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Synthesis of Triazole-Tethered Pyrrolidine Libraries: Novel ECE Inhibitors

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Abstract—The solid-phase synthesis of substituted 1,2,4-triazoles tethered to a 4-mercaptopyrrolidine core **1** is described. This novel class of non-peptidic, Zn^{2+} metallo-protease inhibitors was found to have inhibitory activity for the endothelin converting enzyme (ECE-1). The SAR of the substitution pattern in **1** is discussed.

Endothelin-1 (ET-1) a 21 amino acid peptide, is a potent, long acting vasoconstrictor. High plasma levels are found in many clinical conditions such as congestive heart failure, subarachnoid hemorrhage and pulmonary hypertension.[1] ET is produced from its biologically inactive precursor big-ET by the Zn-endopeptidase, endothelin converting enzyme (ECE). Two homologues are currently known, ECE-1 and ECE-2. The latter does not seem to have a functional importance for the endothelin system.[2] Current understanding is that the inhibition of ECE-1 may allow the specific blockade of the whole ET system, and is therefore an attractive therapeutic approach.

Known Zn-metalloprotease inhibitors have chelating moieties such as acids, mercaptanes, hydroxamic acids, phosphonates and phosphates.[3] Recently, a Novartis group reported on their peptidic, thiol-containing ECE inhibitors.[4] Our lead structure for non-peptidic ECE inhibitors contained the rigid [2*S*,4*R*]-4-mercapto-proline core. Modifications around this core structure led to the identification of triazole-tethered pyrrolidine **1a** which had an IC_{50} of 0.5 μM for human ECE. To further improve the potency of our inhibitors and to elucidate the structure–activity relationship, modifications at three sites were considered (Fig. 1). The invariant thiol moiety in our core structure enabled us to consider a solid phase strategy for the rapid synthesis of compound libraries. To this end, a solid support was prepared by coupling 4-(α,α-diphenylhydroxymethyl)-benzoic acid to benzhydrylamine resin yielding polymer-bound carbinol **2** with a loading of 0.55 mmol/g (based on mass increase).[5] [2*S*,4*R*]-4-Sulfanyl-1-(fluorenylmethoxycarbonyl)-pyrrolidine-2-carboxylic acid[6] was anchored to the solid phase in a 10% TFA/CH_2Cl_2 solvent mixture (Scheme 1). Fmoc group removal under standard conditions allowed for the first element of diversity **4** to be introduced by sulfonylation, urea formation, acylation, carbamoylation and sulfamidation of the *N*-pyrrolidine. This was efficiently achieved when transient silylation was used. Coupling of hydrazine hydrate in the presence of TPTU yielded hydrazide **5** which on further reaction with isothiocyanates furnished intermediates **6**. Typically, cyclization to the 1,2,4-triazole core has been shown to proceed under relatively harsh, basic conditions (NaOH, heat) yielding thiolate product for further alkylation.[7]

We identified a mild, room temperature method for the solid-phase synthesis of triazole **8** where a first step alkylation (R^3-X, DIEA, DMF) allowed for the acid catalyzed (0.06 M TFA/dichloroethane) cyclization step.[8] Pyrrolidine tethered triazoles **1** were liberated from the resin by a 40% TFA/10% triisopropylsilane/dichloroethane mixture (Scheme 2). Crude compounds

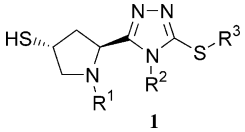

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Figure 1.

