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Efficient Synthesis of 3-Aminocyclobut-2-en-1-ones: Squaramide Surrogates as Potent VLA-4 Antagonists

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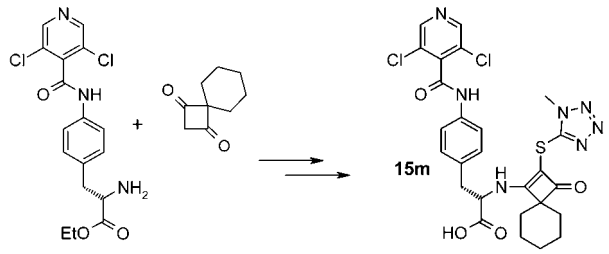
ABSTRACT



A novel series of uniquely functionalized 3-aminocyclobut-2-en-1-ones has been prepared by facile condensation of a variety of cyclobuta-1,3-diones with a phenylalanine-derived primary amine. These systems subsequently lend themselves to substitution at C-2 by reaction with a variety of electrophilic reagents including *N*-halosuccinimides, sulfenyl chlorides, and Eschenmoser's salt. Compounds from this novel series are potent antagonists of VLA-4.

The integrins are a large family of heterodimeric, cell surface receptor proteins which consist of an α and a β subunit. Restricted association of these subunits can give rise to more than 24 characterized integrins, which bind specific peptide ligands of the extracellular matrix.[1] The integrin VLA-4 (very late activating antigen-4, α4β1, CD49d/CD29) is expressed on a variety of leukocytes and through interaction with its endothelial ligand VCAM-1 (vascular cell adhesion molecule) plays an important role in recruitment, trafficking, and infiltration of circulating lymphocytes to sites of inflammation.[2] Accumulating clinical evidence from the use of monoclonal antibodies (i.e., Antegren/Natalizumab) strongly suggests that pharmacological antagonism of VLA-4 is a viable method for alleviating the debilitating tissue damage which arises in autoimmune disorders including rheumatoid arthritis, asthma, multiple sclerosis, and Crohn's disease.[3]

VCAM-1 has been shown to bind VLA-4 through the sequences Ile-Asp-Ser.[4] This binding motif was used as a starting point for early proteomimetic inhibitor design efforts, which initially gave rise to cyclic peptides such as **1** (Figure 1), being derived from this sequence.[5] However, peptides typically make poor drug candidates as they frequently have sub-optimal absorption properties and low metabolic stability, factors which can significantly impede systemic exposure.

N-Acylated phenylalanine derivatives have subsequently emerged from several groups as a major class of potent VLA-4 antagonist.[6] Notable members of this useful class of

(1) Elices, M. J. In *Cell Adhes. Mol. Matrix Proteins*; Mouse, S. A., Ed.; Springer: Berlin, 1999; p 133.
(2) Hemler, M. E. *Annu. Rev. Immunol.* **1990**, *8*, 365.
(3) Seiffge, J. *J. Rheumatol.* **1996**, *23*, 2086. Gordon, F. H.; Lai, C. W. Y.; Hamilton, M. I.; Pounder, R. E. *Gastroenterology* **2001**, *121*, 268. Borchers, M. T.; Crosby, J.; Farmer, S. *Am. J. Physiol.* **2001**, *280*, 813.
(4) Renz, M. E.; Chiu, H. H.; Jones, S.; Fox, J.; Kim, K. J.; Presta, L. G.; Fong, S. *J. Cell Biol.* **1994**, *125*, 1395.
(5) Fotouhi, N.; Joshi, P.; Fry, D.; Cook, C.; Tilley, J. W.; Kaplan, G.; Hanglow, A.; Rowan, K.; Schwinge, V.; Wolitsky, B. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1171.
(6) Porter, J. R. *IDrugs* **2000**, *3*, 788. Tilley, J. W. *Expert Opin. Ther. Pat.* **2002**, *12*, 991.

