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Design and Synthesis of Spiro-cyclopentenyl and Spiro-[1,3]-dithiolanyl Substituted Pyrrolidine-5,5-*trans*-lactams as Inhibitors of Hepatitis C Virus NS3/4A Protease

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Abstract—Using the pyrrolidine-5,5-*trans*-lactam template, we have designed small, neutral, mechanism-based inhibitors of hepatitis C NS3/4A protease. Compound **2b**, with a spiro-cyclobutyl P1 substituent and an isopropyl carbonyl substituent at the lactam nitrogen, has an IC_{50} value in the replicon cell-based assay of 3 μM.

Hepatitis C virus (HCV) infects chronically an estimated 3% of the global human population,[1] often leading to cirrhosis, hepatocellular carcinoma and liver failure in later life.[2] Current therapies are based upon interferon-α alone or in combination with ribavirin. Although sustained response rates are markedly improved using combination therapies, at least 50% of patients fail to show a sustained response. Additionally, current therapies have the disadvantage of frequent and severe side effects.[3] The development of new therapies to treat HCV infection effectively is thus of paramount importance, and is currently an intensive area of research.[4]

HCV is a small, enveloped virus, the genome of which is a 9.5kb single-stranded RNA that encodes for a single polyprotein of 3010–3030 amino acids. Mature non-structural replicative proteins are released from this polyprotein by the action of the viral proteases NS2 and NS3. It has been established that introducing mutations into the NS3 protease region of the HCV genome abolishes infectivity,[5] demonstrating that NS3 protease is thus an essential viral function and should prove to be an excellent target for the development of novel anti-HCV agents.

We recently reported the design and synthesis of a novel class of mechanism-based inhibitors (**1** and **2a**) of the hepatitis C virus NS3/4A protease enzyme based on the α-ethyl,[6] dimethyl and spirocycloalkyl[7] pyrrolidine-5,5-*trans*-lactam template (Fig. 1). The inhibitor **2a** and its close analogues are novel, potent in the replicon cell-based surrogate assay and demonstrate moderate stability in human plasma.

These compounds bear reactive functionality so they are potentially vulnerable to metabolism. Although early studies showed human plasma stability to be acceptable, more recently we have shown that following intravenous administration to dogs, the compounds are rapidly cleared in vivo.[8] Thus, following a strategy developed during the application of this template to inhibition of HCMV protease,[9] we attempted to find a compound that would react with the viral enzyme after binding to the active site, but would be sufficiently stable to hydrolysis by plasma enzymes. Compounds **2a–e** were synthesized from the core template **3**, with the aim of either sterically hindering the approach of the hydrolytic plasma enzymes to the lactam carbonyl, or reducing the reactivity of the lactam carbonyl by making the substituent on the lactam nitrogen less electron withdrawing.

The lactam **3**[7] was deprotonated using lithium hexamethyldisilazide and acylated at 0 °C with a range of acyl- or carbamoyl chlorides, or with methyl chlorothiol formate in moderate to good yields. Acidolysis was followed by diethyl ether trituration in a rapid synthesis fashion. The poor yield of **5c** was probably a consequence of higher

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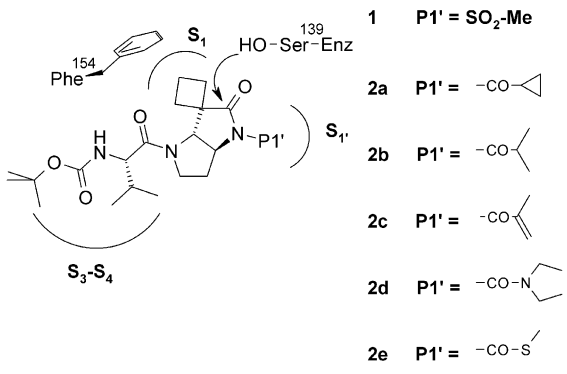


Figure 1. Spirocyclobutyl pyrrolidine-5,5-*trans*-lactam template: potential interactions with protease subsites.

than expected amine trifluoroacetate solubility in ether. In similar fashion, **5d** was obtained less pure than generally observed, due to carryover of an unidentified impurity—this impacted on the isolated yield of **2d** at the next stage. HATU-mediated coupling of Boc-Val, followed by column chromatography or Bond-Elut solid phase cartridge extraction furnished **2a–e** in generally good yields overall.

