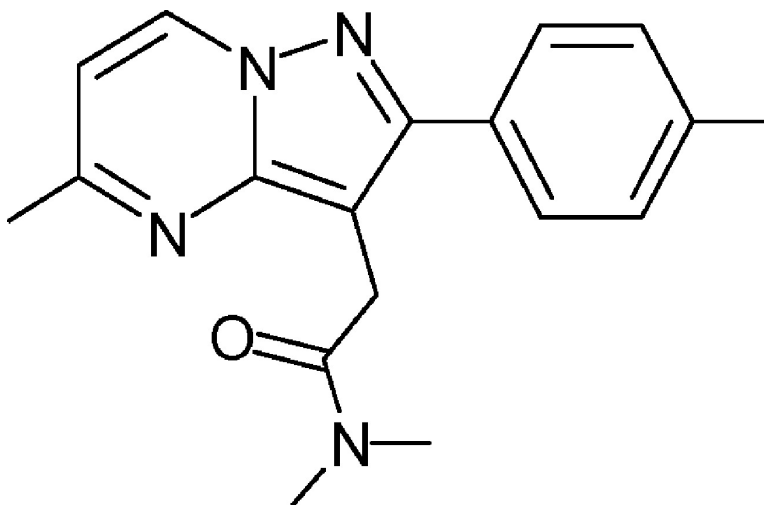


## A Novel Selective GABA $\alpha$ 1 Receptor Agonist Displaying Sedative and Anxiolytic-like Properties in Rodents

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## Brief Articles

### A Novel Selective GABA<sub>A</sub> $\alpha$ 1 Receptor Agonist Displaying Sedative and Anxiolytic-like Properties in Rodents

Silvia Selleri,<sup>§,\*</sup> Fabrizio Bruni,<sup>§</sup> Camilla Costagli,<sup>§</sup> Annarella Costanzo,<sup>§</sup> Gabriella Guerrini,<sup>§</sup> Giovanna Ciciani,<sup>§</sup> Paola Gratterer,<sup>§</sup> François Besnard,<sup>‡</sup> Barbara Costa,<sup>||</sup> Marina Montali,<sup>||</sup> Claudia Martini,<sup>||</sup> Jonna Fohlin,<sup>#</sup> Gaetano De Siena,<sup>#</sup> and Petra Malmberg Aiello<sup>#</sup>

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In our pursuit to identify selective ligands for Bz/GABA<sub>A</sub> receptor subtypes, a novel pyrazolo[1,5-*a*]pyrimidine derivative (**4**), the azaisostere of zolpidem, was synthesized and evaluated in vitro on bovine brain homogenate and on recombinant benzodiazepine receptors ( $\alpha x\beta 2/3\gamma 2$ ,  $x = 1-3, 5$ ) expressed in HEK293 cells. Compound **4** displayed affinity only for  $\alpha 1\beta 2\gamma 2$  subtype ( $K_i = 31$  nM), and in an in-depth, in vivo study it revealed sedative and anxiolytic-like properties without any amnesic and myorelaxant effects in rodents.