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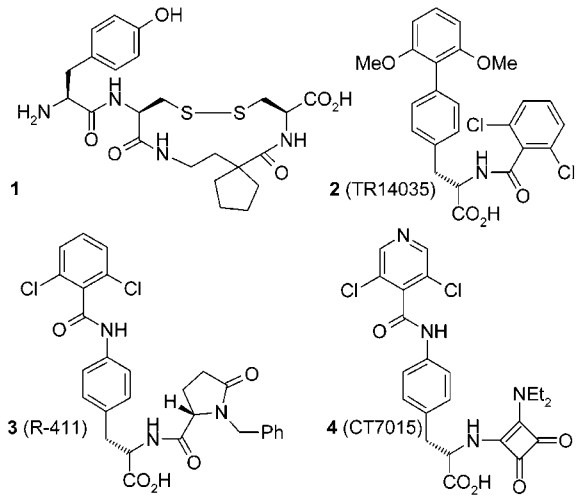


Figure 1. Potent small molecule VLA-4 antagonists.

small molecule are **2**[7] and **3**,[8] which are reportedly undergoing clinical evaluation for the treatment of asthma and are currently in phase 1 and phase 2, respectively.

Of the numerous inhibitors reported in this class, it is clear that a high degree of diversity in the *N*-acyl substituent is tolerated, although these are generally hydrophobic, and that the amino acid α-NH is important for activity. We have recently described the discovery of squaric acid derivatives including CT7015 (**4**) as VLA-4 antagonists[9] that illustrate these observations.

As part of a search for a unique class of VLA-4 antagonists distinct from **4**, we hypothesized that the combination of a hydrophobic spirocyclic substituent, such as that in **1**, in the context of a rigid planar scaffold such as that provided by the 3,4-diamino-3-cyclobuten-1,2-dione (squaramide) group of **4**, would provide potent compounds with an advantageous pharmacokinetic profile. We believed that the 3-amino-2-cyclobuten-1-one analogues typified by **5** would be the most appropriate compounds to test this "hybridization" hypothesis (Figure 2) and investigated the synthesis of these by condensation of an α-amino ester with a cyclobuta-1,3-dione or its enol ether.[10]

We felt that this intriguing structural element, thus far unutilized in the pharmaceutical arena, warranted further

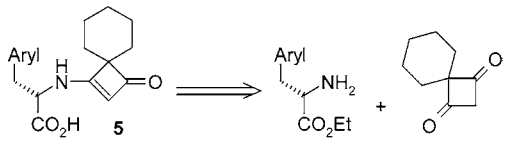
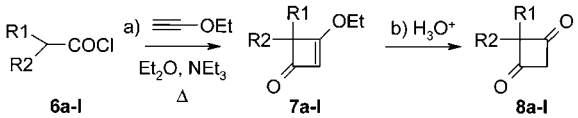


Figure 2. Proposed route to 3-aminocyclobut-2-en-1-ones.

investigation and embarked upon development of a robust method which would grant access to **5** and its analogues.

Accordingly, *gem*-disubstituted 3-ethoxy-2-cyclobutene-1-ones were readily accessed by the cycloaddition of a disubstituted ketene, generated in situ by dehydrohalogenation of the corresponding acid chloride, and ethoxyacetylene, according to a previously recognized phenomenon[11] (Scheme 1). For example, the reaction of cyclohexanecarbonyl

Scheme 1. Synthesis of Cyclobuta-1,3-diones



6a-l **7a-l** **8a-l**

R1	R2		7 yield (%)	8
CH_3	CH_3	**a**	71	91
CH_3	Ph	**b**	45	85
CH_3	Bn	**c**	86	98
(R1=R2)	$-(CH_2)_4-$	**d**	70	99
	$-(CH_2)_5-$	**e**	81	92
	$-(CH_2)_6-$	**f**	87	95
	$-(CH_2)_2NAc(CH_2)_2-$	**g**	67	87
	$-(CH_2)_2O(CH_2)_2-$	**h**	59	87
	$-(CH_2)_2SO_2(CH_2)_2-$	**i**	57	68
	$-CH_2CH=CHCH_2-$	**j**	73	65
	$-(CH_2)_2CHOMe(CH_2)_2-$	**k**	65	94
	$-(CH_2)_2CH^tBu(CH_2)_2-$	**l**	84	90

chloride and NEt_3 (1.5 equiv) in the presence of ethoxyacetylene[12] (2.0 equiv) in Et_2O afforded 3-ethoxyspiro[3.5]-non-2-en-1-one (**7e**) in 81% yield.

The reaction time can be reduced from a reported 21 days at 5 °C[10a] to less than 48 h by heating the reaction to reflux temperature. Although feasible, no apparent dimerization of the intermediate ketenes was observed at this elevated temperature. The novel spirocyclic examples **7g**−**l** were prepared using an analogous transformation with comparable yields being obtained. In all cases examined, the cycloadducts were found to be stable and were conveniently purified by column chromatography on silica gel.

