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Design and synthesis of a new series of 4-alkylated 3-isoxazolol $GABA_A$ antagonists

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Abstract

A number of analogues of the low-efficacy partial $GABA_A$ agonist 5-(4-piperidyl)-3-isoxazolol (4-PIOL), in which the 4-position of the 3-isoxazolol ring is substituted by different groups, were synthesized and tested as $GABA_A$ receptor ligands. While alkyl and benzyl substitution provided affinities and antagonist potencies comparable to those of 4-PIOL, diphenylalkyl and naphthylalkyl substitution resulted in marked increase in both affinity and potency. The 2-naphthylmethyl and the 3,3-diphenylpropyl analogues showed antagonist potencies comparable or markedly higher than that of the standard antagonist SR 95531. Molecular modeling studies exposed a large cavity in the vicinity of the 4-position of 4-PIOL, in which there seems to be additional sites for specific receptor interactions.

Keywords: $GABA_A$ receptor; Antagonists; 4-PIOL; THIP; Muscimol

4-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), inhibits neuronal activity through two classes of GABA receptors, ionotropic $GABA_A$ and $GABA_C$ receptors and metabotropic $GABA_B$ receptors [1,2]. Besides playing an important role in central transmission processes, these receptors, especially $GABA_A$ receptors, are believed to be involved in certain neurological and psychiatric disorders and are therapeutic targets in certain diseases [3,4]. In order to pharmacologically characterize this receptor class, a number of $GABA_A$ ligands (Fig. 1), such as the highly selective $GABA_A$ agonists isoguvacine, muscimol [5] and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) [5,6] and the low-efficacy partial agonist 5-(4-piperidyl)-3-isoxazolol (4-PIOL) [7] have been developed. 2,3′-(Carboxypropyl)-3-amino-6-(*p*-methoxyphenyl)pyridazinium bromide (SR 95531) [8], now used as a standard antagonist for $GABA_A$ receptors, represents another structural class of ligands.

Introduction of alkyl groups into the 4-position of the 3-isoxazolol ring of muscimol severely inhibits interaction with the $GABA_A$ receptor recognition site. This is illustrated by the very low affinity for $GABA_A$ receptor of 4-Me-muscimol ($IC_{50} > 100$ cM) [9] compared with that of muscimol (IC_{50}, 0.006 μM) [10]. In contrast, $GABA_A$ recognition site tolerates introduction of alkyl groups into the 4-position of the 3-isoxazolol ring of 4-PIOL [11,12]. These structure–activity relationships indicate that the binding modes of the $GABA_A$ agonists, muscimol and THIP, and in particular 4-PIOL, are different.

There is strong evidence that an arginine residue at $GABA_A$ receptor recognition site is directly involved in the binding of the anionic part of the receptor ligand [13–15]. Based on this observation, a hypothesis has been proposed concerning the binding modes of the bioactive conformations of muscimol and 4-PIOL as illustrated in Fig. 2 [11]. In these binding modes, the two 3-isoxazolol rings do not overlap. This means that the 4-position of the 3-isoxazolol ring in muscimol does not

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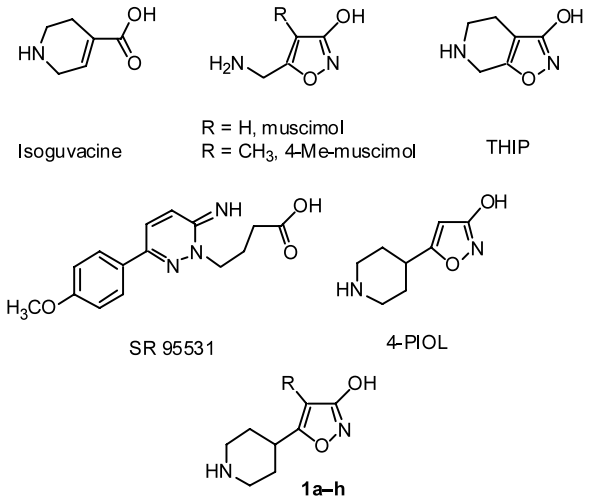


Fig. 1. Structures of $GABA_A$ agonists isoguvacine, muscimol, 4-methylmuscimol and THIP, the $GABA_A$ antagonist SR 95531, the low-efficacy partial $GABA_A$ agonist 4-PIOL and a general structure of the new isoxazolols **1a**–**1h**.

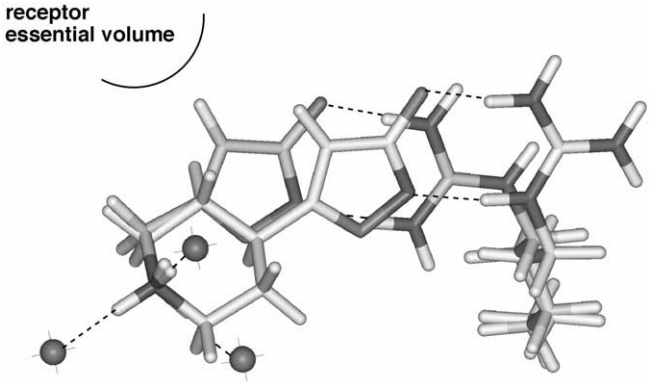


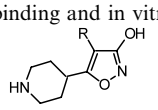
Fig. 2. A pharmacophore model for $GABA_A$ receptor agonists showing the proposed binding modes of muscimol (dark grey bonds) and 4-PIOL (light grey bonds) and their interactions with two different conformations of an arginine residue. The dark grey spheres indicate sites to which the ammonium group in muscimol interacts via hydrogen bonds.

