Bioorganic & Medicinal Chemistry Letters, Vol.2, No.9, pp. 949-954, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

IMPORTANCE OF THE AMIDE BOND OF THIORPHAN IN THE INHIBITOR-ENKEPHALINASE DOCKING PROCESS DEMONSTRATED WITH SOME THIORPHAN ISOSTERES.

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(*Received* 24 *March* 1992)

Abstract : Syntheses and biological activities of four thiorphan isosteres or analogs are described. The central amide linkage is replaced by ketomethylene, aminomethylene, thioamide, and trans-olefinic functionalities. Double chelation mechanism for the inhibitor-enkephalinase docking process is proposed.

The zinc-containing peptidase currently called enkephalinase, neutral endopeptidase or atriopeptidase (recommended names and numbers given by the Enzyme Commission : membrane metalloendopeptidase, EC 3.4.24.11) is responsible for the inactivation of endogenous enkephalins[1], a group of opioid pentapeptides and atrial natriuretic factor[2], a 28-aminoacid polypeptide hormone secreted by the heart. Therefore, inhibition of this peptidase results in therapeutically useful effects in the gastrointestinal, central nervous system and cardiovascular fields. Thiorphan **1** [(RS)-N-[1-oxo-2-(mercaptomethyl)-3-phenylpropyl] glycine] (fig. 1) the first of the enkephalinase inhibitors is also one of the most potent[3]. The importance of the zinc and arginine binding functions (SH, COOH) of thiorphan **1** is illustrated by the marked loss of *in vitro* potency in acetorphan **2** a compound in which they are both esterified (fig. 1), but which is used as a prodrug crossing biological barriers.

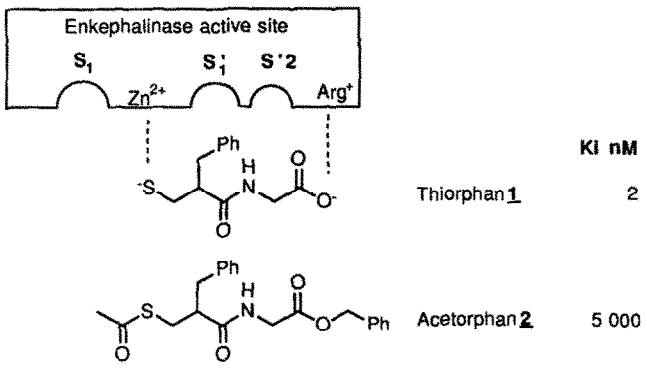


Fig. 1

This work describes an investigation of the influence of various chemical modifications of the amide bond of thiorphan **1** in the inhibitor-enkephalinase docking process. Four different moieties have been used as amide group replacements (fig. 2): the ketomethylene group (-$COCH_2$-); the aminomethylene group (-CH_2NH-); the thioamide group (-CSNH-); and the *trans* olefinic group (-C=C-).

