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DUAL INHIBITION OF ANGIOTENSIN-CONVERTING ENZYME AND NEUTRAL ENDOPEPTIDASE BY TRICYCLIC BENZAZEPINONE THIOLS§

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Abstract: Various thioacyl analogs of CGS 28106, a tricyclic dual inhibitor of angiotensin-converting enzyme and neutral endopeptidase, have been synthesized and their inhibitory potencies evaluated in vitro. The structure-activity relationship supports the proposed hypothesis that, despite its conformational constraints, CGS 28106 can inhibit the two distinct metalloproteases by adopting different binding modes. In addition, the structural features of CGS 28106 confer remarkable oral activity to this dual inhibitor, as measured by its ability to block the angiotensin-I pressor response and to potentiate plasma levels of atrial natriuretic peptide.

Conformationally restricted peptidomimetics have been used extensively to probe the topography of enzyme active sites and to generate potent inhibitors devoid of the typical therapeutic shortcomings of peptides.[1,2] By virtue of their well defined shape complementary to a specific active site, they are expected to display high enzyme selectivity, thereby limiting the risk of adverse biological side-effects. Therefore, it may be surprising at first glance that peptidomimetics would be considered for the design of inhibitors aimed at *two* structurally distinct proteases. In fact, we and others have observed that mercapto-acetyls and -propanoyls, linked to a rigid fused lactam mimicking a dipeptide framework, constitute potent inhibitors of angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP), two zinc metalloproteases implicated in the modulation of vasoactive peptides.[3-9] Using CGS 28106, a recently described dual ACE/NEP inhibitor characterized by a [6H]-azepino indoline nucleus, we have presented arguments supporting alternate binding modes in ACE and NEP (Fig. 1).[10,11]

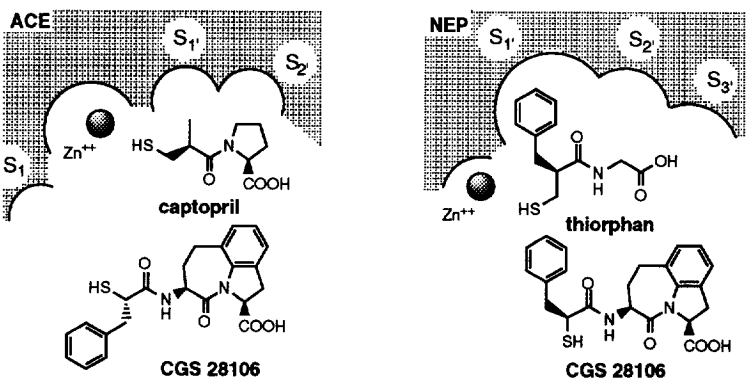
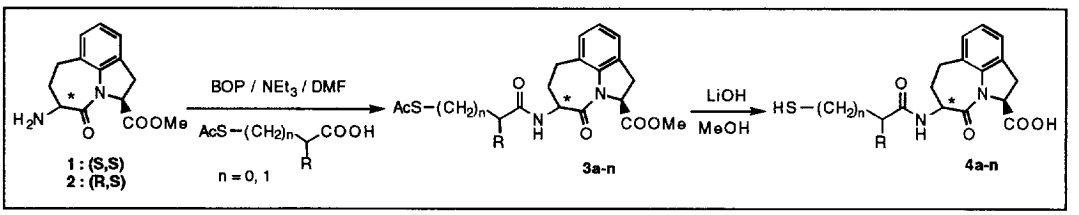


Fig. 1. Proposed binding modes of CGS 28106 in ACE and NEP relative to those of the ACE inhibitor captopril and the NEP inhibitor thiorphan.

This conclusion was based on extensive molecular modeling studies and on the X-ray crystal structure elucidation of CGS 28106 in the active site of thermolysin, a zinc metalloprotease used as a model of NEP.

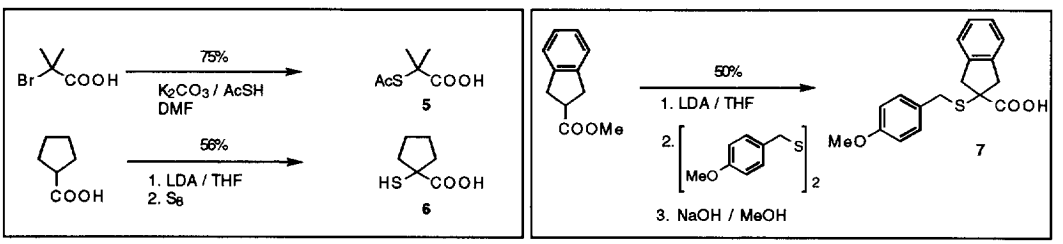
In this letter, we present a complementary study on the SAR of CGS 28106 that focuses on modifications of the mercaptoacyl side chain, and whose results are consistent with the proposed binding hypothesis. In addition, we show that the choice of a [6H]-azepino indoline scaffold not only allows for dual ACE/NEP inhibition in vitro, but confers remarkable oral functional activity to CGS 28106 in vivo.

