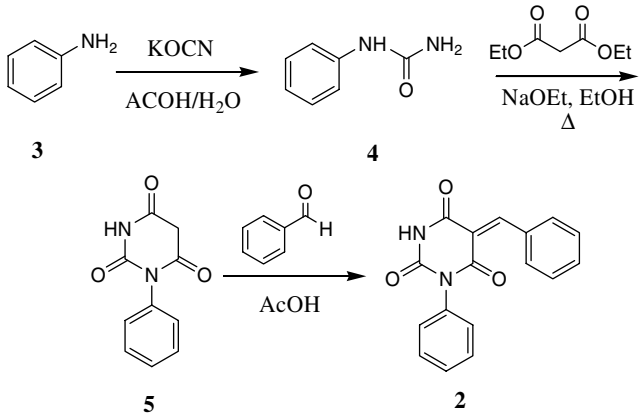
The chemistry leading to the barbituric acids was initiated by the formation of the appropriately substituted urea (Scheme 1, synthesis of compound **2** used as example) via the reaction of potassium cyanate and a substituted amine **3**.[25,26] This provided the urea **4** in 90% yield. This was followed by a two component reaction involving the condensation of a diacid equivalent with the appropriately substituted urea **4** in refluxing sodium ethoxide in ethanol producing the barbituric acid **5** in 40% yield.[27,28] Reaction of the activated methylene with benzaldehyde in the presence of a catalytic amount of acetic acid produced the exocyclic olefin **2** in 85% yield.[29]

We investigated the structure–activity relationships of the barbituric acid for their ability to inhibit the interaction between α4β7 and MAdCAM. Our initial approach included the modifications of three functional groups outlined in Figure 2. These molecules were evaluated in a α4β7-MAdCAM mediated whole cell adhesion assay.[30]



1, LDT **2**

Figure 1. The recognition binding motif sequence **1** of MAdCAM and the barbituric acid **2**.



3 **4** **5** **2**

Scheme 1. General schematic of the synthesis of barbituric acids.

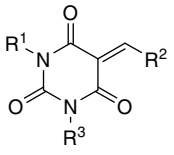


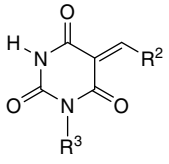
Figure 2. Three sites of diversity (R^1, R^2, and R^3) were investigated.

Initial analogs were synthesized systematically holding R^1 and R^3 constant (R^1 = hydrogen, R^3 = phenyl) and varying the substituent at R^2. From these data we were able to identify certain functionality for R^2 that resulted in more potent molecules. Examples of this are shown in Table 1. The scope of the analogs ranged from small alkyl groups (exemplified by **11**) to larger heterocyclic groups. The optimal functional groups identified were in general heterocyclic, with the analog containing the 3-indolyl group (**9**) being one of the most potent early molecules. Position of the heteroatom proved important as the 2-indolyl **10** was less potent than the 3-indolyl **9**. Aromatic substitutions affecting the electronics of the phenyl containing analogs were inconsequential to the potency of the compounds (exemplified by **14**, **15**, and **16**).

Once the 3-indolyl was identified as an important pharmacophore component, the relative importance of the free N–H hydrogens was investigated, Table 2. Methylation at the indole nitrogen resulted in a decrease in activity (exemplified by **17**). In addition methylation at the barbituric N–H also resulted in decreased potency (exemplified by **18**).

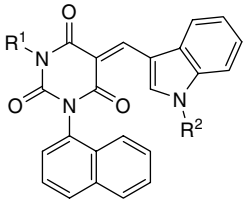
Selected analogs were then evaluated for their selectivity against two additional integrin/cell adhesion molecule pairs to determine the structure–activity trends in selectivity (Table 3). Since we were interested in advancing selective α4β7-MAdCAM selective inhibitors only, we were pleased to find that our molecules proved to be inactive against both the related integrin/cell adhesion molecule pair α4β1-VCAM and the non-related integrin/cell adhesion molecule pair α5β1-fibronectin.