Various attempts to convert enolether **7e** directly to the desired 3-amino derivative (i.e., **5**) by substitution with L-phenylalanine ethyl ester as a test substrate were frustrat-

(7) Sircar, I.; Gudmundsson, K. S.; Martin, R.; Liang, J.; Nomura, S.; Jayakumar, H.; Teegarden, B. R.; Nowlin, D. M.; Cardarelli, P. M.; Mah, J. R.; Connell, S.; Griffith, R. C.; Lazarides, E. *Bioorg. Med. Chem.* **2002**, *10*, 2051.

(8) Chen, L.; Tilley, J.; Trilles, R. V.; Yun, Weiya, Y.; Fry, D.; Cook, C.; Rowan, K.; Schwinge, V.; Campbell, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 137.

(9) Porter, J. R.; Archibald, S. C.; Childs, K.; Critchley, D.; Head, J. C.; Linsley, J. M.; Parton, T. A. H.; Robinson, M. K.; Shock, A.; Taylor, R. J.; Warrellow, G. J.; Alexander, R. P.; Langham, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1051.

(10) Wasserman, Martin, and Moore have each reported the synthesis of a single example of the title compound using this approach, the latter within the context of the preparation of an amino-substituted naphthol, see: (a) Wasserman, H. H.; Piper, J. U.; Dehmlow, E. V. *J. Org. Chem.* **1973**, *38*, 1451. (b) Hasek, R. H.; Gott, P. G.; Martin, J. C. *J. Org. Chem.* **1964**, *29*, 2510. (c) Turnbull, P.; Moore, H. W. *J. Org. Chem.* **1995**, *60*, 644.

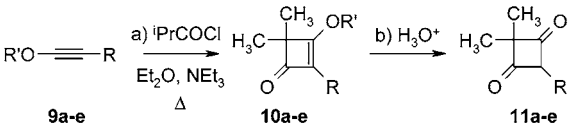
(11) Arens, J. F. *Advances in Organic Chemistry Methods and Results*; Raphael, R. A., Taylor, E. C., Ed.; Interscience Publishers: New York, 1960; Vol. 2, pp 117−212. Martin, J. C. U.S. Patent 3 288 854, 1966.

(12) Ethoxyacetylene was purchased from Lancaster Chemical Co. as an approximately 50%w/w solution in hexanes. Yields **7a**−**l** were calculated based upon the amount of acid chloride used.

ingly unproductive. We were consequently forced to examine formation of the desired vinylogous amides by combination of an amine with a cyclobuta-1,3-dione. To this end, cyclobutenones **7a**−**l** could be hydrolyzed to cyclobutadiones **8a**−**l** in good yield by treatment with 2 M HCl at 20 °C for 24 h. A notable aspect of these conversions is that hydrolysis proceeds without detriment to the four-membered carbocycle, in contrast to analogues lacking geminal substitution at C4 which undergo a facile ring-opening under these conditions to give β-ethoxycrotonic acids.[10a]

In an effort to expand the scope of the ring-forming process, we attempted substitution at the 2-position of the cyclobutenone by employing a variety of substituted alkoxyacetylenes. Consequently, cycloaddition precursors **9a**,**b** were prepared directly by alkylation of the acetylide anion of ethoxyacetylene (1.1 equiv of ^{n}Buli, 2.2 equiv of HMPA) with benzyl bromide and *n*-hexyl iodide, respectively, according to literature procedure.[13] Alkynes **9c**−**e** were prepared by reaction of chloroacetaldehyde dibutylacetal with 2.2 equiv LDA/HMPA followed by alkylation with the appropriate alkyl bromide, RBr.[14] Alkynes **9a**−**e** were also found to undergo cycloaddition with dimethylketene to give enol ethers **10a**−**e** (Scheme 2), and these were hydrolyzed without incident under similar conditions to give diones **11a**−**e**.

Scheme 2. Synthesis of Trisubstituted Cyclobuta-1,3-diones

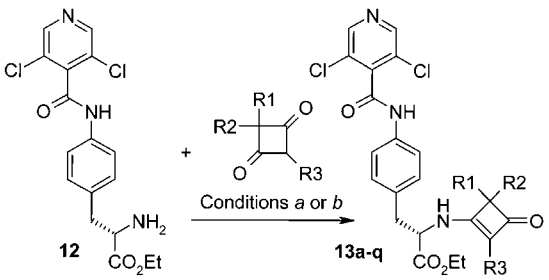


R'	R		**10** yield (%)	**11**
Et	Bn	**a**	75	95
Et	^{n}Hex	**b**	61	98
^{n}Bu	CH_3	**c**	45	85
^{n}Bu	Et	**d**	56	70
^{n}Bu	^{n}Pr	**e**	51	64

In all cases described, the expected regiochemistry of the cycloaddition was complete and no regioisomeric material was detected.