#### Introduction

The GABA<sub>A</sub> receptor is a pentamer consisting of subunits from at least five different families:  $\alpha(1-6)$ ,  $\beta(1-4)$ ,  $\gamma(1-3)$ ,  $\rho(1-3)$ ,  $\delta$ ,  $\epsilon$ ,  $\pi$  and  $\theta$ , among which the  $\alpha$ ,  $\beta$  and  $\gamma$  subunits are not only necessary for the modulation of benzodiazepines (Bzs) but are also the basis of GABAergic neuronal plasticity. This receptor heterogeneity is actually considered promising target sites for the development of new selective therapeutic tools in CNS disorders.<sup>1</sup> It is well-known that Bzs act via a recognition site located between the  $\alpha$  and  $\gamma$  subunits, that is allosterically coupled to the GABA site, and the  $\alpha$  subunit variants seem to determine the different pharmacological effects exerted by Bzs. However, in view of the distinctions that can be made among Bz-ligands based on their binding affinities, it remains difficult to ascribe specific in vivo effects of the above-mentioned ligands to different receptor subtypes. The most prevalent subtype is composed of  $\alpha 1$ ,  $\beta 2$  and  $\gamma 2$  subunits and accounts for more than 50% of all GABA<sub>A</sub> receptors in the brain. This receptor is probably the most investigated because some  $\alpha 1$ -preferring compounds (such as  $\beta$ -CCT, antagonist; CL 218,872, abecarnil, zolpidem and zaleplon, agonists) are available. Nevertheless the role of  $\alpha 1\beta 2\gamma 2$  receptor in mediating different behavioral responses of ligands has not yet been resolved.<sup>2</sup> Recent reports on the effect of anxiolytic-selective anxiolytics such as abecarnil indicated that this compound showed an anxiolytic effect at doses lower than those necessary to induce ataxia or myorelaxation.<sup>3</sup>

On the other hand Belzung et al.<sup>4</sup> have already reported that  $\beta$ -CCT, an  $\alpha 1$ -selective antagonist,<sup>5</sup> abolished the anxiolytic-like action of diazepam in the mouse light/dark box test, while other studies using genetically altered mice, in which GABA/ $\alpha 1$  receptors were rendered insensitive to Bzs (knock-in mice), suggested that the  $\alpha 1$  subunit can mediate the hypnotic-sedative and partially anticonvulsant effects of Bzs.<sup>6</sup> In a recent study, Kralic et al.<sup>7</sup> suggested the compensatory effects in GABA<sub>A</sub> subunit expression as a consequence of knock-out mice technique; in fact, they noted higher sensitivity to the anxiolytic effects of low doses of diazepam in the mouse line lacking  $\alpha 1$ -subunit expression than wild-type ( $\alpha 1^{+/+}$ ) mice, thus revealing functional relationships between GABA<sub>A</sub> subunit expression, receptor function and behavioral responses. On the basis of these facts, our research toward the identification of selective ligands for Bz/GABA<sub>A</sub> subtypes<sup>8</sup> has brought us to the synthesis of a novel pyrazolo[1,5-*a*]pyrimidine derivative (**4**), the azaisostere of zolpidem. The novel compound appears to be endowed with high affinity only for the subtype containing the  $\alpha 1$  subunit and subsequently it was subjected to an in vivo study in rodents.

**Chemistry.** The synthesis of compound **4** was accomplished via the general strategy presented in Scheme 1. Exploiting the base-promoted reactivity of the methylene group of benzoylacetone nitriles, the *p*-tolylacetone nitrile reacted with iodoacetic acid to give the corresponding acetic acid (**1**) in moderate yield (60%) using LiOH·H<sub>2</sub>O as base in 80% EtOH. In the second step, the intermediate **1** reacted in ethanol at reflux with hydrazine hydrate, in the presence of acetic acid, to give the 3-amino pyrazole (**2**) in good yield and purity. Subsequently, the condensation of the 3-amino pyrazole with the 4,4-dimethoxybutan-2-one in EtOH allowed us to obtain the closure of the pyrimidine ring with high

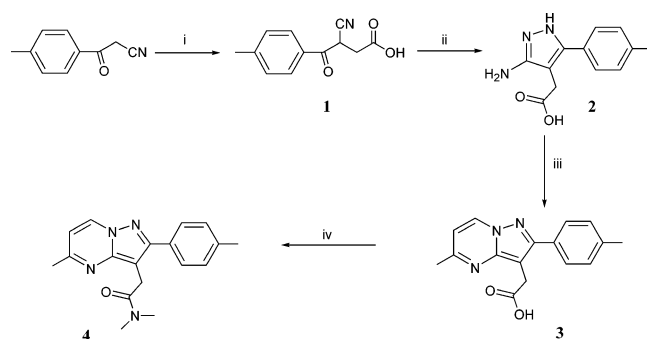
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Scheme 1<sup>a</sup>

<sup>a</sup> (i) Iodoacetic acid/LiOH·H<sub>2</sub>O/EtOH 80%; (ii) N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O/EtOH/AcOH; (iii) 4,4-dimethoxy-2-butanone/EtOH; (iv) NEt<sub>3</sub>/ClCOOEt/THF, NHMe<sub>2</sub>.