With the exception of **4l** (see below), the thiols **4a-n** (Table) were prepared as depicted in Scheme 1. Briefly, the tricyclic benzazepinones **1** or **2**, conveniently prepared in 6 steps from *N*-trifluoroacetyl aspartic acid,[12] were coupled to the desired α- or β-thioacetyl carboxylic acid using the BOP reagent.[13] Subsequently, the desired thiols were obtained by saponification of the methyl ester and thioacetyl groups. The chirality of the α-thiol side chain was secured by using amino acid precursors of opposite absolute configuration.[14] In the case of the β-thiols **4m** and **4n**, chromatographic separation of the diastereomeric thioacetate methyl ester precursors **3m** and **3n** (n = 1, R = CH_2PhPh) was performed. Among the two possible diastereomers, the most potent inhibitor of ACE, **4m**, was assumed to possess the *S,S,S* configuration at the three consecutive chiral centers.



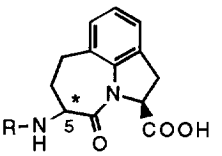
Scheme 1

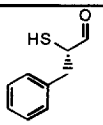
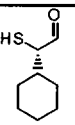
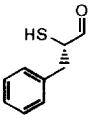
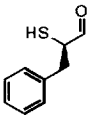
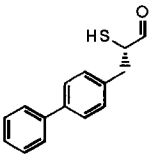
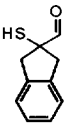
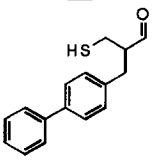
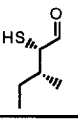
The *gem*-disubstituted α-thiol carboxylic acids **5-7** used to prepare compounds **4j-l** were synthesized as illustrated in Scheme 2. 1-Mercaptocyclopentyl carboxylic acid **6** could be coupled directly to **1**, without the need for protection of the thiol group. The synthesis of **4l** involves the coupling of the acid **7** to **1**, followed by the deprotection of the thiol group with mercuric acetate in trifluoroacetic acid, and finally saponification of the methyl ester.



Scheme 2

Table. In Vitro Inhibition of ACE and NEP by Tricyclic Benzazepinone Thiols.



Cpd	* (C5)	R	IC_{50} (nM)[a] ACE	NEP	Cpd	* (C5)	R	IC_{50} (nM)[a] ACE	NEP
4a (28106)	*S*		40	48	**4h**	*S*		<100	>1000
4b	*R*		62	>1000	**4i**	*S*		ND	>1000
4c	*S*		283	83	**4j**	*S*		>1000	>1000
4d	*S*		89	>1000	**4k**	*S*		253	>1000
4e	*S*		165	>1000	**4l**	*S*		>1000	505
4f	*S*		30	940	**4m**	*S*		77	15
					4n			1120	12
4g	*S*		41	>1000					

[a]NEP activity was measured using the synthetic substrate glutaryl-Ala-Ala-Phe-2 naphthylamide.[15] ACE activity was determined using the substrate hippuryl-His-Leu.[16] ND: not determined.

In the mercaptoacetyl series (α-thiols), the presence of a benzyl substituent in the *(S)*-configuration appears to be optimum for NEP inhibition. As observed in the SAR of thiorphan and its analogs, inversion of configuration at that stereocenter resulted only in a two-fold loss of potency against NEP but a more marked reduction against ACE. Interestingly, as suggested by previous structural studies,[12] the influence of the chirality at C5 in this series was minimal on ACE activity, although it turned out to be critical for NEP inhibition (**4a** vs. **4b**). Replacement of the benzyl substituent in CGS 28106 with a biphenylmethyl substituent was expected to increase the NEP inhibitory potency.[17,18] While this was successfully achieved with the β-thiols **4m-n**, the truncated analog **4d** was devoid of NEP inhibitory potency. The rigid superimposition of thiorphan and CGS 28106 in their bound conformation to thermolysin, as determined by X-ray, may offer an explanation for this difference in activity. This model suggests that, while the corresponding benzyl rings in these two compounds superimpose well, extension to the biphenyl analogs would result in substantial deviations in the overlap of the distal phenyl rings, possibly resulting in unfavorable interactions of **4d** with NEP (Fig. 2.).

