Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 825-830, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

GEM-CYCLOALKYL SUBSTITUTED THIOL INHIBITORS OF NEUTRAL ENDOPEPTIDASE 24.11. SYNTHESIS *VIA* NUCLEOPHILIC OPENING OF 2,2-SPIRO-β-LACTONES

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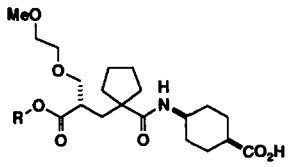
(*Received* 1 *June* 1992; *accepted* 15 *July* 1992)

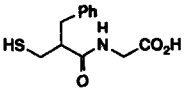
Abstract: Concise syntheses are described of a series of gem-cycloalkyl substituted thiols which are inhibitors of neutral endopeptidase 24.11. The route employs mild closure of strained 2,2-spiro-β-lactones from β-hydroxy-acids using triflic anhydride, followed by *O*-alkyl cleavage with potassium thioacetate.

Neutral endopeptidase EC 3.4.24.11 ('endopeptidase 24.11') is a zinc metalloprotease involved in the metabolic inactivation of atrial natriuretic factor (ANF)[1], a natriuretic peptide hormone secreted from the heart in response to raised extracellular volume[2]. Inhibitors of endopeptidase 24.11 are of considerable interest as potentiators of ANF, and agents of this class may have therapeutic utility[3]. We have recently reported a novel series of geminal-cycloalkylglutaramide derivatives which are potent inhibitors of endopeptidase 24.11[4]. Candoxatril **1** is an orally active prodrug of a member of this inhibitor series, candoxatrilat **2**, which potentiates the natriuretic actions of ANF in animals and man, and is currently under clinical evaluation for the treatment of congestive heart failure[5].

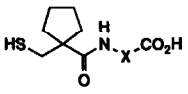


(1) R = 5-indanyl: candoxatril

(2) R = H: candoxatrilat

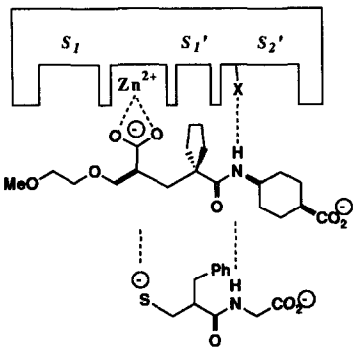


(3) thiorphan



(4)

figure 1

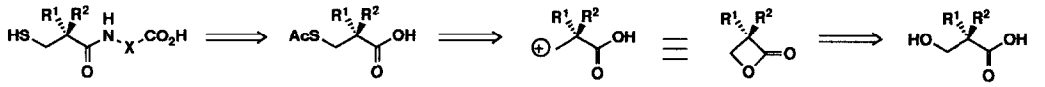


We envisage the binding mode of candoxatrilat to involve a strong interaction between the carboxylate of the inhibitor, and the active site zinc ion of the enzyme, positioning the geminal cycloalkyl ring of the inhibitor in the enzyme S_1' specificity pocket (fig. 1)[4]. The prototypical inhibitor, thiorphan **3**, is believed to bind in a related manner, but employs a thiol zinc ligand, and a benzyl P_1' substituent[6] in order to achieve high affinity for the enzyme. These proposed modes of binding of the two inhibitor types suggested a hybrid series of P_1' cycloalkyl substituted thiols such as **4** which should also satisfy the SAR requirements for high enzyme affinity. We report

here the successful realisation of this design strategy, which has yielded a novel series of potent endopeptidase 24.11 inhibitors, and lends support to the proposed binding model.

1. Chemical Synthesis

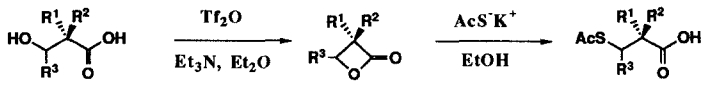
scheme 1



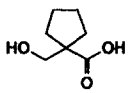
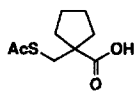
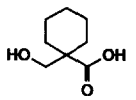
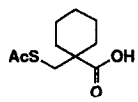
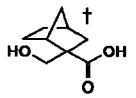
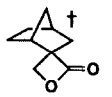
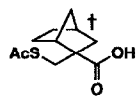
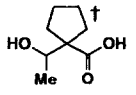
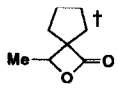
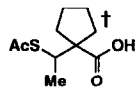
(5) (6) (7) (8) (9)