**Table 1.** Affinity Values at  $\alpha\beta 2/3\gamma 2$  ( $x = 1-3, 5$ )GABA<sub>A</sub>/BZ Subtypes

| compound              | $K_i$ (nM) <sup>a</sup> |            |            |            |
|-----------------------|-------------------------|------------|------------|------------|
|                       | $\alpha 1$              | $\alpha 2$ | $\alpha 3$ | $\alpha 5$ |
| <b>4</b>              | 31 ± 4                  | >10000     | >10000     | >10000     |
| zolpidem <sup>b</sup> | 26.7                    | 156        | 383        | >10000     |
| diazepam <sup>b</sup> | 14                      | 20         | 15         | 11         |

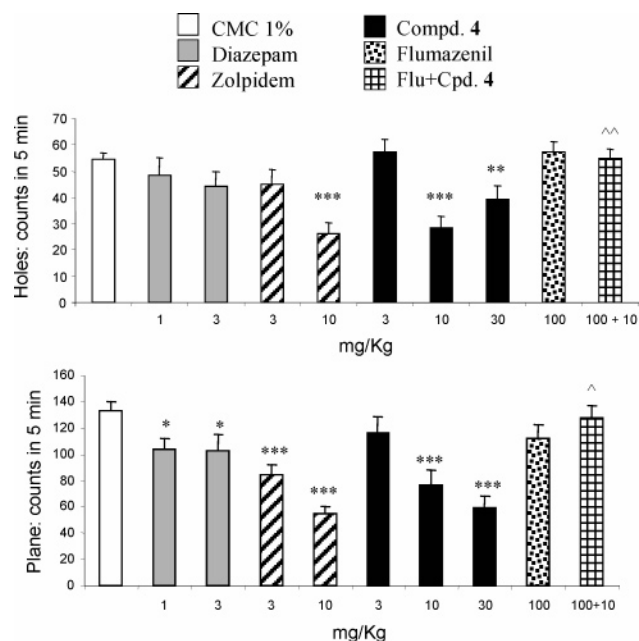
<sup>a</sup>  $K_i$  values represent the means ± SEM derived from three independent experiments, conducted in triplicate. <sup>b</sup> These ligands were employed for comparison purposes in this set of assays, and the reported values were obtained from ref 8.

regioselectivity,<sup>9</sup> affording the 5-methylpyrazolo[1,5-*a*]pyrimidin-3-ylacetic acid (**3**). Finally, compound **3** was converted into a mixed anhydride with ethyl chloroformate, and this intermediate reacted with dimethylamine to afford the amide **4** in good yields and in a short time.

