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GABA_A Receptor Modulation by Etomidate Analogues

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Abstract

Background—Etomidate is a highly potent anesthetic agent that is believed to produce hypnosis by enhancing γ -aminobutyric acid type A (GABA_A) receptor function. We characterized the GABA_A receptor and hypnotic potencies of etomidate analogues. We then used computational techniques to build statistical and graphical models that relate the potencies of these etomidate analogues to their structures in order to identify the specific molecular determinants of potency.

Methods—GABA_A receptor potencies were defined with voltage-clamp electrophysiology using $\alpha_1\beta_3\gamma_2$ receptors harboring a channel mutation (α_1 (L264T)) that enhances anesthetic sensitivity ($n = 36 - 60$ measurements per concentration-response curve). The hypnotic potencies of etomidate analogues were defined using a loss of righting reflexes assay in Sprague Dawley rats ($n = 9 - 21$ measurements per dose-response curve). Three-dimensional quantitative structure-activity relationships were determined *in silico* using comparative molecular field analysis.

Results—The GABA_A receptor and hypnotic potencies of etomidate and the etomidate analogues ranged by 91-fold and 53-fold, respectively. These potency measurements were significantly correlated ($r^2 = 0.72$), but neither measurement correlated with drug hydrophobicity ($r^2 = 0.019$ and 0.005 , respectively). Statistically significant and predictive comparative molecular field analysis models were generated and a pharmacophore model was built that revealed both the structural elements in etomidate analogues associated with high potency and the interactions that these elements make with the etomidate binding site.

Conclusion—There are multiple specific structural elements in etomidate and etomidate analogues that mediate GABA_A receptor modulation. Modifying any one element can alter receptor potency by an order of magnitude or more.

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Conflicts of Interest: The Massachusetts General Hospital has published and submitted patent applications for certain etomidate analogues. Drs. Raines and Husain are inventors of this technology. They and their laboratories have received income related its development. This conflict is being managed by the Partners Healthcare Office for Interactions with Industry. All other authors have no conflicts of interest.

Introduction

Etomidate is a highly selective intravenous anesthetic agent that is widely believed to produce hypnosis by binding to a site (or class of sites) on the γ -aminobutyric acid type A (GABA_A) receptor.^{1–5} Although structural information regarding this site is quite limited, there is growing evidence that it is located at the interface between the GABA_A receptor's α and β subunits in the transmembrane domain.^{6–8} The result of such binding is an enhancement of GABA_A receptor function.^{9,10} Specifically, etomidate binding increases agonist potency for activating (i.e. opening) GABA_A receptors, a process termed “agonist potentiation”. In the absence of agonist, etomidate binding also directly activates GABA_A receptors. Although the agonist potentiating and direct activating actions are measurable using electrophysiological techniques over distinct etomidate concentration ranges (low and high, respectively), it has been proposed that the underlying receptor mechanism for these two enhancing actions is the same (stabilizing the open channel state) and that they reflect etomidate binding to the same receptor site(s).^{10–12} In addition to enhancing GABA_A receptor function, etomidate, etomidate analogues, and other anesthetics can inhibit the receptor's function.^{13–15} This inhibitory action typically occurs only at very high anesthetic concentrations and is likely mediated by a site that is distinct from that which produces enhancement.

Studies of etomidate's two enantiomers indicate that the structural requirements for GABA_A receptor binding and enhancement can be quite specific. Although etomidate's enantiomers have identical physical properties, they differ by 1–2 orders of magnitude in their *in vitro* affinities and potencies for the GABA_A receptor and, consequently, their *in vivo* hypnotic potencies.^{3,13,16} Other small structural modifications around the chiral center similarly impact GABA_A receptor and hypnotic potencies, implying that this region of etomidate's molecular scaffold is a critical determinant of pharmacological activity.¹³ The pharmacological importance of other regions is unknown.

Over the past several years, our laboratory has developed multiple novel etomidate analogues that contain structural modifications in various parts of etomidate's molecular scaffold and exhibit unique pharmacology.^{13,17–20} These compounds include etomidate esters that are ultra-rapidly metabolized, pyrrole analogues devoid of adrenocortical side effects, and achiral analogues with reduced hypnotic and adrenocortical inhibitory potencies. Although it seems likely that only a handful of these analogues have potential value as clinical anesthetic agents, together they can be used as pharmacological tools for structure-activity relationship studies to better define the structural determinants of etomidate's enhancing action on the GABA_A receptor. Such information may also provide clues regarding the nature of the etomidate binding site and ultimately suggest ways of making more potent analogues.

In this study, we quantified the potencies with which etomidate analogues directly activate heteromeric GABA_A receptors. We incorporated a channel mutation into the receptor that stabilizes its open state, thus increasing anesthetic sensitivity and allowing more complete anesthetic concentration-response curves to be generated (1) before reaching aqueous saturation at high anesthetic concentrations and (2) without the potentially confounding

effects of a co-administered agonist.^{10,13,21} We also defined the potencies with which etomidate analogues produce hypnosis in rats. We then used computational techniques to build statistical and graphical models that relate the GABA_A receptor and hypnotic potencies of these etomidate analogues to their physical structures (i.e. steric and electrostatic properties) in three-dimensional space.

Materials and Methods

Animals

All studies were conducted with the approval of and in accordance with the regulations of the Institutional Animal Care and Use Committee at Massachusetts General Hospital (Boston, MA). *Xenopus laevis* adult female frogs were purchased from Xenopus One (Ann Arbor, MI). Adult male Sprague-Dawley rats (300–450 gm) were purchased from Charles River Laboratories (Wilmington, MA).