The compounds demonstrated a range of biochemical potency against a fluorogenic assay system using full-length NS3/4A protein (Table 1). The two most potent compounds were **2a–b**, both of which bear an sp^3 center adjacent to the carbonyl moiety. **2c** (which has an sp^2 center) is markedly less potent even than the close analogue **2b**. The ethyl groups of the urea **2d** are almost certainly too large to be accommodated in the S1′ pocket, hence the compounds are poorly active. The thiocarbamate **2e** demonstrated interesting potency, however, this group does not permit the application of more small, branched groups, so we took the view that it would be difficult to improve upon the modest potency and stability of **2e**, and the series was not pursued. The most potent compounds (i.e., kobs/I > 100 M^{-1} s^{-1}) were tested in the replicon cell-based assay. There was generally a good correlation between biochemical assay potency and replicon potency, although the isopropyl carbonyl analogue **2b** was slightly more potent in the cellular assay than would have been predicted. (**2a** cf **2b** and **2b** cf **2e**- all data is for head-to-head IC_{50} determinations on compounds in the same assay).

We next chose to re-examine the requirement for the spirocyclobutyl P1 substituent. It has been noted that

Table 1. HCV NS3/4A isolated protease potency and replicon cellular assay potency for compounds **2a–e**

Compd	P1′	HCV protease kobs/I (M^{-1} s^{-1})	ELISA replicon IC_{50} (μM)
2a	CO–cyclopropyl	400	4.0
2b	CO–$CH(CH_3)_2$	166	3.1
2c	CO–$C{=}CH_2(CH_3)$	24	NT
2d	CO–$N(C_2H_5)_2$	3.3	NT
2e	CO–SMe	154	7.9

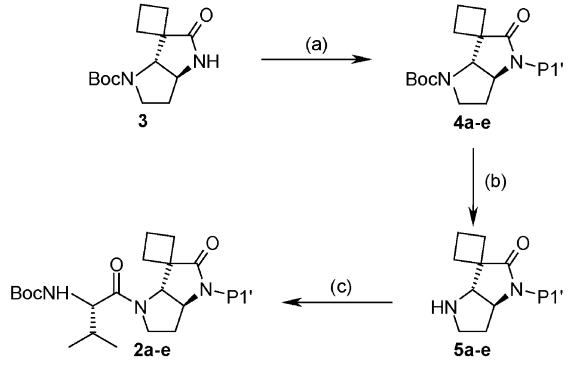
NT, not tested.

the preference for the methanethiol side chain of cysteine at P1 could be driven by interaction of the SH proton with the electron-rich π-cloud of Phe154.[10] Since we wished to retain the stability and synthetic accessibility properties conferred by the spirocyclobutyl substituent, we chose to explore spirocyclopentenyl and dithiolanyl substituents. In the case of the spirocyclopentenyl, it was postulated that the olefin group would be capable of π-π stacking with Phe154.[11] In the case of the diathiolanyl group, we postulated that it could be possible for the sulfur lone-pair electrons to interact with a ring proton of Phe154, the latter having a partial positive character.[12]

Cyclopent-3-enylidene-ethoxymethoxy)-trimethylsilane (**7**) was synthesized as previously described[13] and subject to Lewis acid-mediated acyliminium coupling and hydrolysis to form **8** (Scheme 2). Cyclization proceeded under standard conditions to furnish **9** in excellent yield. Previous work in our group had indicated that removal of the Cbz protecting group under mild hydrogenolytic conditions is difficult (e.g., over Lindlaar catalyst), so the Cbz protecting group was removed using 4 equivalents of TMS iodide in 60% yield and replaced by the Boc protecting group to form **10**. **10** was converted to **11a** by methods analogous to those in Scheme 1.

([1,3]Dithiolan-2-ylidene-ethoxymethoxy)-trimethylsilane (**12**)[14] was subjected to the identical reaction sequence, each step proceeding broadly similarly with respect to reaction yield and compound quality. A noteworthy exception was that the standard base hydrolysis conditions used to remove the trifluoroacetyl protecting group (to produce **13**) unexpectedly caused significant concomitant loss of the ethyl ester protecting group—hence the low yield of **13**. All other reactions proceeded very similarly to the cyclopentenyl series.

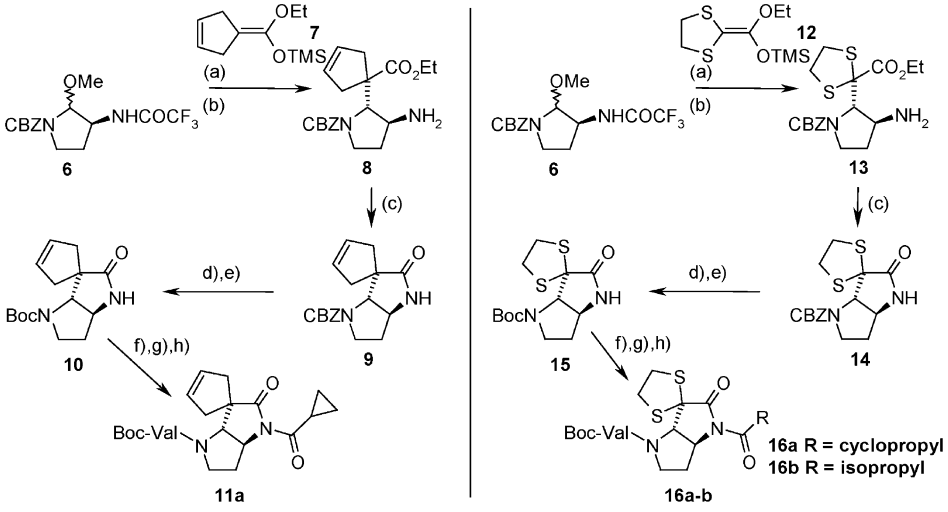
The compounds were assayed biochemically and in the cell-based replicon assay as shown in Table 2. The spirocyclopentenyl compound **11a** was at least an order of