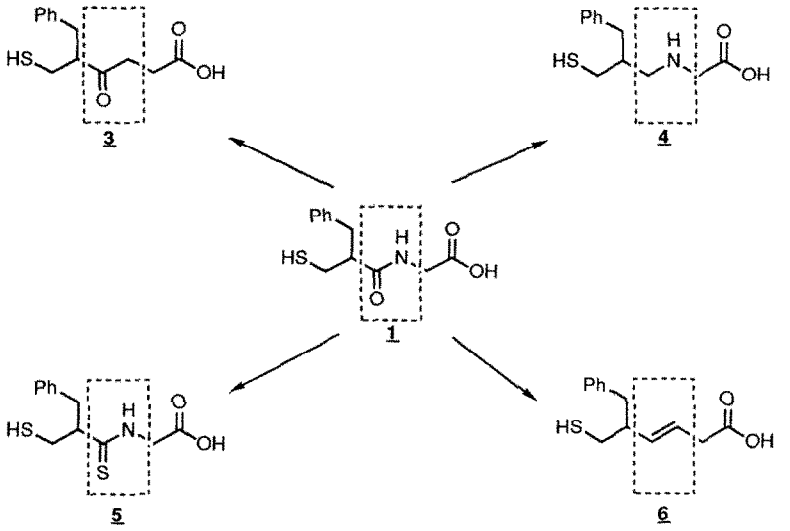
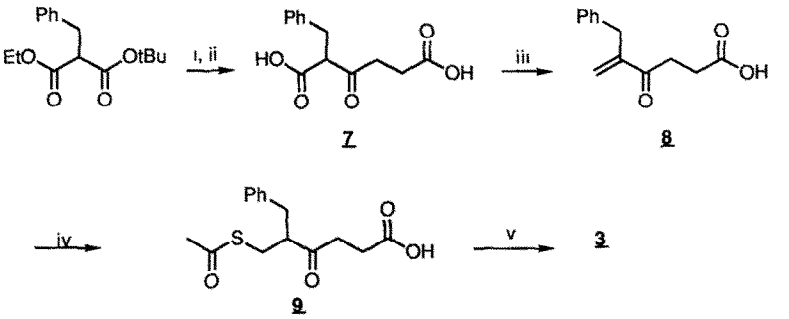


Fig. 2

This paper presents the synthesis and the *in vitro* enkephalinase inhibitory data of these thiorphan isosteres or analogs.

Ketomethylene analog **3** *(scheme 1)*[4].

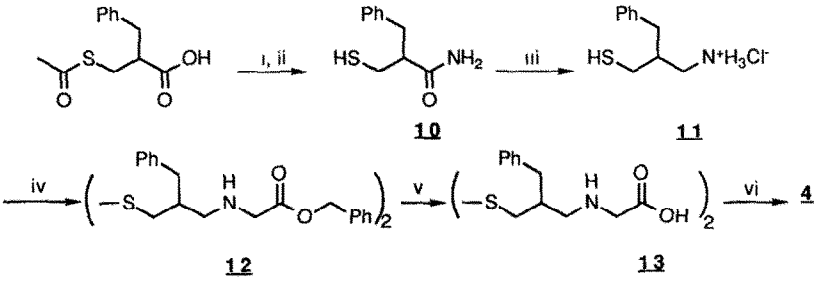
Acylation of ethyl *tert*-butyl 2-benzylmalonate by β-methoxycarbonylpropionyl chloride[5] followed by decarboxylation and basic hydrolysis led to the diacid **7**. The Mannich reaction gave the methylene ketoacid **8**. The Michaël addition of thioacetic acid afforded the thioacetyl acid **9** which was hydrolysed enzymatically[6] to the compound **3**.



Scheme 1 *Reagents* and conditions: i, a $(EtO)_2Mg$, $ClCO(CH_2)_2CO_2Me$, Et_2O reflux, 3 h, b CF_3CO_2H, 12 h (70%); ii, KOH, H_2O, room temp., 12 h (86%); iii, Et_2NH, HCHO 37 wt. % aq, room temp. 12 h (42%); iv, CH_3COSH, 70°C, 12 h (53%); v, enzymatic hydrolysis.

Aminomethylene analog **4** *(scheme 2)*[4].

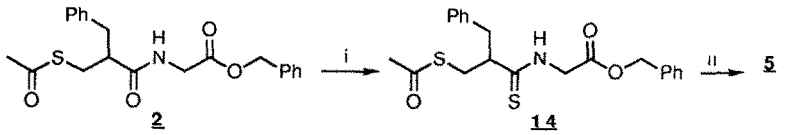
The amidation of 2-(acetylthiomethyl)-3-phenyl propanoic acid[7] by means of DCC and HOBT with ammonium chloride, followed by alkaline hydrolysis led to the mercapto amide **10**. Reduction of this, using diborane furnished the 2-mercaptomethyl-3-phenyl propylamine hydrochloride **11**. After oxidative dimerization in alkaline medium, N-alkylation with benzyl bromoacetate was carried out by using $NEt(iPr)_2$ as base. Hydrolysis of the ester function afforded the acid **13**. Just before use, the disulfide was reduced with dithiothreitol (DTT) to afford the aminomethylene analog **4**.



