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Investigation of glycine α-ketoamide HCV NS3 protease inhibitors: Effect of carboxylic acid isosteres

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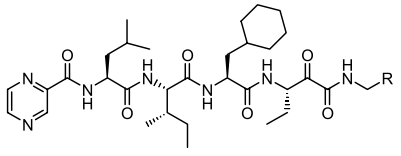
Abstract—The design and synthesis of tetrapeptide-based α-ketoamides containing prime side acid isosteres HCV NS3 protease inhibitors are described. Tetrazole, sulfonic acid, and *N*-sulfonylcarboxamids were demonstrated to be efficient carboxylic acid replacements. Further optimization yielded a series of potent HCV NS3 protease inhibitors with IC_{50} of 0.020–0.060 μM.

1. Introduction

The hepatitis C virus (HCV) is the major etiologic agent of transfusion-associated and community-acquired non-A, non-B hepatitis. About 3% of the world population are infected by HCV.[1] In most cases, HCV establishes a persistent infection resulting in chronic hepatitis and cirrhosis, which can lead to hepatocellular carcinoma. Current therapies used include treatment with interferon-α (INF-α) alone and in combination with ribavirin.[2] These therapies have limited efficacy and are frequently accompanied by side effects. Consequently, there is a clear need to develop more effective therapeutics for the treatment of HCV-associated hepatitis. Among the multiple approaches, HCV NS3 protease has been studied extensively and well characterized.[3] Kolykhalov et al. demonstrated that NS3 protease was required for HCV infection in Chimpanzee, hence provided preclinical validation of this target.[4] Furthermore, the availability of crystal structures of NS3 protease makes it an attractive target for small-molecule computer-assisted inhibitor design.[5]

Early studies from our laboratory showed that an α-ketoamide of glycine (**1**) was an effective inhibitor of HCV NS3 protease.[6] Docking of **1** in the active site of the protease indicated that the carboxylic acid moiety can engage in hydrogen bonding with Arg 109 and Lys 136 of the enzyme.[6a] In an attempt to further investigate this lead compound, we investigated replacing the acid with functional groups that can maintain a similar hydrogen bonding network on the prime side (structure **2**). In this communication, we wish to report results based on prime side investigations.



1: R = CO_2H, IC_{50} = 0.23 μM
2: R = carboxylic acid isosteres

2. Results and discussion

Simple sulfonic acid (**2a**, Table 1) gave comparable potency (IC_{50} = 0.22 μM)[7] to **1**. Tetrazole, a well known carboxylic acid isosteres, was also effective, resulting in only a twofold loss of activity (**2b**). The *N*-(methylsulfonyl)glycinamide analog **2c** was two orders of magnitude less potent than the acid **1**, however, the triflouromethylsulfonyl analog **2d** restored most of the potency. Compared to the methyl analog **2c**, phenyl- and benzylsulfonyl glycinamides gave only modest potency (8.9 and 12 μM, respectively). Naphthyl further improved potency to 1.2 μM, indicating that further SAR work in this area was warranted.

Molecular modeling of the *N*-phenylsulfonylglycinamide analog **2e** in the active site of NS3 protease revealed that the sulfonamide group appear to interact

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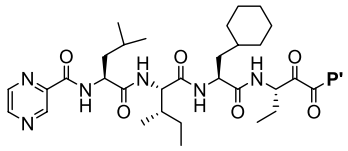
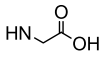
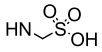
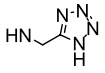
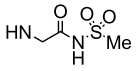
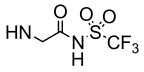
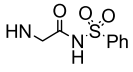
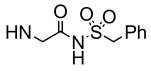
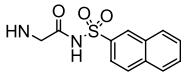
Table 1. SAR of analogs with acid isostere as prime groups



Compound	P′	IC_{50} (μM)
1		0.23
2a		0.22
2b		0.46
2c		25
2d		0.47
2e		8.9
2f		12
2g		1.2

with the polar side chain of Lys 136 near the S1′ site of the enzyme (Fig. 1). The phenyl group appears to point to an open space, which explains the SAR observed for compounds **2e–g**. To further explore the binding site of this region, a series of substituted *N*-phenylsulfonylglycinamide analogs were designed and evaluated. As shown in Table 2, chloro substitution at the meta-position of the phenyl ring (**2h**) improved the potency by 35-fold over the un-substituted counterpart (**2e**), to give a 0.25 μM inhibitor. Nitro and phenyl groups (**2i** and **2j**) at the meta-position also improved the potency, both under 1 μM. The para-congeners of the nitro and phenyl groups (**2k** and **2l**) were also prepared and tested. However, they are less potent than their meta-congeners. Introduction of a second substituent to the remaining meta-position of the phenyl group proved beneficial to potency. Despite the difference in electronic and steric properties, both (benzamido)sulfonyl and chloro substitutions gave comparable potency, and both improved the IC_{50} value to about 0.1 μM. Additionally, the 2,5-disubstituted phenylsulfonylglycinamide derivative **2o** (0.11 μM) provided equal potency as compared to **2m**