Yields (%)

	a	b	c	d	e
4	73	44	92	51	48
5	99	72	42	92	80
2	76	100	97	25	98

Scheme 1. Elaboration of core template. Reagents and conditions: (a) LiHMDS; acyl chloride (**4a–c**), Et_2NCOCl (**4d**) or MeSCOCl; THF, 0°C; (b) TFA/DCM 1:1, diethyl ether trituration; (c) HATU, DIPEA, RCO_2H, MeCN or DMF.



Compound number	Yield (%)	Compound number	Yield (%)
8	72	**13**	22
9	93	**14**	98
10	52	**15**	34
11a	42	**16a**	93
		16b	42

Scheme 2. Synthesis of cyclopentenyl and dithiolanyl pyrrolidine-5,5-*trans*-lactams. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, DCM, −5 °C; (b) K_2CO_3, EtOH, 70 °C; (c) *t*BuMgCl, THF, 5 °C; (d) TMS-I (4 equiv), MeCN, 0 °C; (e) Boc_2O (1.3 equiv), THF; (f) LiHMDS; acyl chloride, THF, 0 °C; (g) 4 M HCl in dioxane; (h) HATU, DIPEA, RCO_2H, MeCN.

Table 2. HCV NS3/4A isolated protease potency and replicon cellular assay potency for compounds **11a–c** (data for **2a** and **2b** reproduced for comparison)

Compd	P1	P1′	Calculated logP[15]	HCV protease kobs/I (M^{-1} s^{-1})[16]	ELISA replicon IC_{50} (μM)[17]	Medium stability (% turnover at 4 h)[18]
11a	Cyclopentenyl	CO-cyclopropyl	2.9	19	NT	NT
16a	Dithiolanyl	CO-cyclopropyl	3.2	621	>100	>50
16b	Dithiolanyl	CO-isopropyl	3.7	362	17.0	>50
2a	Cyclobutyl	CO-cyclopropyl	2.4	400	4.0	27
2b	Cyclobutyl	CO-isopropyl	3.0	166	3.1	5

NT, not tested.

magnitude less potent than any of the other compounds examined and was not progressed to the cell-based assay. Interestingly, although **2b** was the least biochemically potent of the remaining compounds, it displayed the highest cellular potency. Conversely, the most potent compound in the NS3/4A assay (**16a**) was essentially inactive in the replicon assay. From the data presented, it appears that the excellent biochemical potency conferred by the cyclopropyl carbonyl lactam substituent (**2a** cf **16a**) translates less well to replicon potency than is the case for the isopropyl carbonyl substituent (**2b** cf **16b**). Clearly, in addition to biochemical potency, other factors contribute to cellular potency. All the compounds examined in the replicon assay were calculated to be of similar lipophilicity (clogP 2.4–3.7) and are broadly within the same molecular weight range (433–485), leading us to suggest that cellular permeability was unlikely to be the most important factor. In the absence of any other factor to account for the trends seen in the data, we speculated that, although the dithiolanyl moiety confers excellent biochemical activity, those compounds bearing this substituent are too inherently unstable in cellular systems to be of utility.

This hypothesis is confirmed by inspection of the in vitro stability behavior of the four compounds, also shown in Table 2. Although **2b** is of modest biochemical potency, it is clearly the most stable compound of the four examined- thus demonstrating the greatest cellular activity. Evidently, in this series, a prerequisite for cellular activity is that compounds possess both stability and biochemical potency. It should be borne in mind that there is a considerable difference in swept volume for the isopropyl moiety compared to the cyclopropyl moiety. The interatomic distance for the beta carbons is calculated to be almost double for the isopropyl group −2.54 Å compared to 1.53 Å for the cyclopropyl group. The results presented are consistent with a hypothesis that the smaller substituent is more readily accommodated in the S1′ subsite of the NS3/4A protease, but that the larger substituent reduces the rate of non-specific hydrolysis of the lactam ring by steric hindrance.

Additionally, the larger isopropyl is less electronegative than cyclopropyl, providing an electrostatic contribution to stability.[19] The application of these observations will be the subject of future communications.

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