Table 1. Potency of inhibitors



Compound	R^2	R^3	α4β7/MAdCAM IC_{50} (μM)
2	Ph	Ph	2.2
6	Cynamoyl	Ph	8.6
7	2-Furyl	Ph	1.3
8	*N*-CH_3-3-indoyl	Ph	4.1
9	3-Indoyl	Ph	0.9
10	2-Indoyl	Ph	10
11	3-Indoyl	*t*-Butyl	>20
12	3-Indoyl	2-Biphenyl	0.26
13	3-Indoyl	Naphthyl	0.06
14	Phenyl	4-ChloroPh	1.0
15	4-CF_3Ph	Ph	1.3
16	Ph	3,5-DichloroPh	1.6

Table 2. Potency of certain 3-indoyl substituted inhibitors



Compound	R^1	R^2	α4β7/MAdCAM IC_{50} (μM)
13	H	H	0.06
17	H	CH_3	0.18
18	CH_3	H	5.5

Table 3. Selectivity of certain potent inhibitors α4β1/VCAM

Compound	α4β7/MAdCAM IC_{50} (μM)	α4β1/VCAM IC_{50} (μM)	α5β1/FN IC_{50} (μM)
7	1.3	>20	>20
12	0.26	>20	>20
13	0.06	>20	>20

The pharmacokinetic properties in mouse of compound **13** were examined, and it showed an oral bioavailability of 62%, a *t*1/2 of 4.1 h, a clearance of 71 mL/min/kg, a volume of distribution of 24.8 L/kg, and an AUC of 170 mg min/mL. The pharmacokinetics of this molecule was not suitable for its evaluation in the DSS (dextran sulfate sodium) derived mouse model of inflammatory bowel disease.[31] Further optimization of this chemical series is ongoing and may result in molecules with more suitable exposure profiles to enable a proof of concept study with this class of molecules.