Etomidate and Etomidate Analogues

Figures 1 and 2 show the structures of etomidate and the etomidate analogues used in this study, and indicate their names and compound numbers (1 – 23). The structures of etomidate and metomidate are shown in the top row of figure 1. Etomidate was purchased from Bachem Americas (Torrance, CA) and metomidate was synthesized by Aberjona Laboratories (Beverly, MA) using the general approach previously reported for etomidate.²² The second row in figure 1 shows the structures of S-etomidate, dihydrogen etomidate, and cyclopropyl etomidate. These etomidate analogues, which have chiral center modifications, were synthesized as previously described.¹³ The third row in figure 1 shows the structures of the pyrrole etomidate analogues carboetomidate and 4-pyridine carboetomidate. Both compounds were synthesized by Aberjona Laboratories. The last row in figure 1 shows the structures of racemic pentafluoroetomidate, S-pentafluoroetomidate, and R-pentafluoroetomidate. The first two compounds were synthesized by Aberjona Laboratories (Beverly, MA) using the approach previously reported for etomidate using racemic 1-pentafluorophenylethanol or R-1-pentafluorophenylethanol, respectively, in place of 1-phenylethanol.²² R-pentafluoroetomidate was not synthesized because the necessary chiral alcohol reagent (S-1-pentafluorophenyl ethanol) was not commercially available. However, potency values for this enantiomer were estimated from those determined for racemic pentafluoroetomidate and S-pentafluoroetomidate. The structures of the etomidate esters used in this study are shown in figure 2. They were synthesized as previously described.^{17,19}

GABA_A Receptor Electrophysiology

Oocytes were harvested from frogs and injected with messenger RNA encoding the α_1 (L264T), β_3 , and γ_2 subunits of the human GABA_A receptor (5 ng of messenger RNA total at a subunit ratio of 1:1:3). After RNA injection, oocytes were incubated in ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH=7.4) containing 0.1 mg/mL of ciprofloxacin and 0.05 mg/mL of gentamicin for 18 to 48 hours at 18°C before electrophysiological study. Electrophysiological recordings were performed at room temperature using the whole cell two-electrode voltage-clamp technique as previously described.²⁰ In each experiment, the peak amplitude was defined as the difference between

the baseline current prior to etomidate analogue infusion and the single highest point in the current trace during infusion. This amplitude was then normalized to the peak amplitude measured in control currents elicited by 100 μ M GABA (a concentration of GABA that maximally activates GABA_A receptors) in the same oocyte. For each etomidate analogue, the concentration-mean response data were fit to a Hill equation with minima and maxima constrained to 0% and 100%, respectively, using equation 1 as previously described:¹³

$$\text{Normalized Peak Amplitude} = 100 / (1 + (\frac{EC_{50}}{[\text{analogue}]})^n)$$

where EC₅₀ is the etomidate analogue concentration that evokes a peak current amplitude that is half that evoked by 100 μ M GABA, [analogue] is the etomidate analogue concentration, and n is the Hill coefficient.

Rat Loss of Righting Reflexes Assay

The hypnotic potencies of etomidate analogues were assessed in rats using a loss of righting reflexes (LORR) assay.¹⁷ In brief, the desired dose of analogue in dimethyl sulfoxide vehicle (0.1–0.3 ml) was rapidly injected through an intravenous catheter placed in a tail vein followed by a normal saline flush. Immediately afterward, rats were turned supine. A rat was judged to have LORR if it failed to turn itself back onto all four paws within 5 sec after drug administration. For each analogue, the median effective dose (ED₅₀) for LORR was determined from a data set of at least 24 separate doses using the method of Waud.²³ For *in silico* modeling studies, each ED₅₀ value expressed in mg/kg was converted to units of μ M/kg using the analogue's molecular weight.

Determination of Octanol:Water Partition Coefficients

The octanol:water partition coefficient of each etomidate analogue was determined as previously described.¹³

Comparative Molecular Field Analysis (CoMFA)

The low energy conformer of etomidate and each etomidate analogue was defined using MMFF94 force field utilizing an energy gradient convergence criterion of 0.01 kcal/mol with SybylX2.1.1 (Certara, St. Louis, MO). Partial electronic charges were calculated by the software using the Gasteiger-Hückel method.²⁴ Using etomidate's structure as the template, each etomidate analogue was then automatically aligned *in silico* to overlay its molecular shape, hydrogen-bonding capacities, and electrostatic properties with Surflex-Sim (Certara L.P.). When an imidazole ring was present in an etomidate analogue, these alignments were further optimized by manually aligning this ring with that of etomidate.

Classic CoMFA interaction fields (steric and electrostatic fields) were calculated with SybylX2.1.1 using the default settings. The steric and electrostatic interaction energies were calculated on grid points of a regularly spaced three-dimensional lattice with a sp³ carbon probe atom having a charge of +1 and a van der Waals radius of 1.52 Å. The grid size had the software's default resolution of 2 Å. Cutoffs were applied to both the steric and electrostatic interactions energies at 30 kcal/mol. CoMFA region focusing was used to

enhance the resolution of the model and improve its predictive power. In this procedure, the weightings of lattice points in the grid are modified by the software in an iterative fashion to enhance or reduce each point's contribution to subsequent analysis.

The partial least-squares approach was used to derive the three-dimensional quantitative structure-activity relationship, and cross-validation was performed using the leave-one-out method.²⁵ The optimum number of components (NC) that produced the lowest standard error of predictions (SDEP) was determined and the cross-validation correlation coefficient (q^2) was calculated. A coefficient of determination (r^2) was then defined from the relationship between the predicted and experimental potencies using linear regression.

Pharmacophore Analysis

The five molecules with the lowest GABA_A receptor EC₅₀s (i.e. the five most potent GABA_A receptor direct activators) were extracted from the alignment prepared for the GABA_A receptor CoMFA analysis and a pharmacophore model was constructed with Unity (Certara L.P.). Such models describe the common features present in these ligands and the molecular interactions that they make with the protein that are important determinants of potency. For this analysis, we required that such interactions be present in at least four out of the five high potency molecules in the dataset. We used a maximum volume constraint of 1 Å around each pharmacophore point. Thus in some cases, a pharmacophore point on the ligand could not be associated with one at the receptor binding site because the latter's volume exceeded the 1 Å cutoff (i.e. there was too much uncertainty regarding the location of the receptor pharmacophore point). Aromatic and hydrophobic ligand domains were identified utilizing rules in SybylX2.1.1 software. We then used MOLCAD (Cetara L.P.) to visualize the surface features and physical properties required for molecular recognition.