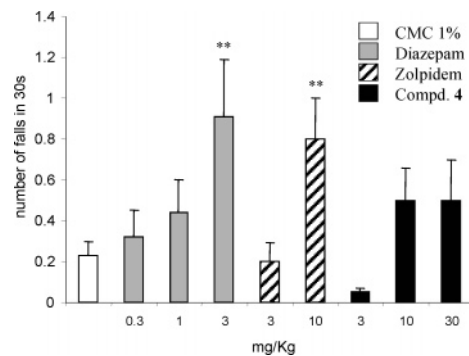
## Results and Discussion

Compound **4** was tested for its ability to displace [<sup>3</sup>H]-Ro15-1788 binding from bovine brain membranes, showing a relatively low affinity for the homogenate ( $K_i = 255 \pm 20$  nM). Nevertheless, the following binding studies on recombinant rat  $\alpha\beta 2/3\gamma 2$  ( $x = 1-3, 5$ ) GABA<sub>A</sub>/Bz receptor subtypes have displayed a complete selectivity of compound **4** for the  $\alpha 1$  subtype in comparison with zolpidem (Table 1). On the basis of these results, the effects of the new compound were evaluated in rodents and compared with diazepam, a nonsubtype selective Bz ligand, and with zolpidem, an  $\alpha 1$ -preferring agonist. Eight different behavioral tests were used: hole board, rota rod, grip strength meter test for mouse motility and muscle relaxation; light/dark choice and lick suppression tests for anxiolytic-like actions. Likewise, it was checked if the new molecule could be active against chemically induced convulsions, impair mouse short-term memory in passive avoidance paradigm, and potentiate sleep time caused by ethanol.

Since the sedative actions of Bzs are connected with activation of  $\alpha 1$ -subtype receptors,<sup>6</sup> compound **4** was first of all studied on mouse spontaneous motility and curiosity using the hole board test. As seen in Figure 1, compound **4** caused dose-dependent highly significant sedative effects on the mouse's movements on the plane and diminished curiosity for the holes. Flumazenil significantly prevented all the effects of compound **4** on mouse spontaneous motility. Thus, we can agree with



**Figure 1.** The effect of compound **4** on mouse spontaneous motility in comparison to diazepam, zolpidem and vehicle, carboxymethylcellulose 1% (CMC), in the hole board test. The upper figure represents the cumulative counts for holes and the lower figure the cumulative counts for plane. The test was performed 30 min after the administration of compound **4** (po), 20 min after diazepam and zolpidem (po), 40 min after Flumazenil (ip). Each column represents the mean ± S. E. M. of 9–18 mice, except the control group ( $n = 43$ ). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  versus control mice.  $\wedge p < 0.01$ ,  $\wedge\wedge p < 0.001$  versus compound **4** (10 mg/kg po)-treated mice (ANOVA, followed by Fisher's LDS test).

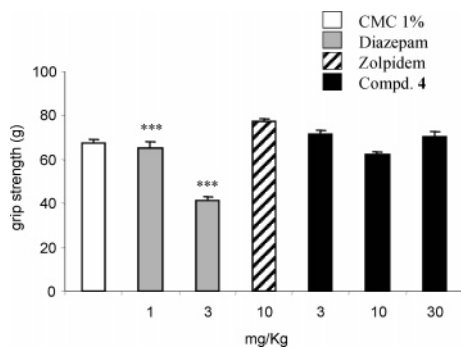


**Figure 2.** The effect of compound **4** on mouse motor coordination in comparison to diazepam, zolpidem and vehicle, carboxymethylcellulose 1% (CMC), in the rota-rod test. Compound **4** was administered 25 min while diazepam and zolpidem were administered 15 min before the test. \*\* $p < 0.01$  versus control mice. (ANOVA, followed by Fisher's LDS test).

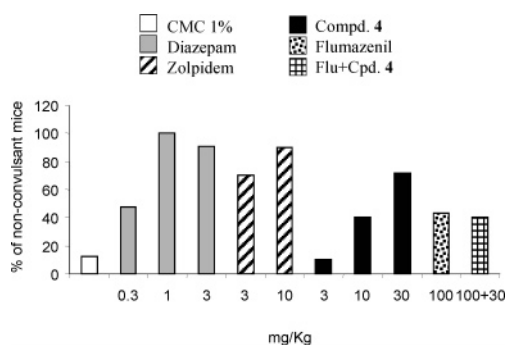
the authors cited above that the activation of  $\alpha 1$ -subtype receptor is responsible for mouse sedative effects.

With both the reference molecules, the deleterious effects on motor coordination are seen at higher doses than in the hole board test and not at all with compound **4** (Figure 2).