References and notes

1. Butcher, E. C. *Cell* **1991**, *67*, 1033.
2. Springer, T. A. *Cell* **1994**, *65*, 301.
3. Brezinschek, R. I.; Brezinschek, H.-P.; Lazarovits, A. I.; Lipsky, P. E.; Oppenheimer-Marks, N. *Am. J. Pathol.* **1996**, *149*, 1651.
4. Berlin, C.; Berg, E. L.; Briskin, M. J.; Andrew, D.; Kilshaw, P. J.; Holzmann, B.; Weissman, I. L.; Hainann, A.; Butcher, E. C. *Cell* **1993**, 74.
5. Bargatze, R. F.; Jutila, M. A.; Butcher, E. C. *Immunity* **1995**, *3*, 99.
6. Briskin, M.; Winsor-Hines, D.; Shyan, A.; Cochran, N.; Bloom, S.; Wilson, J.; Butcher, E. C.; Kassam, N.; Mackay, C. R.; Newman, W.; Ringler, D. *J. Am. J. Pathol.* **1997**, *151*, 97.
7. Picarella, D.; Hurlbut, P.; Rottman, J.; Shi, X.; Butcher, E.; Ringler, D. *J. Immunol.* **1997**, *158*, 2099.
8. Hesterberg, P. E.; Winsor-Hines, D.; Briskin, M. J.; Soler-Ferran, D.; Merrill, C.; Mackay, C. R.; Newman, W.; Ringler, D. J. *Gastroenterology* **1996**, *111*, 1373.
9. Briskin, M. J.; McEvoy, L. M.; Butcher, E. C. *Nature* **1993**, *363*, 461.
10. Briskin, M. J.; Rott, L.; Butcher, E. C. *J. Immunol.* **1996**, *156*, 719.
11. Shroff, H.; Schwender, C.; Baxter, Brookfield A.; Payne, L.; Cochran, N.; Gallant, D.; Briskin, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1601.
12. Shroff, H.; Schwender, C.; Dottavio, D.; Yang, L.; Briskin, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2495.
13. Dubree, N.; Artis, D.; Castanedo, G.; Marsters, J.; Sutherlin, D.; Caris, L.; Clark, K.; Keating, S.; Beresini, M.; Chiu, H.; Fong, S.; Lowman, H.; Skelton, N.; Jackson, D. *J. Med. Chem.* **2002**, *45*, 3451, cyclic peptide.
14. Boer, J.; Gottschling, D.; Schuster, A.; Semmrich, M.; Holzmann, B.; Kessler, H. *J. Med. Chem.* **2001**, *44*, 2586.
15. Georgette, M. C.; Fredrick, C. S.; Nathan, J. P. D.; John, B. N.; Caris, L.; Clark, K.; Keating, S.; Beresini, M.; Chiu, H.; Fong, S.; Marsters, J.; Jacksona, D.; Sutherlina, D. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2913.
16. Harriman, G.; Schwender, C.; Gallant, D.; Cochran, N.; Briskin, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1497.
17. Lawson, E. C.; Kinney, W. A.; Santulli, R. J.; Fisher, C. M.; Damiano, B. P.; Maryanoff, B. E. *Lett. Drug Des. Discov.* **2005**, *2*, 601.
18. Jackson, D. *Curr. Pharm. Des.* **2002**, *8*, 99.
19. Dyatkin, A. B.; Gong, Y.; Miskowski, T. A.; Kimball, E. S.; Prouty, S. M.; Fisher, M. C.; Santulli, R. J.; Schneider, C. R.; Wallace, N. H.; Hornby, P. J.; Diamond, C.; Kinney, W. A.; Maryanoff, B. E.; Damiano, B. P.; He, W. *Bioorg. Med. Chem.* **2005**, *13*, 6693, phe analogs.
20. Castanedo, G. M.; Sailes, F. C.; Dubree, N. J. P.; Nicholas, J. B.; Caris, L.; Clark, K.; Keating, S. M.; Beresini, M. H.; Chiu, H.; Fong, S.; Marsters, J. C.; Jackson, D. Y.; Sutherlin, D. P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2913.
21. Gong, Y.; Barbay, J. K.; Dyatkin, A. B.; Miskowski, T. A.; Kimball, E. S.; Prouty, S. M.; Fisher, E. C.; Santulli, R. J.; Schneider, C. R.; Wallace, N. H.; Ballentine, S. A.; Hageman, W. E.; Masucci, J. A.; Maryanoff, B. E.; Damiano, B. P.; Andrade-Gordon, P.; Hlasta, D. J.; Hornby, P. J.; He, W. *J. Med. Chem.* **2006**, *49*, 3402.
22. Carson, K.; Schwender, C.; Shroff, H.; Cochran, N.; Gallant, D.; Briskin, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 711.
23. Locardi, E.; Boer, J.; Modlinger, A.; Schuster, A.; Holzmann, B.; Kessler, H. *J. Med. Chem.* **2003**, *46*, 5752.
24. Engelhardt, B.; Briskin, M. J. *Eur. J. Immunol.* **2005**, *35*, 2268.
25. Yogeeswari, P.; Sriram, D.; Brahmandam, A.; Sridharan, I.; Thirumurugan, R.; Stables, J. P. *Med. Chem. Res.* **2003**, *12*, 57.
26. Laudien, R.; Mitzner, R. J. *Chem. Soc., PT 2* **2001**, *11*, 2226.
27. Oguz, S. F.; Dogan, I. *Tetrahedron: Asymmetry* **2003**, *14*, 1857.
28. Jursic, B. S.; Neumann, D. M. *Tetrahedron Lett.* **2001**, *42*, 8435.
29. Naya, S.; Nitta, M. *Tetrahedron* **2003**, *59*, 3709.
30. To evaluate these compounds, a cell adhesion assay involving the B-cell lymphoma RPMI 8866 cells, and soluble human MAdCAM-IG chimera was used in a 96-well plate format. RPMI 8866 cells have previously been shown to bind avidly to MAdCAM. RPMI 8866 cells were fluorescently labeled by preincubation with BCECF stain (Molecular Probes), washed, and resuspended in assay buffer containing 2% fetal calf serum and 2 mM Mg^{2+}. Compounds were tested in HBSS/2% FCS/25 mM Hepes buffer at 2.5 106 cells per mL. The typical assay consisted of a final volume of 200 mL containing 50 mL of cells at 1.25 10^5 cells per well. Adhesion assays for MAdCAM were washed on an automatic plate washer using a buffer

consisting of 50 mM Tris/150 mM NaCl/2 mM $MnCl_2$, pH 7.2, in a wash volume of 500 mL for two wash cycles. Assays were then read on an Idexx fluorescent plate reader at 485/535 nm. Inhibition was determined by the number of cells adhering to the MAdCAM lined plates in the presence and absence of an inhibitor and IC_{50} values were determined using Kaleidagraph (Adelbeck Software) and are reported as an average of several determinations.

31. Cooper, H. S.; Murthy, S. N. S.; Shah, R. S.; Sedergran, D. J. *Lab. Invest.* **1993**, *69*, 238.