Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 17, pp. 2053-2058, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00367-8

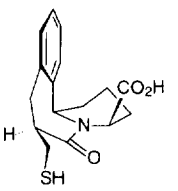
SYNTHESIS OF CONSTRAINED THIORPHAN ANALOGS AS INHIBITORS OF NEUTRAL ENDOPEPTIDASE[1]

Douglas W. Beight,* Shujaath Mehdi, Jack R. Koehl, and Gary A. Flynn
Hoechst Marion Roussel, 2110 East Galbraith Rd., Cincinnati, OH 45215

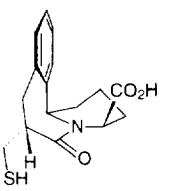
Abstract: Constrained derivatives of thiorphan were prepared and found to be potent inhibitors of neutral endopeptidase 24.11. Copyright © 1996 Elsevier Science Ltd

Neutral endopeptidase 24.11 (NEP, EC 3.4.24.11), a zinc proteinase, plays an important role in the degradation of a number of physiologically important peptides,[2] such as atrial natriuretic peptide[3] (ANP) and the enkephalins.[4] It has been shown that inhibition of NEP in the periphery potentiates the desirable natriuretic and diuretic effects of ANP that could be useful in the treatment of hypertension or congestive heart failure. NEP inhibition in the CNS results in naloxone reversible analgesic effects in prestressed animals.[4c,d] An active site model for NEP[5] has been proposed based on the observed trend of NEP inhibition for the three highly constrained tricyclic thiol containing inhibitors MDL 28,102,[6] MDL 28,067, [6] and MDL 27,855. These three compounds differ only in the positioning of the zinc coordinating thiol group relative to the tricyclic scaffold (Figure 1). The fit of thiorphan to MDL 27,855 bound to this NEP model suggested that a bridged analog **1** would position the phenyl ring similarly to that of MDL 27,855 while allowing the thiol group to coordinate with the zinc atom. The fit to this model also suggested that the ethylene bridged analog **1b** would be preferred to the methylene bridged analog **1a**. This paper describes the synthesis and the in vitro evaluation of methylene and ethylene bridged analogs of thiorphan.[1,7]

