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Heterocycles |*Hot Paper*|

Synthesis of Highly Substituted 1,2-Diazetidin-3-ones, Small-Ring Scaffolds for Drug Discovery

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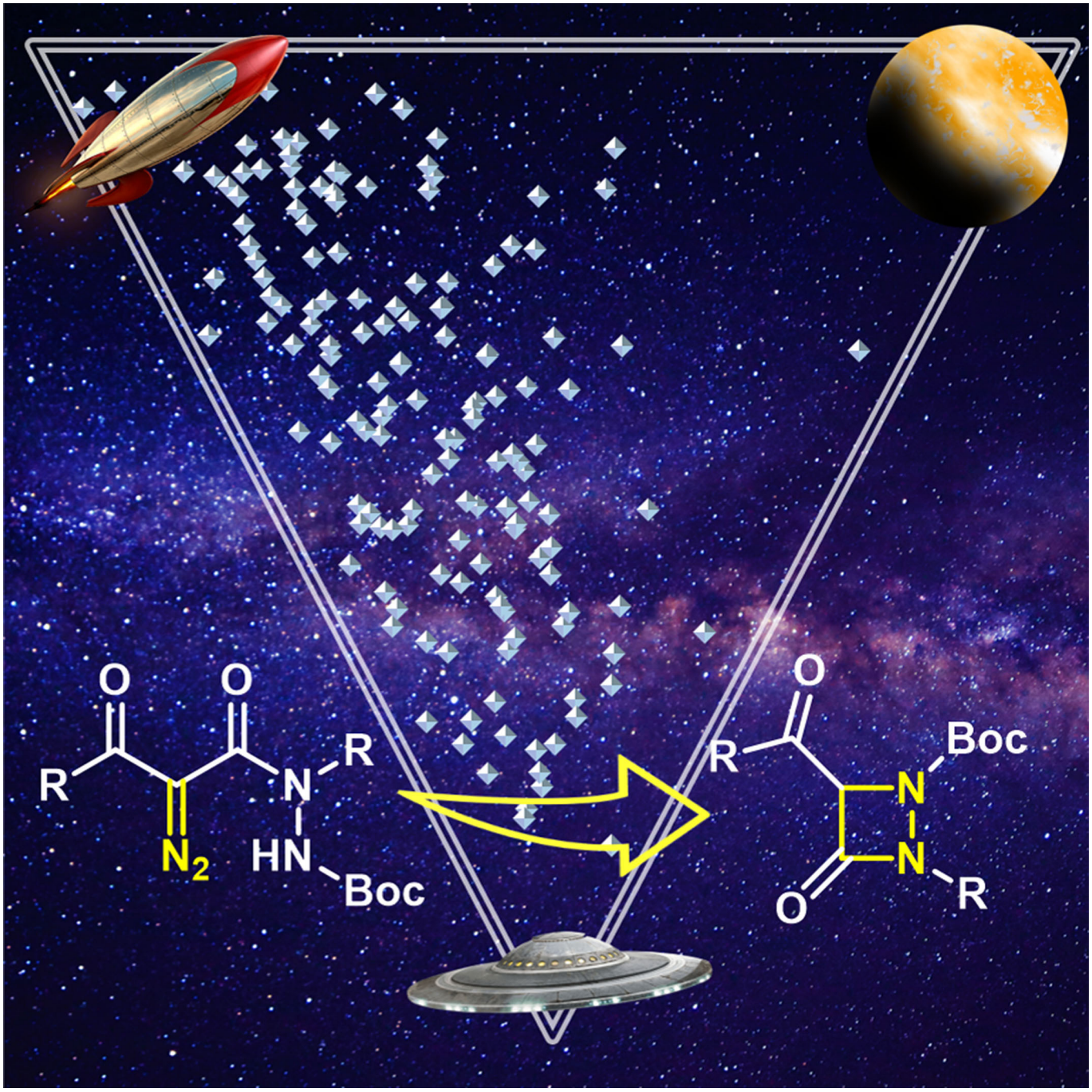

