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STUDIES ON THE STRUCTURAL FEATURE OF S'1 SUBSITE OF NEPRILYSIN (EC.3.4.24.11) : STEREOCHEMICAL REQUIREMENT FOR THE ENZYME-INHIBITOR DOCKING PROCESS

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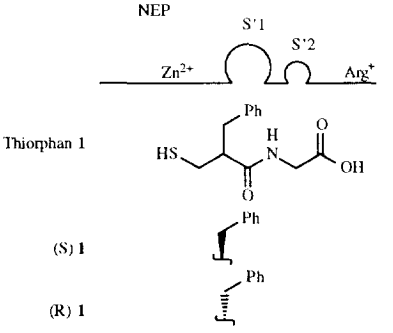
Abstract : The preferred conformation of thiorphan during the inhibitor-neprilysin docking process was investigated. A series of achiral inhibitors were tested. This study led to the design of a potent inhibitor, in which the ethylenic bond bears the aryl residue of P'1. Copyright © 1996 Elsevier Science Ltd

Neprilysin (NEP ; EC.3.4.24.11) plays a central role both in the inactivation of endogenous enkephalins[1] and atrial natriuretic peptides[2] (ANF). The inhibition of NEP remains thus a major challenge for potential therapeutic uses both in analgesia[3] and gastroenterology[4] as well as in cardiovascular applications[5]. The lead compound, thiorphan[6] (fig. 1) was designed as a potent inhibitor, by way of its ability to i) interact with the zinc atom present at the active site of NEP, ii) recognize the S'1 subsite (according to Schechter and Berger[7]) through a benzyl group at the P'1 position, iii) involve hydrogen bonds by means of its amide group[8], and iv) interact with a positively charged arginine by the terminal carboxylate.

Figure 1 :



Results reported herein are based on previous observations[9,10] that both isomers of thiorphan were able to inhibit NEP at a similar nanomolar range (fig. 1).

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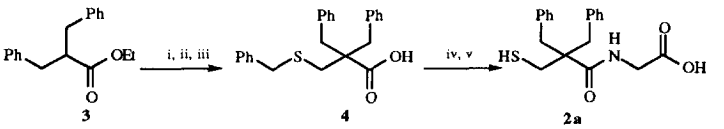
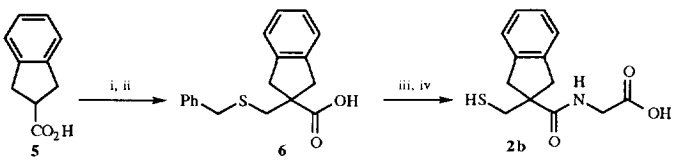
As part of our program for the elucidation of the structural feature of the S'1 subsite of NEP, we were interested in looking for an explanation of the equipotency of the two enantiomers of thiorphan[10]. For this purpose, we describe the synthesis and NEP inhibition of a series of achiral mercaptoacids[11] **2a-d.**

Chemistry :

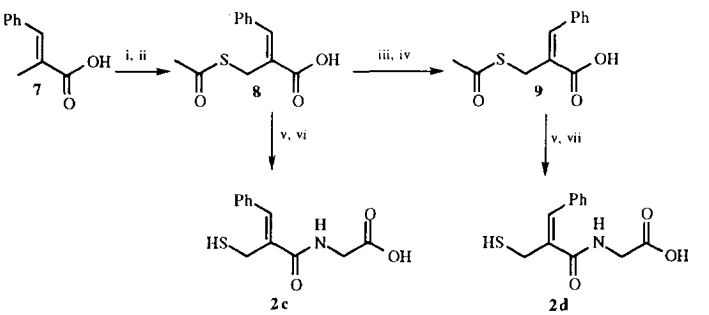
The synthetic methods used in this study are reported below :



Reagents : i) LDA ; ii) $PhCH_2SCH_2Br$; iii) KOH/EtOH 5M reflux (51%) ; iv) $H_2NCH_2CO_2CH_2Ph$, DCC, HOBT ; v) Na/NH_3 (16%)



Reagents : i) LDA ; ii) $PhCH_2SCH_2Br$ (35%) ; iii) $H_2NCH_2CO_2CH_2Ph$, DCC, HOBT ; iv) Na/NH_3 (57%)



Reagents : i) $(PhCOO)_2$, NBS, CCl_4, reflux (57%) ; ii), CH_3COSH, K_2CO_3, H_2O (67%) ; iii) hν, EtOH, 16 h.; iv) 0.4 eq.dicyclohexylamine (32%) ; v) $H_2NCH_2CO_2CH_2Ph$, DCC, HOBT (80%) ; vi) NaOH, H_2O/MeOH, under argon, room temp. 2 h. (70%) ; vii) LiOH, THF/H_2O (75/25), under argon, 0°C 1 h. (64%).