Statistical Analysis

All data are reported as mean $\pm$ SD. Sample sizes were chosen based on our previous experience.¹³ Hypothesis testing was two-tailed and a P value <0.05 was considered to be statistically significant. Curve fitting to define GABA_A receptor EC₅₀s and Rat LORR ED₅₀s and their respective standard deviations were performed using Igor Pro 6.1 (Wavemetrics, Lake Oswego, OR). Linear regressions of logarithm-transformed data (GABA_A receptor EC₅₀s and Rat LORR ED₅₀s versus hydrophobicity) were performed using Igor Pro 6.1. Statistical differences between the EC₅₀s of two compounds were assessed using an extra sum-of-squares F test with Prism 6 (Graphpad, La Jolla, CA). The sample sizes for data shown in all figures are indicated in the text. CoMFA statistics (NC, SDEP, q^2 , and r^2) were calculated by SybylX2.1.1 software. There was no lost or missing data.

Results

Direct Activation of GABA_A Receptors by Etomidate Analogues

All etomidate analogues directly activated $\alpha_1(L264T)\beta_3\gamma_2$ GABA_A receptors expressed in oocytes in a concentration-dependent manner. Figure 3A shows representative electrophysiological traces recorded upon perfusing a single oocyte with the diastereomeric

etomidate ester pair R-isopropyl-methoxycarbonyl metomidate and S-isopropyl-methoxycarbonyl metomidate at concentrations of 1 μM (top) and 10 μM (bottom). At 1 μM , the R- and S- diastereomers evoked currents having peak amplitudes of 0.041 μA and 0.29 μA , respectively. At 10 μM , they evoked respective currents having peak amplitudes of 0.36 μA and 0.90 μA , respectively. For the two diastereomers, figure 3B plots the concentration-response relationship for peak current activation (mean $\pm$ SD, $n=6$ oocytes per data point). For both diastereomers, the normalized peak current response increased with concentration. At a near saturating aqueous concentration (100 μM), S- (but not R-) isopropyl-methoxycarbonyl metomidate evoked currents that approximated those evoked by a maximally-activating concentration (100 μM) of GABA. A fit of these concentration-response curves to equation 1 yielded EC_{50} 's for direct activation of $2.6 \pm 0.3 \mu\text{M}$ and $46 \pm 6 \mu\text{M}$ for S- and R-isopropyl-methoxycarbonyl metomidate, respectively ($p<0.0001$).

Figure 4A plots the concentration-response relationships for peak current activation by etomidate and carboetomidate, a pyrrole etomidate analogue that does not suppress adrenocortical function. The normalized peak current amplitude increased with both etomidate and carboetomidate concentrations. However similar to R-isopropyl-methoxycarbonyl metomidate, carboetomidate's relatively low potency and aqueous solubility limited our studies to carboetomidate concentrations that activated (at most) only $68 \pm 6\%$ of GABA_A receptors. From these concentration-response curves, we determined that carboetomidate's potency for directly activating GABA_A receptors is approximately $1/8^{\text{th}}$ that of etomidate with EC_{50} s of $13.8 \pm 0.9 \mu\text{M}$ for carboetomidate and $1.83 \pm 0.28 \mu\text{M}$ for etomidate ($p<0.0001$).

Figure 4B plots the concentration-response relationships for peak current activation by the fluorinated etomidate analogue S-pentafluoroetomidate. This figure also plots this relationship for racemic pentafluoroetomidate, which contains equal quantities of the R- and S- enantiomers. The EC_{50} for the S-enantiomer was 22-fold higher than that of the racemic mixture ($166 \pm 25 \mu\text{M}$ versus $7.6 \pm 0.6 \mu\text{M}$, respectively; $p<0.0001$). This implies that essentially all of the directly activated current recorded during application of the racemic mixture was attributable to the R-enantiomer and that R-enantiomer's EC_{50} is $\sim 3.8 \mu\text{M}$ (i.e. one-half the EC_{50} of the racemic mixture).

Table 1 gives the GABA_A receptor EC_{50} 's for etomidate and all of the etomidate analogues that we have characterized to date, along with their physical properties (i.e. molecular weights, molecular volumes, and octanol:buffer partition coefficients) and their hypnotic ED_{50} s measured in Sprague Dawley rats. We have reported some of these values previously, and provide the references for these published values in the table.

Although hydrophobicity has long been considered to be an important determinant of *in vitro* and *in vivo* anesthetic potency, figure 5 shows that the correlation between the GABA_A receptor potencies of etomidate and etomidate analogues and their octanol:buffer partition coefficients is rather poor.^{26–28} A linear fit of the logarithm-transformed values of this relationship yielded a slope that is not significantly different from zero (0.13 ± 0.20 , $p = 0.5271$) and an r^2 of only 0.019. An analogous linear fit of the logarithm-transformed relationship between of hypnotic potency in rats and octanol:buffer partition coefficient

similarly yielded a slope that was not significantly different from zero (0.09 ± 0.30 , $p = 0.7562$) and an r^2 of only 0.005 (data not shown).

Three-Dimensional Quantitative Structure-Activity Analysis of Etomidate Analogue Potency

We used CoMFA to identify the structural elements in our etomidate analogues (figure 6) that account for their widely ranging (91-fold) GABA_A receptor potencies. Figure 7 shows the final alignment of etomidate and the 21 different etomidate analogues along with the resulting CoMFA contour maps. Figure 7A shows the steric contour map depicting where bulky substituents increase (green) or decrease (yellow) GABA_A receptor potency. Figure 7B shows the electrostatic contour map depicting where electronegative substituents (red) or electropositive substituents (blue) increase GABA_A receptor potency. The model had good predictive ability as assessed by leave-one-out cross-validation with values of $q^2=0.458$, $NC=4$, $SDEP=0.458$. Figure 8 shows the correlation between the GABA_A receptor EC₅₀ values predicted by the CoMFA model and the experimentally derived GABA_A receptor EC₅₀ values. The model explained 91.2% of the variance in the observed activities of the etomidate analogues with values of $r^2=0.912$, $NC=4$, $SDEP=0.227$.

The identical alignment was used to identify the structural features of our etomidate analogues that account for their widely ranging (53-fold) hypnotic potencies in rats. Figure 9A shows the steric contour map depicting where bulky substituents increase (green) or decrease (yellow) hypnotic potency. Figure 9B shows the electrostatic contour map depicting where electronegative substituents (red) or electropositive substituents (blue) increase hypnotic potency. Figure 10 shows the correlation between the hypnotic ED₅₀ values predicted by the CoMFA model and the experimentally derived hypnotic ED₅₀ values. The CoMFA model explained 97.3% of the variance in the observed hypnotic potencies ($r^2=0.973$, $NC=5$, $SDEP=0.093$) and showed good predictive capability with leave-one-out validation ($q^2=0.521$, $NC=5$, $SDEP=0.377$).