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Abstract: 1,2-Diazetidin-3-ones are readily accessible, small ring scaffolds that upon functionalization have the potential to produce diverse 3-dimensional structures for drug discovery. Thus, treatment of diazo hydrazides, obtained from simple hydrazides and malonyl half ester derivatives, followed by diazo transfer, with catalytic amounts of rhodium(II) acetate dimer results in intramolecular carbenoid N–H insertion to give 1,2-diazetidin-3-ones. Although subsequent functionalization reactions could be hampered by the lability of the 4-membered ring, a wide range of new derivatives was available by deprotection at N-1, and subsequent amide or urea formation. The structures of four four-membered rings was confirmed by X-ray crystallography; the compounds showed modest growth inhibitory activity in mammary carcinoma cells.

Introduction

Four-membered rings are increasingly prevalent as bioactive scaffolds in drug discovery programmes.[1] Not only do they add structural novelty in under-explored areas of chemical space, they can also contribute to enhanced physicochemical properties. This has been amply illustrated by the dramatic increase in interest in oxetane ring systems in recent years.[2] One four-membered ring that attracted our attention is the 1,2-diazetidin-3-one core structure (Figure 1).[3] Although known for almost a 100 years, this small ring system remains largely unexplored in medicinal chemistry, but has recently attracted renewed attention following the discovery that they act as potent inhibitors of the serine hydrolase protein phosphatase methylesterase-1 by acylation of the key serine residue.[4] Notwithstanding their potential liability toward ring opening, these densely functionalized small rings possess well-defined vectors, and form a worthwhile low molecular weight scaffold for drug discovery.

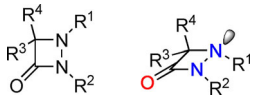

Figure 1. The 1,2-diazetidin-3-one scaffold.

To examine the potential molecular and structural diversity of the 1,2-diazetidin-3-one scaffold a virtual library was enumerated using LLAMA (Lead-Likeness And Molecular Analysis).[5] A virtual library of 177 compounds based on 3 core scaffolds was shown to have suitable molecular properties as lead compounds for drug discovery (RMM = 369, ALogP = 1.11, pTSA = 104 Å^2). Additionally, the 1,2-diazetidinone fragment is highly distinctive from existing fragment databases. The Murko framework[6] of the 1,2-diazetidinone core was not found as a substructure in a random 2% sample of the ZINC database of commercially available compounds.[7] Furthermore, examining the molecular shape of the virtual library using a PMI plot indicates that diverse three-dimensional molecular shape can be achieved by functionalization of the 1,2-diazetidinone core (Figure 2).

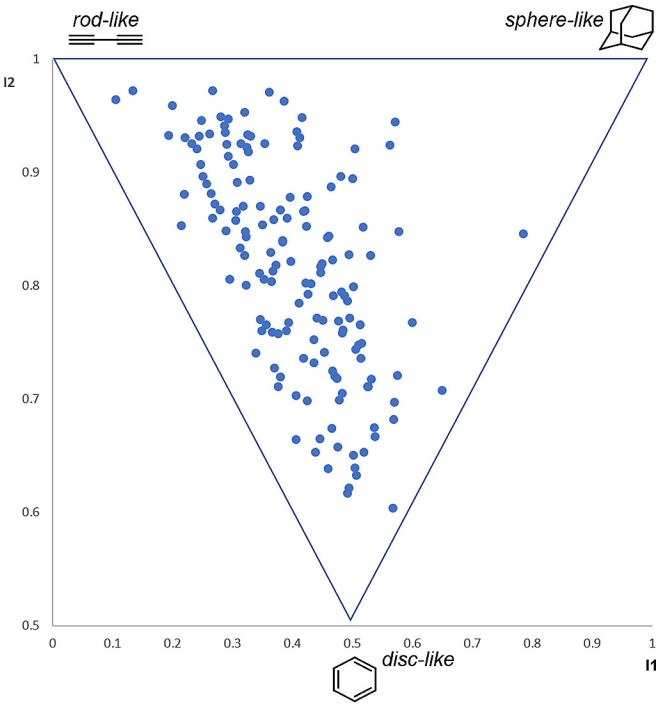

Figure 2. Principal moments of inertia (PMI) plot of the prepared scaffolds, generated using LLAMA.