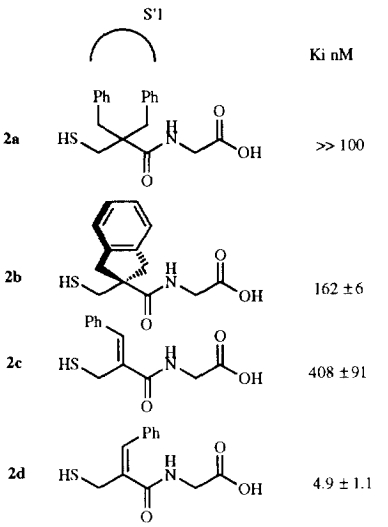
Compounds **2a** and **2b** were obtained as follows : ester **3** or acid **5** were deprotonated using LDA as base and alkylated with bromomethyl benzylsulfide[12] to afford the acid derivatives **4** or **6**. After condensation with glycine benzyl ester in the presence of DCC and HOBT, the target compounds, mercapto acids **2a** and **2b** were obtained by sodium/ammonia deprotection. Ethylenic compounds **2c** and **2d** were readily obtained from α–methylcinnamic acid **7**, the bromination of which by means of NBS[13], followed by substitution using thioacetate ion led to the Z acid **8**[14]. After UV irradiation in ethanol, the mixture of stereoisomeric acids **8** (Z) and **9** (E) (Z/E = 6/4) thus obtained was purified using 0.4 eq. of

dicyclohexylamine in diethyl ether. The insoluble dicyclohexylamine salt of the acid **9** (E) was collected and then acidified to give the acid **9** (E)[14]. The synthesis of the inhibitors **2c** and **2d** was easily performed by coupling the glycine benzyl ester to the appropriate acid **8** or **9** by the classical DCC/HOBT method followed by saponification.

Results and discussion :

The first study was to examine the compound **2a** (fig. 2) in which two benzyl groups are connected to the backbone of the molecule. The aim was to decide if the S'1 subsite, which can accomodate the benzyl group of either (R) thiorphan or (S) thiorphan (fig. 1) could tolerate the simultaneous presence of the two substituents.

Figure 2 :



Ki values are the mean ± SEM from three independent experiments performed in triplicate.

Compound **2a** appeared to be too sterically hindered to fit with the S'1 subsite. In a second set of experiments we decided to prepare and test new compounds with only one aryl substituent (as in thiorphan), but in a fixed position (**2b-d** "rigidified" analogs of thiorphan). The purpose of the study was to elucidate which conformation the benzyl group of thiorphan actually adopts with respect to the backbone of the inhibitor when present at the active site of NEP.

As shown by the results (fig. 2), compounds **2b** and **2c** are very poor inhibitors with respect to thiorphan. In contrast, compound **2d** exhibits a Ki value very similar to that of (RS) thiorphan **1**. This observation shows that in the docking process, the benzyl group of thiorphan must adopt a conformation in which the phenyl ring and the amide bond are in a cisoid orientation. This conformation can be obtained either by (S)-**1** or (R)-**1**, this justifying the equipotency of these two compounds for the inhibition of NEP.

In conclusion, this study has several consequences :

i- The high affinity of **2d** opens up a new route for the design of NEP inhibitors. Of interest is that such ethylenic compounds are achiral, thus avoiding enantiomeric separation. Some ethylenic inhibitors are under biological evaluation[15].

ii- Ethylenic compound **2d** is a poor inhibitor for ACE (Ki = 380 nM). This has to be taken into account when designing either specific or mixed NEP-ACE inhibitors[16].

iii- Finally, it clarifies through experimental evidence the preferred conformation of the inhibitor during the docking process.

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11 **Assay of NEP activity :** NEP activity of 1 ng of the recombinant human enzyme was evaluated with 25 µM succinyl-Ala-Ala-Phe-amidomethylcoumarin as the substrate [16].

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14 **(Z)-2-acetylthiomethyl-3-phenyl propenoic acid 8** : m.p. 114°C ; ^{1}H NMR ($CDCl_3$/TMS) : 9.5 (m, 1H, -OH), 8.0 (s, 1H, ethylenic), 7.5 (s, 5H, aromatic), 4.1 (s, 2H, -CH_2S-), 2.3 (s, 3H, CH_3CO-)
(E)-2-acetylthiomethyl-3-phenyl propenoic acid 9 : m.p. 57°C ; ^{1}H NMR ($CDCl_3$/TMS) : 9.6 (m, 1H, -OH), 7.5 (s, 5H, aromatic), 7.2 (s, 1H, ethylenic), 3.85 (s, 2H, -CH_2S-), 2.3 (s, 3H, CH_3CO-).The configurations of acids **8** and **9** were confirmed by NOEDS experiments. In this series of compounds the E derivatives exhibit a NOE effect between the CH_2S protons and the ethylenic proton, while the Z isomers do not show this effect under the same conditions.

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