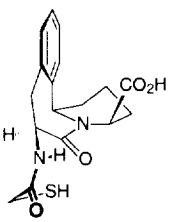
Figure 1



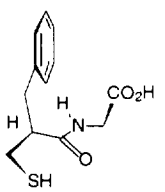
MDL 28,102
K_i (NEP) > 300 nM



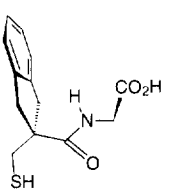
MDL 28,067
K_i (NEP) = 45 nM



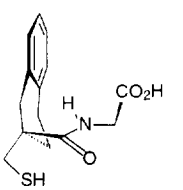
MDL 27,855
K_i (NEP) = 5 nM



(S) -Thiorphan
K_i (NEP) = 1.9 nM



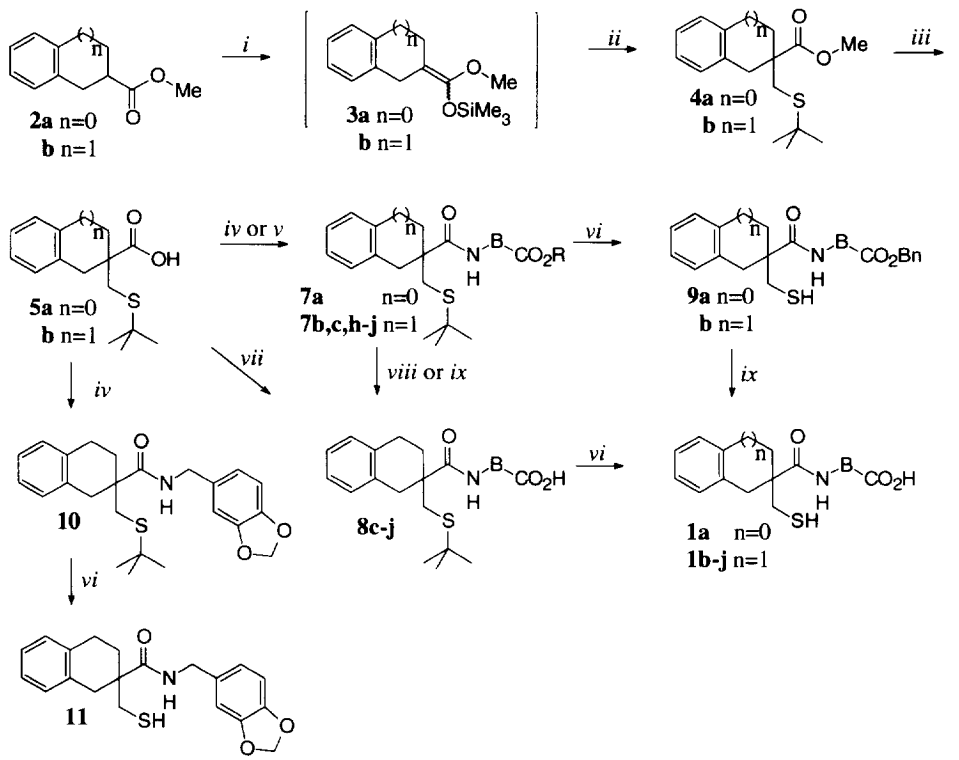
1a



1b

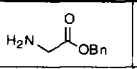
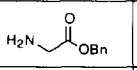
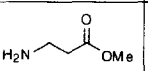
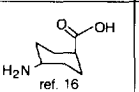
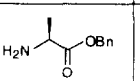
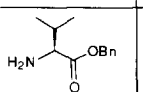
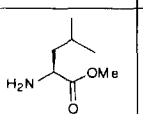
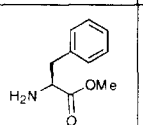
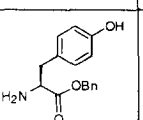
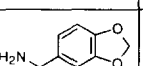
We envisioned the key step for the preparation of bridged inhibitors **1** to be the alkylation of bicyclic ester **2** to give the alkylated ester **4** (Scheme 1). However, alkylation of the lithium enolate of indane ester **2a**[8,9] with chloromethyl-*para*-methoxybenzyl sulfide[10] was unsuccessful. In addition, chloromethyl-*para*-methoxybenzyl sulfide was unstable to Lewis acid conditions and yielded only products from the decomposition of the chloromethyl sulfide using a Lewis acid promoted alkylation of **3a**.[11] Therefore, the *tert*-butyl group was investigated as a more stable yet readily removable protecting group for sulfur using the Lewis acid approach. Treatment of the trimethylsilylketene acetal **3a**, derived from indane ester **2a**, with *tert*-butyl chloromethyl sulfide[12] and catalytic $ZnBr_2$ produced the desired alkylated ester **4a** in 61% yield. This methodology was also applied to tetrahydronaphthalene **2b** to afford racemic ester **4b** in 71% yield. Esters **4a,b** were saponified in quantitative yield with lithium hydroxide in refluxing methanol to give the corresponding acids **5a,b** that were used to prepare a series of constrained inhibitors.

Scheme 1



(*i*) LDA, TMSCl, THF, -78 °C; (*ii*) *tert*-butylchloromethyl sulfide, $ZnBr_2$, CH_2Cl_2;[11] (*iii*) LiOH, MeOH, reflux, 18 h; (*iv*) oxalyl chloride then **6**, Et_3N, CH_2Cl_2; (*v*) EDC, **6**, CH_2Cl_2;[13] (*vi*) $Hg(OAc)_2$, TFA, then H_2S;[14] (*vii*) oxalyl chloride then **6**, $NaHCO_3$, acetone, H_2O;[15] (*viii*) LiOH, MeOH; (*ix*) 10% TfOH, Me_2S.