Despite the effects already studied with the hole board and rota rod apparatus, we wanted to observe specific effects on myorelaxation using the grip strength meter test. It is worthwhile to note that in our experiments only diazepam in all these tests caused dose-dependent significant effect, while the two  $\alpha 1$ -subtype receptor agonists exhibited no muscle relaxant effect (Figure 3).



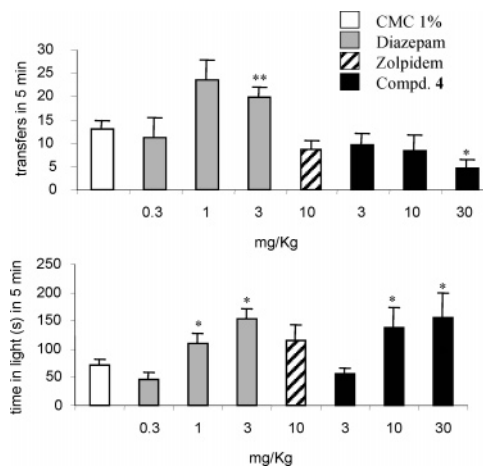
**Figure 3.** The effect of compound 4 on muscle relaxation in comparison to diazepam, zolpidem and vehicle, carboxymethylcellulose 1% (CMC), in the mouse grip strength test. Compound 4 was administered 35 min, while diazepam and zolpidem were administered 25 min before the test. \*\*\* $p < 0.01$  versus control mice (ANOVA, followed by Fisher's LDS test).



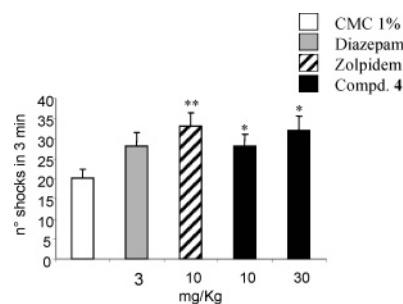
**Figure 4.** Anticonvulsant effects of compound 4 against PTZ-induced convulsions in mice in comparison to diazepam, zolpidem and vehicle, carboxymethylcellulose 1% (CMC). Each column represents the mean of 10–20 mice, except the control group ( $n = 48$ ). Compound 4 was administered (po) 30 min, while diazepam and zolpidem (po) were administered 20 min before the injection of PTZ (90 mg/kg sc). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  versus control mice ( $\chi^2$ -test).

Anticonvulsant activity was studied using pentyletetrazole (90 mg/kg sc) for chemically induced convulsions, and on the basis of the results reported in Figure 4, it seems likely that the compounds having high affinity for  $\alpha 1$ -subtype-containing receptors are involved in anticonvulsant activity.<sup>6b,7</sup>

A further approach to study the effects of compound 4 was to verify if this  $\alpha 1$ -subtype selective ligand had any anxiolytic-like activity in the mouse light/dark choice test (Figure 5). At a dose of 3 mg/kg, compound 4 had no effect while at the higher doses (10 and 30 mg/kg po) about one-half of the animals spent all the time in the lit compartment. As expected, also the number of transfers from one compartment into the other was dose-dependently and significantly decreased with compound 4 to a much higher extent than with the reference molecules. This fact is often considered to reflect more the animal's state of sedation rather than a real anxiolytic-like activity. Since Dawson and Tricklebank<sup>10</sup> reported that locomotor-dependent anxiolytic tests, such as the light/dark box or the elevated plus maze, may be inappropriate methods to determine the potential anxiolytic effects of compounds that decrease motor activity or induce sedation, we performed additional experiments using the punished drinking conflict (Vogel's) test in rats (Figure 6). Also in this test



**Figure 5.** Anxiolytic-like effects of compound 4 in the mouse light/dark box test in comparison to diazepam, zolpidem and vehicle, carboxymethylcellulose 1% (CMC). In the upper figure the columns represent the number of transfers from one compartment into the other, and in the lower figure the columns represent the time spent in light. Compound 4 was administered (po) 30 min, while diazepam and zolpidem (po) were administered 20 min before the test. Each column represents the mean  $\pm$  SEM of 8–25 mice. \* $p < 0.05$ , \*\* $p < 0.01$  versus control mice (ANOVA, followed by Fisher's LDS test).



