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DESIGN AND SYNTHESIS OF A NEW CLASS OF CONFORMATIONALLY CONSTRAINED INHIBITORS TO PROBE THE ACTIVE SITES OF THERMOLYSINE AND NEUTRAL ENDOPEPTIDASE 24.11

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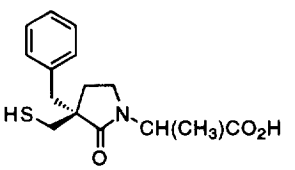
Abstract: The design as well as the synthesis, resolution, and biological evaluation of the neutral endopeptidase 24.11 inhibitors, (*1S,2R,5S*)-(-)-[(2-mercapto-5-phenyl-cyclopentanecarbonyl)-amino]-acetic acid and (*1R,2S,5R*)-(+)-[(2-mercapto-5-phenyl-cyclopentanecarbonyl)-amino]-acetic acid are described. Copyright © 1996 Elsevier Science Ltd

Neutral endopeptidase (EC 3.4.24.11, NEP), a mammalian zinc metallopeptidase, is physiologically involved in the inactivation of the endogenous opioid pentapeptides, methionine-enkephalin and leucine-enkephalin, present in the central nervous system (CNS). NEP is also present in the kidney and involved in the metabolism of the atrial natriuretic factor (ANF), a 28-amino-acid cardiac hormone. The inhibition of NEP in the CNS and in the periphery results in naloxone analgesic effects and natriuretic and diuretic effects respectively.[1] Thus, there is considerable interest in the synthesis of neutral endopeptidase inhibitors as new analgesics and antihypertensive agents.



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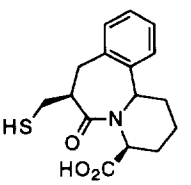

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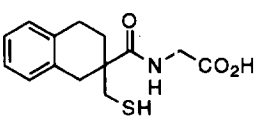

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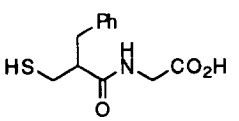

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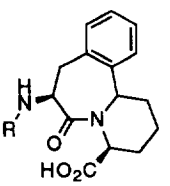

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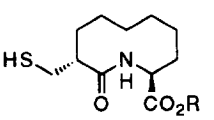

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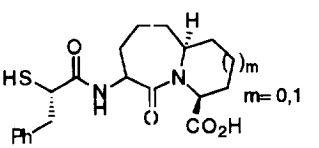

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On the other hand, the investigation of the active site topology of NEP by the synthesis of conformationally restricted inhibitors has been the subject of less widespread attention. Indeed, few groups have reported the synthesis of rigid inhibitors of NEP. The first reported[2] rigid analogs (**1-3**) were structurally derived from thiorphan (**4**), a well studied and very potent NEP inhibitor.[1] More recently,[3] the synthesis of restricted PheAla dipeptide mimetics **5** of the HisLeu portion of angiotensin I and the PheLeu portion of the Leu-enkephalin as well as the macrocyclic lactam **6** and bicyclic lactam **7** allowed more insight into the topology of the active site of NEP.

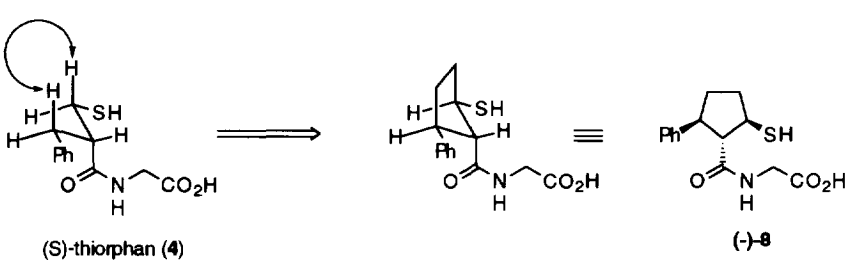
In this report, we present the design and synthesis of a new class of conformationally constrained analogs of thiorphan (**4**) based on the three dimensional structure of the latter which has been cocrystallized in the active site of thermolysine (EC 3.4.24.4, TLN), a well studied bacterial zinc-endopeptidase.[4] The comparative biological evaluation of these new "rigid" inhibitors on NEP and TLN reveals some important details on the similarities and distinctions between the active sites of these two enzymes..

1. Design

A knowledge of the topology of the active site of NEP would be of considerable benefit in the design and synthesis of potent and specific inhibitors. Unfortunately, the three-dimensional structure of NEP is unknown. Despite the absence of crystallographic data, several studies[3,5] using substrate-derived inhibitors have nevertheless permitted to propose an active site model for NEP. On the other hand, the structure of NEP has been related to the well studied bacterial zinc-endopeptidase thermolysine (TLN), since both exhibit similar specificity with regard to substrates and inhibitors.[6] The amino acid sequence analysis[7] in combination with site-directed mutagenesis have shown the presence of equivalent zinc binding residues as well as substrate binding and catalytic residues in a similar sequence in both enzymes.[8] The difference is the presence of an additional ionic interaction in the case of NEP between Arg 102 and the carboxyl group of thiorphan (**4**) which is absent in TLN.[9]