Synthesis of general structure **5** required access to β-acetylthioacid **6** (scheme 1), which could be coupled subsequently to amino ester fragments and hydrolysed to give the free thiol inhibitors. In turn, we envisaged generation of structure **6** from a synthon **7** by nucleophilic attack of thioacetate. We selected 2,2-substituted β-lactone **8** as the expression of **7**, since there is good precedent for *O*-alkyl versus *O*-acyl cleavage of lactones by soft nucleophiles[7]. Furthermore, ring opening would yield the free carboxylic acids directly. β-Lactones are commonly prepared either *via* cycloaddition of ketenes to carbonyl compounds[8], or by intramolecular esterification of β-hydroxy carboxylic acids[9]. We chose the latter route, which is reportedly most effective for α-substituted β-hydroxy acids, where cyclisation rather than competing β-elimination takes place to relieve steric compression. The necessary β-hydroxy acids **9** were readily accessible *via* reaction of carboxylic acid dianions with aldehydes[10].

table 1



(9) (8) (6)

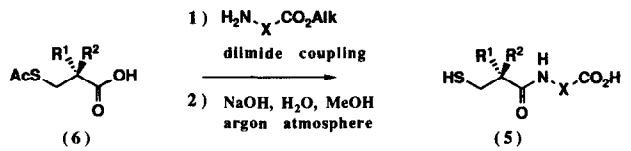
			% yield		% yield
a			71		30
b			87		36
c			50		23
d			94		0

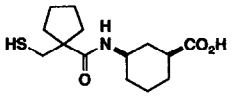
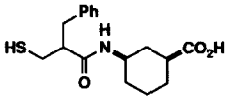
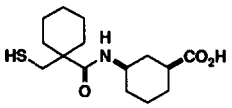
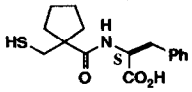
† *Mixture of stereoisomers*

Cyclisation of the β-hydroxy acids **9(a)-(d)** to give the strained lactones could not be accomplished satisfactorily *via* the standard procedure employing phenylsulphonyl chloride as dehydrating agent[11] as a result of low yields and difficulties in isolation of the products. We have therefore developed a novel and much improved method, which utilises trifluoromethanesulphonic anhydride and triethylamine in ether to effect the desired lactonisation[12] (table 1). This procedure is particularly convenient, since the triethylammonium triflate byproduct of the reaction separates from the ether as a denser, immiscible phase, allowing simple isolation of the product β-lactones after separation of phases, base wash and evaporation. This procedure allowed isolation of examples **8a** and **8b**[13] in good yield. The more strained examples **8c** and **8d**, were not stable to base wash and were used directly in the next reaction.

Treatment of the lactones **8a-d** with potassium thioacetate in ethanol (table 1) provided the desired β-acetylthio acids **6a-c** in modest yield[14], but failed to produce the highly substituted example **6d**, presumably as a result of steric hindrance. Use of alternative solvents, such as dimethylacetamide, did not increase yields significantly. The carboxylic acids **6a** and **6b** were converted to the desired thiol inhibitors **5(i)-(iv)** as depicted in table 2, *via* diimide coupling to the appropriate amino ester, followed by base hydrolysis under argon atmosphere to avoid facile conversion to the homodisulphide[14]. Yields of inhibitors over these final two steps are summarised in table 2:

table 2



		yield %	IC_{50} (nM)			yield %	IC_{50} (nM)
5(i)		61*	215	5(iv)		38*	180
5(ii)		43*	310	3	thiorphan		40
5(iii)		69†	220	2	candoxatrilat		38

* *Prepared from racemic cis-3-amino-cyclohexanecarboxylic acid benzyl ester.* † *Prepared from S-phenylalanine ethyl ester*

2. Biological Results and Discussion

Activities of inhibitors versus rat kidney neutral endopeptidase EC.3.4.24.11[15] are shown in table 2. The *cis*-3-amino-cyclohexane carboxyl substituent[4] (examples **5(i), 5(ii)** and **5(iv)**) was employed as a standard P_2' substituent to explore the SAR for geminal substitution at P_1' in this novel thiol series. Results for examples **5(i)** and **5(ii)** demonstrate that both gem-cyclopentyl and gem-cyclohexyl substituents yield effective inhibitors. Substitution of an S-phenylalanine for the cis-3-amino-cyclohexane carboxylic acid also yields a potent enzyme inhibitor **5(iii)**, demonstrating that use of the gem-cyclopentyl P_1'-substituent is not restricted to inhibitors bearing highly constrained *cis*-amino-cyclohexane-carboxyl groups. Importantly, the potency of these gem-cycloalkyl substituted inhibitors is comparable to that of the benzyl substituted analogue **5(iv)**[16]. Although examples **5(i)-(iii)** appear equipotent as inhibitors of neutral endopeptidase 24.11, as a group, they appear slightly less potent than the prototypical thiol inhibitor, thiorphan, and the glutaramide inhibitor candoxatrilat[17].