Subsequent reaction of cyclobuta-1,3-diones **8a**−**i** with the phenylalanine derivative **12** was found to proceed with suprising speed when mixed in THF at room temperature, to give the desired vinylogous amide derivatives **13a**−**l** in <24 h[15] (Scheme 3, conditions a). Contrastingly, those reactions involving C-2 susbstituted cyclobuta-1,3-diones **11a**−**e** were wholly unreactive with **12** under these conditions and required heating at 100 °C in nitromethane for 48 h to achieve complete conversion to the corresponding analogues **13m**−**q** (Scheme 3, conditions b).

Scheme 3. Condensation of a Phenylalanine-Derived α−Amino Ester with Cyclobuta-1,3-diones



R1	R2	R3		yield (%) **13**	
CH_3	CH_3	H	*a*	**a**	93
CH_3	Ph	H	*a*	**b**	59
CH_3	Bn	H	*a*	**c**	89
-$(CH_2)_4$-		H	*a*	**d**	86
-$(CH_2)_5$-		H	*a*	**e**	95
-$(CH_2)_6$-		H	*a*	**f**	86
-$(CH_2)_2$NAc$(CH_2)_2$-		H	*a*	**g**	67
-$(CH_2)_2$O$(CH_2)_2$-		H	*a*	**h**	91
-$(CH_2)_2$SO$_2(CH_2)_2$-		H	*a*	**i**	23
-CH_2CH=CHCH_2-		H	*a*	**j**	59
-$(CH_2)_2$CHOMe$(CH_2)_2$-		H	*a*	**k**	87
-$(CH_2)_2$CH^{t}Bu$(CH_2)_2$-		H	*a*	**l**	84
CH_3	CH_3	Bn	*b*	**m**	59
CH_3	CH_3	^{n}Hex	*b*	**n**	72
CH_3	CH_3	Me	*b*	**o**	70
CH_3	CH_3	Et	*b*	**p**	73
CH_3	CH_3	^{n}Pr	*b*	**q**	75

We speculate that this may either be a steric phenomenon or alternatively that the C2 substituent stabilizes the relatively unreactive enolic form of the dione, reducing the relative concentration of the reactive dicarbonyl tautomer. Interestingly, the condensation reaction of *meso*-diones **8b, c** and **k**, which contain prochiral centers, gave inseparable diastereomeric products with the reaction showing only weak diasteroselectivity (<30%). Contrastingly, dione **8l** reacted to give compound **13l** as a single isomer, the stereochemistry of which we were unable to determine conclusively.

With the intention of providing access to novel C-2 substituted versions of **5**, we surveyed the reactivity of **13e** to a variety of electrophiles and the results of this study are summarized in Scheme 4. In keeping with the observation that **7a** undergoes bromination,[10a] vinylogous amide **13e** was found to be rapidly convertible to the 2-bromo analogue **14c** in 95% yield, by reaction with bromine or NBS in THF at room temperature (Scheme 4). Chlorination and iodination are achieved using NCS and NIS respectively and fluorination was performed using Selectfluor[16] to give **14b**, **14d**, and **14a**, respectively. The thioalkyl substituents of **14e**,**f** were introduced by reaction with the appropriate sulfenyl chlorides, which were produced in situ by reaction of the corresponding disulfide and SO_2Cl_2[17] prior to addition of **13f**. Compound **14b** can arise as a minor byproduct of these reactions, presumably through chlorination of **13e** by un-

(13) Pons, J.-M.; Kocienski, P. *Tetrahedron Lett.* **1989**, *30*, 1833. **9a** and **9b** were prepared in 99% and 92% yields, respectively.

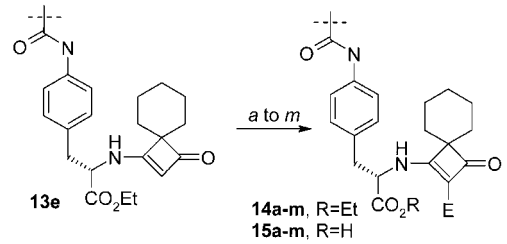
(14) A modification of the method reported in: Newman, M. S.; Geib, J. R.; Stalick, W. M. *Org. Prep. Proced. Int.* **1972**, *4*, 89.