Visual comparison of the contour maps for *in vitro* GABA_A receptor potency (figure 7) and *in vivo* hypnotic potency (figure 9) reveals certain similarities. For example, in both steric contour maps (figures 7A and 9A), there is a yellow contour near the chiral carbon located between the phenyl and imidazole rings indicating that the S-form is sterically disfavored. There is a similar yellow contour near the spacer located between the two ester moieties of etomidate esters indicating that the R-form is sterically disfavored. The “steric penalty” for being in the disfavored enantiomeric form at either of these chiral centers was a reduction in receptor and hypnotic potencies of as much as 1 – 2 orders of magnitude (Table 1). For both steric contour maps, there is also a green contour around the alkyl groups of the etomidate ester spacer (represented as R₂ in figure 6) indicating that bulky substituents in this region increase both receptor and hypnotic potencies. In both electrostatic contour maps (figures 7B and 9B), there is a red contour around the conserved carboxylate ester showing favored negative charge related to the carbonyl oxygen and a blue contour around the distal ester of etomidate esters showing favored positive charge relating to the carbonyl carbon.

Three-Dimensional Pharmacophore Analysis of Etomidate Analogue

To better understand the drug-receptor interactions that mediate high GABA_A receptor potency, we built a pharmacophore model using the five most potent GABA_A receptor direct activators shown on Table 1. Because four out of five of these compounds have an additional carboxylate ester moiety (they are etomidate esters), we allowed a 20% miss rate (1 out of 5 molecules) to allow optional pharmacophore features to be included in the final model. This model, along with the five most potent compounds, is shown in figure 11. The model identified four common features and one optional feature in these compounds. The first feature is the basic nitrogen in the imidazole ring, which was identified as a hydrogen bond acceptor for a donor in the receptor. This interaction explains why etomidate's potency is 1 – 2 orders of magnitude higher than those of the pyrrole analogues carboetomidate and 4-pyridine carboetomidate. The second feature is the phenyl ring, which is hydrophobic and explains the 24-fold higher potency of carboetomidate as compared to 4-pyridine carboetomidate. The third and fourth features are the two conserved oxygens that form the ester adjacent to the imidazole ring. Similar to the imidazole nitrogen, the carbonyl oxygen of this ester was identified as a hydrogen bond acceptor for a donor in the receptor. The distance between these two donors in the receptor was determined to be 9.36 Å by the model. The final feature is the distal ester found in the etomidate esters, which was identified as optional hydrogen bond acceptor. The van der Waals surface of these five most potent compounds was then calculated and their lipophilic potentials were mapped onto that surface. Figure 12 shows that surface, along with the structures of those five most potent compounds, the pharmacophore model, and the CoMFA contours for GABA_A receptor potency. As the yellow contours denote regions where bulky substituents reduce potency (presumably because steric hindrance reduces binding affinity), they are considered to represent (part of) the lining of the etomidate binding pocket. Conversely, the green contour denotes the only region in our receptor model where bulky substituents increase potency. As this bulk is in the form of hydrophobic alkyl groups in all of our compounds (R₂ in figure 6), we conclude that this part of the binding pocket is relatively hydrophobic and spacious as it can even accommodate an isopropyl group, which was the largest alkyl group that we studied.

Discussion

Etomidate is widely believed to produce its hypnotic effects by binding to the GABA_A receptor and enhancing its function. This conclusion is most strongly supported by studies showing that (1) etomidate's R- and S- enantiomers produce hypnosis with potencies that correlate with their GABA_A receptor affinities and potencies; and (2) an amino acid mutation that abolishes etomidate's ability to enhance GABA_A receptor function (N265M on the beta subunit) significantly reduces the etomidate sensitivities of transgenic mice containing that mutation.^{3,8,13,16,29} This amino acid, along with others, may contribute to an etomidate binding site on the GABA_A receptor's open state that is located in the membrane-spanning receptor domains between the alpha and beta subunits.^{5–7}

There have been few studies to define the anesthetic structural features required for high affinity binding to the GABA_A receptor's etomidate binding site and/or modulation of the

receptor's function. Competition studies using an etomidate photoaffinity label indicate that propofol and barbiturates bind to that site with lower affinity than R-etomidate, and the steroid anesthetic alphaxalone does not bind to that site at all.⁸ Thus, this binding site can distinguish among classes of general anesthetics. The *in vitro* electrophysiological data presented in this study demonstrate that even within a single anesthetic class (i.e. etomidate), this site exhibits considerable selectivity as evidenced by the widely (91-fold) ranging GABA_A receptor potencies of our etomidate analogues that could not be simply explained by their different hydrophobicities. The widely ranging GABA_A receptor potencies of our analogues were matched by a similarly large (53-fold) range in their hypnotic potencies, and a logarithmic plot of GABA_A receptor potency versus hypnotic potency (figure 13) showed a significant correlation ($r^2 = 0.72$) between these two potency measurements. The slope of this relationship was significantly different from zero ($p < 0.0001$) and near unity (1.22 ± 0.18), indicating that (in general) when an etomidate analogue's GABA_A receptor potency doubled its hypnotic potency also doubled. These findings are consistent with a direct cause-and-effect relationship between GABA_A receptor enhancement by etomidate analogues and the production of *in vivo* hypnosis.

Our computational studies provide additional details regarding the specific anesthetic structural features present in high potency modulators of the GABA_A receptor function, and define the important interactions that etomidate analogues make with amino acids that form the etomidate binding site on the receptor. For example, our studies show that the basic nitrogen in the imidazole ring and the carboxylate ester adjacent to that ring are important hydrogen bond acceptors for donors in the receptor. Although we do not know the identity of these amino acids, it is tempting to speculate that one of these donor amino acids is β_3 N265. This could explain the results of allosteric modeling studies indicating that mutating this asparagine to a methionine (which is not a hydrogen bond donor) reduces etomidate's affinity for open state GABA_A receptors by orders of magnitude whereas mutating it to a serine (which can be hydrogen bond donor) reduces etomidate's affinity by only half.^{30–32} It was concluded from those studies that the reduced etomidate sensitivity of the serine-containing mutant (β_3 N265S) measured in electrophysiological studies is primarily due to a reduction in etomidate's efficacy rather than its binding affinity. Our computational studies also indicated that (1) the phenyl ring engages in a hydrophobic interaction with hydrophobic amino acid residues in the binding site; (2) there is chiral selectivity around the carbon located between the phenyl and imidazole rings; and (3) in the case of etomidate esters, there is a hydrogen bond interaction between the distal carboxylate ester and a donor on the protein, and chiral selectivity around the spacer that links that distal ester to the etomidate pharmacophore.