In addition to being an attractive scaffold for further functionalization, 1,2-diazetidin-3-ones are aza-analogues of the well known β-lactam antibiotics. Despite being discovered 90 years ago, β-lactam antibiotics continue to play a key role in clinical practice and remain one of the best-investigated families of antibiotics. Their mode of action and susceptibility to resistance via β-lactamase enzymes have been widely studied, resulting in the chemical synthesis and biological evaluation of numerous derivatives and analogues.[8] Much of the interest in analogues has focused on the modification of the ring system of naturally occurring β-lactams such as the peni-

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cillins and cephalosporins. Such analogues often incorporate an additional nitrogen atom in the ring, and include 2-azapenems,[9] 3-azacephams,[9b] and 1-azacephems (Figure 3).[10] 1,2-Diazetidin-3-ones represent an alternative aza-β-lactam analogue that places the additional nitrogen in the four-membered ring itself.

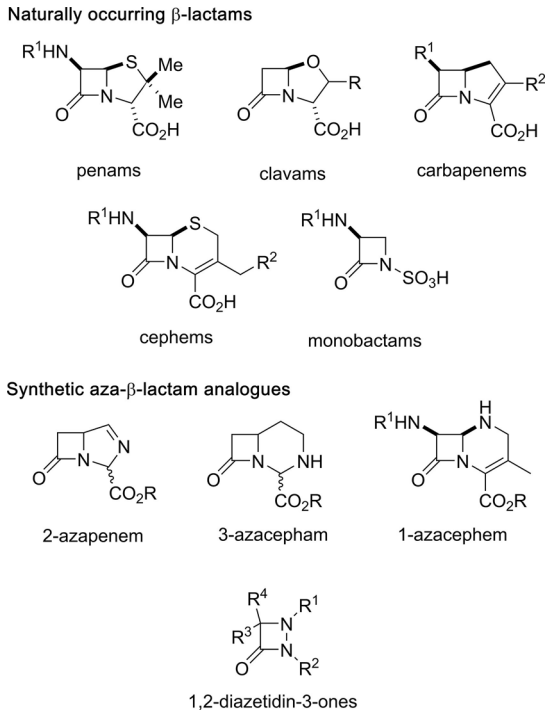
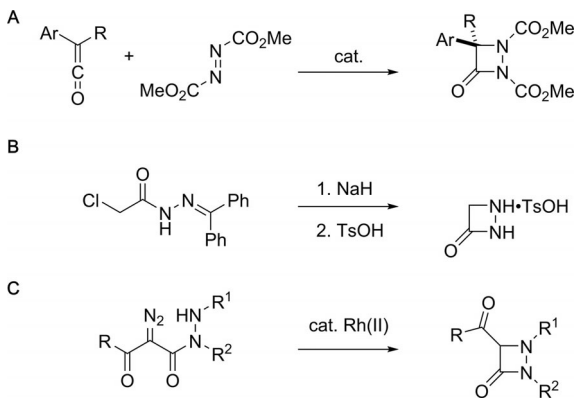


Figure 3. Naturally occurring β-lactams and some aza-analogues.

1,2-Diazetidin-3-ones were originally prepared almost a century ago by Staudinger by reaction of azo compounds with ketenes.[11] The process has been subject of renewed interest with the development of enantioselective versions using asymmetric nucleophilic catalysis (Scheme 1 A),[12] although it remains somewhat limited in its substrate scope. In contrast, in seminal work Taylor and co-workers described versatile routes to 1,2-diazetidin-3-ones based on the cyclization of benzophenone chloroacetylhydrazone, followed by treatment with 4-toluenesulfonic acid to give the parent ring system for further elaboration (Scheme 1 B).[13] Our own contributions centered on the intramolecular N–H insertion reaction of rhodium carbene intermediates derived from diazocarbonyl compounds (Scheme 1 C).[14]



Scheme 1. Routes to 1,2-diazetidin-3-ones.