The synthetic route outlined above has therefore provided efficient access to a novel series of neutral endopeptidase inhibitors, which constitute a hybrid of the earlier, successful designs represented by candoxatrilat and thiorphan. A conceptually related inhibitor series has been described recently in the patent literature[18], but no details of biological activity were reported. The high affinity of the present inhibitor series for endopeptidase 24.11 lends support to the hypothesis that candoxatrilat and thiorphan bind in a manner whereby their gem cyclopentyl- and benzyl-groups respectively occupy a similar position in the enzyme inhibitor complex. This is further supported by the similar potencies of the gem-cyclopentyl- and benzyl-substituted inhibitors **5(i)** and **5(iv)** respectively. Conformational analysis of these two compounds illustrates that the low energy conformations of **5(iv)** are readily accessible to the more conformationally mobile **5(i)**.

The slightly greater affinity of thiorphan and candoxatrilat for endopeptidase 24.11 highlights the importance of optimising the P_2' substituent of the inhibitor in each case to achieve maximum complementarity to the enzyme. The concise synthetic route described above to key intermediates such as 2-acetylthiomethyl-cyclopentanecarboxylic acid **6a**, sets the scene for wider exploration of the SAR of this novel inhibitor series.

3. Typical experimental procedures

6-Oxa-5-oxo-bicyclo(4.3.0)octane, 8a. Trifluoromethanesulphonic anhydride (2.39ml, 13.90mmol) in dry diethyl ether (5ml) was added slowly to a stirred solution of 1-hydroxymethyl-cyclopentanecarboxylic acid (2g, 13.90mmol) in diethyl ether (15ml), cooled to between 0 and 8°C under an atmosphere of nitrogen. Triethylamine (3.86ml, 2.77mmol) in diethyl ether (5ml) was added slowly to the reaction, and once the exotherm had subsided, the the reaction flask was sealed and stored at 6°C in a refrigerator overnight. The reaction was stirred at room temperature for 2 hrs and and the ethereal layer decanted off. The triethylammonium triflate phase was triturated twice with ether, the combined ethereal phases washed with sodium bicarbonate (1.5%), water, dried (Na_2SO_4), and the solvent evaporated at room temperature to yield the title compound as an oil (1.24g, 71%), ν_{max} 1815 cm^{-1}.

1-Acetylthiomethyl-cyclopentanecarboxylic acid, 6a. A solution of the lactone above (0.75g, 5.95mmol) in dioxan (4ml) was added dropwise to a stirred solution of potassium thioacetate (1.02g, 8.90mmol) in ethanol at 5-10°C under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for 18 hr, then concentrated by evaporation at room temperature, and the residue dissolved in aqueous sodium bicarbonate (5%). The aqueous phase was washed with diethyl ether, acidified with conc. HCl to pH 1-2, extracted with diethyl ether, the organic phase washed with brine, dried (Na_2SO_4), and evaporated to yield a solid. Recrystallisation from pentane gave the title compound as granules (0.54g, 30%), m.p. 81°C. Found: C, 53.33; H, 6.89. $C_9H_{14}SO_3$ requires C, 53.44; H, 6.98%.

***Cis*-3-{N-[thiomethyl-1-cyclopentanecarbonyl]amino}cyclohexanecarboxylic acid benzyl ester.** N-methylmorpholine (0.33ml, 3.00mmol) was added dropwise with stirring to a solution of **6a** (0.20g, 1 mmol), racemic *cis*-3-aminocyclohexanecarboxylic acid benzyl ester (0.4g, 1mmol), 1-hydroxybenztriazole (0.14g, 1mmol), and 1-ethyl-3-(dimethylaminopropyl)-carbodiimide (0.38g, 2mmol) in dry dichloromethane (8ml) cooled to 8°C and the reaction mixture stirred for 20hr. The reaction was then washed with water, dried (Na_2SO_4) and evaporated to yield the crude product, which was purified by chromatography over silica gel, eluting with mixtures of ethyl acetate/hexane to yield the title compound as needles (0.34g, 81%) m.p. 128-129°C. Found: C, 66.43; H, 7.58; N, 3.04. $C_{23}H_{31}NO_4S$ requires C, 66.15; H, 7.48; N, 3.36%.

***Cis*-3-{N-[thiomethyl-1-cyclopentanecarbonyl]amino}cyclohexanecarboxylic acid, 5(i).** Aqueous sodium hydroxide (2.33ml, 1M, 2.33mmol) was added dropwise to a degassed, stirred suspension of the ester above (325mg, 0.78mmol) in methanol (15ml) at room temperature, and the resulting solution stirred for 24hr under an atmosphere of argon. Additional aqueous sodium hydroxide (1.5ml) was added, and stirring continued for a further 24hr. The reaction mixture was then acidified to pH 2 with aqueous hydrochloric acid (2M) at 10°C, extracted with dichloromethane, the extracts washed with brine, dried (Na_2SO_4) and evaporated to yield a powder which was recrystallised from ethyl acetate/hexane to give the title compound as a solid (0.165g, 74%) m.p. 185-186°C. Found: C, 59.05; H, 8.06; N, 4.77. $C_{14}H_{23}NO_3S$ requires C, 58.91; H, 8.12; N, 4.91%.