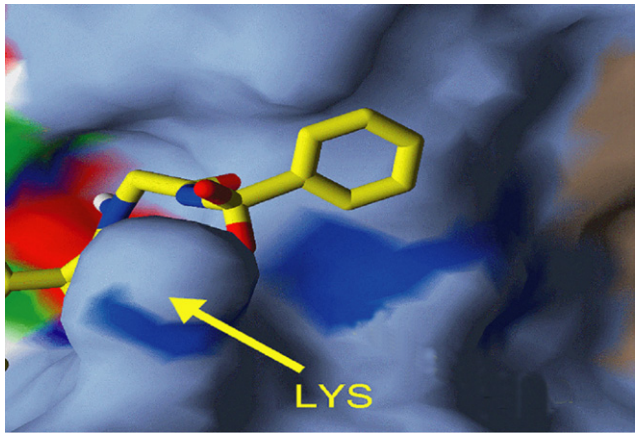
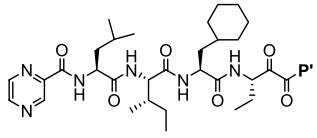
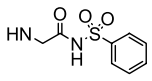
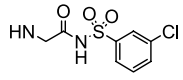
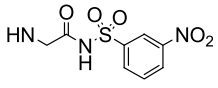
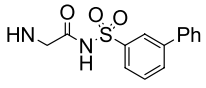
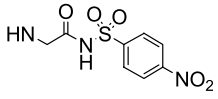
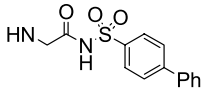
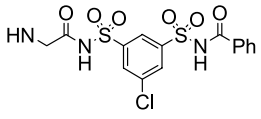
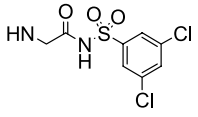
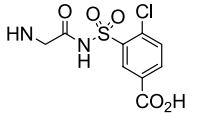
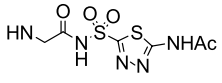


Figure 1. Sectional view of a computer-generated model of **2e** (in stick presentation) in the active site of HCV NS3 protease (in surface presentation).

Table 2. SAR of analogs with *N*-sulfonylglycinamide as prime groups

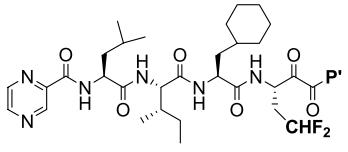


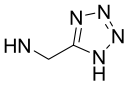
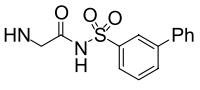
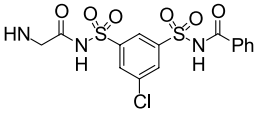
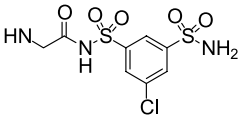
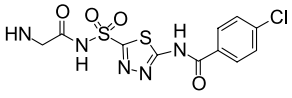
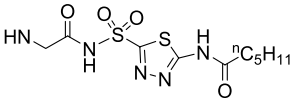
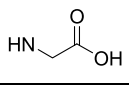
Compound	P′	IC_{50} (μM)
2e		8.9
2h		0.25
2i		0.41
2j		0.64
2k		0.92
2l		2.4
2m		0.12
2n		0.13
2o		0.11
2p		0.25

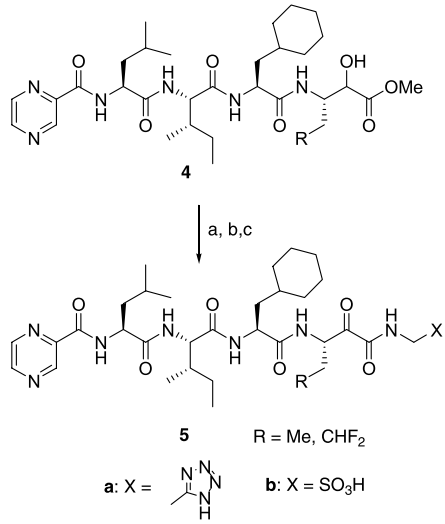
and **2n**. Besides *N*-phenylsulfonylglycinamide, the electron rich *N*-thiadiazolesulfonylglycinamide was also well tolerated and provided an inhibitor **2p** with IC_{50} of 0.25 μM. The enhanced potency for the substituted analogs can be explained by the hydrophobic interactions gained from the substitution groups on the phenyl. Figure 1 also reviewed that the para-position of the phenyl is closer to the enzyme surface compared to the meta-position, which explains the better tolerance of a meta-substitution.