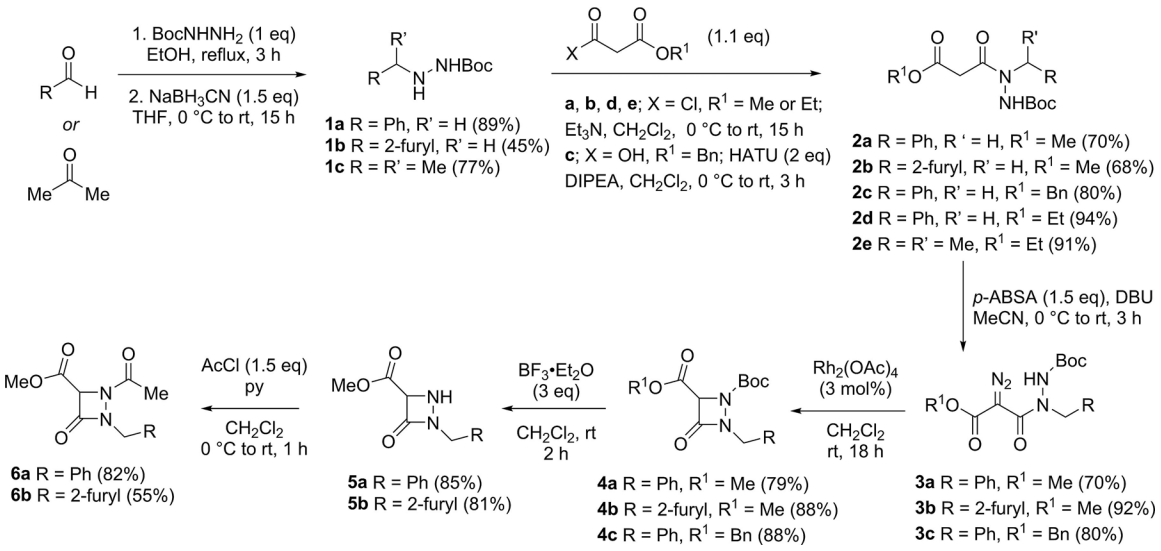
Despite the development of successful synthetic routes,[3] 1,2-diazetidinones remain poorly investigated, and attempts to further develop their chemistry have often foundered due to their lability and inherent ring strain. We now report a detailed study on the synthesis of a range of 1,2-diazetidin-3-one scaffolds using the versatile rhodium carbene methodology, followed by their subsequent derivatization reactions, and preliminary biological evaluation.

Results and Discussion

The synthesis began with the reaction between benzaldehyde and *tert*-butyl carbazate followed by reduction with cyanoborohydride to form the hydrazine **1 a**. Treatment with methyl malonyl chloride and triethylamine in dichloromethane at room temperature gave the desired 1,3-dicarbonyl compound **2 a** in 70% yield (Scheme 2). Subsequent diazo group transfer reaction using *p*-acetamidobenzenesulfonyl azide (*p*-ABSA)[15] in the presence of DBU afforded diazocarbonyl compound **3 a** in good yield. The diazocarbonyl compound represents the key substrate for the overall strategy, and treatment with a catalytic amount of rhodium(II) acetate dimer (3 mol%) induced metallocarbene formation and intramolecular N–H insertion providing the 1,2-diazetidin-3-one **4 a** as a single product in 79% yield after chromatographic purification. The same synthetic sequence was applied to the hydrazine **1 b** derived from furfural, and delivered the 1,2-diazetidin-3-one **4 b** in comparable fashion. Likewise the corresponding benzyl ester **4 c** was prepared as outlined in Scheme 2. As expected, the 1,2-diazetidin-3-ones exhibited a high frequency carbonyl stretch at about 1800 cm^{-1} in their IR spectra.