Table 1*: Yields[a] and conditions[b] for *Scheme 1.

			coupling reaction			first deprotection			final product			
entry	amine **6**	n	method	product	yield	method	product	yield	method	product	yield	K_i(nM)
a		1	A	**7a**	97%[c]	D	**9a**	94%	F	**1a**	48%	10
b		2	A	**7b**	87%	D	**9b**	94%	F	**1b**	50%	0.4
c		2	A	**7c**	65%	E	**8c**	93%[c]	D	**1c**	88%	0.2
d	H_2N-$(CH_2)_6$-CO_2H	2	B	**8d**	52%		NA[d]		D	**1d**	84%	0.6
e	 ref. 16	2	B	**8e**	59%		NA		D	**1e**	94%	0.6
f		2	C	**7f**	48%	F	**8f**	83%	D	**1f**	15%[e]	0.3
g		2	C	**7g**	55%	F	**8g**	NI[f]	D	**1g**	78%	2.0
h		2	A	**7h**	72%	E	**8h**	100%[c]	D	**1h**	88%	7.0
i		2	A	**7i**	72%	E	**8i**	97%[c]	D	**1i**	94%	4.0
j		2	A	**7j**	78%	E	**8j**	92%	D	**1j**	80%	0.5
k		2	A	**10**	100%		NA		D	**11**	52%	14
l	(R)-Thiorphan			NA			NA			NA		1.6
m	(S)-Thiorphan			NA			NA			NA		1.9

[a]All compounds represent chromatographed yields unless otherwise specified. [b]Method A: **5**, oxalyl chloride, **6**, Et_3N, CH_2Cl_2. Method B: **5**, oxalyl chloride, **6**, $NaHCO_3$, acetone-water.[15] Method C: **5**, **6**, EDC, CH_2Cl_2.[13] Method D: $Hg(OAc)_2$, TFA, then H_2S.[14] Method E: LiOH, MeOH. Method F: 10% TfOH in Me_2S. [c]Not purified. [d]NA = Not applicable. [e]Insolubility in ether led to a loss of material during workup. [f]NI = Not isolated due to partial loss of the *tert*-butyl group on sulfur.

Acids **5a,b** were coupled to a variety of amines **6**[16] either by their acid chlorides or by EDC mediated[13] condensations. Intermediate esters **7** were first hydrolyzed with lithium hydroxide in methanol or by 10% trifluoromethanesulfonic acid (TfOH) in dimethyl sulfide to yield acids **8**. Dealkylation of the sulfide with mercuric acetate/hydrogen sulfide in trifluoroacetic acid[14] (TFA) provided the targeted thiols **1**. Alternatively, sulfide esters **7a,b** could be selectively deprotected with mercuric acetate/hydrogen sulfide in TFA to give thiol intermediates **9a,b** that could then be hydrolyzed with 10% TfOH in dimethyl sulfide to give acids **1a,b**. Reaction conditions and yields for Scheme 1 and inhibition constants for NEP are summarized in Table 1.

Compounds were assayed for NEP activity according to methods reported previously.[17] The following observations were made from binding constants in Table 1. Constraint of the benzyl side chain of thiorphan by the methylene bridged analog **1a** reduced activity compared to thiorphan, while the ethylene bridged analog **1b** exhibited enhanced NEP binding affinity relative to thiorphan. Examination of the enantiomers of **1b**[18] showed that the (+) enantiomer completely inhibited NEP at 10 nM whereas no inhibition of NEP was observed by the (−) enantiomer at 10 nM. In contrast, the enantiomers of thiorphan were reported to be nearly equipotent.[19] These results supported the importance of the orientation for this P_1' residue.