The computational modeling approach utilized in the present studies (CoMFA) to define ligand-protein interactions are distinct from – but complimentary to – computational docking studies.^{33–36} In the CoMFA approach, there are no *a priori* assumptions regarding the nature of the protein binding site. Instead, the molecular (i.e. steric and electrostatic) interactions between a set of ligands and their protein binding site are identified from the relationship between ligand molecular structure and binding affinity, the latter often quantified experimentally using a functional assay.^{37–39} This technique allows one to

construct a pharmacophore model that explains the observed biological activity and provides information about the molecular forces involved in binding. There are several important limitations of this approach. These limitations include potential uncertainty regarding the alignment of ligands, particularly when the structures of ligands in a data set vary widely. Additionally, the conformation of a ligand may change upon protein binding. Consequently, the low energy states calculated by CoMFA may differ from the actual protein-bound states. Such inherent limitations may explain why the correlations that we observed between model-predicted and experimental potency values were good, but not perfect. In contrast, docking studies attempt to fit a ligand into a defined protein binding site *in silico*. They are typically agnostic to ligand potency and can utilize a single ligand, but require a high-resolution structural model of the binding site to calculate the interaction energy between ligand and protein. Unfortunately, the structures of open-state heteromeric GABA_A receptors have not yet been defined at high resolution. However, progress in that direction is being made through the construction of homology models that seek to approximate the receptor's structure by utilizing structurally similar proteins.^{33,34} With further refinement and validation of these models or the production of a high-resolution heteromeric GABA_A receptor crystal structure, it may be possible to unambiguously identify amino acids at the etomidate binding site that are the important determinants of receptor (and therefore hypnotic) potency and account for our pharmacophore model.

In summary, we have measured the GABA_A receptor potencies of a series of etomidate analogues and found that their potencies range by 91-fold. The receptor potencies of these analogues correlated with their hypnotic potencies but not with their hydrophobicities. CoMFA indicated that there are multiple structural elements in these etomidate analogues that define their GABA_A receptor potencies. These include the imidazole nitrogen and carboxylate ester (which act as hydrogen bond acceptors), the phenyl ring (which can engage in hydrophobic interactions), the chiral carbon located between the phenyl and imidazole rings (the R configuration has higher potency), and the chiral carbon located between the etomidate pharmacophore and the distal ester in etomidate esters (the S configuration has higher potency). Modifying any of one of these structural elements can alter receptor potency by an order of magnitude or more.

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References

1. Reynolds DS, Rosahl TW, Cirone J, O'Meara GF, Haythornthwaite A, Newman RJ, Myers J, Sur C, Howell O, Rutter AR, Atack J, Macaulay AJ, Hadingham KL, Hutson PH, Belelli D, Lambert JJ, Dawson GR, McKernan R, Whiting PJ, Wafford KA. Sedation and anesthesia mediated by distinct GABA(A) receptor isoforms. *J Neurosci*. 2003; 23:8608–17. [PubMed: 13679430]
2. Belelli D, Lambert JJ, Peters JA, Wafford K, Whiting PJ. The interaction of the general anesthetic etomidate with the gamma-aminobutyric acid type A receptor is influenced by a single amino acid. *Proc Natl Acad Sci U S A*. 1997; 94:11031–6. [PubMed: 9380754]