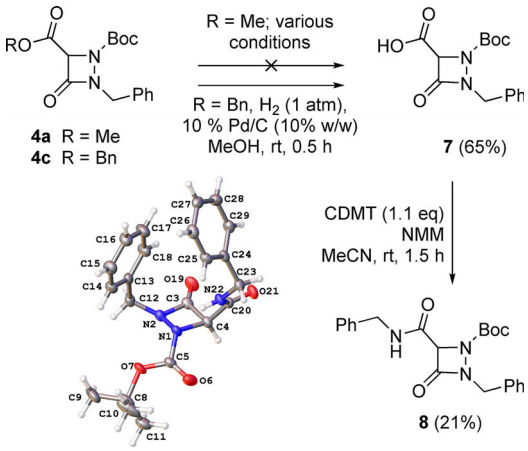
With the 4-membered heterocycle in hand, it was a priority to establish its ability to participate in a range of functionalization reactions. Initial attempts to remove the Boc-protecting group under standard acidic conditions were unsatisfactory, but exposure of aza-β-lactams **4 a** and **4 b** to boron trifluoride etherate[16] resulted in clean removal of the nitrogen protecting group to deliver the 1,2-diazetidin-3-ones **5** in which N-1 is free for further elaboration. Exemplar acylation reactions at N-1 using acetyl chloride proceeded smoothly to provide derivatives **6** (Scheme 2).

In parallel, and to expand the structural variation in the 1,2-diazetidinones through functional group interconversion, we attempted to hydrolyze the 4-carboxylate ester in 1,2-diazetidinone **4 a** to the corresponding carboxylic acid **7**. Standard alkaline hydrolysis conditions resulted in rapid consumption of ester **4 a**, but no carboxylic acid **7** could be isolated. Likewise use of Me_3SiOK or Me_3SnOH as reagents was unsuccessful. In all cases, a complex mixture of products was observed, suggesting that the 1,2-diazetidin-3-one ring is unstable under



Scheme 2. Synthesis of 1-Boc-1,2-diazetidinones, and their deprotection and re-acylation.

such conditions. Therefore, we switched our attention to the corresponding benzyl ester **4c** in the expectation that it could be cleaved under non-nucleophilic conditions by hydrogenolysis. In the event, this was the case, and the carboxylic acid **7** could be isolated in 65% yield after hydrogenolysis over palladium on charcoal (Scheme 3). However, the acid **7** proved to

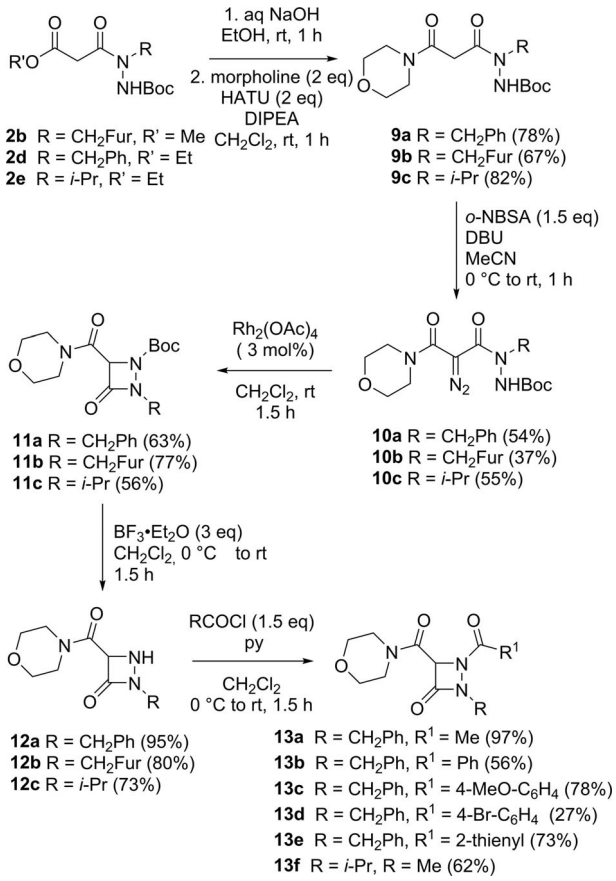


Scheme 3. Formation of 1,2-diazetidinone-4-carboxylic acid, and amide formation; X-ray crystal structure of amide **8** (CCDC 1829702).