Scheme 2*Reagents and conditions*: i, NH_4Cl, NEt_3, DCC, HOBT, THF, $CHCl_3$, room temp.,6 h (56%); ii, NaOH, H_2O, MeOH, under argon, room temp., 2 h (75%); iii, a B_2H_6, THF reflux, under argon,48 h, b 3N HCl, (95%); iv, a NaOH, H_2O, MeOH, room temp., 3 days, (74%), b $(iPr)_2NEt$, $BrCH_2CO_2CH_2Ph$, THF, -20°C to room temp. over 0.5 h then room temp., 12 h (34%); v, NaOH, H_2O, THF, room temp., 12 h (78%); vi, dithiothreitol.

Thioamide isostere **5** *(sheme 3)*[4].

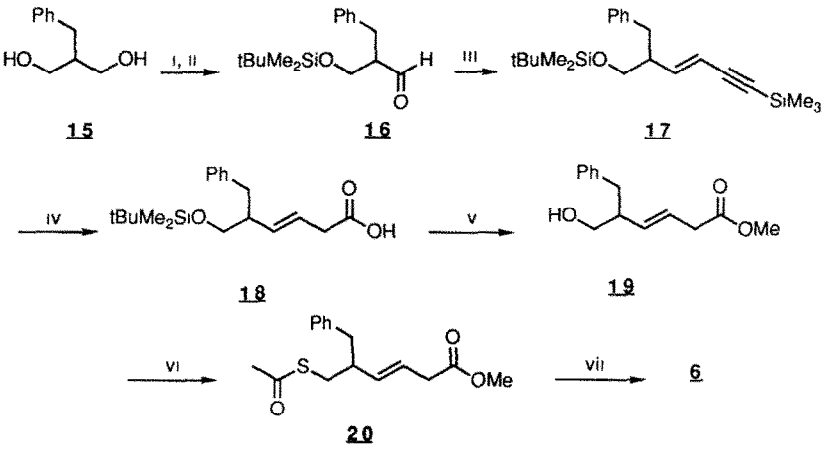
Acetorphan **2**[7] was treated with Lawesson's reagent[8] and gave thioamide **14** which was hydrolysed to the thioamide isostere **5** under argon.



Scheme 3 *Reagents and conditions*: i, Lawesson's reagent, toluene reflux, 6 h (80%); ii, NaOH, H_2O, MeOH, under argon, room temp. 3 h (75%).

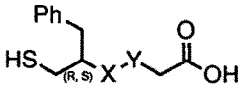
trans-Carbon-carbon double bond isostere **6** *(scheme 4)*[4].

The starting diol **15** was obtained through reduction of the diethyl benzylmalonate using $LiAlH_4$ (73%). Mc Dougal's monosilylation[9] followed by oxidation of the alcohol using pyridinium chlorochromate (PCC) gave the aldehyde **16**. For the conversion of **16** into the β-γ unsaturated acid **18**, we adapted the procedure of Hann et al.[10] in the synthesis of the isosteric Leu^5-enkephalin analogue. Reaction between the Wittig ylide and the aldehyde **16** afforded the silylated trans-enyne **17**. Treatment of the enyne with one equivalent of dicyclohexylborane followed by oxidation with alkalin hydrogen peroxide at 0°C led to the acid **18**. Desilylation and esterification of **18** with HCl-MeOH afforded the *trans* olefinic ester **19**. Mitsunobu type reaction[11] using thioacetic acid gives the acetylthio ester **20** which was hydrolysed enzymatically[6] to the required pure *trans*-carbon-carbon double bond isostere **6**.