3. Tomlin SL, Jenkins A, Lieb WR, Franks NP. Stereoselective effects of etomidate optical isomers on gamma-aminobutyric acid type A receptors and animals. *Anesthesiology*. 1998; 88:708–17. [PubMed: 9523815]
4. Forman SA. Clinical and molecular pharmacology of etomidate. *Anesthesiology*. 2011; 114:695–707. [PubMed: 21263301]
5. Ge R, Pejo E, Gallin H, Jeffrey S, Cotten JF, Raines DE. The pharmacology of cyclopropyl-methoxycarbonyl metomidate: A comparison with propofol. *Anesth Analg*. 2014; 118:563–7. [PubMed: 24557104]
6. Li GD, Chiara DC, Sawyer GW, Husain SS, Olsen RW, Cohen JB. Identification of a GABAA receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. *J Neurosci*. 2006; 26:11599–605. [PubMed: 17093081]
7. Chiara DC, Dostalova Z, Jayakar SS, Zhou X, Miller KW, Cohen JB. Mapping general anesthetic binding site(s) in human $\alpha 1\beta 3$ γ -aminobutyric acid type A receptors with [(3)H]TDBzl-etomidate, a photoreactive etomidate analogue. *Biochemistry*. 2012; 51:836–47. [PubMed: 22243422]
8. Chiara DC, Jayakar SS, Zhou X, Zhang X, Savechenkov PY, Bruzik KS, Miller KW, Cohen JB. Specificity of intersubunit general anesthetic-binding sites in the transmembrane domain of the human $\alpha 1\beta 3\gamma 2$ γ -aminobutyric acid type A (GABAA) receptor. *J Biol Chem*. 2013; 288:19343–57. [PubMed: 23677991]
9. Hill-Venning C, Belelli D, Peters JA, Lambert JJ. Subunit-dependent interaction of the general anaesthetic etomidate with the gamma-aminobutyric acid type A receptor. *Br J Pharmacol*. 1997; 120:749–56. [PubMed: 9138677]
10. Rusch D, Zhong H, Forman SA. Gating allosterism at a single class of etomidate sites on $\alpha 1\beta 3\gamma 2$ L GABA A receptors accounts for both direct activation and agonist modulation. *J Biol Chem*. 2004; 279:20982–92. [PubMed: 15016806]
11. Forman SA. Monod-Wyman-Changeux allosteric mechanisms of action and the pharmacology of etomidate. *Curr Opin Anaesthesiol*. 2012; 25:411–8. [PubMed: 22614249]
12. Guitchounts G, Stewart DS, Forman SA. Two etomidate sites in $\alpha 1\beta 2\gamma 2$ γ -aminobutyric acid type A receptors contribute equally and noncooperatively to modulation of channel gating. *Anesthesiology*. 2012; 116:1235–44. [PubMed: 22531336]
13. Pejo E, Santer P, Jeffrey S, Gallin H, Husain SS, Raines DE. Analogues of etomidate: Modifications around etomidate's chiral carbon and the impact on in vitro and in vivo pharmacology. *Anesthesiology*. 2014; 121:290–301. [PubMed: 24777068]
14. Tomlin SL, Jenkins A, Lieb WR, Franks NP. Preparation of barbiturate optical isomers and their effects on GABA(A) receptors. *Anesthesiology*. 1999; 90:1714–22. [PubMed: 10360871]
15. Davies PA, Kirkness EF, Hales TG. Modulation by general anaesthetics of rat GABAA receptors comprised of alpha 1 beta 3 and beta 3 subunits expressed in human embryonic kidney 293 cells. *Br J Pharmacol*. 1997; 120:899–909. [PubMed: 9138697]
16. Belelli D, Muntoni AL, Merrywest SD, Gentet LJ, Casula A, Callachan H, Madau P, Gemmell DK, Hamilton NM, Lambert JJ, Sillar KT, Peters JA. The in vitro and in vivo enantioselectivity of etomidate implicates the GABAA receptor in general anaesthesia. *Neuropharmacology*. 2003; 45:57–71. [PubMed: 12814659]
17. Cotten JF, Husain SS, Forman SA, Miller KW, Kelly EW, Nguyen HH, Raines DE. Methoxycarbonyl-etomidate: a novel rapidly metabolized and ultra-short-acting etomidate analogue that does not produce prolonged adrenocortical suppression. *Anesthesiology*. 2009; 111:240–9. [PubMed: 19625798]
18. Cotten JF, Forman SA, Laha JK, Cuny GD, Husain SS, Miller KW, Nguyen HH, Kelly EW, Stewart D, Liu A, Raines DE. Carboetomidate: A pyrrole analog of etomidate designed not to suppress adrenocortical function. *Anesthesiology*. 2010; 112:637–44. [PubMed: 20179500]
19. Husain SS, Pejo E, Ge R, Raines DE. Modifying methoxycarbonyl etomidate inter-ester spacer optimizes in vitro metabolic stability and in vivo hypnotic potency and duration of action. *Anesthesiology*. 2012; 117:1027–36. [PubMed: 22929736]
20. Pejo E, Cotten JF, Kelly EW, Le Ge R, Cuny GD, Laha JK, Liu J, Lin XJ, Raines DE. In vivo and in vitro pharmacological studies of methoxycarbonyl-carboetomidate. *Anesth Analg*. 2012; 115:297–304. [PubMed: 21965364]

21. Ge RL, Pejo E, Haburcak M, Husain SS, Forman SA, Raines DE. Pharmacological studies of methoxycarbonyl etomidate's carboxylic acid metabolite. *Anesth Analg*. 2012; 115:305–8. [PubMed: 22052979]
22. Godefroi EF, Janssen PA, Vandereycken CA, Vanheertum AH, Niemegeers CJ. DI-1-(1-Arylkyl)Imidazole-5-Carboxylate Esters. A Novel Type of Hypnotic Agents. *J Med Chem*. 1965; 8:220–3. [PubMed: 14332665]
23. Waud DR. On biological assays involving quantal responses. *J Pharmacol Exp Ther*. 1972; 183:577–607. [PubMed: 4636393]
24. Gasteiger J, Marsili M. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. *Tetrahedron*. 1980; 36:3219–28.
25. Stone M. Cross-validatory choice and assessment of statistical predictions. *J R Stat Soc Series B Stat Methodol*. 1974; 36:111–47.
26. Meyer H. Theorie der alkoholnarkose. *Arch Exp Pathol Pharmacol*. 1899; 42:109–18.
27. Barker JL. Activity of CNS depressants related to hydrophobicity. *Nature*. 1974; 252:52–4. [PubMed: 4372540]
28. Peoples RW, Ren H. Inhibition of N-methyl-D-aspartate receptors by straight-chain diols: Implications for the mechanism of the alcohol cutoff effect. *Mol Pharmacol*. 2002; 61:169–76. [PubMed: 11752218]
29. Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA(A) receptor beta3 subunit. *FASEB J*. 2003; 17:250–2. [PubMed: 12475885]
30. Stewart DS, Pierce DW, Hotta M, Stern AT, Forman SA. Mutations at beta N265 in γ -aminobutyric acid type A receptors alter both binding affinity and efficacy of potent anesthetics. *PLoS One*. 2014; 9:e111470. [PubMed: 25347186]
31. Desai R, Ruesch D, Forman SA. Gamma-amino butyric acid type A receptor mutations at beta2N265 alter etomidate efficacy while preserving basal and agonist-dependent activity. *Anesthesiology*. 2009; 111:774–84. [PubMed: 19741491]
32. Gregoret LM, Rader SD, Fletterick RJ, Cohen FE. Hydrogen bonds involving sulfur atoms in proteins. *Proteins*. 1991; 9:99–107. [PubMed: 1755867]
33. Bertaccini EJ, Yoluk O, Lindahl ER, Trudell JR. Assessment of homology templates and an anesthetic binding site within the γ -aminobutyric acid receptor. *Anesthesiology*. 2013; 119:1087–95. [PubMed: 23770602]
34. Franks NP. Structural comparisons of ligand-gated ion channels in open, closed, and desensitized states identify a novel propofol-binding site on mammalian γ -aminobutyric acid type A receptors. *Anesthesiology*. 2015; 122:787–94. [PubMed: 25575161]
35. Bu W, Pereira LM, Eckenhoff RG, Yuki K. Stereoselectivity of isoflurane in adhesion molecule leukocyte function-associated antigen-1. *PLoS One*. 2014; 9:e96649. [PubMed: 24801074]
36. Shanmugasundararaj S, Zhou X, Neunzig J, Bernhardt R, Cotten JF, Ge R, Miller KW, Raines DE. Carboetomidate: An analog of etomidate that interacts weakly with 11 β -hydroxylase. *Anesth Analg*. 2013; 116:1249–56. [PubMed: 23492967]
37. Sear JW. What makes a molecule an anaesthetic? Studies on the mechanisms of anaesthesia using a physicochemical approach. *Br J Anaesth*. 2009; 103:50–60. [PubMed: 19435782]
38. Sewell JC, Sear JW. Derivation of preliminary three-dimensional pharmacophoric maps for chemically diverse intravenous general anaesthetics. *Br J Anaesth*. 2004; 92:45–53. [PubMed: 14665552]
39. Sewell JC, Raines DE, Eger EI 2nd, Laster MJ, Sear JW. A comparison of the molecular bases for N-methyl-D-aspartate-receptor inhibition versus immobilizing activities of volatile aromatic anesthetics. *Anesth Analg*. 2009; 108:168–75. [PubMed: 19095845]

Figure 1.