be unstable, and decomposed within a few hours of its preparation. ^{1}H and ^{13}C NMR spectroscopic analysis of the resulting degradation products did not provide evidence for decomposition by a simple process such as decarboxylation. Nevertheless, a range of amide coupling reactions was investigated using benzylamine as a model amine nucleophile. The first amide coupling agent evaluated was HATU which enabled the formation of amide **8**, the structure of which was confirmed by X-ray crystallography, in poor yield of 21%. Use of EDCI, CDI or PyBOP was unsuccessful, whereas other coupling agents such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM), prepared in situ from 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and *N*-methylmorpholine (NMM), and propylphosphonic anhydride (T3P) resulted in similar yields for amide **8**. Direct amidation attempts using the trimethylaluminum reagent, DABAL-Me_3[17] also gave disappointing results. Presumably the low yields in amide coupling reactions again reflect the instability of 1,2-diazetidinone-4-carboxylic acid.

Given the instability of the 1,2-diazetidinone-4-carboxylic acid, and the resulting poor yields in amide formation, we investigated the alternative strategy in which the amide is formed before the construction of the 4-membered ring. Thus, hydrazine derivatives **2b**, **2d** and **2e** (Scheme 2) were hydrolyzed to the corresponding acids in near quantitative yields, and coupled to morpholine as a representative amine to give the amides **9** (Scheme 4). Diazo transfer proceeded smoothly using *o*-nitrobenzenesulfonyl azide (*o*-NBSA),[18] the original conditions employing *p*-ABSA being unsatisfactory. The resulting diazocarbonyl compounds **10** underwent rhodium(II) catalyzed N–H insertion to give the 1,2-diazetidin-3-ones **11**. As before, treatment with boron trifluoride etherate successfully removed the *N*–1 protecting group in good to excellent yield, and the resulting amine **12** underwent amide coupling with a range of representative acid chlorides to give amides **13a–f** (Scheme 4). The structures of amides **13a**, **13b** and **13d** were confirmed by X-ray crystallography (Figure 4). In a similar manner, 1,2-diazetidinone **12a** reacted with isocyanates to give a range of ureas **14** (Scheme 5).

With a range of novel 1,2-diazetidin-3-ones in hand, it remained to carry out preliminary biological evaluation. The compounds were tested against a range of bacterial and fungal strains as part of the CO-ADD (Australia) antimicrobial screen, but were for the most part inactive.[19] Compounds **13e** and **14b** showed activity antifungal activity against *Cryptococcus neoformans var. grubii* showing 91 and 51% inhibition of growth, respectively after 2 h incubation. Further structure–activity relationship studies are ongoing.



Scheme 4. [Fur = 2-furyl] Synthesis of 1,2-diazetidinone-4-carboxamides.

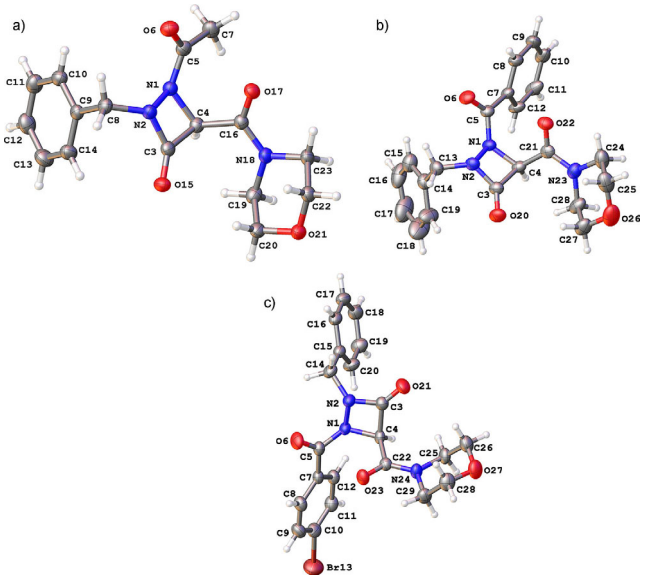
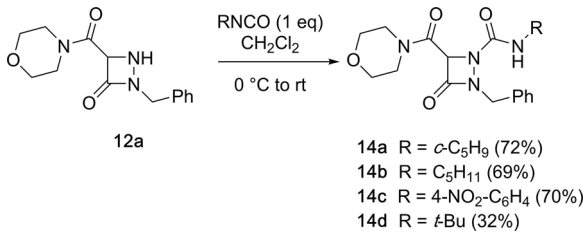