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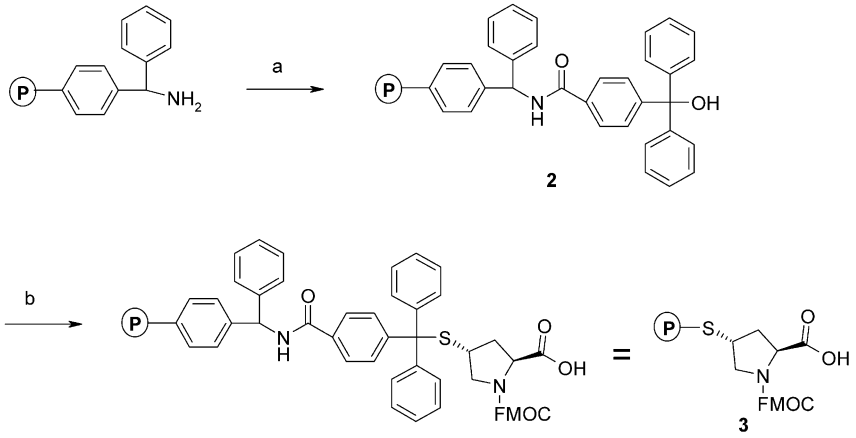
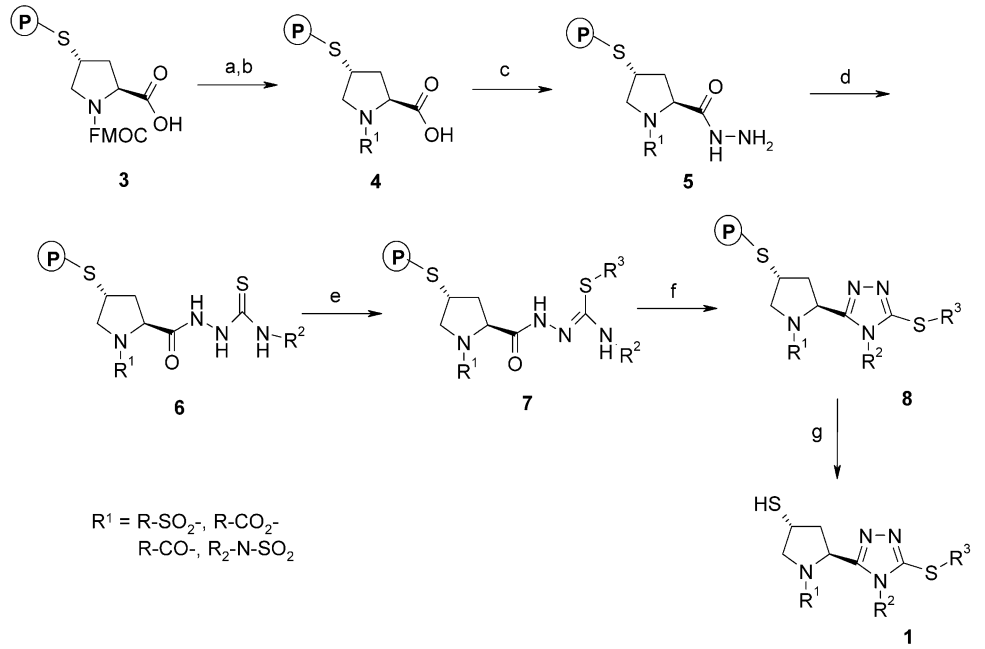
were purified using high-throughput preparative RP-HPLC and characterized by standard analytical techniques.[9]

Compounds were tested for their inhibition of hECE-1.[11] The results are summarized in Tables 1–3. Modifications at the *N*-pyrrolidine group R^1 are listed in Table 1. Replacing the 2-naphthylsulfonamide **1a** by lipophilic sulfonamides (**1b**–**1d** and **1f**) led to potent inhibitors, also butylcarbamate **1e** showed interesting hECE-1 inhibition. With the more polar 4-fluoro-phenylsulfonamide **1g** or methylsulfonamide **1h**, as well as the urea **1i** the inhibitory activity was low. Introducing the dimethylsulfamide **1k** was found to be detrimental to the inhibitory activity. The best compound was still the original 2-naphthyl substituted **1a**, suggesting that the two moieties at the [1,2,4]-triazole residue had to be optimized to decrease the IC_{50}. Modifications at the R^3-sulfanyl moiety were investigated (Table 2). Replacement of the methyl by a propyl group **1l** resulted in an increase in inhibitory activity, whereas branched alkyl groups (**1m** or **1n**) were less favorable. The influence of the R^2 substituent was then examined (Table 3). Replacing the 4-phenyl group by methyl (**1s**, $R^2 = Me$) resulted in a significant drop of the inhibitory activity. It was clear that a lipophilic group was required in this position. The activity could be tuned slightly with changes to the substitution pattern (**1p**–**r**). Inserting a methylene