**Figure 6.** Anxiolytic-like effects of compound 4 in the rat lick suppression test in comparison to diazepam, zolpidem and vehicle, carboxymethylcellulose 1% (CMC). The columns represent the number of shocks taken during 3 min. All the substances were administered po 30 min before the test. Each column represents the mean  $\pm$  SEM of 4–6 rats. \* $p < 0.05$ , \*\* $p < 0.01$  versus control rats. (ANOVA, followed by Fisher's LDS test).

compound 4 was, like zolpidem, significantly effective. Therefore, on the basis of our results and in consideration of the very high  $\alpha 1$  selectivity of compound 4, we may assume that the anxiolytic-like effect observed in rodents could be due to activation of the  $\alpha 1$  subtype-containing GABA<sub>A</sub> receptors.

Although Bzs are considered safe, it is well-known that these drugs potentiate the depressant effect of ethanol and cause amnesic effects; for this reason our next step was to verify whether compound 4 influences ethanol-induced sleep time and impairs mouse short-term memory in passive avoidance paradigm. In our experiments (Table 2) even 0.3 mg/kg po diazepam was able to increase 2-fold (highly significantly) the duration of loss of the righting reflex induced by ethanol with respect to controls, while compound 4, at the highest sedative dose used (30 mg/kg po), demonstrated a minor effect. It has been suggested that the  $\alpha 5$  subunit is involved in the interaction of Bzs with alcohol;<sup>11</sup> since compound 4 does not bind the  $\alpha 5$  subunit (Table 1), its effect is comprehensible.



**Table 2.** Ethanol Potentiation

| treatment, po | dose, mg/kg | no. of mice | time of loss of the righting reflex (min) <sup>a</sup> |
|---------------|-------------|-------------|--|
| CMC 1%        | 10 mL       | 22          | 66.1 ± 7.4   |
| diazepam      | 0.3         | 8           | 127.4 ± 14.5**   |
|               | 1           | 12          | 129.3 ± 10.0**   |
|               | 3           | 14          | 149.5 ± 9.3**  |
| <b>4</b>      | 30          | 8           | 104.9 ± 14.62*   |

<sup>a</sup> Treatment has been performed 30 min before the ethanol 4 g/kg ip-injection. \*\**p* < 0.001; \**p* < 0.01 in comparison with control mice. (ANOVA, followed by Fischer posthoc test for multiple comparison).

**Table 3.** Effect on Mouse Memory in the Pass-Through Avoidance Test

| 0 h treatment, po | dose, mg/kg | no. of mice | 24 h retention test, % of mice not entering into the dark compartment in 120 s <sup>a</sup> |
|-------------------|-------------|-------------|---|
| CMC 1%            | 10 mL       | 10          | 40  |
| zolpidem          | 10          | 10          | 50  |
|                   | 30          | 10          | 70  |
| <b>4</b>          | 10          | 10          | 40  |
|                   | 30          | 10          | 50  |

<sup>a</sup> Treatment has been performed immediately after the training test. \**p* < 0.05 ( $\chi^2$ -test).

The pass-through passive avoidance paradigm was used to detect influence of compound **4** on mouse memory in comparison with zolpidem. As seen in Table 3, both zolpidem- and compound **4**-treated mice remembered the unpleasant experience of falling into cold water 24 h before, in equal manner to controls. Different effects on human memory with Bzs and zolpidem have already been noted in clinical pharmacology.<sup>12</sup> Nowadays, a probable role of reverberant circuit in hippocampus, long-term potentiation, is tied to learning and memory processes; in particular the  $\alpha 5$  subunit, being primarily expressed in the hippocampus and representing in this region 20% of all GABA<sub>A</sub> receptors, can play a role in cognitive performance.<sup>13</sup>