Scheme 4 *Reagents and conditions*: i, NaH, THF, room temp, 0.75 h then tBu-Me_2SiCl, THF, room temp., 0.5 h (68%) ii, PCC, CH_2Cl_2, room temp., 2 h (61%); iii, $Ph_3P^+CH_2-C\equiv C-SiMe_3$ Br$^-$, nBuLi, THF, - 70°C 0.75 h then **16** THF, -70°C 0.5 h, and room temp. 0.5 h (59%); iv, a dicyclohexylborane, THF, 0°C, 1 h, b MeOH, 2N NaOH, H_2O_2, 0°C, 1 h (77.5%); v, HCl, MeOH reflux, 2 h (50%); vi, a Ph_3P, diethyl azodicarboxylate, THF, 0°C, 0.5 h, b CH_3COSH, then addition of **19** THF, 0°C 2 h then room temp. vii, enzymatic hydrolysis.

The compounds (**3**, **4**, **5**, **6**) were evaluated as inhibitors of enkephalinase[12]. Some preliminary biological results are reported in table1. A number of conclusions may be drawn from these results.



Compounds	X-Y	Ki (nM)
Ketomethylene analog **3**	-$COCH_2$-	64
Aminomethylene analog **4**	-CH_2NH-	1000
Thioamide isostere **5**	-CSNH-	275
Trans carbon-carbon double bond isostere **6**	-C=C- (E)	1000
Thiorphan **1**	-CONH-	2

Table 1. Inhibitory constants (Ki) of thiorphan and its isosteres or analogs

The thiorphan isosteres or analogs (**3**, **4**, **5**, **6**) exhibit loss of potency in comparison with thiorphan **1**, indicating that the modification of the amide function is not consistent with full biological activity.

The virtually identical activity of the ketomethylene analog **3** (Ki = 64 nM) with that of the N-methyl thiorphan (Ki = 57 nM)[14] suggests that an intermolecular hydrogen bond, e. g. drug receptor interaction, from the amide hydrogen of thiorphan **1** is involved at the active site of the receptor.

However, the thioamide isostere **5** exhibits a poor activity although the NH acidity was increased. This observation supports a mechanism involving an hydrogen bonding interaction of the amide carbonyl of thiorphan **1** : indeed, the sulfur atom of a thioamide is larger and has reduced hydrogen bonding capability[15], compared to the oxygen of an amide, factors that could decrease binding to the enzyme. Consistent with these results is the fact that the *trans*-carbon-carbon double bond isostere **6** (suppresion of the amide group interactions) and the aminomethylene analog **4** (suppresion of the amide carbonyl interaction and possible effect of charge, since the aminomethylene is likely to be protonated under assay conditions) poorly inhibits enkephalinase.

As a corollary to these observations it is clear that intermolecular hydrogen bonds, e. g. drug receptor interaction, from the two heteroatoms of the amide group of thiorphan **1** are involved at the active site of enkephalinase[16].

The inhibitory activity of the ketomethylene analog **3** suggests that the hydrogen bond involving the amide carbonyl in thiorphan **1** is of primary importance in the inhibitor-ENK docking process. This result leads us to conclude that taking advantage of this interaction may constitute a new direction in the search of new enkephalinase inhibitors.

Acknowledgement : T. M. and N. N. thank Bioprojet for generous financial support.

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6 **Enzymatic hydrolysis**. Mouse kidneys were homogenized with ultra turrax (Kinematica, Littau, Switzerland) for 1 min in 2 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged immediately for 20 min at 15, 000 x g at 4°C. The pellet was resuspended in 70 mL of buffer, and the resulting suspension used as enzyme source. The particulate fraction (990 µL) was incubated at 37°C for 30 min in the presence of the protected inhibitors (10 µL, 10^{-2}M).
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12 **Assay of Enkephalinase Activity**. Mouse striatal membranes were used as the enzyme source. Enkephalinase activity was evaluated with succinyl-Ala-Ala-Phe-amidomethylcoumarin as the substrate[13].
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