Scheme 1. Synthesis was performed on 42 mmol scale (loading of PS-BHA-NH_2·HCl = 0.93 mmol/g): (a) 4-(α,α-diphenylhydroxymethyl)benzoic acid 1.5 equiv, *O*-(1,2-dihydro-2-oxopyridyl-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumtetrafluoroborate (TPTU) 1 equiv, DIEA 3 equiv, DMF (250 mL), rt, 1 h; (b) [2*S*,4*R*]-4-sulfanyl-1-(fluorenylmethoxycarbonyl)-pyrrolidine-2-carboxylic acid, 1.2 equiv, TFA (80 mL), CH_2Cl_2 (500 mL), rt, 1 h.



Scheme 2. Parallel solid-phase synthesis was performed on 500 mg scale, starting from resin **3**: (a) 20% piperidine/DMF 2×10 min; (b) TMS-Cl 6 equiv, DIEA 6 equiv, CH_2Cl_2 (6 mL), argon, then, RSO_2-Cl or RNCO or RCOCl or ROCOCl or R_2NSO_2-Cl 1.5–2 equiv, DIEA 3 equiv, CH_2Cl_2 or DMF (6 mL), rt, 1–2 h, argon, then i^{i}PrOH; (c) TPTU 2 equiv, DIEA 4 equiv, DMF (7 mL), rt, 15 min, wash resin, then, hydrazine hydrate 5 equiv, DMF (7 mL), rt, 1–6 h; (d) R^2NCS 2 equiv, DIEA 1 equiv, DMF, (5 mL), rt, 16 h; (e) R^3-X 5–10 equiv, DIEA 15 equiv, DMF (5 mL), rt, 16 h; (f) 0.06 M TFA/1,2-dichloroethane (6 mL), rt, 16 h; (g) 60% TFA/10% triisopropylsilane/1,2-dichloroethane (6 mL), rt, 0.5 h.

spacer led to the identification of the most potent analogue **1o** with an IC_{50} of 150 nM.

In summary, we developed an efficient solid phase method for the synthesis of triazole-tethered pyrrolidines. By applying this methodology we improved on the original naphthylsulfonamide **1a**, with three small libraries, to [3*R*,5*S*]-5-[4-(4-fluoro-benzyl)-5-methylsulfanyl-4H-[1,2,4]triazol-3-yl]-1-(naphthalene-2-sulfonyl)-pyrrolidine-3-thiol **1o** with an IC_{50} of 150 nM.

Table 1. Modifications at the pyrrolidine substituent R^1; R^2 = Ph, R^3 = Me

Compd	Yield[a] (%)	ECE-1 inhibition IC_{50} (μM)[b]	R^1
1a	24	0.5	2-Naphthyl-SO_2
1b	23	1.2	4-Propyl-PhSO_2
1c	9	1.2	4-Cl-PhSO_2
1d	42	1.3	4-CF_3-PhSO_2
1e	39	2.7	*n*-BuOCO
1f	5	4.4	4-Me-PhSO_2
1g	21	8.95	4-F-PhSO_2
1h	15	15.5	MeSO_2
1i	17	17.7	4-F-PhNHCO
1k	28	28%	$(Me)_2NSO_2$

[a]Overall yield of purified product based on 500 mg (0.28 mmol) of initial resin **3**.
[b]All ECE activity measurements were performed in triplicate;[10] % = % inhibition at 10 μM.