## Conclusions

It is well-known that the  $\alpha 1$ -containing receptors play an important role in sedation and hypnosis/sleep control and therefore  $\alpha 1$ -selective ligands can represent valid aids in the treatment of insomnia. In addition the use in therapy of hypnotic drugs endowed with slight anxiolytic properties does not represent any disadvantage. A different situation exists for anxiolytic drugs, in which an eventual sedative effect certainly represents a serious side effect, thus the identification of  $\alpha 2$ -selective agonist remains the best target for anxiolysis.

In the opinion of these authors it is not surprising that apparent inconsistencies have been noted by other authors in investigations of the  $\alpha 1$  role employing genetic knock-in and knock-out techniques, as well as binding methods with suitably  $\alpha$ -subtype-selective ligands.

In fact, discrepancy in the role of  $\alpha 1$  could be revised in consideration of its natural abundancy (more than 50% of the entire  $\alpha$ -population) and its distribution in the brain. It would seem unthinkable to ascribe to the  $\alpha 1$  subunit only a hypnotic effect, but rather its role may be seen as two distinct aspects:

- Involvement in a physiological regulating role, thus responsible for a mediating effect on the other subunits (GABA to GABA network);<sup>8</sup>

- A direct involvement in pharmacological responses. In fact, the stoichiometry of the receptor can be two  $\alpha$ -, two  $\beta$ -, one  $\gamma$ -subunits or two  $\alpha$ -, one  $\beta$ -, two  $\gamma$ -subunits;<sup>1</sup> thus Bz/GABA<sub>A</sub> receptor sensitivity strictly depends on its morphology.

On the basis of all these considerations the authors suggest compound **4**, endowed with very high  $\alpha 1\beta 2\gamma 2$  subtype selectivity and sedative and anxiolytic-like properties in rodents, as a new useful tool for revising the role of the  $\alpha 1$  subunit in the Bz/GABA<sub>A</sub> system.

## Experimental Section

**Chemistry.** Melting points were determined on a Gallenkamp apparatus and were uncorrected. The structures of all compounds were supported by their <sup>1</sup>H NMR spectra (recorded with a Varian Gemini 200 instrument, chemical shifts reported in  $\delta$  (ppm) using DMSO-*d*<sub>6</sub> or CDCl<sub>3</sub> as solvent).

**3-Cyano-4-(4-methylphenyl)-4-oxo-butanoic Acid (1).** To a suspension of LiOH·H<sub>2</sub>O (10 mmol) in EtOH (50 mL) was added of 3-oxo-3-(4-methylphenyl)propanenitrile<sup>14</sup> (10 mmol) previously dissolved in EtOH (30 mL), and subsequently a solution of iodoacetic acid (12.5 mmol) and LiOH·H<sub>2</sub>O (12.5 mmol) in 70% EtOH (50 mL) was dripped into the reaction mixture; it was refluxed under magnetic stirring for 6 h. After cooling, evaporation of the solvent under reduced pressure gave a residue, that was treated with ice-water (100 mL), and the resulting mixture was washed with diethyl ether (60 mL × 3). Acidification of the aqueous phase with concentrated hydrochloric acid causes the separation of a solid product which was isolated by filtration. The compound was purified by silica gel column chromatography [Tol/EtOAc/AcOH 8:2:1 v/v, as eluent]. Ivory crystals, yield 56%, mp 116–118 °C, <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 2.45 (s, 3H, CH<sub>3</sub>), 2.92–3.06 (m, 1H, CH<sub>2</sub>), 3.28–3.42 (m, 1H, CH<sub>2</sub>), 4.70–4.78 (m, 1H, CH), 7.35 (d, 2H, Ph), 7.95 (d, 2H, Ph). Anal. (C<sub>12</sub>H<sub>11</sub>NO<sub>3</sub>) C, H, N.