Chemical structures of etomidate analogues, their names, and their numerical designations. Top row shows the parent compounds etomidate and metomidate. Second row compounds are etomidate analogues with modifications around the chiral center. Third row compounds are pyrrole etomidate analogues. Fourth row compounds are fluorinated etomidate analogues.

Figure 2.

Chemical structures of etomidate esters, their names, and their numerical designations. The etomidate pharmacophore is displayed in the box (top). The varying spacer that links the metabolically labile ester moiety to the etomidate pharmacophore is shown below the box.

Figure 3.

Direct activation of $\alpha_1(\text{L264T})\beta_3\gamma_2$ γ -aminobutyric acid type A (GABA_A) receptors expressed in oocytes by R-isopropyl-methoxycarbonyl metomidate and S-isopropyl-methoxycarbonyl metomidate. (A) Electrophysiological traces recorded upon perfusing an oocyte expressing $\alpha_1(\text{L264T})\beta_3\gamma_2$ GABA_A receptors with 1 μM (top) or 10 μM (bottom) S- or R-isopropyl-methoxycarbonyl metomidate. The same oocyte was used to obtain all 4 traces. (B) S- and R-isopropyl-methoxycarbonyl metomidate concentration-response relationships for $\alpha_1(\text{L264T})\beta_3\gamma_2$ GABA_A receptor direct activation. Each data point is the mean value ($\pm$ SD) obtained from 6 different oocytes. In each panel, the chemical structures are shown as insets with the differences between analogues highlighted in red. The curves are fits of the two data sets to a Hill equation yielding half-maximal direct activating concentrations of $2.6 \pm 3 \mu\text{M}$ and $46 \pm 6 \mu\text{M}$ for S- and R-isopropyl-methoxycarbonyl metomidate, respectively ($p < 0.0001$).

Figure 4.

(A) Etomidate and carboetomidate concentration-response relationships for $\alpha_1(\text{L264T})\beta_3\gamma_2$ γ -aminobutyric acid type A (GABA_A) receptor direct activation. Each data point is the mean value ($\pm$ SD) obtained from 4 – 6 different oocytes. Data for etomidate was taken from reference ¹³. The curves are fits of the two data sets to a Hill equation yielding half-maximal direct activating concentrations of $1.83 \pm 0.28 \mu\text{M}$ and $13.8 \pm 0.9 \mu\text{M}$ for etomidate and carboetomidate, respectively ($p < 0.0001$). (B) Racemic and S- pentafluoroetomidate concentration-response relationships for $\alpha_1(\text{L264T})\beta_3\gamma_2$ GABA_A receptor direct activation. Each data point is the mean value ($\pm$ SD) obtained from 6 different oocytes. The curves are fits of the two data sets to a Hill equation yielding half-maximal direct activating concentrations of $7.6 \pm 0.6 \mu\text{M}$ and $166 \pm 25 \mu\text{M}$, respectively ($p < 0.0001$). In each panel, the chemical structures are shown as insets with the differences between analogues highlighted in red.

Figure 5. Relationship between the half-maximal direct activating concentration of etomidate and etomidate analogues (EC_{50}) and their octanol-water partition coefficients. The dashed line is a linear fit of the logarithm-transformed data. The slope of the fitted line was 0.13 ± 0.20 μM and the coefficient of determination (r^2) was 0.019. The name and structure of each numbered compound is given in Table 1.

Figure 6.

Generic structure for all etomidate analogues. The boxes highlight the location where the molecular structure of the analogues varied. Red boxes highlight structural elements found only in the etomidate ester series of compounds. The asterisks show carbons that were chiral centers in some of our compounds.

Figure 7.

Alignment of etomidate and etomidate analogues and comparative molecular field analysis contour maps for direct activation of $\alpha_1(L264T)\beta_3\gamma_2$ γ -aminobutyric acid type A (GABA_A) receptors. (A) Steric field contour map. The green contours represent regions where bulky substituents increase analogue potency whereas yellow contours represent regions where they decrease potency. (B) Electrostatic contour map. The blue contours represent regions where electropositive substituents increase analogue potency whereas red contours represent regions where electronegative substituents increase potency.

Figure 8.

Relationship between the half-maximal direct activating concentration (EC₅₀) predicted by our comparative molecular field analysis (CoMFA) model and that determined experimentally from our electrophysiological studies. The experimental EC₅₀ for each analogue was defined using $\alpha_1(\text{L264T})\beta_3\gamma_2$ γ -aminobutyric acid type A (GABA_A) receptors expressed in oocytes. The dashed line is a linear fit of the logarithm-transformed data. The slope of the fitted line was 0.912 ± 0.063 and the coefficient of determination (r^2) was 0.91. The name and structure of each numbered compound is given in Table 1.

Figure 9.

Alignment of etomidate and etomidate analogues and comparative molecular field analysis contour maps for loss of righting reflexes in rats. (A) Steric field contour map. The green contours represent regions where bulky substituents increase analogue potency whereas yellow contours represent regions where they decrease potency. (B) Electrostatic contour map. The blue contours represent regions where electropositive substituents increase analogue potency whereas red contours represent regions where electronegative substituents increase potency.

Figure 10.

Relationship between the median effective dose that produces loss of righting reflexes in rats (ED₅₀) predicted by our comparative molecular field analysis (CoMFA) model and that determined experimentally in rats. The dashed line is a linear fit of the logarithm-transformed data. The slope of the fitted line was 0.972 ± 0.036 and the coefficient of determination (r^2) was 0.97. The name and structure of each numbered compound is given in Table 1.