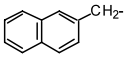
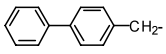
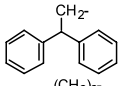
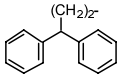
correspond to the 4-position in the 3-isoxazolol ring of 4-PIOL during interaction of muscimol and 4-PIOL with the receptor recognition site.

A number of analogues of 4-PIOL have been synthesized with substituents in the 4-position of the 3-isoxazolol ring in order to further investigate the steric tolerance of this position [12]. Substituents of different size and structural flexibility such as alkyl, phenylalkyl, diphenylalkyl and naphthylalkyl were explored.

Pharmacological characterization of the synthesized compounds was carried out using receptor binding assays and by electrophysiological experiments using whole-cell patch-clamp techniques. The results from these studies are exemplified in Table 1 [12]. Substitution

Table 1
Receptor binding and in vitro electrophysiological data



	R	[^{3}H]muscimol binding[a] K_i (μM)	Electrophysiology[b] IC_{50} (μM)
4-PIOL	H	9.1	110
1a	CH_3CH_2-	6.3	26
1b	$CH_3(CH_2)_3$-	7.7	3.0
1c		3.8	4.0
1d		0.10	0.48
1e		0.049	0.37
1f		0.4	0.71
1g		0.36	0.81
1h		0.068	0.02
	SR 95531	0.074	0.24

[a] Standard receptor binding on rat brain synaptic membranes, $n = 3$. [b] Two electrode voltage-clamp recordings on human $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors expressed in oocytes.

of the 4-position with alkyl or benzyl groups, as for compounds **1a**–**1c**, resulted in affinities and potencies comparable with those of 4-PIOL. Interestingly, introduction of more bulky groups such as biphenylalkyl, diphenylalkyl and naphthylalkyl groups, as exemplified by the 4-biphenylmethyl, 1-naphthylmethyl, 2-naphthylmethyl, 2,2-diphenylethyl and the 3,3-diphenylpropyl analogues (**1d**–**1h**), is not only tolerated but resulted in a marked increase both in affinity and potency, as shown in Table 1.

Using cultured cerebral cortical neurones in the electrophysiological testing, the pharmacology of the 4-PIOL analogues in the absence or presence of the specific $GABA_A$ receptor agonist isoguvacine was studied [12]. The results demonstrated that the structural modifications led to a change in the pharmacological profile of the compounds from moderately potent low-efficacy partial $GABA_A$ receptor agonist activity to a potent and selective antagonist effect. The 2-naphthylmethyl and the 3,3-diphenylpropyl analogues, **1e** and **1h**, respectively, showed an antagonist potency comparable to or markedly higher than that of the standard $GABA_A$ antagonist SR 95531. Only the methyl and ethyl analogues retained detectable ability to induce an agonist effect shown on cultured cerebral neurones [12].

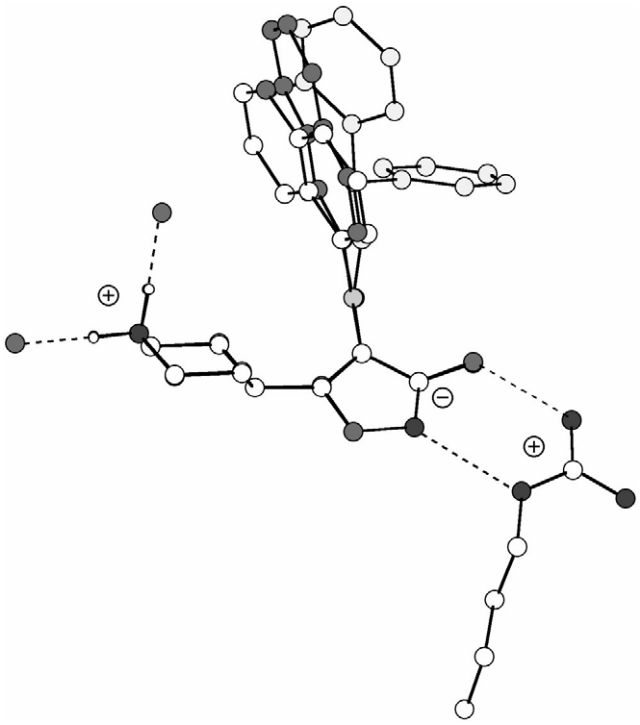


Fig. 3. Superimposition of compounds **1d** (unfilled atoms), **1e** (dark grey atoms) and **1h** (light grey atoms) in their proposed bioactive conformations.

These structure–activity studies seem to support the proposed hypothesis concerning the distinct binding mode of 4-PIOL, implying that the 4-position in 4-PIOL does not correspond to the 4-position in muscimol (Fig. 2). Thus, a cavity of considerable binding capacity seems to exist at the 4-PIOL recognition site of $GABA_A$ receptor. Molecular modeling studies of the two high-affinity compounds containing a 2-naphthylmethyl and a 3,3-diphenylpropyl substituent, **1e** and **1h**, respectively, and the less active 1-naphthylmethyl analogue, **1d**, indicate that this proposed binding cavity may be exploited in two directions (Fig. 3). In both these positions, an aromatic ring seems to be highly favourable for the receptor affinity.

This series of alkylated and arylalkylated 4-PIOL analogues has provided new information on the agonist and competitive antagonist binding site in $GABA_A$ receptor and is going to be exploited in further development of the pharmacophore model. In addition, these compounds could serve as useful tools for studies of $GABA_A$ receptor structure and mechanisms.

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