Previously, we reported that when the ethyl P1 group of compound **1** was replaced with 2,2-difluoroethyl group, potency was enhanced.[8] Therefore, the 2,2-difluoroethyl P1 group was incorporated to tetrazole and sulfonylglycinamide series. As shown in Table 3, the tetrazole derivative **3a** was a 40 nM inhibitor, about 10-fold more potent than the ethyl counterpart (**2b**). The difluoroethyl P1 also enhanced the potency of sulfonylglycinamide series (**3b–3f**), all gave IC_{50} less than 100 nM. Among them, the two thiadiazolesulfonyl analogs were most potent, with IC_{50} value of 20 nM. Compared to the 2,2-difluoroethyl P1 optimized carboxylic acid inhibitor **3g**, the acid isosteres analogs (**3a–3f**) provided equal potent or slightly better HCV NS3 protease inhibitors.

Table 3. SAR of analogs with CH_2CHF_2 as P1 group



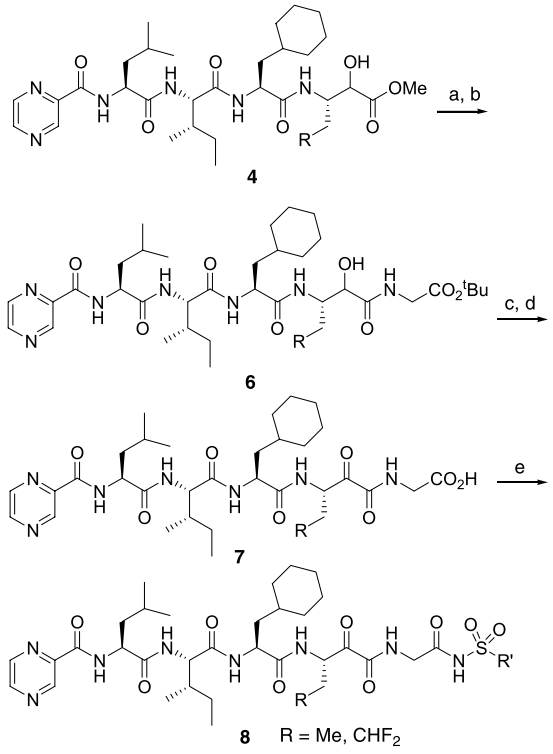
Compound	P′	IC_{50} (μM)
3a		0.040
3b		0.060
3c		0.060
3d		0.050
3e		0.020
3f		0.020
3g		0.060



Scheme 1. Reagents and conditions: (a) LiOH, THF, H_2O, 0 °C, 2 h (81–87%); (b) NH_2CH_2X, BOP, DIEA, DMF, 0–25 °C, 2 h (61–74%); (c)Dess–Martin oxidation, CH_2Cl_2, 25 °C, 4 h (65–84%).

3. Synthesis

The synthesis of tetrazole and sulfonic acid analogs is outlined in Scheme 1. Starting from intermediate **4**, which was prepared from cyclohexylalanine as described previously,[8a] saponification followed by coupling with 5-(aminomethyl)tetrazole or aminomethanesulfonic acid provided the corresponding α-hydroxy amides. Dess–



Scheme 2. Reagents and conditions: (a) LiOH, THF, H_2O, 0 °C, 2 h (87–91%); (b) HATU, HOBt, TMP, H-Gly-O^{t}Bu, 0 °C to rt, 2 h (65–87%); (c) Dess–Martin oxidation, CH_2Cl_2, rt, 8 h (50–83%); (d) TFA, CH_2Cl_2, rt, 1 h (74–91%); (e) NH_2SO_2R', EDCI, DMAP, DMF, 0 °C to rt, 2 h (56–87%).

Martin oxidation provided the corresponding α-keto-amides **5a** and **5b** in good yields.

The synthesis of the *N*-sulfonylglycinamide analogs is illustrated in Scheme 2. Hydrolysis of **4** followed by coupling with glycine *t*-butyester gave intermediate **6**. Dess–Martin oxidation yielded the corresponding α-keto-amides, which was hydrolyzed under acidic conditions to yield acid **7**. Treatment of **7** with various sulfonamides in the presence of EDCI and DMAP provided the desired α-keto sulfonylglycinamides **8**.

In summary, acid isosteres such as sulfonic acid, tetrazole, and *N*-sulfonylglycinamide were investigated as carboxylic acid replacement using a tetrapeptide-based α-keto-amide template. All three types of acid isosteres were efficient carboxylic acid replacements. Incorporation of the optimized difluoroethyl P1 group yielded a series of potent HCV NS3 protease inhibitors with IC_{50} of 20–60 nM.

Acknowledgments

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References and notes

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4. (a) Kolykhalov, A. A.; Agapov, E. V.; Blight, K. J.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *Science* **1997**, *277*, 570; (b) Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. *J. Virol.* **2000**, *74*, 2046.
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