Acknowledgement.

We gratefully acknowledge the assistance of Dr P.L. Barclay in determination of inhibitory activity *versus* endopeptidase 24.11, and Drs J.C. Danilewicz and M.J. Wythes in helpful discussions.

4. References

1 Stephenson, S.L. and Kenny, A.J., *Biochem. J.*, **1987**, *241*, 237.

2 Lang, R.E., Tholken, H., Ganten, D., Luft, F.C., Ruskoaho, H. and Unger, Th., *Nature*, **1985**, *314*, 264.

3 (a)Sybertz, E.J., *Cardiovascular Drug Rev.*, **1990**, *8*, 71. (b)Roques, B.P. and Beaumont, A., *Trends in Pharmacological Sciences*, **1990**, *11*, 245. (c)Granger, J.P., *Circulation*, **1990**, *82(1)*, 313.

4 Danilewicz, J.C., Barclay, P.L., Barnish, I.T., Brown, D., Campbell, S.F., James, K., Samuels, G.M.R., Terrett, N.K., and Wythes, M.J., *Biochem. Biophys. Res. Comm.*, **1989**, *164*, 58.

5 Northbridge, D.B., Jardine, A.G., Alabaster, C.T., McConnell, J., Dilly, S.G., Lever, A.G., *Lancet*, **1989**, *8663*, 591.

6 (a) Roques, B.P., Fournie-Zaluski, M.-C., Soroca, E., Lecompte, J.M., Malfroy, B., Llorens, C., and Schwartz, J.-C., *Nature*, **1980**, *288*, 286; (b) Llorens, C., Gacel, G., Swerts, J.-P., Perdrisot, R., Fournie-Zaluski, M.-C., Schwartz, J.C., and Roques, B.P., *Biochem. Biophys. Res. Comm.*, **1980**, *96*, 1710.

7 (a) Node, M., Nishide, K., Ochiai, M., Fuji, K. and Fujita, E., *J. Org. Chem.*, **1981**, *46*, 5163. (b) Trost, B.M. and Kunz, R.A., *J. Org. Chem.*, **1974**, *39*, 2648. (c) Arnold, L.D., May, R.G. and Vederas, J.C., *J. Am Chem. Soc.*, **1988**, *110*, 2237.

8 Borrman, D. and Wegler, R., *Chem. Ber.*, **1966**, *99*, 1245.

9 Waldemar, A., Baeza, J. and Liu, J., *J. Am. Chem. Soc.*, **1972**, *94*, 2000.

10 Pfeffer, P.E., Kinsel, E. and Silbert, L.S., *J. Org. Chem.*, **1972**, *37*, 1256.

11 Krapcho, A.P. and Jahngen, E.G.E., *J.Org.Chem.*, **1974**, *39*, 1650.

12 See typical experimental procedure provided.

13 All the β-lactones exhibited a characteristic i.r. carbonyl stretch in the 1815-1820 cm^{-1} region, and ^1H nmr signal at δ4.2 for *O*-alkyl proton(s).

14 Compounds exhibited elemental analyses consistent with their structures.

15 Endopeptidase activity was measured using [1-^{14}C-glycine]Hip-Phe-Arg as substrate. Microvilli (10-50μg) resuspended in 150μl 50mM HEPES buffer pH 8.0 containing 150mM NaCl and 0.6M Na_2SO_4 were pre-incubated for 15 min at 25°C in the presence or absence of inhibitors. The enzyme assay was started by addition of 100μl [1-^{14}C-glycine]Hip-Phe-Arg (7.5mM, specific activity 0.025μCi/mol), and tubes incubated at 37°C for up to 60min. The reaction was terminated by addition of 250ml 1M HCl, and enzyme activity assessed by the appearance of radioactive products (i.e. [1-^{14}C-glycine]hippuric acid) extracted into 1.5ml ethyl acetate.

16 Example **5(iv)** was prepared from racemic *cis*-3-amino-cyclohexanecarboxylic acid benzyl ester and 2-acetylthiomethyl-3-phenyl-propionic acid using the typical amide coupling and subsequent base hydrolysis procedures described above.

17 Barclay, P.L., Danilewicz, J.C. and Samuels, G.M.R., *Lancet*, **1990**, *336*, 754.

18 Neustadt, B.R., WO 9109840-A, 1991; *Chem. Abs.*, 1991, **115(25)**, 280556d.