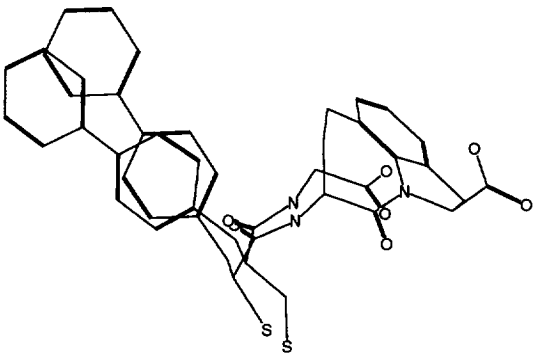


Fig. 2. Thiorphan and CGS 28106 in their bound conformation to thermolysin superimposed and modified by addition of a phenyl ring to the 4-position of the benzyl substituent

The biphenyl groups of **4d** and **4m** were tolerated for ACE inhibition. Replacement of the benzyl group in **4a** with other aliphatic groups (e.g., **4e-h**) had a relatively minor impact on ACE inhibition but caused a drastic loss in inhibitory activity against NEP. Complete removal of this substituent (**4i**) resulted in a total loss of NEP inhibitory activity. Considering the critical importance of the hydrophobic interaction of the P_1' substituent in potent NEP inhibitors and the secondary role of a P_1 side-chain in thiol-based ACE inhibitors, this observation is also consistent with the proposed hypothesis.[10] In contrast to the SAR determined with open-chain mercaptoacyl dipeptides,[19] introduction of a cyclopentyl group in the side-chain (**4k**) resulted in a loss of activity in both proteases, an observation consistent with the distinct binding modes proposed for these two series of dual inhibitors.[10] Similar effects were produced with other geminal disubstitutions (**4j**, **4l**).

One important initial consideration in the design of the [6H]-indoline nucleus as an attractive Ala-Pro mimetic was that this scaffold could be well-suited to provide oral activity, while being readily accessible synthetically.[12] To test this possibility and demonstrate functional ACE and NEP inhibition in vivo, experiments with CGS 28106 were conducted in conscious rats. ACE inhibition was assessed with the

angiotensin-I (AI) pressor test, in which CGS 28106 (10 mg/kg) efficiently blocked the increase in blood pressure produced by AI. Remarkably, within experimental error, there was little difference between the inhibitory profiles obtained during a 6 hour period after intravenous or oral administration, suggesting a high degree of oral bioavailability (Fig. 3). In this test, the effects of CGS 28106 as an ACE inhibitor were comparable to those of captopril in magnitude of response and duration of action. Next, the ability of CGS 28106 to protect exogenously infused atrial natriuretic peptide (ANP) was evaluated as a measure of NEP inhibition in vivo. One hour after administration of CGS 28106 (10 mg/kg) to rats, the circulating ANP levels were significantly increased relative to those measured in vehicle-treated animals. Moreover, there was again little difference in the responses observed after iv versus po administration, corroborating the hypothesis of a high degree of oral bioavailability for this compound (Fig. 4.).

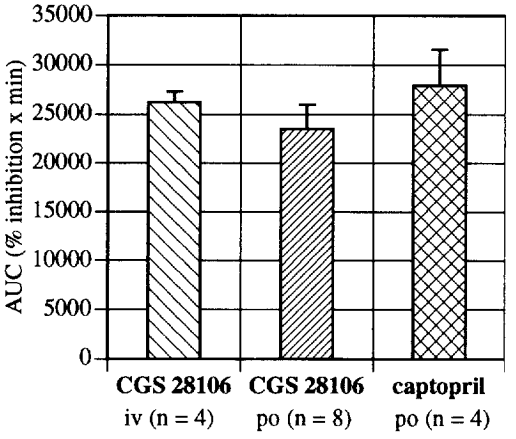


Fig. 3. AI-pressor response inhibition measured for 6 h (area under the curve) with CGS 28106 and captopril (10 mg/kg).

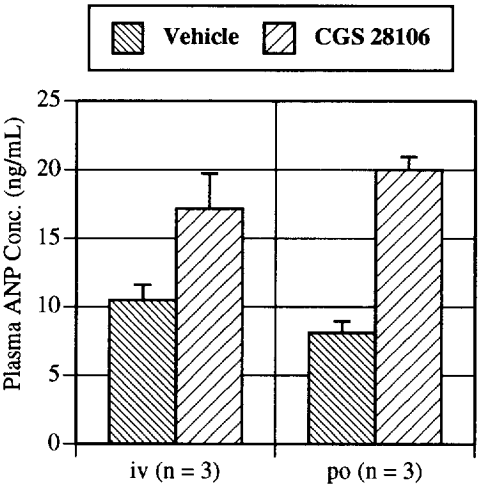


Fig. 4. Increase in plasma ANP concentration produced by CGS 28106 1 h after dosing (10 mg/kg).

In conclusion, SAR studies with CGS 28106 support the binding hypothesis presented previously on the basis of modeling studies and X-ray data in the active site of thermolysin. NEP inhibition, which is well-known to be highly sensitive to the nature of the $P_{1'}$ substituent, is the most affected by structural modifications of the benzyl group in CGS 28106. Although some analogs displayed higher inhibitory potency as ACE or NEP inhibitors, CGS 28106 remained the most potent dual inhibitor in this series. In addition, the tricyclic benzazepinone scaffold is not only readily accessible, but also provides CGS 28106 with remarkable oral functional activity, a feature which compensates for its relatively modest in vitro inhibitory potencies and alleviates the need of derivatization to a prodrug. The pharmacological characterization of CGS 28106 as an orally active dual ACE/NEP inhibitor will be reported elsewhere.

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