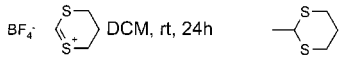
(15) Contrastingly, the reaction of **4** with cyclopenta-1,3-dione required several days for completion under these conditions.

(16) Banks, R. E.; Murtagh, V.; Tsiliopoulous, E. *J. Fluorine Chem.* **1991**, *52*, 389.

(17) Still, I. W. J.; Kutney, G. W.; McLean, D. *J. Org. Chem.* **1982**, *47*, 560.

Scheme 4. Reaction of **13e** with Electrophiles



	reagents and conditions	E	yield **14**(%)
a	Selectfluor™, THF, 70°C, 48h	F	89
b	NCS, THF, rt, 30min	Cl	80
c	Br_2, NEt_3, THF, 0°C, 20min	Br	95
d	NIS, THF, rt, 5min	I	66
e	$ClSCH_3$, THF/DCM, rt, 20min	SMe	94
f	ClS^{i}Pr, THF/DCM, rt, 20min	S^{i}Pr	87
g	ClSPh, THF/DCM, rt, 30min	SPh	81
h	ClSePh	SePh	87
i	$CH_2NMe_2^+ I^-$	CH_2NMe_2	89
j	$Pb(OAc)_4$, DCM, -40°C, 4h	OH	56
k	 DCM, rt, 24h		87
l			89
m			89

reacted SO_2Cl_2, although this side-reaction could be avoided by using an excess of disulfide during the sulfenyl chloride preparation step.

Compounds **14g**,**h** were prepared in an analogous fashion by reaction with commercially available phenylsulfenyl and -selenenyl chloride. A dimethylaminomethyl group can be introduced onto **13e** by reaction with Eschenmoser's salt [$H_2C{=}N(Me)_2^+$ I^-]. Hydroxylation of the unsubstituted vinylic carbon was achieved by oxidation with $Pb(OAc)_4$ to give compound **14j**. π-Electrophiles are also found to react with the nucleophilic amino-cyclobutenone system as evidenced by reaction of **13e** with 1,3-dithienium tetrafluoroborate to give **14k**. The heterocyclic sulfenyl chlorides used in reactions **l** and **m** were prepared in-situ directly from the corresponding heterocyclic mercaptans by reaction with NCS in THF[18] and these were found to react predictably with **13e** to give **14l**,**m**.

The ability of vinyl iodide **14d** to act as a participant in a Stille-type reaction would allow more convenient parallel synthesis of C2-substituted compounds, thereby circumventing the need to synthesize individual acetylenes. To this end, **14d** undergoes a $Pd(PPh_3)_4$-catalyzed Stille coupling with 3-tributylstannylpyridine and tributylstannyl-1,4-pyridazine in 41% and 53% yield, respectively, as representative examples of this strategy.

As a prelude to biological testing, ethyl esters **14a**−**m** were required to be converted to their corresponding acids **15a**−**m**, and this could be achieved by reaction with 1M LiOH in THF, in high yield (>85%). It is noteworthy that the aminocyclobutenone core of all compounds were found to be stable under these basic conditions (with the exception of **14j**), an indication of the "amidic" stabilization of the enaminone moiety. The ability of carboxylic acids **15a**−**m** to disrupt binding of VLA4 to VCAM was evaluated in a cell based assay using E6.1 Jurkat cells (human T-cell line) and immobilized VCAM-1 in the presence of 1%BSA (bovine serum albumin), and all demonstrated an $IC_{50} < 1$ μM. Notably compound **14m** has an IC_{50} of 3 nM in this assay. A fuller analysis of the biological properties of compounds **15a**−**m** and their congeners shall be reported in due course.

In summary, we have developed the synthesis of a variety of highly functionalized 3-amino-2-cyclobutenones by condensation of an amine with a cyclobuta-1,3-dione as the key step. These uncommon functional groups have subsequently shown potential application as squaramide isosteres in a biological context.

Acknowledgment. We thank Dr. Richard Taylor and Barbara Mason for providing spectral and analytical data and Dr. Tony Shock and Paul Hale for cell assay data.

Supporting Information Available: Preparative methods and spectral and analytical data for compounds **7e**−**l**, **13a**−**q**, and **14a**−**m**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) 1-Methyltetrazole-5-sulfenyl chloride has been previously prepared by chlorination of the mercaptan with chlorine. See: Postle, S. R. U.S. Patent 4 387 158, 1983.