The 25- to 50-fold difference in activity between **1a** and **1b** led us to concentrate our efforts on making analogs of the more potent **1b**. Thus the P_2' region of **1b** was modified in an attempt to maximize binding. Extension of the terminal carboxylic acid group from the amide linkage, as in **1c-e**, had little effect on inhibition. Substitution of large lipophilic amino acids for glycine in **1b** resulted in weaker inhibition constants for NEP, although a tyrosine for phenylalanine substitution was well tolerated. Replacement of the P_2' amino acid with a neutral methylene-dioxybenzyl group **11** resulted in a 35-fold loss of activity suggesting that a carboxyl group might be necessary for optimal inhibition.

In summary, conformationally restricted thiorphan analogs were designed based on molecular modeling comparisons with previously reported constrained inhibitors. The Lewis acid mediated approach to bicyclic intermediates **4a,b** allowed efficient preparation of a variety of NEP inhibitors. Results showed that positioning of the P_1' phenyl ring was important. The enhanced potency of the ethylene bridged inhibitor **1b** relative to the methylene bridged inhibitor **1a** was consistent with the postulated conformation of thiorphan bound at the active site of the NEP model.

Experimental procedure for the preparation of ***4b***: To a solution of 2.31 g (16.5 mmol) diisopropylamine in 10 mL dry THF cooled to -70 °C was added 6.6 mL (16.5 mmol) 2.5 M n-butyl lithium in hexanes dropwise by syringe. The resultant solution was allowed to stir 20 min under an atmosphere of argon after which time 2.85 g (15.1 mmol) ester **2b** in 10 mL THF was added dropwise over 30 min. The resultant solution was allowed to stir 30 min at -70 °C then 1.90 mL (15.1 mmol) trimethylsilylchloride was added all at once. The mixture was allowed to warm to ambient temperature and was concentrated in vacuo. The residue

was stirred in 25 mL dry CH_2Cl_2 under argon and 2.3 mL (12.5 mmol) *tert*-butylchloromethyl sulfide was added followed by 0.5 g anhydrous $ZnBr_2$. The mixture was allowed to stir 45 min at ambient temperature after which none of the ketene acetal **3b** remained by GC (150 °-250 °C @ 15 °C/min, 5 m x 0.53 mm column, methyl silicone gum packing.) The mixture was partitioned between saturated $NaHCO_3$ solution and CH_2Cl_2. The organic solution was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 5-10% ethyl acetate in hexane. Concentration of the appropriate fractions yielded 2.57 g (71%) of the desired alkylated ester **4b** which crystallized on standing. ^1H NMR (300 MHz, $CDCl_3$, δ) 7.18 (m, 4H), 3.68 (s, 3H), 3.25 (d, J = 16.4 Hz, 2H), 2.86 (d, J = 11.5 Hz, 1H), 2.85 (d, J = 16.4 Hz, 1H), 2.82 (t, J = 6.8 Hz, 2H), 2.76 (d, J = 11.5 Hz, 1H), 2.18 (dt, J_1 = 13.6 Hz, J_2 = 6.8 Hz, 1H), 1.97 (dt, J_1 = 13.6 Hz, J_2 = 6.8 Hz, 1H), 1.27 (s, 9H). ^{13}C NMR ($CDCl_3$, δ) 175.6, 135.0, 134.3, 129.2, 128.6, 125.9, 52.0, 46.1, 42.0, 36.9, 35.4, 30.7, 30.1, 26.0. MS(EI) m/e 292(m^+), 202(base), 143, 129, 57.

Acknowledgement: The authors wish to thank Ron George for the separation of the enantiomers of **1b**.

