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(±)-2-Amino-2-Thiazoline-4-Ethanoic Acid; A novel, Specific $GABA_A$ Receptor Agonist

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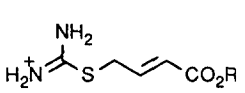
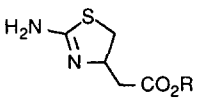
Abstract: Intramolecular Michael cyclization led directly to 2-amino-2-thiazoline-4-ethanoic acid, a new and potent member of the limited group of specific $GABA_A$ receptor agonists.

2-Amino-2-thiazoline-4-ethanoic acid is unknown, although the corresponding aromatic thiazole features in the 7β-acylamino side chain of certain β-lactam antibiotics. We now describe a brief entry to the title compound, and its biological activity as a GABA mimetic.

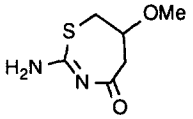
Methyl or ethyl 4-bromobut-2-enoate reacted in acetone with thiourea to gie the isothiouronium bromides (1) and (2), quantitatively. These were readily converted to the 2-amino-2-thiazoles (3) and (4) ($NaHCO_3$, acetone) in high yield, via a 5-exo Trig Michael annelation. The sequence could be performed in a one-pot reaction.



1, R = Me
2, R = Et



3, R = Me
4, R = Et
5, R = H



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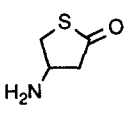

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An alternative cyclization structure (6) could not immediately be precluded on the basis of i.r. or n.m.r. spectroscopy, but was discounted because of the appearance in the E.I. mass spectrum of $(M\text{-}CH_2CO_2R)^+$ as the base peak. 400MHz n.m.r. spectroscopy allowed interpretation of the complex five-proton region region in (3), (H-4, H-5a, H-5b, H-6a and H-6b); δ^1H ($CDCl_3$) 2.72 (2H, ddd, H-6a and H-6b, $J_{H\text{-}6a,H\text{-}6b}$ 17.0, $J_{H\text{-}6a,H\text{-}4}$ 9.0, $J_{H\text{-}6b\text{-}H\text{-}4}$ 4.5), 3.22 (2H, ddd, H-5a and H-5b, $J_{H\text{-}5a,H\text{-}5b}$ 11.5, $J_{H\text{-}5a,H\text{-}4}$ 8.8, $J_{H\text{-}5b,H\text{-}4}$ 7.6), 4.61 (1H, m, H-4). Alkaline hydrolysis of (4) and (5) (NaOH, MeOH) and acidification gave (5) as the racemate.[1] Acidic hydrolysis gave the novel thiolactone (7)[2] as the hydrochloride. This thiolactone is a β-aminoacid variant of related acetamide and propionamide homocysteine lactone derivatives isolated from Streptomyces TU 2476.[3] Hydrolysis of (7) has been shown to give an isomer of homocysteine,[4] an intermediate in the metabolic conversion of methionine to cysteine. Our route constitutes an efficient preparation of racemic β-amino thiolactones.

Because of its structural similarity of the inhibitory neurotransmitter γ-aminobutyric acid (GABA),

2-aminothiazoline-4-ethanoic acid was examined in several tests of central GABA-ergic activity.[5,6] The effects on binding of [^{3}H]-GABA to either $GABA_A$ or $GABA_B$ receptors were investigated in homogenates of rat cerebral cortex. A dose-dependent inhibition of [^{3}H]-GABA binding to $GABA_A$ receptors gave an IC_{50} value of 0.5 ± 0.15 μM (n=6). This compares favourable with IC values for GABA and the selective $GABA_A$ agonists THIP and isoguvacine of 0.1 μM and 0.49 μM respectively. In contrast, (5) was essentially inactive in displacing [^{3}H]-GABA binding to $GABA_B$ sites. In keeping with the ability of $GABA_A$ receptor agonists to enhance [^{3}H]-benzodiezapine binding, 100 μM (5) induced a maximal 142 ± 20% (n=3) stimulation of [^{3}H]-diazepam binding to rat cortical membranes with EC_{50} of 3.4 ± 0.82 μM (n=3). This compares with a maximal stimulation of binding of 156 ± 15% (n=4) and an EC_{50} of 3.1 ± 0.65 μM (n=4) for the $GABA_A$ agonist isoguvacine.

In electrophysiological experiments (5) depressed the CA1 population spike in rat hippocampal slice, with EC_{50} 28.3 μM (n=11) compared with EC_{50} values for isoguvacine and THIP of 13 ± 1 μM (n=26) and 55 ± 10 μM (n=7) respectively. The specific $GABA_A$ receptor antagonist bicuculline induced parallel shifts to the right of the dose response curve for (5).

In summary, these results indicate that (±)-2-aminothiazoline-4-ethanoic acid is a new and selective $GABA_A$ receptor agonist of comparable potency to THIP and isoguvacine. The flat, aromatic thiazole analogue is only marginally active in comparison. It is noteworthy that the synthetic route is of general applicability, since 2-, 3- and 4-substituted variants of bromocrotonate afford similar cyclizations with thiourea, the 2- and 4-alkyl substituents leading to diastereoisomeric mixtures. Attempts to resolve (5) by classical crystallization methods with, for example, alkaloid bases, were unsuccessful, possibly due to equilibration with the open-chain form. This problem is, however, currently being addressed in order that a chiral topology for the $GABA_A$ receptor may be defined.

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