Figure 4. X-Ray crystal structures of 1,2-diazetidin-3-ones a) **13a**, b) **13b** and c) **13d** (CCDC 1829700, 1829701, 1829703 respectively).

The compounds were also screened against two cancer cell lines: MCF-7 (mammary carcinoma) and HCT116 (colon carcinoma), using the MTT assay. The results are summarized in Table 1, and show that the compounds were largely inactive against colon carcinoma cells, but showed modest growth inhibitory activity in mammary carcinoma cells.



Scheme 5. Synthesis of 1,2-diazetidin-3-one ureas.

Table 1. Growth inhibitory activity of 1,2-diazetidin-3-ones in MCF-7 and HCT-116 carcinoma cells lines.

Compound	GI_{50} mean ± SEM [μM] MCF-7	HCT-116
5a	72.7 ± 12.3	> 100
5b	76.0 ± 7.51	> 100
6a	82.0 ± 18.0	> 100
6b	95.3 ± 6.69	> 100
12b	54.3 ± 4.18	> 100
13a	64.0 ± 3.51	> 100
13b	67.3 ± 6.06	> 100
13c	26.3 ± 21.9	41.3 ± 23.9
13d	49.3 ± 10.7	> 100
13e	52.3 ± 2.60	> 100
13f	66.3 ± 12.9	> 100
14c	57.3 ± 9.70	> 100

MCF-7 breast cancer cells; HCT-116 colon cancer cells; cells were exposed to experimental agents for 48 h before viability was assessed by MTT assay. Mean ± SEM (standard error of the mean) of ≥ 3 independent trials are shown; $n = 4$ per concentration per trial.

Conclusion

A range of 1,2-diazetidine-3-ones has been synthesized using intramolecular N–H insertion of a rhodium carbenoid as a key step. Although some attempts at subsequent derivatization could be thwarted by the lability of the 4-membered ring, a range of derivatives was accessed by functionalization of the pendant carboxylate group prior to cyclization. The compounds show modest biological activity.

Experimental Section

Full experimental details are given in the Supporting Information, together with copies of NMR spectra.

CCDC 1829702, 1829700, 1829701, and 1829703 contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Keywords: 4-membered rings · carbene insertion · drug discovery · heterocycles · lead-oriented synthesis

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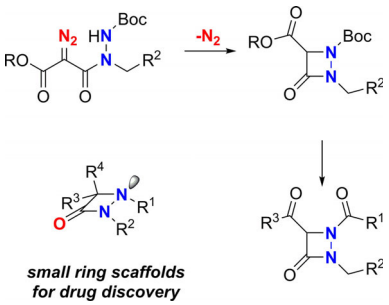
FULL PAPER

Small rings to populate chemical space: 1,2-Diazetidin-3-ones, readily obtained through intramolecular rhodium carbene N–H insertion, are useful scaffolds for drug discovery.



Heterocycles

*M. S. Santos, A. Nortcliffe, W. Lewis, T. D. Bradshaw, C. J. Moody**

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Synthesis of Highly Substituted 1,2-Diazetidin-3-ones, Small-Ring Scaffolds for Drug Discovery

1,2-Diazetidin-3-ones remain an under-investigated small-ring heterocycle. Nevertheless their diverse structures offer the potential to explore chemical space as illustrated by the principal moments of inertia "triangle" in rod-like (rocket), sphere-like (planet) and disc-like (flying saucer) regions. Inspired by the unexpected three-dimensionality of substituted 1,2-diazetidin-3-ones, the synthesis and functionalization of a range of these strained four-membered rings are reported; for more details, see Full Paper by C. J. Moody et al. on page ■■ ff.