References

1. Beight, D. W.; Mehdi, S.; Koehl, J. R.; Flynn, G.A. Presented in preliminary form at the 203rd National Meeting of the American Chemical Society, Washington, DC, August 1992.
2. Laragh, J. H.; Brenner, B. M. *Hypertension: Pathophysiology, Diagnosis, and Management, Second Edition*; Raven: New York, 1995, pp 3099-3114.
3. For a review on ANF metabolism see: Schwartz, J. C.; Gros, C.; Lecomte, J. M.; Bralet, J. *Life Sci.* **1990**, *47*, 1279.
4. (a) Malfroy, B.; Swerts, J. P.; Guyon, A.; Roques, B. P.; Schwartz, J. C. *Nature* (London) **1978**, *276*, 523. (b) Roques, B. P.; Fournié-Zaluskie, M. C.; Soroca, E.; Lecomte, J. M.; Malfroy, B.; Llorens, C.; Schwartz, J. C. *Nature* (London) **1980,** *288*, 286. (c) Greenberg, R.; O'Keefe, E. H. *Life Sci.* **1982**, *31*, 1185. (d) Chipkin, R. C.; Latranyi, M. B.; Iorio, L. C. *Life Sci.* **1982,** *31*, 1189.
5. (a) Flynn. G. A.; Beight, D. W.; Warshawsky, A. M.; Burkholder, T. P.; Mehdi, S.; Giroux, E. L.; Dage, R. C. Presented at the 203rd National Meeting of the American Chemical Society, San Francisco, CA, April 1992. (b) Flynn, G. A.; Beight, D. W.; Mehdi, S.; Koehl, J. R.; Giroux, E. L.; French, J. F.; Hake, P. W.; Dage, R. C. *J. Med. Chem.* **1993**, *36*, 2420.
6. Flynn, G. A.; Beight, D. W.; Huber, E. W.; Bey, P. *Tetrahedron Lett.* **1990,** *31*, 815.
7. (a) Neustadt, B. World Patent WO 91/09840. (b) Flynn G. A.; Beight, D. W.; US Patent 5,252,601, **1993**.
8. Gross H.; Höft, E. *Angew. Chem.* **1969**, *79*, 358.
9. For the preparation of **3a** see: Tomiyama, T.; Wakabayashi, S.; Yokata, M. *J. Med. Chem.* **1989**, *32*, 1988.

10. Turchaninova, L.P.; Voronkov, M. G.; Shipov, A. G.; Korchevin, N. A.; Baukov, Y. I.; Deryagina, E. N. *Zh. Obshch. Khim.* **1989**, *59*, 722. *Experimental procedure for preparation of chloromethyl sulfides:* To a mixture of 15.9 mL (125 mmol) TMSCl and 1.5 g (50 mmol) paraformaldehyde at 0 °C was added 7.0 mL (50 mmol) *para*-methoxybenzyl mercaptan. The mixture was stirred 2 h while warming to 20 °C, then distilled, bp 90-96 °C/0.1 torr. Yield: 3.5 g (34%).
11. Paterson, I.; Fleming, I. *Tetrahedron Lett.* **1979**, 993.
12. See ref 10. bp 80-86 °C/75 torr. Yield: 60%.
13. Spevak, W.; Nagy, J. O.; Charych, D. H.; Shaefer, M. E.; Gilbert, J. H.; Bednarski, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 1146.
14. Nishimura, O.; Kitada, C.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 1576.
15. Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids;* John Wiley and Sons: New York, **1961**, pp 891-895.
16. For the preparation of **6e** see: (a) Pearlman W. M. *Org. Synth. Collect. Vol. 5*; Baumgarten, H. E., Ed.; Wiley: New York, 1973; p 760. (b) Johnston, T.P.; McCaleb G. S.; Clayton, S. D.; Frye, J. L.; Krauth, C. A.; Montgomery, J. A. *J. Med. Chem.* **1977**, *20*, 279.
17. French, J. F.; Flynn, G. A.; Giroux, E. L.; Mehdi, S.; Anderson, B.; Beach, D. C.; Koehl, J. R.; Dage, R. C. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 180.
18. The enantiomers of **1b** were separated on a cyclodextrin column. The (+) enantiomer eluted first and was purified to ~96% ee. The (-) enantiomer was purified to ~86% ee.
19. Benchetrit, T.; Fournié-Zaluskie, M. C.; Roques, B. P. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 1034.

(*Received in USA* 22 *April* 1996; *accepted* 24 *July* 1996)