The three-dimensional structure of (*S*)-thiorphan (**4**) bound to TLN that has been determined crystallographically was used as a template in the design of the conformationally restricted inhibitors. The X-ray structure[4] shows that the carbon skeleton of thiorphan (**4**) is in an extended conformation where the hydrogens of the benzylic position eclipse both hydrogens located on the carbon bearing the thiol group and the hydrogen α to the amide carbonyl is antiperiplanar to two of the neighboring eclipsed hydrogens. In order then to retain and rigidify the active conformation of thiorphan (**4**), particularly as pertains to the thiol and phenyl groups, the remaining eclipsed pair of hydrogens has been replaced with an ethylene link (Scheme 1). The resulting inhibitor is a trisubstituted cyclopentane derivative in which the neighboring substituents are all *trans* to one another.

Scheme 1



(S)-thiorphan (**4**) (-)-**8**

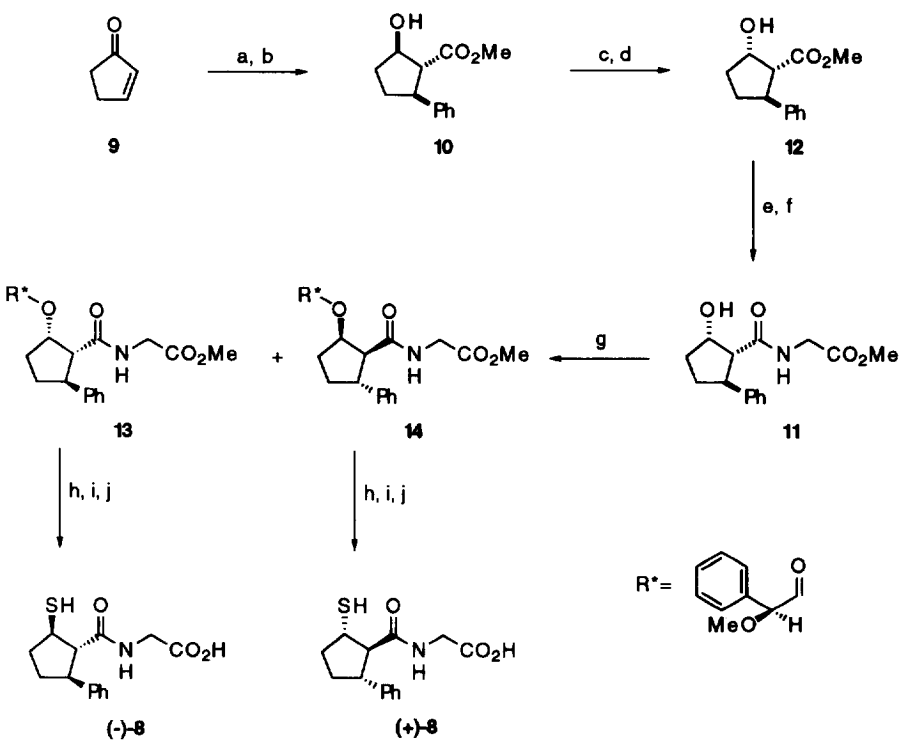
It is important to note here that the strategy of rigidification is particularly aimed at exploring the topological relationship between the zinc atom and the hydrophobic pocket at the active sites of the two enzymes.

In order to further evaluate the effects of the rigidification of thiorphan (**4**), we designed a synthesis which could give access to the enantiomerically pure inhibitor (-)-**8** as well as its enantiomer (+)-**8**.

2. Synthesis

The two rigid thiorphan (**4**) analogs were prepared in ten steps starting from the commercially available 2-cyclopenten-1-one (**9**), (Scheme 2). The key steps of this synthesis are the stereoselective preparation of the hydroxy-ester **10** by a $Zn(BH_4)_2$ reduction as well as the resolution of the alcohol **11** with (*S*)-(+)-methoxy phenylacetic acid.

Scheme 2



(a) Ph_2CuLi, Et_2O then $NCCO_2Me$ (60%) (b) $Zn(BH_4)_2$, Et_2O (65%) (c) DEAD, Ph_3P, $PhCO_2H$, THF (76%) (d) NaOMe, MeOH (56%) (e) KOH 10%, EtOH (90%) (f) Glycine methyl ester hydrochloride, HOBT, DCC, Et_3N, CH_2Cl_2 (68%) (g) (*S*)-(+)-Methoxyphenylacetic acid, DCC, DMAP, CH_2Cl_2 then separation (88%) (h) NaOMe, MeOH (30% and 34%) (i) DEAD, Ph_3P, CH_3COSH, THF (49% and 21%) (j) NaOH 10%, MeOH (97% and 86%).