**3-Amino-5-(4-methylphenyl)pyrazol-4-ylacetic Acid (2).** To a solution of **1** (10 mmol) in EtOH (50 mL) were added hydrazine hydrate (20 mmol, 0.97 mL) and acetic acid (1 mL), and the reaction mixture was refluxed for 6 h. After cooling, evaporation of the solvent, under reduced pressure, gave a residue that was purified by silica gel column chromatography [CHCl<sub>3</sub>/MeOH, 10:1 v/v, as eluent]. White crystals, yield 44%; mp 226–227 °C; <sup>1</sup>H NMR (DMSO)  $\delta$ : 2.30 (s, 3H, CH<sub>3</sub>), 3.30 (s, 2H, CH<sub>2</sub>), 7.20 (d, 2H, Ph), 7.38 (d, 2H, Ph). Anal. (C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**2-(4-Methylphenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-ylacetic Acid (3).** To a solution of **2** (1 mmol) in EtOH (6 mL) was added 4,4-dimethoxy-2-butanone (1.1 mmol), and the mixture was refluxed under stirring for 4 h. The progress of reaction was monitored by TLC. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography [CHCl<sub>3</sub>/MeOH/ACOH 10:0.5:0.1 v/v, as eluent]. Ivory crystals, yield 34%; mp 163–164 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$ : 2.42 (s, 3H, p-CH<sub>3</sub>), 2.66 (s, 3H, 5-CH<sub>3</sub>), 4.03 (s, 2H, CH<sub>2</sub>), 6.71 (d, 1H, H-6, *J*<sub>H6-H7</sub> = 6.96 Hz), 7.29 (d, 2H, Ph), 7.62 (d, 2H, Ph), 8.65 (d, 1H, H-7, *J*<sub>H7-H6</sub> = 6.96 Hz). Anal. (C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

***N,N*-Dimethyl-[2-(4-methylphenyl)-5-methylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide (4).** Compound **3** (1 mmol) and triethylamine (3.5 mmol) were dissolved in 5 mL of dry THF and cooled to –10 °C. After stirring for 30 min, to this solution was added ethyl chloroformate (1.1 mmol), and then the reaction suspension was stirred for 1 h at –10 °C before the addition of 1.1 mmol of the suitable amine. The suspension was allowed to warm to room temperature under magnetic stirring for 5 h. The reaction was quenched by introducing 5 mL of H<sub>2</sub>O, and the resulting mixture was extracted with ether (2 × 10 mL); the ethereal extracts were washed successively with aqueous 5% HCl (5 mL) and dried over Na<sub>2</sub>SO<sub>4</sub>. The crude acetamide (**4**) was obtained by removing the solvent under reduced pressure, and the residue was purified by column chromatography. [CHCl<sub>3</sub>/MeOH 10: 1 v/v, as eluent]. Ivory

crystals, yield 34%; mp 168–170 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ: 2.41 (s, 3H, p-CH<sub>3</sub>), 2.58 (s, 3H, 5-CH<sub>3</sub>), 3.01 (s, 3H, N(CH<sub>3</sub>)<sub>2</sub>), 3.17 (s, 3H, N(CH<sub>3</sub>)<sub>2</sub>), 3.93 (s, 2H, CH<sub>2</sub>), 6.63 (d, 1H, H-6, *J*<sub>H6-H7</sub> = 7.0 Hz), 7.30 (d, 2H, Ph), 7.67 (d, 2H, Ph), 8.50 (d, 1H, H-7, *J*<sub>H7-H6</sub> = 7.0 Hz). Anal. (C<sub>18</sub>H<sub>20</sub>N<sub>4</sub>O) C, H, N.

**Acknowledgment.** This research was supported by funds of the Italian Ministry of University and Scientific Research (MURST).

**Supporting Information Available:** Elemental analyses data, binding studies, and pharmacological methods. This material is available free of charge via the Internet at: <http://pubs.acs.org>.

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