Table 2. Modifications at the [1,2,4]-triazole substitution pattern; R^1 = 2-naphthylSO_2, R^2 = Ph

Compd	Yield[a] (%)	ECE-1 inhibition IC_{50} (μM)[b]	R^3
1l	44	0.28	Propyl
1a	24	0.5	Me
1m	45	0.70	Cyclopropyl-methyl
1n	33	0.72	*i*-Butyl

[a]Overall yield of purified product based on 500 mg (0.28 mmol) of initial resin **3**.
[b]All ECE activity measurements were performed in triplicate;[10] % = % inhibition at 10 μM.

Table 3. Modifications at the [1,2,4]-triazole substitution pattern; R^1 = 2-naphthylSO_2, R^3 = Me

Compd	Yield[a] (%)	ECE-1 inhibition IC_{50} (μM)[b]	R^2
1o	18	0.15	4-F-Benzyl
1p	8	0.25	2,3,5,6-Tetra-fluoro-Ph
1q	10	0.36	4-CF_3-Ph
1a	24	0.5	Ph
1r	7	1.2	3-Cl-4F-Ph
1s	10	43%	Me

[a]Overall yield of purified product based on 500 mg (0.28 mmol) of initial resin **3**.
[b]All ECE activity measurements were performed in triplicate,[10] % = % inhibition at 10 μM.

Acknowledgements

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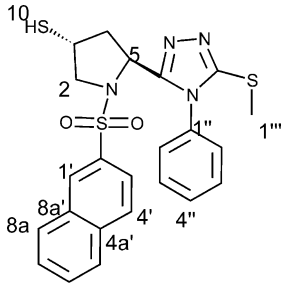

1a: ^{1}H NMR ($CDCl_3$) 1.38 (d, H-10), 2.00 and 2.40 (m, H_2-4), 2.68 (s, H_3-1‴), 3.16 and 3.62 (dd, H_2-2), 3.50 (m, H-3), 5.00 (dd, H-5), 7.45 (br s, H-2″ and H-6″), 7.61 (dd, H-7′), 7.63–7.66 (m, H-6′, H-3″, H-4″, H-5″), 7.70 (dd, H-3′), 7.62 (d, H-5′), 7.95 (d, H-4′, H-8′), 8.27 (d, H-1′); ^{13}C NMR ($CDCl_3$) 14.9 (C-1‴), 35.4 (C-3), 43.3 (C-4), 53.5 (C-5), 57.5 (C-2), 123.2 (C-3′), 128.0 (C-7′), 128.4 (C-5′, C-2″, C-6″), 129.5 (C-6′), 129.7 (C-1′), 129.8 (C-4′ or C-8′), 130.0 (C-4′ or C-8′), 130.6 (C-3″, C-5″), 131.2 (C-4″), 132.5 (C-8a′), 132.8 (C-1″), 133.9 (C-2′), 135.5 (C-4a′), 154.5 (C-9), 156.0 (C-6).
10. ECE inhibitors were measured in triplicate at 5–7 concentrations in the range of 10 μM–10 pM. IC_{50} values were calculated after logit/log transformation of the percent inhibition data with a best fit regression model. All assays were calibrated with phosphoramidon as internal standard inhibitor. Phosphoramidon, under the conditions of the assay showed an IC_{50} of 1.0 ± 0.2 μM (M ± SD, n = 30). IC_{50} values of unknown compounds were accepted when the IC_{50} (x)

measured for phosphoramidon in the assay was $0.8 < x < 1.2\,\mu M$.

11. Human endothelin-converting enzyme (ECE-1) has three isoforms with distinct subcellular localization. For a description of our ECE assay, see: Schweizer, A.; Valdenaire, O.; Nelböck, P.; Deuschle, U.; Dumas, J.-P.; Edwards, M.; Stumpf, J. G.; Löffler, B.-M. *Biochem. J.* **1997**, *328*, 871.