Addition of lithium diphenyl cuprate (prepared from phenyllithium and copper iodide) to 2-cyclopenten-1-one (**9**) and subsequent trapping of the resulting anion with methyl cyanoformate[10] afforded the Michael adduct

in 60% yield. The latter was stereoselectively reduced in 65% yield using zinc borohydride[11] in a 29:1 ratio mixture in favor of the all-*trans* product **10**. After only two steps, all the stereocenters of the target were in place. In order to introduce the mercaptan group, a double inversion of the hydroxyl group was therefore required. Following the Mitsunobu procedure,[12] the hydroxyl group of **10** was converted into the epimeric benzoate ester in 76% yield which was subsequently hydrolyzed to the corresponding alcohol **12** in 56% yield. Saponification of the carbomethoxy ester **12** with sodium hydroxide in methanol generated the corresponding carboxylic acid in 90% yield. The latter was then coupled with glycine methyl ester hydrochloride producing the hydroxy-amide **11** in 68% yield.

The racemic hydroxy-amide **11** was resolved by coupling with (*S*)-(+)-methoxyphenylacetic acid to form the pair of diastereoisomers **13** and **14** in 88% yield that was easily separated by flash chromatography. The mandelic ester was also used to determine the absolute stereochemistry of the diastereoisomeric pair.[13] Removal of the chiral auxiliary was achieved with sodium methoxide in low yield to afford back the free hydroxy group. The Mitsunobu reaction using thiol acetic acid as the nucleophile[14] produced the fully protected inhibitors which were deprotected with sodium hydroxide in methanol to furnish respectively the mercaptocarboxylic acid inhibitors **(-)-8** and **(+)-8** in their optically pure form.[15]

3. Biological Tests and Discussion

Activities of the inhibitors have been measured[16] on rat kidney NEP and are reported as IC_{50} in Table 1. The analogs of thiorphan (**4**): **(-)-8** and **(+)-8** are less potent inhibitors for both enzymes than thiorphan (**4**) itself.[6c] The effect of the rigidification of thiorphan (**4**) on the potency of the ligand is more important in the case of NEP than TLN, which is not unexpected; the potency of the rigid inhibitors **(-)-8** and **(+)-8** is more than 220 times lower than that of thiorphan (**4**) in the case of NEP. In the case of TLN, the impact of rigidification is much less dramatic; the potency decreases by factors of 16 and 60 for **(-)-8** and **(+)-8** respectively. *This observation reflects a difference in the active conformation of thiorphan (4) for the two enzymes and shows that the strategy of cyclization is valuable since the potency of the inhibitor (-)-8, which is derived from the potent (S)-thiorphan (4), is only slightly affected.* The difference in the active conformation of thiorphan (**4**) for the two enzymes can probably be mostly related to a slight difference in the three-dimensional positioning of the zinc atom versus the hydrophobic pocket (see below).

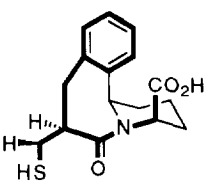
The IC_{50} measurements also show that the active site of NEP is able to accommodate both enantiomers of **8** with no discrimination and the same observation had been made for thiorphan.[6c] In the case of TLN, the enzyme is able to differentiate between both enantiomers which is reflected in the difference in potency by a factor of 4 in favor of **(-)-8** over **(+)-8**. The enzyme also makes a slight differenciation between the enantiomers of thiorphan. Finally, the results also support the proposed large hydrophobic pocket for NEP.[17] This large pocket allows both enantiomers to fit into the active site of NEP with little discrimination contrary to TLN, where the hydrophobic cavity is tighter and permits less adjustment, whence the greater selectivity toward the inhibitors and the slight loss in potency of **(-)-8** compared to (*S*)-thiorphan (**4**). Another point which needs consideration is the conformation required of the cyclopentane ring to adopt the active conformation in order to maximize the

interactions with the enzyme. The envelope form in which all the substituents are pseudo equatorial is certainly expected to be an allowed conformation and the favored conformation for the inhibition of TLN by **(-)-8**, since it corresponds to the extended conformation of (*S*)-thiorphan.

Table 1

	IC_{50}	
Inhibitors	NEP (nM)	TLN (μM)
(S)-Thiorphan (**4**)	1,9	1,8
(R)-Thiorphan (**4**)	1,6	3,0
(-)-**8**	430	29
(+)-**8**	480	110

Finally, *a most interesting observation that can be made about our constrained model (-)-8 is that it can overlap perfectly with the constrained model 2 of Flynn*[3a] *which, as pointed out above, was designed from totally different premises.* Furthermore, both compounds show similar IC_{50} values (430 for **(-)-8** and >300 for **2**[3a]) which substantiates the observation and what's more, the "rigid" model of Flynn[3a] was amenable to dramatic improvement for NEP inhibition by modification at the thiol bearing site which bodes well for future work.



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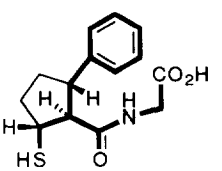

(-)-8

In conclusion, we have provided a new "rigid" model for the investigation of the topological relationship between NEP and TLN, particularly as pertains to the relative disposition of the zinc atom versus the hydrophobic pocket. The next step in this endeavor will be the synthesis and evaluation of analogs modified at the thiol bearing site in order to further refine the probe.

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