Figure 11.

Pharmacophore model of etomidate analogues overlaying the 5 compounds having the highest γ -aminobutyric acid type A (GABA_A) receptor potencies. The hydrophobic regions are blue, the aromatic regions are orange, the acceptor atom – donor site interactions are green. For the etomidate esters, there is also an acceptor atom – donor site interaction that is shown in purple. The dotted lines show interactions where a pharmacophore point on the ligand could be associated with one on the receptor binding site because the volume constraint of the latter also fell within the 1 Å limit. The distance between donor site 1 and donor site 2 is 9.36 Å.

Figure 12.

Pharmacophore model of etomidate analogues with the merged surface of the 5 compounds having the highest γ -aminobutyric acid type A (GABA_A) receptor potencies. The surface colors are based on lipophilic potential ranging from brown (highly lipophilic) to blue (highly hydrophilic), z-clipping was applied to the surface to match the GABA_A receptor comparative molecular field analysis contour maps. The dotted lines show interactions where a pharmacophore point on the ligand could be associated with one on the receptor binding site because the volume constraint of the latter also fell within the 1 Å limit.

Figure 13.

Relationship between *in vitro* γ -aminobutyric acid type A (GABA_A) receptor and *in vivo* hypnotic potencies of etomidate and etomidate analogues. Each compound's GABA_A receptor potency is defined as the half-maximal direct activating concentration (EC₅₀) and its hypnotic potency is defined as the median effective dose that produces loss of righting reflexes in rats (ED₅₀). The dashed line is a linear fit of the logarithm-transformed data. The slope of the fitted line was 1.22 ± 0.18 and the coefficient of determination (r^2) was 0.72.

Table 1

Molecular and Pharmacological Properties of Etomidate and Etomidate Analogues

Compound #	Compound Name	Molecular Weight (g/mol)	Molecular Volume [†] (Å)	Octanol:Buffer Partition Coefficient	GABA _A Receptor EC ₅₀ [#] (μM)	Hypnotic ED ₅₀ (mg/kg) [*]
1	Etomidate	244.29	239.89	731 ± 72 (13)	1.83 ± 0.28 (13)	0.47 ± 0.17 (13)
2	Metomidate	230.26	229.88	380 ± 48	4.4 ± 0.7	0.73 ± 0.50
3	S-etomidate	244.29	247.38	711 ± 90 (13)	57.0 ± 5.1 (13)	5.2 ± 0.9 (13)
4	Dihydrogen etomidate	230.26	224.38	388 ± 32 (13)	161 ± 13 (13)	5.2 ± 1 (13)
5	Cyclopropyletomidate	256.3	254.28	458 ± 45 (13)	67.6 ± 8.1 (13)	5.2 ± 0.7 (13)
6	Carboetomidate	243.3	251.06	15,000 ± 3,700 (20)	13.8 ± 0.9	7.7 ± 0.8 (18)
7	4-Pyridine carboetomidate	244.29	755.12	753 ± 63	335 ± 36	25.0 ± 0.3
8	Racemic pentafluoroetomidate	334.24	N/A	860 ± 260	7.6 ± 0.6	1.7 ± 0.4
9	S-pentafluoroetomidate	334.24	268.04	880 ± 220	167 ± 25	11.1 ± 0.8
10	R-pentafluoroetomidate	334.24	276.62	870 [‡]	3.8 [‡]	0.9 [‡]
11	Methoxycarbonyl metomidate	288.3	284.33	159 ± 15 (19)	38 ± 2	11.1 ± 0.8 (19)
12	S-methyl-methoxycarbonyl metomidate	302.33	294.77	330 ± 16 (19)	9.4 ± 0.8	3.5 ± 0.4 (19)
13	R-methyl-methoxycarbonyl metomidate	302.33	293.08	380 ± 15 (19)	8.2 ± 0.9	9.6 ± 1.9 (19)
14	Dimethyl-methoxycarbonyl metomidate	316.35	303.45	660 ± 110 (19)	1.6 ± 0.4	0.72 ± 0.16 (19)
15	Cyclopropyl methoxycarbonyl metomidate	314.34	302.96	420 ± 11 (19)	3.8 ± 0.4 (5)	0.69 ± 0.04 (19)
16	(S)-isopropyl-methoxycarbonyl metomidate	330.38	328.88	2,860 ± 67 (19)	2.6 ± 0.3	1.2 ± 0.2 (19)
17	(R)-isopropyl-methoxycarbonyl metomidate	330.38	337.5	3,830 ± 310 (19)	46 ± 6	3.6 ± 0.8 (19)
18	Methoxycarbonyl etomidate	302.33	309.63	190 ± 25 (19)	20 ± 2	5.3 ± 1.5 (19)

Compound #	Compound Name	Molecular Weight (g/mol)	Molecular Volume [†] (Å)	Octanol:Buffer Partition Coefficient	GABA _A Receptor EC ₅₀ [#] (μM)	Hypnotic ED ₅₀ [*] (mg/kg)
19	α-(S)-methyl-methoxycarbonyl etomidate	316.35	317.55	530 ± 170 (19)	11 ± 2	3.1 ± 0.4 (19)
20	α-(R)-methyl-methoxycarbonyl etomidate	316.35	310.3	670 ± 120 (19)	35 ± 12	5.2 ± 0.5 (19)
21	α-Dimethyl-methoxycarbonyl etomidate	330.38	328.51	2,240 ± 150 (19)	5.5 ± 0.6	2.4 ± 1 (19)
22	β-(R)-methyl-methoxycarbonyl etomidate	316.35	319.02	500 ± 24 (19)	11 ± 1	3.5 ± 0.6 (19)
23	β-Dimethyl-methoxycarbonyl etomidate	330.38	326.65	1,580 ± 40 (19)	1.7 ± 0.2	1.9 ± 0.3 (19)

[†] Van der waals volume calculated by SybylX2.1.1

[#] EC₅₀ = Concentration that evokes a peak current amplitude that is 50% of that produced by 100 μM GABA. Hill coefficients averaged 0.83 ± 0.22 (range: 0.539 – 1.54)

^{*} ED₅₀ = Dose that produces loss of righting reflexes in 50% of rats.

[†] Value estimated from studies using the racemic mixture and the S-enantiomer.
Numbers in parentheses are the references for previously published values.